Investigational New Drug

ALN-PCSSC

The Orion HoFH Study

An Open-Label, Single-Arm, Multicenter Pilot Study to Evaluate Safety, Tolerability, and Efficacy of ALN-PCSSC in Subjects with Homozygous Familial Hypercholesterolemia

Protocol No.: **MDCO-PCS- 16-02 "ORION-2"**
U.S. IND No.: **127589**
EudraCT No.: **2016-003376-49**
Amended Protocol Dated: **07 November 2016**
Original Protocol Dated: **02 September 2016**

Development Phase: II

Sponsor: The Medicines Company
8 Sylvan Way
Parsippany, NJ 07054

[redacted]

Issue Date: Date: 07 November, 2016

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This study will be conducted in compliance with Good Clinical Practice (GCP) and protection of the subject as required by the regulations and directives in operation at this time.

PROCEDURES IN CASE OF EMERGENCY

Emergency Contact Information

Role in Study	Name:	Telephone Number:
[redacted]		

PROTOCOL SYNOPSIS

Name of Sponsor/Company: The Medicines Company
Name of Finished Drug: ALN-PCS$_{SC}$
Name of Active Ingredient: ALN-60212
Title of Study: An open-label, single-arm, multicenter pilot study to evaluate safety, tolerability, and efficacy of ALN-PCS$_{SC}$ in subjects with homozygous familial hypercholesterolemia
Phase of Development: II
Study Centers: Multi-center study
Central Facilities: [redacted]
Number of Subjects: 8-10 subjects
Principal Investigator: [redacted]
Study Period: The estimated study period will be approximately 12 months from first subject enrolled in the study to last subject completed.

Objectives:
Primary
To characterize the effect of 90 and 180 days of subcutaneous ALN-PCS$_{SC}$ on the percentage change from Day 1 in low-density lipoprotein cholesterol (LDL-C) in subjects with homozygous familial hypercholesterolemia
 	
Secondary
– To assess the effect of ALN-PCS$_{SC}$ on:
  – Absolute change and percentage change in LDL-C from Day 1 to each subsequent visit until Day 180 or final visit
  – Absolute change and percentage change in PCSK9
  – Absolute change and percentage change in total cholesterol, triglycerides, HDL-C, non-HDL-C, VLDL-C, Apo-A1, Apo-B and Lp(a) from Day 1 to each subsequent visit until Day 180 or final visit
– To evaluate the safety and tolerability of ALN-PCS$_{SC}$ in subjects with homozygous familial hypercholesterolemia
 	
Exploratory:
– To evaluate the formation of Anti-drug antibodies (ADA) to ALN- PCS$_{SC}$
– To assess response of LDL-C by underlying causal mutations of homozygous familial hypercholesterolemia (HoFH)

Methodology: Hypotheses: The primary hypothesis is that ALN-PCS$_{SC}$, when used in combination with maximally tolerated statin therapy with or without ezetimibe, will be well-tolerated, will suppress PCSK9 synthesis assessed by the reduction in circulating PCSK9 >70% and will result in reduction of LDL-C, defined as mean percent change from Day 1 following 90 days and 180 days of treatment in subjects with homozygous familial hypercholesterolemia. This is a pilot study. If the trial meets a defined mean LDL-C reduction of ≥ 15% this will guide the decision to initiate a subsequent larger Phase III trial.

Study Design: This study will be a Phase II, open label, single arm, multicenter pilot study in subjects with homozygous familial hypercholesterolemia. Informed consent (and verbal assent if subject is < 18 years of age) will be obtained from subjects before the initiation of any study-specific procedures.

Subjects who meet study inclusion/exclusion criteria will be instructed to continue to follow a NCEP Adult Treatment Panel III (or comparable) diet [Appendix C] and be required to maintain their current lipid lowering drug therapy for the duration of the study.

In this study, 8-10 subjects will be enrolled and receive open label ALN-PCS$_{SC}$ 300mg SC. Dosing interval will be determined by PCSK9 level at Days 60 or 90 or rate of change of PCSK9 between Days 60 and 90.

On Day 1, eligible subjects will be enrolled and receive the first SC administration of ALN-PCS$_{SC}$. After first study drug administration, the subject will be observed in the clinic for at least 4 hours post injection before being discharged. Subjects will return at day 14 and day 30 and then at monthly intervals. If a second dose of study drug is deemed necessary (depending on the degree of suppression of serum PCSK9 as compared to baseline), subjects will receive this dose at Day 90 or 104, based on PCSK9 levels from the previous visit.

Study visits will include collection of AE and SAE data, vital signs, ECGs, concomitant medication, and laboratory tests. See Section 6.1 for a detailed list of assessments. The study also includes collection of biomarker (pharmacodynamics) samples for efficacy and, where approved by the independent ethics committee and/or institutional review board (IEC/IRB) and applicable regulatory and other authorities, all subjects will be invited to consent to pharmacogenetics analyses, unless underlying causal mutations of HoFH are well documented by a validated specialized laboratory.

Efficacy assessments will include measurement of the effects of ALN-PCS$_{SC}$ on levels of LDL-C, other lipoproteins including total cholesterol (TC), triglycerides, high-density lipoprotein cholesterol (HDL-C), non-HDL-C, very low-density lipoprotein cholesterol (VLDL-C), apolipoprotein A1 (Apo-A1), apolipoprotein B (Apo-B), lipoprotein(a) [Lp(a)], and PCSK9.

Formation of anti-drug antibodies (ADA) will be assessed on Day 1 (prior to and 4 hours after the injection) and on subsequent visits until the end-of-study (EOS).

The EOS visit and the last estimation of lipids will occur at Day 180. Following Day 180 subjects will continue in the study until the observed LDL-C reduction is less than 20% of the absolute reduction from baseline to Day 90. Subjects will be encouraged to complete all planned visits. The decision to initiate a Phase III study will be defined as an observed mean 15% or greater reduction in LDL-C. A number of factors may influence the proportion of subjects achieving this metric, in particular the specific LDL receptor mutation(s) or other causative mutations of HoFH studied. Given the small sample size, such issues will be taken into careful consideration prior to making the final decision.

The efficacy and safety/ tolerability data from this study will be reviewed by a Safety Committee (SC) on an ongoing basis, and the results will be used to guide the decision to initiate a phase III study. A recommendation may be taken to stop or amend the study at any of these reviews.

Diagnosis and Main Criteria for Selection:

Inclusion Criteria:

1. Males and females, ≥ 12 years of age with a diagnosis of homozygous familial hypercholesterolemia by genetic confirmation or a clinical diagnosis based on a history of an untreated LDL-C concentration >500 mg/dl (13 mmol/L) together with either xanthoma before 10 years of age or evidence of heterozygous familial hypercholesterolemia in both parents.
2. Stable on a low-fat diet
3. Stable on their pre-existing, lipid-lowering therapies (such as statins, cholesterol-absorption inhibitors, bile-acid sequestrants, or combinations thereof) for at least 4 weeks with no planned medication or dose change for the duration of study participation
4. Fasting central lab LDL-C concentration >130 mg/dl (3.4 mmol/L) and triglyceride concentration < 400 mg/dL (4.5 mmol/L),
5. Bodyweight of 40 kg or greater at screening.
6. Subjects should be willing and able to give written informed consent before initiation of any study-related procedures (if the subject is less than 18 years of age, written consent will be obtained from their guardian or legally authorized representative, with verbal assent from the child).

Exclusion criteria:

1. LDL or plasma apheresis within 8 weeks prior to the screening visit, and no plan to receive it during the study because of the attendant difficulty in maintaining stable concentrations of LDL-C while receiving apheresis.
2. Use of Mipomersen or Lomitapide therapy within 5 months of screening.
3. Previous treatment with monoclonal antibodies directed towards PCSK9 within 8 weeks of screening.
4. New York Heart Association (NYHA) class III or IV heart failure or last known left ventricular ejection fraction < 30% or any cardiac arrhythmia within past 3 months that is not controlled by medication.
5. Myocardial infarction, unstable angina, percutaneous coronary intervention (PCI), coronary artery bypass graft (CABG) or stroke within 3 months of enrollment
6. Planned cardiac surgery or revascularization
7. Uncontrolled severe hypertension: systolic blood pressure >180 mmHg or diastolic blood pressure >110 mmHg despite anti-hypertensive therapy.
8. Poorly controlled diabetes mellitus, i.e., glycated hemoglobin A1c (HbA1c) >10.0%.
9. Estimated glomerular filtration rate (eGFR) < 30 ml/min/1.73m^2
10. Active liver disease defined as any known current infectious, neoplastic, or metabolic pathology of the liver or unexplained alanine aminotransferase (ALT), aspartate aminotransferase (AST), elevation > 3x the upper limit of normal (ULN), at screening confirmed by a repeat measurement at least 1 week apart.
11. Creatine kinase (CK) > 5x ULN without a known cause
12. Other serious comorbid disease in which the life expectancy of the subject is shorter than the duration of the trial (e.g., acute systemic infection or other serious illnesses).
13. Any history of malignant disease, with the exception of treated basal-cell carcinoma occurring >5 years before screening.
14. Females who are pregnant or nursing, or who are of childbearing potential (includes adolescent females who have reached menarche and are sexually active) and unwilling to use at least two methods of contraception (e.g., oral contraceptives, barrier methods, approved contraceptive implant, long- term injectable contraception, intrauterine device) for the entire duration of the study. Exemptions from this criterion:
    a. Women >2 years postmenopausal (defined as 1 year or longer since their last menstrual period) AND more than 55 years of age
    b. Postmenopausal women (as defined above) and less than 55 years of age with a negative pregnancy test within 24 hours of enrollment
    c. Women who are surgically sterilized at least 3 months prior to enrollment
    d. Adolescent females who have not reached menarche

15. Males who are unwilling to use an acceptable method of birth control during the entire study period (e.g., condom with spermicide).
16. Known history of alcohol and/or drug abuse within 5 years.
17. Any condition that according to the investigator could interfere with the conduct of the study, such as but not limited to:
    a. Inappropriate for this study, including subjects who are unable to communicate or to cooperate with the investigator.
    b. Unable to understand the protocol requirements, instructions and study-related restrictions, the nature, scope, and possible consequences of the study (including subjects whose cooperation is doubtful due to drug abuse or alcohol dependency).
    c. Unlikely to comply with the protocol requirements, instructions, and study-related restrictions (e.g., uncooperative attitude, inability to return for follow-up visits, and improbability of completing the study).
    d. Have any medical or surgical condition, which in the opinion of the investigator would put the subject at increased risk from participating in the study.
    e. Involved with, or a relative of, someone directly involved in the conduct of the study.
18. Any clinically significant disease or condition affecting a major organ system, including but not limited to gastrointestinal, renal, hepatic, endocrinologic, pulmonary, neurological, metabolic or cardiovascular disease.
19. Any surgical or medical condition which, in the judgment of the Investigator, might interfere with the pharmacokinetics, distribution, metabolism, or excretion of the study drug (if applicable).
20. Treatment with other investigational medicinal products or devices within 30 days or 5 half-lives, whichever is longer, prior to the administration of the study drug, planned use of investigational medicinal products or devices.
21. Previous participation in this study or any preceding study with ALN-PCS$_{SC}$.
22. Hypersensitivity to any of the ingredients of the study drug being used.

Test Drug, Dose and Mode of Administration, Batch Number(s):

The study drug (ALN-PCS$_{SC}$) will be administered by subcutaneous (SC) injection as a formulation of ALN-60212. It will be supplied as a sterile 100 mg vial (200 mg/mL solution) for SC injection.

ALN-PCS$_{SC}$ will be administered as a single SC injection of 300mg on Day 1. If mean serum PCSK9 levels are not suppressed by > 70% at Day 60 or 90, as compared to baseline, a second dose of 300mg will be administered at the next visit at Day 90 or 104 respectively.

Duration of Treatment: The expected duration of the subjects' involvement in the study will be approximately 208 days, which includes screening, study drug administration, and the follow-up period through EOS. However additional follow-up visits may occur every 30 days until Day 300 for subjects whose LDL-C reduction is less than 20% of the absolute reduction from baseline to Day 90 (for a maximum duration up to 328 days). If ALN-PCS$_{SC}$ is found to be effective in lowering LDL-C in HoFH, a subsequent study may follow for subjects participating in the study.

Reference Therapy, Dose and Mode of Administration: There is no reference therapy in this study.

Criteria for Evaluation:

Efficacy:

Primary Endpoint: Percentage change in LDL-C.

Secondary Endpoint(s):

- Absolute change and percentage change in LDL-C
- Absolute change and percentage change in PCSK9 levels
- Absolute change and percentage change in other total cholesterol, triglycerides, HDL-C, non-HDL-C, VLDL-C, Apo-A1, Apo-B and Lp(a)

Exploratory Endpoint(s):

- Anti-drug antibodies (ADA) to ALN-PCS$_{SC}$
- Response of LDL-C reduction by underlying causal mutations of HoFH

Safety: AEs, SAEs, vital signs, clinical laboratory values (hematology, HbA1c, coagulation testing, biochemistry, high sensitivity C-reactive protein (hsCRP), and urinalysis), and electrocardiograms (ECGs) will be collected at specified visits through to the EOS visit. In addition, ADA will be evaluated for the study drug.

Statistical Methods:

The primary analysis set is the modified Intent-to-Treat set (mITT), which will include all enrolled subjects who have received at least 1 dose of study drug.

Unless specified otherwise, the mITT will be the default analysis set in this study.

Summary statistics for continuous variables will include the number of subjects, mean, median, standard deviation or standard error, minimum and maximum. For categorical variables, the frequency and percentage will be given.

The primary endpoint is the percent change from Day 1 in LDL-C following 90 and 180 days of treatment.

Summary statistics and 95% CI of this primary endpoint will be provided. Changes of LDL-C from Day 1 by scheduled visits will also be summarized. Response rate of subjects with 15% or greater reduction in LDL-C from Day 1 following 90 and 180 days of treatment will be calculated.

Safety summaries will include the incidence of adverse events, summaries of laboratory parameters, vital signs, ECGs and anti-drug antibodies.

Sample Size

The sample size was chosen based on clinical considerations.

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LIST OF ABBREVIATIONS

ADA	anti-drug antibodies
AE	adverse event
AESI	Adverse Event of Special Interest
ALP	alkaline phosphatase
ALT	alanine aminotransferase
Apo-A1	apolipoprotein A1
Apo-B	apolipoprotein B
aPTT	activated partial thromboplastin
ASCVD	atherosclerotic cardiovascular disease
AST	aspartate aminotransferase
BUN	total protein urea
CABG	coronary artery bypass graft
CHD	coronary heart disease
CK	creatine kinase
CRP	C-reactive protein
CTCAE	Common Terminology Criteria for Adverse Events
CV	Cardiovascular
dL	deciliter(s)
EC	Ethics Committee
ECG	electrocardiogram
eCRF	Electronic Case Report Form
ED50	effective dose of a drug for 50% of the population receiving the drug
ED80	effective dose of a drug for 80% of the population receiving the drug
EDC	electronic data capture
eGFR	estimated glomerular filtration rate
EOS	end of study
FDA	Food and Drug Administration
GalNAc	N-acetylgalactosamine
GCP	Good Clinical Practice
GGT	gamma glutamyl transferase
GLP	Good Laboratory Practice
GPV	Global Pharmacovigilance Department
HbA1c	glycated hemoglobin A1C
HDL-C	high density lipoprotein cholesterol
HoFH	homozygous familial hypercholesterolemia
hsCRP	high sensitivity C-reactive protein
IB	Investigator's Brochure
IC50	concentration of the drug required for 50% inhibition in vitro
ICH	International Conference on Harmonisation of Technical

	Requirements for Registration of Pharmaceuticals
IEC	Independent Ethics Committee
IFN-γ	interferon-gamma
IL6	interleukin 6
INR	International normalized ratio
IRB	Institutional Review Board
ISR	Injection site reaction
ITT	intent-to-treat
kg	kilograms(s)
LDL-C	low density lipoprotein cholesterol
LDLR	low density lipoprotein receptor
LNP	lipid nanoparticles
LP(a)	lipoprotein a
MCH	mean cell hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
MD	multiple dose
MDCO	The Medicines Company
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram(s)
MI	myocardial infarction
mITT	Modified intent-to-treat
mL	milliliter(s)
mm	millimeter(s)
mmHg	millimeters of mercury
mmol	Millimole
mRNA	messenger ribonucleic acid
NCEP	National Cholesterol Education Program
NHP	nonhuman primate
NOAEL	no observed adverse effect level
NYHA	New York Heart Association
PCI	percutaneous coronary intervention
PCS	potentially clinical significant
PCSK9	proprotein convertase subtilisin/kexin type 9
pH	-log[H+]
PT	prothrombin time
RBC	red blood cells
RNA	ribonucleic acid
RNAi	ribonucleic acid interference
SAD	single ascending dose

SAE	serious adverse event
SAP	Statistical Analysis Plan
SC	Safety Committee, subcutaneous
SD	standard deviation
siRNA	small interfering ribonucleic acid
TBIL	total bilirubin
TC	total cholesterol
TEAE	treatment emergent adverse event
TNF-α	tumor necrosis factor-alpha
TTR	Target transthyretin
ULN	upper limit of normal
USA	United States of America
VLDL-C	very low density lipoprotein cholesterol
WBC	white blood count
WHO	World Health Organization

1. INTRODUCTION

This protocol describes a study to assess the safety, tolerability and efficacy of ALN-PCSSC in subjects with homozygous familial hypercholesterolemia (HoFH).

This study will be conducted in compliance with Good Clinical Practices (GCP) including the Declaration of Helsinki and all applicable regulatory requirements.

1.1. Background

1.1.1. Disease Overview

According to the World Health Organization (WHO), atherosclerotic cardiovascular disease (ASCVD), comprised mainly of coronary heart disease (CHD) and stroke, is the leading cause of death worldwide, resulting in 17 million deaths annually [WHO Cardiovascular Statistics, 2011]. Data from the INTERHEART case-control study estimates that 45% of myocardial infarctions (MI) in Western Europe and 35% of myocardial infarctions in Central and Eastern Europe are due to abnormalities in blood lipids [Yusef et al, 2004]. In particular, elevated LDL-C has been shown in multiple studies to be one of the major risk factors for CHD with a continuous and graded relationship between plasma LDL-C concentration and CHD risk. For every 30 mg/dL (0.78 mmol/L) change in LDL-C, the relative risk for CHD changes by approximately 30% [Grundy et al, 2004]. In addition, a large meta-analysis of 21 statin studies concluded that for every 1 mmol/L (39 mg/dL) reduction in LDL-C (with statin therapy) there is an approximate 22% reduction in cardiovascular events [CTTC et al, 2010]. While statins are the treatment of choice for hyperlipidemia and the primary and secondary prevention of ASCVD there is still a need for additional lipid-lowering therapies for patients who do not reach target LDL-C levels or sufficient percent reductions in LDL-C to attenuate their ASCVD risk. Furthermore, in many patients, statin therapy cannot be optimized as patients are either intolerant of statins due to side effects (most commonly muscle pain, myopathy myositis) or because of other adverse effects such as elevations in liver enzymes. These limitations of contemporary therapy are particularly relevant among patients with pre-existing ASCVD such as diabetes, or patients with familial hypercholesterolemia who are at the highest risk of future cardiovascular (CV) events, and hence require the most intensive and aggressive management of hypercholesterolemia [Davidson et al, 2005; Nag et al, 2007; Reiner et al, 2011; Stone et al, 2014]. Among these high-risk subjects, less than 50% achieved the target LDL-C goal of <100 mg/dL at 6 months post-statin treatment despite close monitoring and drug regimen optimization [Foley et al, 2003; Kearney et al, 2008; CTTC et al, 2010; Foody et al, 2010]. Thus, there remains a clear unmet medical need for lowering LDL-C, especially in certain patient populations.

1.1.2. PCSK9 Biology and Target Rationale

Proprotein convertase subtilisin kexin type 9 (PCSK9) is a member of the subtilisin serine protease family. Proprotein convertase subtilisin kexin type 9 is predominantly expressed by the liver and is critical for the down regulation of hepatocyte low-density lipoprotein receptor (LDLR) expression [Mousavi et al, 2009]. LDL-C levels in plasma are markedly elevated in humans with gain of function mutations in PCSK9, classifying them as having severe familial

hypercholesterolemia [Abifadel et al, 2003]. Data from genetic association studies have identified loss of function alleles in human PCSK9 that result in lower PCSK9 protein levels and lower LDL-C levels [Zhao et al, 2006; Hooper et al, 2007; Horton et al, 2009]. In one published study, heterozygous individuals (carrying a single copy of a loss of function PCSK9 mutation) had significantly lower LDL-C with median levels of approximately 70 mg/dL (1.81 mmol/L) [Cohen et al, 2006]. Over a 15-year period of retrospective data analysis, this sustained lowering in LDL-C levels translated to an 88% lower risk of risk for CHD. Follow-up publications describe two adult individuals who are compound heterozygous for loss of function alleles of PCSK9. These individuals lack detectable plasma PCSK9 protein, have LDL-C levels ≤20 mg/dL, and yet are otherwise healthy [Zhao et al, 2006; Hooper et al, 2007]. Additionally, recent human clinical trials with PCSK9 blocking antibodies have shown significant lowering of LDL-C in healthy volunteers and across a range of high CV risk populations and with elevated LDL-C both with and without statins [Banerjee et al, 2012; Dias et al, 2012; Milazzo et al, 2012; Raal et al, 2012; Roth et al, 2012; Stein et al, 2012; Sullivan et al, 2012; Hooper et al, 2013]. Thus, the overall scientific and clinical data suggests that PCSK9 is a well-validated drug target whose inhibition results in significant LDL-C lowering without otherwise negatively impacting overall health, most recently culminating in the approval of two monoclonal agents to inhibit PCSK9.

1.1.3. Mechanism of RNA Interference

Ribonucleic acid (RNA) interference (RNAi) is a naturally occurring cellular mechanism for regulating gene expression that is mediated by small interfering RNAs (siRNAs). Typically, synthetic siRNAs are 19-base to 25-base pair double-stranded oligonucleotides in a staggered duplex with a two- to four-nucleotide overhang at one or both of the 3' ends. Such siRNAs can be designed to target an endogenous messenger RNA (mRNA) transcript of a given gene. When introduced into cells, the guide (or antisense) strand of the siRNA loads into an enzyme complex called the RNA-Induced Silencing Complex. This enzyme complex subsequently binds to its complementary mRNA sequence, mediating cleavage of the target mRNA and the suppression of the target protein encoded by the mRNA [Elbashir et al, 2001].

Since unmodified siRNAs are rapidly eliminated and do not achieve significant tissue distribution upon systemic administration [Soutschek et al, 2004], various formulations are currently used to target their distribution to tissues, and to facilitate uptake of siRNAs into the relevant cell type. One approach that has been used successfully in vivo, in animal models (including in rodents and nonhuman primates [NHP]) and humans employs intravenous delivery of siRNA in lipid nanoparticle (LNP) formulations [Soutschek et al, 2004; Morrissey et al, 2005; Geisbert et al, 2006; Judge et al, 2006; Zimmermann et al, 2006; Coelho et al, 2013; Tabernero et al, 2013]. Another approach for liver-specific gene silencing is subcutaneously administered siRNA conjugated to a N-acetylgalactosamine (GalNAc) carbohydrate ligand [Ashwell and Morell, 1974]. Conjugation of a triantennary GalNAc ligand to an siRNA enables hepatocyte binding and subsequent cellular uptake via the asialoglycoprotein receptor, resulting in engagement of the RNAi pathway and down regulation of hepatic proteins. Single and multiple doses of subcutaneously administered siRNA-GalNAc conjugates have been used to target transthyretin (TTR) mRNA for the treatment of TTR-mediated amyloidosis. ALN-TTRCSC has been found to be generally safe and well tolerated in Phase I and Phase II clinical trials in over 40 healthy volunteers and 18 subjects with familial amyloidotic cardiomyopathy and senile

systemic amyloidosis (ALN-TTRSC-001; EudraCT 2012-004203-12; and ALN-TTRSC-002; EudraCT 2013-002856-33).

1.2. ALN-PCS$_{SC}$, an RNAi Therapeutic for Hypercholesterolemia

ALN-PCS$_{SC}$ Solution for Injection (subcutaneous [SC] use) is comprised of the PCSK9 siRNA, ALN-60212, formulated in phosphate buffer. The PCSK9 siRNA is a chemically synthesized double stranded oligonucleotide covalently linked to a ligand containing GalNAc residues. This synthetic investigational RNAi therapeutic has been designed to suppress the liver production of PCSK9 when administered via SC injection. Inhibition of PCSK9 synthesis through an RNAi mechanism has the potential to lower tissue and circulating plasma PCSK9 protein levels, resulting in higher expression of LDLR in the liver, and consequently lower LDL-C levels in the blood stream. The initial proposed indication for ALN-PCS$_{SC}$ is the treatment of subjects with hypercholesterolemia who are not achieving therapeutic LDL-C goals despite maximally tolerated lipid-lowering therapy, or for subjects who are intolerant of statins.

1.2.1. Nonclinical Studies

The safety pharmacology and toxicology of ALN- PCS$_{SC}$ was evaluated in a series of in vitro and in vivo nonclinical studies.

The drug substance in ALN-PCS$_{SC}$ (ALN-60212) is designed to match the human and cynomolgous monkey mRNA transcripts for PCSK9, sharing a substantial partial match to the rat PCSK9 mRNA. ALN-60212 was informatically identified from a large collection of possible siRNAs targeting PCSK9 based on its predicted potency and selectivity. ALN-PCS$_{SC}$ reduced the expression of PCSK9 in Hep3B liver cells with an IC$_{50}$ of 20pM and inhibited hPCSK9 serum protein levels in a Tg mouse model with a single dose ED$_{50}$ of approximately 2 mg/kg and an ED$_{80}$ dose of approximately 6 mg/kg. In single and multi-dose regimen studies in cynomolgus monkeys, ALN-PCS$_{SC}$ exhibited dose-dependent sustainable suppression of PCSK9 protein that was paralleled by lowering of serum LDL-C with the same kinetics. The time to reach PCSK9 and LDL-C nadir was approximately 20 days and there was no difference in time to nadir between different dose levels. In single dose studies the maximal mean PCSK9 and LDL-C inhibition was 85% and 68%, respectively, which was observed at the two highest doses of ALN-PCS$_{SC}$ administered (6 and 10 mg/kg); however, the duration of PCSK9 silencing and LDL-C lowering was markedly extended at the higher 10 mg/kg dose. In multi-dose studies the maximal mean PCSK9 and LDL-C inhibition was 93% and 74%, respectively, which was similar to those observed in the single dose study. Following discontinuation of ALN-PCS$_{SC}$ administration, recovery of PCSK9 levels was slow, returning to baseline after 100 days in the single dose study and only showing partial recovery between doses in the multi-dose regimen. There were no ALN-PCS$_{SC}$ related changes to the levels of HDL-C or triglycerides in any of the groups. As expected, total cholesterol levels were reduced by up to 30% reflecting the decrease in LDL-C.

Good Laboratory Practice (GLP) compliant repeat dose studies of 4 and 15 weeks duration have been conducted in rats and monkeys. These studies included toxicokinetic analysis of ALN-PCS$_{SC}$ in plasma and in tissues including the liver which is the target organ, and the kidney which is the main organ of elimination.

The GLP 4 week toxicology studies in rats and monkeys involved every other week administration of ALN-PCS$_{SC}$ at dose levels of 10, 50 and 250 mg/kg and once a week administration of 10 mg/kg for rats and 30 mg/kg for monkeys. The GLP 15 week toxicology studies in rats and monkeys involved once monthly administration of ALN-PCS$_{SC}$ at dose levels of 10, 50 and 250 mg/kg and once every other week administration of 125 mg/kg for rats and 25 mg/kg for monkeys. Each toxicology study also included an 8 week treatment free recovery period for all dose groups. ALN-PCS$_{SC}$ was well tolerated in all studies and there were no dose limiting toxicities. The most common findings were related to the pharmacological effects of ALN-PCS$_{SC}$ on lipid profiles. There were consistent decreases in low density lipoprotein cholesterol (LDL-C) and total cholesterol which were expected. Histopathological findings included vacuolation in hepatocytes of rats and lymph node macrophages of monkeys and the presence of basophilic granules in hepatocytes of monkeys and kidneys of rats. These microscopic findings were not associated with changes in clinical pathology parameters and are consistent with class effects of oligonucleotides and were not considered adverse. In a non-GLP dose range finding (DRF) study conducted in monkeys, ALN-PCS$_{SC}$ did not stimulate pro-inflammatory cytokines, activate complement or impact coagulation. ALN-PCS$_{SC}$ also did not stimulate pro-inflammatory cytokines following single dose administration to mice. In a cardiovascular and respiratory study in telemetered conscious cynomolgus monkeys ALN-PCS$_{SC}$ had no immediate or delayed effects on clinical observations, qualitative or quantitative ECG parameters, hemodynamic parameters, respiration rate, or body temperature at any dose level. In addition, ALN-PCS$_{SC}$ did not induce gene mutations or chromosomal damage in a battery of in vitro and in vivo genotoxicity studies.

Further information is in the Investigator's Brochure (IB).

1.2.2. Clinical Studies

One Phase I study has been conducted to date (Study ALN-PCS$_{SC}$ -001). This was a randomized, single-blind, placebo-controlled, single-dose escalation and multiple-dose study of ALN-PCS$_{SC}$ administered SC to subjects with elevated LDL-C. The study was conducted in two phases: a single ascending dose (SAD) phase and a multiple dose (MD) phase. During the SAD phase, 24 subjects were assigned to either receive placebo or one of five doses of ALN-PCS ranging from 25 mg to 800 mg. Those who received doses of at least 100 mg saw their LDL-C drop at least 40%; at the 500 mg dose, LDL-C levels dropped as much as 78%. At 140 days after the treatment was given, subjects still had an average LDL-C reduction of about 40%.

In the MD phase, 45 subjects received multiple doses of either ALN-PCS (125mg weekly x4, 250 mg bi-weekly x2, 300 mg and 500 mg twice given 1 month apart) or placebo. These subjects had maximal LDL-C reductions of 80% and average LDL-C reductions of 50% to 60%. To date, the drug appears to be generally safe and well tolerated. One subject on statin comedication had elevated liver enzymes with ALT >4x the upper limit of normal (ULN), which resolved on stopping the statin.

One Phase II dose finding study (MDCO-PCS-15-01) is currently ongoing. This study is a placebo-controlled, double-blind, randomized trial to compare the effect of different doses of ALN-PCS$_{SC}$ given as single or multiple subcutaneous injections in subjects with high cardiovascular risk and elevated low-density lipoprotein cholesterol (LDL-C). Randomization has been completed and follow up is ongoing. An interim analysis will be performed after all

patients have completed the Day 90 visit in order to select the dose for subsequent studies. A regular review of safety is performed by an independent Data Monitoring Committee and to date no safety concerns have been reported and no changes to the protocol have been requested.

1.2.3. Known and Potential Risks and Benefits

Subjects taking part in this clinical study will receive guideline recommended standard of care as background therapy (including maximally-tolerated statin therapy and/or other LDL-C lowering therapies) when administered ALN-PCS$_{SC}$. Reduction of LDL-C has been associated with reduced CV risk both by epidemiology and in controlled clinical trials. The safety profile observed to date is considered acceptable for this clinical trial.

An expanded risk-benefit summary is provided in the IB.

1.3. Study Rationale

Homozygous Familial Hypercholesterolemia (HoFH) is a rare and life-threatening disease characterized by markedly elevated circulating levels of low-density lipoprotein cholesterol (LDL-C) and accelerated, premature atherosclerotic cardiovascular disease (ACVD). The first major CV events often occur during adolescence, although angina pectoris, myocardial infarction and death have been reported in early childhood. Historically thought to affect one in a million, new research indicates that HoFH prevalence is likely to be higher, with as many as one in 160,000 to 300,000 people affected. There may be as many as 40,000 people world-wide with HoFH. Due to the severity of the condition, early diagnosis and initiation of lipid-lowering treatment is critical. However, even at the highest doses of the most efficacious statins, only modest reductions in LDL C plasma levels, of 10-25%, are observed. Combination of statin plus ezetimibe can produce a further decrease in LDL-C levels of 10-15%. Combination with other lipid-lowering treatments such as ALN- PCS$_{SC}$ may therefore provide an important adjunctive therapy in HoFH [Cuchel et al 2014].

Previous studies using PCSK9-targeting siRNAs formulated in LNPs (ALN-PCS02) or using PCSK9 antibodies, and one Phase I study with ALN-PCS$_{SC}$ in which subjects received single-doses ascending from 25 mg to 800 mg, have demonstrated that substantial lowering of PCSK9 is safe and well-tolerated in humans. Doses for the Phase I study were calculated using the rat and monkey NOAELs based on body weight and body surface area (mg/m^2). Collectively, the results of nonclinical studies with ALN-PCS$_{SC}$ supported a starting dose of 25 mg for subjects participating in the SAD phase of the previous Phase I study.

In Phase I Study ALN-PCS$_{SC}$-001, clinically significant reductions in LDL-C levels were seen with single ALN-PCS$_{SC}$ doses as low as 25 mg with larger decreases seen with higher doses and a plateau at 300 mg. Subjects at ALN-PCS$_{SC}$ doses of 300 mg or higher had maximal LDL-C reductions of up to 78.1% and average least squares mean group nadir reductions of 50 to 59%. In the MD phase, 45 subjects received multiple doses of either ALN-PCS (125 mg weekly x4, 250 mg biweekly, 300 mg and 500 mg twice given 1 month apart) or placebo. Subjects at ALN-PCS$_{SC}$ doses of 300 mg or higher had maximal LDL-C reductions of 83% and average least squares mean group nadir LDL-C reductions of 53.4% to 59.9% at all dose levels tested.

Based on the interim results of the Phase I Study ALN-PCS$_{SC}$ -001, where maximum effect on LDL-C was seen by 84 days and a significant effect was still observed at 180 days, this study has

been designed to test the efficacy and duration of effect of 300mg of ALN- PCS$_{SC}$ in subjects with HoFH. This will allow dose selection for a subsequent Phase III study in HoFH. The dosing regimens used in this study are therefore fully supported by the findings of the Phase I study.

1.4. Study Population

This study will enroll male and female subjects, ≥ 12 years of age with a diagnosis of homozygous familial hypercholesterolemia.

2. TRIAL OBJECTIVES AND PURPOSE

2.1. Primary Objective(s)

To characterize the effect of 90 and 180 days of subcutaneous ALN-PCS$_{SC}$ on the percentage change from Day 1 in low-density lipoprotein cholesterol (LDL-C) in subjects with homozygous familial hypercholesterolemia

2.2. Secondary Objective(s)

- To assess the effect of ALN-PCS$_{SC}$ on:
  - Absolute change and percentage change in LDL-C from Day 1 to each subsequent visit until Day 180 or final visit
  - Absolute change and percentage change in PCSK9
  - Absolute change and percentage change in total cholesterol, triglycerides, HDL-C, non-HDL-C, VLDL-C, Apo-A1 Apo-B and Lp(a) from Day 1 to each subsequent visit until Day 180 or final visit
- To evaluate the safety and tolerability of ALN-PCS$_{SC}$ in subjects with homozygous familial hypercholesterolemia

2.3. Exploratory Objective(s)

- To evaluate the formation of Anti-drug antibodies (ADA) to ALN-PCS$_{SC}$
- To assess response of LDL-C by underlying causal mutations of HoFH

3. TRIAL DESIGN

3.1. Type/Design of Trial

This study will be a Phase II, open label, single arm, multicenter pilot study in subjects with homozygous familial hypercholesterolemia. Informed consent (and verbal assent if subject is < 18 years of age) will be obtained from subjects before the initiation of any study-specific procedures.

Subjects who meet study inclusion/exclusion criteria will be instructed to continue to follow a National Cholesterol Education Program (NCEP) Adult Treatment Panel III (or comparable) diet [Appendix C] and will be required to maintain their current lipid lowering drug therapy for the duration of the study.

In this study, 8-10 subjects will be enrolled and receive open label ALN-PCS$_{SC}$ 300mg SC. Dosing interval will be determined by PCSK9 level at Day 60 or 90 or rate of change of PCSK9 between Days 60 and 90.

On Day 1, eligible subjects will be enrolled and receive the first SC administration of ALN-PCS$_{SC}$. After first study drug administration, the subject will be observed in the clinic for at least 4 hours post injection before being discharged. Subjects will return at Day 14 and Day 30 and then at monthly intervals. If a second dose of study drug is deemed necessary (if mean serum PCSK9 levels are not suppressed by > 70% at Day 60 or 90, as compared to baseline), subjects will receive this dose at Day 90 or 104 respectively, based on PCSK9 levels from the previous visit.

Study visits will include collection of AE and SAE data, vital signs, ECGs, concomitant medication, and laboratory tests. See Section 6.1 for a detailed list of assessments. The study also includes collection of biomarker samples and, where approved by the independent ethics committee and/or institutional review board (IEC/IRB) and applicable regulatory and other authorities, all subjects will be invited to consent to pharmacogenetics analyses, unless underlying causal mutations of HoFH are well documented by a validated specialized laboratory.

Efficacy assessments will include measurement of the effects of ALN-PCS$_{SC}$ on levels of LDL-C, other lipoproteins including total cholesterol (TC), triglycerides, high-density lipoprotein cholesterol (HDL-C), non-HDL-C, very low-density lipoprotein cholesterol (VLDL-C), apolipoprotein A1 (Apo-A1), apolipoprotein B (Apo-B), lipoprotein(a) [Lp(a)], and PCSK9.

Formation of anti-drug antibodies (ADA) will be assessed on Day 1 (prior to and 4 hours after the injection) and on subsequent visits until the end-of-study (EOS).

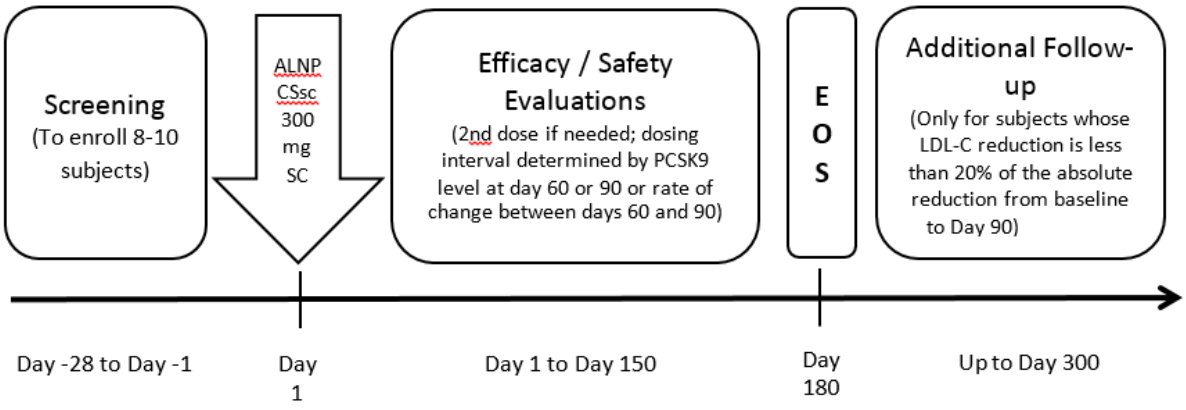
The EOS visit and the last estimation of lipids will occur at Day 180. Following Day 180 subjects will continue in the study until the observed LDL-C reduction is less than 20% of the absolute reduction from baseline to Day 90. Subjects will be encouraged to complete all planned visits.

The decision to initiate a Phase III study will be defined as an observed mean 15% or greater reduction in LDL-C. A number of factors may influence the proportion of subjects achieving this metric, in particular the specific LDL receptor mutation(s) or other causative mutations of HoFH

studied. Given the small sample size for this part of the study, such issues will be taken into careful consideration prior to making the final decision.

The efficacy and safety/ tolerability data from this study will be reviewed by a Safety Committee (SC) on an ongoing basis, and the results will be used to guide the decision to initiate a Phase III study. A recommendation may be taken to stop or amend the study at any of these reviews.

3.2. Schematic Diagram of Trial Design



3.3. Primary Endpoint

The primary endpoint of this trial is percentage change in LDL-C.

3.4. Secondary Endpoint(s)

The secondary endpoints of this trial are:

- Absolute change and percentage change in LDL-C
- Absolute change and percentage change in PCSK9 levels
- Absolute change and percentage change in total cholesterol, triglycerides, HDL-C, non-HDL-C, VLDL-C, Apo-A1, Apo-B, and Lp(a)

3.5. Exploratory Endpoint(s)

The exploratory endpoints of this trial are:

- Anti-drug antibodies (ADA) to ALN-PCS$_{SC}$
- Response of LDL-C reduction by underlying causal mutations of HoFH

3.6. Measures to Minimize/Avoid Bias

This is an unblinded, open label study. The primary endpoint is based on an assessment of LDL-C, which is a measurement that is not likely to be subject to interpretation bias.

3.7. Safety Committee (SC)

A Safety Committee (SC) will review efficacy and safety data and serious adverse events on an ongoing basis, and the results will be used to guide the decision to initiate a Phase III study. A recommendation may be taken to stop or amend the study at any of these reviews.

4. SUBJECT POPULATION

4.1. Number of Subjects

In this study, 8-10 subjects will be enrolled at 2-5 multi-national centers.

4.2. Inclusion Criteria

Subjects may be included in the study if they meet all of the following criteria:

1. Males and females, ≥ 12 years of age with a diagnosis of homozygous familial hypercholesterolemia by genetic confirmation or a clinical diagnosis based on a history of an untreated LDL-C concentration >500 mg/dl (13 mmol/L) together with either xanthoma before 10 years of age or evidence of heterozygous familial hypercholesterolemia in both parents.
2. Stable on a low-fat diet.
3. Stable on their pre-existing, lipid-lowering therapies (such as statins, cholesterol-absorption inhibitors, bile-acid sequestrants, or combinations thereof) for at least 4 weeks with no planned medication or dose change for the duration of study participation.
4. Fasting central lab LDL-C concentration >130 mg/dl (3.4 mmol/L) and triglyceride concentration < 400 mg/dL (4.5 mmol/L).
5. Bodyweight of 40 kg or greater at screening.
6. Subjects should be willing and able to give written informed consent before initiation of any study-related procedures (if the subject is less than 18 years of age, written consent will be obtained from their guardian or legally authorized representative, with verbal assent from the child). The subject should be willing to comply with all required study procedures.

4.3. Exclusion Criteria

Subjects will be excluded from the study if any of the following exclusion criteria apply prior to enrollment:

1. LDL or plasma apheresis within 8 weeks prior to the screening visit, and no plan to receive it during the study because of the attendant difficulty in maintaining stable concentrations of LDL-C while receiving apheresis.
2. Use of Mipomersen or Lomitapide therapy within 5 months of screening.
3. Previous treatment with monoclonal antibodies directed towards PCSK9 within 8 weeks of screening.
4. New York Heart Association (NYHA) class III or IV heart failure or last known left ventricular ejection fraction < 30% or any cardiac arrhythmia within past 3 months that is not controlled by medication.
5. Myocardial infarction, unstable angina, percutaneous coronary intervention (PCI), coronary artery bypass graft (CABG) or stroke within 3 months of enrollment.

6. Planned cardiac surgery or revascularization.
7. Uncontrolled severe hypertension: systolic blood pressure >180 mmHg or diastolic blood pressure >110 mmHg prior to enrollment despite anti-hypertensive therapy.
8. Poorly controlled diabetes mellitus, i.e., glycated hemoglobin A1c (HbA1c) >10.0% prior to enrollment.
9. Estimated glomerular filtration rate (eGFR) < 30 ml/min/1.73m2.
10. Active liver disease defined as any known current infectious, neoplastic, or metabolic pathology of the liver or unexplained alanine aminotransferase (ALT), aspartate aminotransferase (AST), elevation > 3x the upper limit of normal (ULN), at screening confirmed by a repeat measurement at least 1 week apart.
11. Creatine kinase (CK) > 5x ULN without a known cause.
12. Other serious comorbid disease in which the life expectancy of the subject is shorter than the duration of the trial (e.g., acute systemic infection, or other serious illnesses).
13. Any history of malignant disease, with the exception of treated basal-cell carcinoma occurring >5 years before screening.
14. Females who are pregnant or nursing, or who are of childbearing potential (includes adolescent females who have reached menarche and are sexually active) and unwilling to use at least two methods of contraception (e.g., oral contraceptives, barrier methods, approved contraceptive implant, long- term injectable contraception, intrauterine device) for the entire duration of the study. Exemptions from this criterion:
    a. Women >2 years postmenopausal (defined as 1 year or longer since their last menstrual period) AND more than 55 years of age
    b. Postmenopausal women (as defined above) and less than 55 years old with a negative pregnancy test within 24 hours of enrollment
    c. Women who are surgically sterilized at least 3 months prior to enrollment
    d. Adolescent females who have not reached menarche
15. Males who are unwilling to use an acceptable method of birth control during the entire study period (e.g., condom with spermicide).
16. Known history of alcohol and/or drug abuse within 5 years.
17. Any condition that according to the investigator could interfere with the conduct of the study, such as but not limited to:
    a. Inappropriate for this study, including subjects who are unable to communicate or to cooperate with the investigator.
    b. Unable to understand the protocol requirements, instructions and study-related restrictions, the nature, scope, and possible consequences of the study (including subjects whose cooperation is doubtful due to drug abuse or alcohol dependency).
    c. Unlikely to comply with the protocol requirements, instructions, and study-related restrictions (e.g., uncooperative attitude, inability to return for follow-up visits, and improbability of completing the study).

d. Have any medical or surgical condition, which in the opinion of the investigator would put the subject at increased risk from participating in the study.
e. Involved with, or a relative of, someone directly involved in the conduct of the study.

18. Any clinically significant disease or condition affecting a major organ system, including but not limited to gastrointestinal, renal, hepatic, endocrinologic, pulmonary, neurological, metabolic or cardiovascular disease.
19. Any surgical or medical condition which, in the judgment of the Investigator, might interfere with the pharmacokinetics, distribution, metabolism, or excretion of the study drug (if applicable).
20. Treatment with other investigational medicinal products or devices within 30 days or 5 half-lives, whichever is longer, prior to the administration of the study drug/device, planned use of investigational medicinal products or devices.
21. Previous participation in this study or any preceding study with ALN-PCS$_{SC}$.
22. Hypersensitivity to any of the ingredients of the study drug being used.

Subjects excluded for any of the above reasons may not be re-screened for participation at any time if the exclusion characteristic has changed.

4.4. Withdrawal Criteria

All subjects have the right to withdraw at any point during treatment without prejudice. The investigator can discontinue any subject at any time if medically necessary. It will be documented whether or not each subject completed the clinical study. If for any subject study treatment or observations were discontinued, the reason will be recorded and the Sponsor should be notified promptly. Reasons that a subject may discontinue participation in a clinical study are considered to constitute one of the following:

- Adverse event(s)
- Death
- Subject withdrew consent
- Physician decision
- Lost to follow-up

Upon occurrence of a serious or intolerable AE, the investigator or designee will make every possible attempt to confer with the Sponsor and the SC before discontinuing the subject.

It is imperative to obtain complete follow-up data for all subjects whether or not they receive their study treatment or have discontinued ALN-PCS$_{SC}$. All data collected up until the time of subject withdrawal is to be entered into the electronic case report form (eCRF). In addition, every attempt should be made to collect follow-up information except for those subjects who specifically withdraw consent to release of such information. All procedures and laboratory specimens or tests requested for evaluation following administration of ALN-PCS$_{SC}$ should be carried out when possible whether or not a subject continues to receive treatment according the protocol. Subjects will not be replaced in this trial.

4.4.1. Withdrawal from Study Medication

In the event a subject withdraws or is withdrawn from the study medication (eg, receives first injection and not second injection if indicated), the investigator will inform the Medical Monitor and the Sponsor immediately. If there is a medical reason for withdrawal, the subject will remain under the supervision of the investigator for protocol-specified safety follow up procedures. The SC may be notified.

It is imperative to obtain complete follow-up data for all enrolled subjects whether or not they receive their treatment or have discontinued study drug.

5. TREATMENT OF SUBJECTS

5.1. Study Medication

5.1.1. ALN-PCSSC

ALN-PCSSC is a synthetic, chemically modified small interfering ribonucleic acid (siRNA) targeting proprotein convertase subtilisin kexin type 9 (PCSK9) messenger ribonucleic acid (mRNA [ALN-60212]) with a covalently attached triantennary N-acetylgalactosamine (GalNAc) ligand. Study drug (ALN-PCSSC) information is described below.

Table 1: Investigational Product

Product Name:	ALN-PCSSC
Dosage Form:	Solution for Injection
Unit Dose	100 mg vial (200 mg/mL)
Route of Administration	SC use
Physical Description	Clear, colorless to pale yellow solution essentially free of particulates
[redacted]	

Study drug preparation: The pharmacist or designee will prepare the study drug under aseptic conditions. On study drug dosing days, the pharmacist or designee will withdraw the required amount of study drug into one syringe to be administered to the subject on that day. The procedure for preparing study drug and the volume to be loaded into the syringe is provided in the Pharmacy Manual.

Study drug administration: Subjects will be administered ALN-PCSSC 300mg by SC injection Study drug injection will be administered by qualified clinical study site staff under the supervision of the investigator or designee and the injection site will be marked and mapped for later observation. The site of injection is the abdomen. If more than one injection is necessary, the first on Day 1 should be on one side of the abdomen and the second on Day 90 or 104 on the opposite side of the abdomen. Do not inject into areas of active skin disease or injury such as sunburns, skin rashes, inflammation, or skin infections.

If a local reaction around the injection site occurs, photographs of the injection site should be obtained at first presentation and at each of the follow-up visits until the injection site reaction resolves. Injection site reactions must be reported as described in Section 8.2.3. Detailed instructions for study drug administration are found in the Pharmacy Manual.

5.1.2. Packaging and Labeling

Study drug will be provided by Catalent Pharma Solutions. Medication labels will comply with regulatory requirements. The storage conditions for the study drug will be described on the medication label.

The ALN-PCS$_{SC}$ Solution for Injection (subcutaneous use) is packaged in 2 mL glass vials with a fill volume of no less than 0.55 mL. The container closure system consists of a Type I glass vial, a Teflon-faced 13 mm stopper, and a flip-off aluminum seal.

5.1.3. Storage

ALN-PCS$_{SC}$ will be stored in a secure refrigerator or at the appropriate conditions as specified in the Pharmacy Manual. Access should be strictly limited to the investigator, pharmacists, and their designees. No special procedures for the safe handling of ALN-PCS$_{SC}$ are required.

5.1.4. Accountability

The investigator or designee must maintain an inventory record of study drug received and administered to assure the regulatory authorities and the Sponsor that the study drug will not be dispensed to any person who is not a subject under the terms and conditions set forth in this protocol. Study drug accountability forms and/or specific instructions can be found in the Pharmacy Manual.

The study drug supplied for use in this study is to be prescribed only by the principal investigator or designated sub-investigators and may not be used for any purpose other than that outlined in this protocol.

During the study, all used study drug containers (eg, empty vials/bottles) will be kept until the monitor has reviewed the accountability records.

All unused study drug will be destroyed on site (or returned to the packaging and labeling facility for destruction if destruction on site is not possible) once the study drug has been inventoried and the monitor has reviewed the accountability records. In the event that the study drug needs to be returned for any other reason, the site will receive a written request listing the drug lot number(s) to be returned and the reason for the return request.

5.1.5. Product Complaints

Sites are required to report any product complaints to MDCO immediately but no later than 24 hours from the time of awareness, by phone or e-mail as follows:

Product Complaint: Is defined as any written, electronic, or oral communication that alleges deficiencies related to the identity, durability, reliability, quality, safety, effectiveness or performance of a product, after it is released for distribution (EU DIR 2001/83/EC). (Derived from Ref US 21 CFR 211.198)

There are two types of Product Complaints:

Technical Quality Complaints: A report of dissatisfaction with product with regard to its efficacy, strength, integrity, purity, or quality; thus a potential failure to meet product specifications. Examples include:

- An indication that there is an unexpected physical change in the drug product such as discoloration, change in shape of the drug product, presence of particulates or any other physical change that might indicate contamination, a manufacturing defect or any other event that might indicate a compromise in product quality.
- An indication that the content does not meet its labeled volume, count, etc.
- An indication that there is an unexpected physical change in any part of the container (this includes the bottle, any part of the seal, the cap or the label).
- An indication that the product is mislabeled.
- An indication that there is an unexpected physical change of the product or container once the product is diluted or reconstituted (the container includes the vial, bag, IV line, syringe or any other item that is in contact with the product).
- An indication that the product is falsified, tampered with or adulterated.
- An indication that the product did not meet its pharmacologic effect, i.e. lack of efficacy.
- Medical Device Incidents

Preference Complaints: A report of dissatisfaction with service, delivery, packaging or other preference.

5.2. Concomitant Medications

5.2.1. Required Concomitant Medication(s) and Diet

Subjects who meet inclusion/exclusion criteria will be instructed to continue to follow a NCEP Adult Treatment Panel III (or comparable) diet and be required to maintain their current lipid lowering drug therapy for the duration of the study.

5.2.2. Prohibited Concomitant Medications

The following medications/treatments are not permitted to be added during the study:

- Medications used to lower LDL-C (eg, statins, ezetimibe, lomitapide, mipomersen, niacin, colesevelam, bile acid absorption inhibitors, monoclonal antibodies directed towards PCSK9).

5.2.3. Permitted Concomitant Medication(s)

The following medications/treatments are permitted during the study:

- Hormone replacement therapy
- Lipid-lower medications; subjects on lipid-lower medications (such as statins and/or ezetimibe) should be on a stable dose for ≥30 days before screening with no planned medication or dose change during study participation
- Prescription medications prescribed to treat pre-existing medical conditions such as diabetes and hypertension
- Prescription or nonprescription medications, when necessary to treat an AE, at the discretion of the investigator

5.3. Medical Management Guidelines

If a local reaction around the injection site occurs, photographs of the injection site should be obtained at first presentation and at each of the follow-up visits until the injection site reaction resolves. Injection site reactions must be reported as described in Section 8.2.3. Detailed instructions for study drug administration are found in the Pharmacy Manual.

5.4. Restrictions

Subjects will have to comply with the following restrictions during the study:

- Fasted for at least 8 hours for all visits for fasting lipids and glucose blood samples
- Blood donation will not be allowed at any time during the study
- Must refrain from unaccustomed strenuous physical exercise for 48 hours before the screening and any study visit until the follow-up has been completed

5.5. Blinding

Not applicable, this is not a blinded study.

6. SCHEDULE AND SEQUENCE OF PROCEDURES

The Schedule of Events/Assessments (Section 6.1) summarizes the study assessments by time point.

This study consists of four periods: Screening, Treatment, End of Study and Additional Follow-up.

- The Screening period occurs prior to administration of study drug and consists of confirming eligibility.
- The Treatment period occurs from the time of enrolling the subject, collecting Day 1 assessments and study drug administration through Day 90/104.
- The End of Study visit occurs at Day 180.
- The Additional Follow-up period (if necessary) occurs from end of study through Day 300.
- The maximum duration of a subject's participation is from screening/informed consent to end of study (last follow-up) and is expected to be up to 328 days.

6.1. Schedule of Events/Assessments

	Screening	Treatment Phase								End of Study (EOS)	Additional Follow-Up [p]
Study Day	**-28 to -1**	**Day 1**	**FU1 Day 14 (± 2d)**	**FU2 Day 30 (± 3d)**	**FU3 Day 60 (± 3d)**	**FU4 Day 90 (± 3d) Dose 2? [a]**	**FU4X Day 104 (± 3d) Dose 2? [a]**	**FU5 Day 120 (± 3d)**	**FU6 Day 150 (± 3d)**	**FU7 Day 180 (± 3d)**	**FU8 (Day 210) (± 3) FU9 (Day 240) (± 3) FU10 (Day 270) (± 3) FU11 (Day 300) (± 3)**
Informed consent	X										
Medical History and prior meds	X										
Physical Examination & full neurologic exam	X									X	
Inclusion / Exclusion Criteria	X	X									
Pharmacogenetic sample [o]	X										
Enrollment		X									
Vital Signs [b]	X	X	X	X	X	X	X	X	X	X	
12 Lead ECG [c]		X								X	
Hematology, Coagulation [d, f]	X	X	X	X	X	X	X	X	X	X	X
Biochemistry, Inflammatory markers [d, f]	X	X	X	X	X	X	X	X	X	X	X
HbA1c	X	X				X				X	X [n]
Urinalysis [e,]	X	X [g]								X	X
Pregnancy test [e, h]	X	X [h]	X	X	X	X [h]	X [h]	X	X	X	X [i]
ADA antibodies [j]		X [j, k]		X					X [k]	X [k]	X [k]
Lipids / lipoproteins [m]	X	X	X	X	X	X	X	X	X	X	X
Biomarker (stored samples)[q]	X	X	X	X	X	X	X	X	X	X	X
Study drug admin		X				X [a]	X [a]				
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X
AE reporting		X	X	X	X	X	X	X	X	X	X
SAE reporting		X	X	X	X	X	X	X	X	X	X

ADA = anti-drug antibodies; AE = adverse event; ECG = electrocardiogram; FU=follow-up; EOS = end of study; hsCRP=high sensitivity C-reactive protein; IL6=interleukin 6; IFN-γ=interferon gamma; LDL-C = low-density lipoprotein cholesterol; PCSK9 = proprotein convertase subtilisin/kexin type 9; SAE = serious adverse event; TNF-α=tumor necrosis factor alpha.

[a] Day 104 visit only for those subjects who receive a second dose of study drug. Dose #2 may be given at Day 90 or 104 depending on PCSK9 levels as compared to baseline

[b] Vital signs: blood pressure and heart rate will be measured prior to injection and at 4 hours after injection.

[c] ECG is performed prior to the injection on Day 1.

[d] Hematology, chemistry (including lactate, bicarbonate, glucose, HbA1c, liver and renal function, hsCRP, IL6, IFN-γ, and TNF-α), and coagulation testing. Blood samples for determination of laboratory values will be performed prior to study drug injection where relevant. All laboratory testing will be performed with subjects in a fasted state.

[e] Lab tests performed in participating institution's laboratory. Results must be available before the start of study drug injection on Day 1 to confirm subjects meet eligibility criteria.

[f] Lab tests performed by study's designated Central Lab facility from enrollment to EOS. In addition, subjects in whom LDL-C reduction is less than 20% of the absolute reduction from baseline to Day 90 will continue to be followed up on a monthly visit schedule either until this level has been reached or until Day 300.

[g] Urinalysis collection is prior to the injection on Day 1.

[h] Urine pregnancy test performed at each visit for females of childbearing potential. In addition, postmenopausal women who are less than 55 years of age will have a pregnancy test within 24 hours of enrollment and prior to any study drug injection. Results must be available prior to the injection on Day 1 and, if a second dose of study drug is required, on Day 90/104.

[i] Females of childbearing potential will have a pregnancy test at each additional follow-up visit until LDL-C reduction is less than 20% of the absolute reduction from baseline to Day 90.

[j] Two ADA samples will be drawn on Day 1: one before the injection and one 4 hours after the injection.

[k] Additional aliquots of plasma and serum will be collected at each time point and stored for future analyses.

[l] For subjects in whom LDL-C reduction is less than 20% of the absolute reduction from baseline to Day 90, formation of ADA will be assessed at the last visit.

[m] Efficacy parameters (Lipids / lipoproteins) will include LDL-C, total cholesterol, triglycerides, HDL-C, non-HDL-C, very low-density lipoprotein (VLDL-C), apolipoprotein A1 (Apo-A1), apolipoprotein B (Apo-B), lipoprotein(a) [Lp(a)], and PCSK9.

[n] Day 270 only.

[o] Sample may be taken during screening or anytime during study to be processed and stored, but only after separate consent has been signed.

[p] Additional Follow-Up visits only for those subjects in whom LDL-C reduction is less than 20% of the absolute reduction from baseline to Day 90.

[q] 3 aliquots of plasma and 3 aliquots of serum will be stored for efficacy and safety biomarker analysis if needed. All samples will be destroyed 1 year following the last subject's last visit in the study.

6.2. General Conduct of the Trial

Written informed consent will be obtained for this study by the principal investigator or sub-investigator from all subjects before the performance of any protocol-specific procedure (if the subject is less than 18 years of age, written consent will be obtained from their guardian or legally authorized representative, with verbal assent from the child).

Please see Section 6.1 for detailed schedule, and Section 7 for listings of all tests required in each panel.

6.3. Screening Period (Days –28 to –1)

The following procedures will be performed within 28 days prior to enrollment:

All screening laboratory tests will be analyzed at the Central Lab, with the exception of urinalysis and pregnancy test, which will be done in-house at the participating institution's laboratory using the testing materials provided by the Central Lab. The results of all screening laboratory tests should be reviewed prior to enrollment. If these results confirm an exclusion criterion or suggest any contraindication to treatment with ALN-PCS$_{SC}$, and/or other required ancillary medication(s), the subject must not be enrolled.

1. Review of inclusion/exclusion criteria to confirm subject eligibility.
2. Documentation of:
    a. Demography: age, gender and ethnic origin
    b. Medical history
    c. Concomitant medications
3. Full physical examination including height, weight and full neurological examination (as described in Appendix A).
4. Vital signs (blood pressure and heart rate).
5. Blood samples drawn for screening clinical laboratory tests and results reviewed prior to enrollment. All laboratory testing will be performed with subjects in a fasted state.
    a. Biochemistry
    b. Inflammatory markers
    c. Lipids/lipoproteins
    d. Hematology
    e. Coagulation
    f. HbA1c
    g. Biomarker (stored samples)
    h. Pharmacogenetics (stored samples, only if separate consent has been signed)
6. Urinalysis (local), using standardized supplies from Central Lab.
7. Urine pregnancy test (local), using standardized supplies from Central Lab, for females of childbearing potential. In addition, postmenopausal women who are less than 55 years of age must have a negative pregnancy test within 24 hours of enrollment.

6.4. Enrollment (Day 1)

Enrollment should only occur once subject eligibility is confirmed.

The following procedures will be performed prior to the injection of study drug:

1. Review of inclusion/exclusion criteria
2. Vital signs (blood pressure and heart rate will be measured prior to injection and at 4 hours after injection)
3. 12-lead ECG
4. Blood samples drawn for clinical laboratory tests (Central Lab):
   a. Biochemistry
   b. Inflammatory markers
   c. Lipids/lipoproteins
   d. Hematology
   e. Coagulation
   f. HbA1c
   g. Anti-ALN-PCS$_{SC}$ (ADA) antibodies (prior to and 4 hours after the injection)
   h. Biomarkers (stored samples)
5. Urinalysis (local)
6. Urine pregnancy test (local), for females of childbearing potential. In addition, postmenopausal women less than 55 years of age will have a pregnancy test within 24 hours of enrollment. Results must be available prior to the first injection of study drug
7. Concomitant medications
8. AE reporting
9. SAE reporting
10. Enrollment (only after subject eligibility is confirmed)

If eligible, the subject will receive the first injection (SC) of study drug and will be observed in the clinic for at least 4 hours post-injection before being discharged. The site of injection is the abdomen. The injection site will be marked and mapped for later observation

The following procedures will be performed 4 hours after the injection:

11. Vital signs: blood pressure and heart rate
12. Blood samples drawn for Anti-ALN-PCS$_{SC}$ (ADA) antibodies
13. Concomitant medications
14. AE reporting
15. SAE reporting

6.5. Follow-up Visits FU 1-6

Subjects will return to the study center after study drug administration for Follow-Up Visits according to the Schedule of Events/Assessments (Section 6.1) until the end of the study. Procedures will be as follows:

1. Vital signs (blood pressure and heart rate will be measured prior to injection and at 4 hours after injection)
2. Blood samples drawn for clinical laboratory tests:
   a. Biochemistry
   b. Inflammatory markers
   c. Lipids/lipoproteins
   d. Hematology
   e. Coagulation
   f. HbA1c (only at Days 90 and 180 and Day 270 if applicable)
   g. Anti-ALN-PCS$_{SC}$ (ADA) antibodies (only at Day 30 and Day 150)
   h. Biomarkers (stored samples)
3. Urine pregnancy test (local) for females of childbearing potential. In addition, on Day 90 or 104 (if a second dose of study drug is required), postmenopausal women less than 55 years of age will have a pregnancy test and results must be available prior to the injection
4. Concomitant medications
5. AE reporting
6. SAE reporting
7. Study drug administration **ONLY** for subjects requiring a second dose of study drug (depending on the degree of suppression of serum PCSK9 as compared to baseline).

   If a second dose is deemed necessary, the subject will receive the injection (SC) at **Day 90 or 104**, based on the PCSK9 levels from the previous visit. The site of injection is the abdomen (on the opposite side than the injection on Day 1). The injection site will be marked and mapped for later observation

6.6. End of Study (EOS) Visit (FU 7)

A subject's participation in the study is complete when all procedures at the last study visit have been completed and all ongoing SAEs have been followed to resolution (refer to Section 8.1.1).

Procedures will be as follows:

1. Vital signs (blood pressure and heart rate)
2. Physical examination (including full neurological examination as described in Appendix A)
3. 12-lead ECG
4. Blood samples drawn for clinical laboratory tests:
   a. Biochemistry
   b. Inflammatory markers

c. Lipids/lipoproteins
d. Hematology
e. Coagulation
f. Anti-ALN-PCS$_{SC}$ (ADA) antibodies
g. Biomarkers (stored samples)

5. Urinalysis (local)
6. Urine pregnancy test (local) for females of childbearing potential
7. Concomitant medications
8. AE reporting
9. SAE reporting

6.7. Additional Follow-Up Visits (FU 8-11, if needed)

These visits are only for those subjects in whom LDL-C reduction is less than 20% of the absolute reduction from baseline to Day 90. Procedures will be as follows:

1. Blood samples drawn for clinical laboratory tests:
   a. Biochemistry
   b. Inflammatory markers
   c. Lipids/lipoproteins
   d. Hematology
   e. Coagulation
   f. HbA1c (only at Day 270)
   g. Anti-ALN-PCS$_{SC}$ (ADA) antibodies (only at last visit)
   h. Biomarkers (stored samples)
2. Urinalysis (local)
3. Urine pregnancy test (local). Females of childbearing potential will have a pregnancy test at each additional follow-up visit until LDL-C reduction is less than 20% of the absolute reduction from baseline to Day 90
4. Concomitant medications
5. AE reporting
6. SAE reporting

7. PROTOCOL ASSESSMENTS

7.1. Assessment of Safety

7.1.1. Adverse Events

Subjects will be carefully monitored for adverse events by the investigator during the designated study period (see Section 6.1 for details).

7.1.2. Demographics and Medical History

Baseline demographic information will be collected during screening, and will include age, sex and race/ethnicity.

Relevant medical history includes all ongoing medical or surgical issues. Remote medical and surgical history > 5 years from the time of screening should only be included if considered relevant to the study.

7.1.3. Vital Signs

Vital signs will include heart rate and blood pressure.

7.1.4. Electrocardiograms

12-Lead ECGs will be collected at Day 1 and at the EOS visit only, unless clinically indicated.

7.1.5. Physical Examination

The physical examination should include a focused examination, which may include general, respiratory, cardiovascular, abdominal, extremities, and recording of height, weight.

The physical examination should also include a full neurologic evaluation as described in Appendix A.

7.1.6. Clinical Laboratory Assessments

Specimens will be obtained at the time points in the Schedule of Assessments (Section 6.1). Additional aliquots of plasma or serum will be collected at each time point and stored for any clinically indicated efficacy or safety analyses to be conducted at the end of the study.

Subjects will be in a fasted state for all clinical laboratory assessments. Screening lab tests will be performed by the Central Lab, with the exception of urinalysis and pregnancy test, which will be done in-house at the participating institution's laboratory using testing materials supplied by the Central Lab. Results from these screening tests must be available before the start of study drug injection on Day 1 to confirm subjects meet eligibility criteria. Details regarding the processing, shipping, and analysis of samples will be provided in the Laboratory Manual. Note: Efficacy laboratory assessments (e.g., LDL-C and PCSK9) are described in Section 7.2.

Laboratory assessments will include:

Biochemistry: AST, ALT, alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), uric acid, total bilirubin (TBIL), direct bilirubin, indirect bilirubin, lactate, bicarbonate, sodium, creatine kinase (CK), albumin, total protein urea (BUN), creatinine, potassium, chloride, glucose (fasting), inorganic phosphate, eGFR, calcium, and tryptase (as required).

Inflammatory markers: hsCRP (fasting), IL6, IFN-γ, and TNF-α

Hematology: hemoglobin, hematocrit, erythrocytes, reticulocytes, platelet counts, mean cell hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), white blood cell count, differential blood count.

Coagulation: prothrombin time (PT), international normalized ratio (INR), activated partial thromboplastin (aPTT).

HbA1c: hemoglobin A1c

Urinalysis: Urinalysis will be performed at the time points defined in the Schedule of Assessments and evaluated by dipstick analyses at the investigational site local lab (a standardized dipstick test will be supplied by the Central Lab). Urinalysis will be performed from a sample of mid-stream urine. In case of abnormal results, microscopy and other assessments will be performed at the local lab. The following parameters will be assessed: Nitrite, protein, glucose, ketone, urobilinogen, bilirubin, red blood cells (RBC)/erythrocytes, white blood count (WBC)/leukocytes, pH, urine sediment (microscopic examination will be performed in the event of abnormalities).

Urine pregnancy: Urine pregnancy testing will be conducted locally at the visits specified in the Schedule of Assessments (a standardized pregnancy test will be supplied by the Central lab).

7.1.7. Anti-ALN-PCSSC Antibodies

Additional sample for analysis of the induction of antibodies will be collected at the time points in the Schedule of Assessments (Section 6.1).

Aliquots of serum samples will be obtained and frozen, to permit future analysis of the effect of ALN-PCSSC on the expression of these exploratory biomarkers. Biological samples for biomarker research will be retained on behalf of the Sponsor for one year following the last subject's last visit in the study. Details regarding the collection, processing, storage, and shipping will be in the Study Laboratory Manual.

7.2. Assessment of Efficacy

Specimens for assays of lipids/lipoproteins will be obtained at the time points in the Schedule of Assessments (Section 6.1). Subjects will be in a fasted state for all efficacy laboratory assessments. Parameters to be assessed will include: total cholesterol (TC), triglycerides, LDL-C, HDL-C, non-HDL-C, very low-density lipoprotein (VLDL-C), apolipoprotein A1 (Apo-A1), apolipoprotein B (Apo-B), lipoprotein(a) [Lp(a)], and PCSK9.

7.2.1. Change in LDL-C

The primary efficacy endpoint is the percentage change in LDL-C.

In addition, this study will assess absolute and percentage change in LDL-C as a secondary efficacy endpoint.

Blood samples for determination of LDL-C (β-quantification) concentrations will be collected at the time points in the Schedule of Assessments. Details regarding the collection, processing, shipping, and storage of the samples will be provided in a Laboratory Manual.

7.2.2. Change in Lipids/Lipoproteins

Secondary efficacy assessments will include measurement of the effects of ALN-PCS$_{SC}$ on levels of lipids and lipoproteins including total cholesterol, triglycerides, LDL-C, HDL-C, non-HDL-C, VLDL-C, Apo-A1, Apo-B, Lp(a), and PCSK9.

- Absolute change and percentage change in PCSK9 levels
- Absolute change and percentage change in other lipids, and apolipoproteins
- **Biomarkers** (stored samples): Additional aliquots of plasma and serum will be collected at each time point and stored for additional analyses, including future analysis of biomarkers of CV risk. These samples will be retained for 1 year following the last subject's last visit in the study.

Plasma samples will be analyzed using a validated enzyme linked immunosorbent assay to determine PCSK9 protein concentration. Full details of the analytical methods used will be described in a separate bioanalytical report.

7.3. Assessment of Pharmacogenetics

A blood sample will be collected, preferably during screening, only from subjects who sign a separate consent for pharmacogenetics. Samples will be processed as described in the Laboratory Manual and stored. Genetic assessment will be performed by an accredited laboratory. This assessment will determine if there is a different response for LDL-C lowering based on the type of mutation(s).

8. ADVERSE EVENTS

8.1. Definitions

8.1.1. Adverse Event

Adverse Event (AE): Any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (eg, an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Planned hospital admissions and/or surgical operations for an illness or disease that existed before the study drug was given or the subject was randomized in a clinical study are not to be considered AEs.

Adverse events or abnormal test findings will be followed until the event (or its sequelae) or the abnormal test finding resolves or stabilizes at a level acceptable to the Sponsor/Investigator.

8.1.2. Serious Adverse Event

A serious adverse event (SAE) is any untoward medical occurrence that at any dose:

- Results in death,
- Is life-threatening, i.e., the subject was, in the opinion of the investigator, 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),
- Results in a significant, persistent or permanent change, impairment, damage or disruption in the subject's body function/structure, physical activities and/or quality of life,
- Requires in-subject hospitalization or prolongs hospitalization,
- Is a congenital anomaly/birth defect, or
- Is another medically significant event where medical and scientific judgement should be exercised in deciding whether other situations should be considered serious reactions, such as important medical events that might not be immediately life threatening or result in death or hospitalization but might jeopardize the subject or might require intervention to prevent one of the other outcomes listed above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization or development of dependency or abuse. Any suspected transmission via a medicinal product of an infectious agent is also considered a serious adverse reaction.

8.1.3. Medication Errors

Medication error refers to any unintended error in the dosing and/or administration of the study drug as per instructions in the protocol. Medication errors generally fall into 4 categories as follows:

- wrong study drug;
- wrong dose (including dosing regimen, strength, form, concentration, amount, including above the maximum or under the minimum recommended dose);
- wrong route of administration;
- wrong subject (i.e. not administered to the intended subject)

8.2. EVALUATING ADVERSE EVENTS

8.2.1. AE Severity

The severity of an AE will be assessed by the investigator. The investigator should ensure that any subject experiencing an AE receives appropriate medical support until the event resolves.

Adverse events (AE) will be graded on a 3-point scale and reported as indicated on the case report form. The intensity of an AE is defined as follows:

1 = Mild: Discomfort noticed, but no disruption to daily activity.

2 = Moderate: Discomfort sufficient to reduce or affect normal daily activity.

3 = Severe: Inability to work or perform normal daily activity.

A distinction should be drawn between serious and severe AEs. Severity is an estimate or measure of the intensity of an AE, while the criteria for serious AEs are indications of adverse subject outcomes for regulatory reporting purposes. A severe AE need not necessarily be considered serious and a serious AE need not be considered severe. For example, nausea that persists for several hours may be considered severe nausea, but not an SAE. On the other hand, a myocardial infarction (MI) that may be considered minor could also be an SAE if it prolonged hospitalization.

8.2.2. Study Drug Causality

The relationship of an AE to study treatment (i.e. ALN-PCS$_{SC}$) will be assessed with consideration to the following criteria:

- Temporal relationship to the initiation of study medication
- Response of the event to withdrawal of study medication
- AE profile of concomitant therapies
- Clinical circumstances during which the AE occurred
- Subject's clinical condition and medical history

Categorization of causality will be designated by the investigator as stated below:

Reasonable possibility - There are facts (evidence) or arguments to suggest a causal relationship between the event and the study drug.

No Reasonable possibility - There are few to no facts (evidence) or arguments to suggest a causal relationship between the event and the study drug.

8.2.3. Adverse Event of Special Interest (AESIs)

An adverse event of special interest (serious or nonserious) is one of scientific and medical concern specific to the Sponsor's study drug or program, which warrants ongoing monitoring and rapid communication by the investigator to the sponsor. Such an event might warrant further investigation in order to characterize and understand it.

The following AESI(s) have been identified for the study drug ALN-PCS$_{SC}$ in this protocol.

- Injection site reactions (ISR)

Injection site reactions including individual signs or symptoms at the injection site reported following study drug administration will be collected as an AESI.

The grade (severity) of injection site reaction will be determined by the Common Terminology Criteria for Adverse Events (CTCAE) criteria of Injection Site Reaction (General disorders and administration site conditions) (see Table 2).

Table 2: Common Terminology Criteria for Adverse Events (CTCAE) of Injection Site Reaction

Grade I: Tenderness with or without associated symptoms (eg, warmth, erythema, itching)
Grade II: Pain; lipodystrophy; edema; phlebitis
Grade III Ulceration or necrosis; severe tissue damage; operative intervention indicated
Grade IV Life-threatening consequences; urgent intervention indicated
Grade V Death

Reference: Common Terminology Criteria for Adverse Events (CTCAE), Version 4.03, Published 14 June 2010.

As indicated in Section 5.3 and Section 8.2.3, photographs of ISR should be obtained either by the investigator or another health care professional at first presentation and at each of the follow-up visits until the injection site reaction resolves, if possible.

Potential anaphylactic reactions will be assessed using the Sampson Criteria (Appendix B).

8.2.4. Special Situations

Collection, evaluation, and/or reporting of safety information is required by the regulatory authorities in special situations.

The special situations not previously defined include the following:

- Suspected transmission via a medicinal product of an infectious agent
- Drug interactions
- Occupational exposure

8.3. PROCEDURE FOR ADVERSE EVENT REPORTING

8.3.1. Procedure for Non-Serious Adverse Event Recording

All non-serious AEs that occur during the designated study period from enrollment up to EOS must be assessed and recorded on the source documents and eCRF, regardless of causal relationship to the study drug.

8.3.2. Procedure for Serious Adverse Event Reporting

All SAEs that occur during the designated study period from enrollment up to EOS must be reported to the Sponsor's Global Pharmacovigilance Department (GPV) within 24 hours of awareness of the event using the provided study specific SAE/AESI Report Form. In addition to completing the SAE/AESI Report Form, each SAE/AESI must be entered on the appropriate page of the case report form (CRF).

The investigator must assess the causality for each SAE/AESI.

The Sponsor will contact the investigator, if necessary, to clarify any of the event information. The investigator should provide any follow-up information for the event to the Sponsor on an updated SAE/AESI report form as soon as it becomes available.

If the investigator is notified of a SAE/AESI that occurs post-study period, that he or she wishes to report to the Sponsor (e.g., an event suspected to be causally related to study drug), the event should be reported through the process described above.

Where appropriate, if required by local regulations or procedures, the investigator should report these events to the Institutional Review Board (IRB)/Ethics Committee (EC) and/or national regulatory authority in addition to the Sponsor.

8.3.3. Procedure for Medication Error Reporting For Study Drug

Medication errors with or without an associated AE need to be recorded as medication errors in the eCRF as described in Section 8.1.3.

Medication errors with an associated SAE need to be recorded as medication errors in the eCRF and reported to the Sponsor's Global Pharmacovigilance Department as described in Section 8.3.2.

8.3.4. Procedure For Reporting Adverse Events Of Special Interest (AESIs)

Injection Site Reaction has been identified as an AESI for the study product(s) in this protocol as per Section 8.2.3. The SAE/AESI form should be utilized for reporting the AESI even if a serious outcome may not apply, and the form should indicate that the reported event is an AESI. Non-serious AESIs should be reported to the Sponsor within 72 hours and serious AESIs should

be reported to the Sponsor within 24 hours. In both instances, the reporting procedure provided in Section 8.3.2 should be followed.

8.3.5. Procedure For Reporting Pregnancies Exposure

Occurrences of pregnancy in a study subject or study subject's partner should be reported within 24 hours using the Pregnancy Reporting Form. In cases where a pregnancy occurs with a Serious Adverse Event, the Serious Adverse Event reporting form should be used to report the SAE/AESI and the Pregnancy Reporting form should be used to report the pregnancy. When a pregnancy occurs without any concurrent SAE, the Pregnancy Reporting Form may be submitted alone. The pregnancy must be followed through to outcome of pregnancy. Any pregnancy discovered from the time of consent to follow-up needs to be reported.

8.3.6. Procedure For Reporting Special Situations

The occurrence of a Special Situation event, defined in Section 8.2.4, must be reported to the Sponsor as per Section 8.3.2, Procedure for Serious Adverse Event Reporting. Note: The Special Situations event does not need to be serious to be reported on the SAE/AESI Report form.

9. DATA COLLECTION

An electronic data capture (EDC) system which is 21 CFR Part 11 compliant will be used for this trial. All users will be trained on the technical features of the EDC as well as the content of the eCRF by qualified personnel prior to gaining access to the EDC. A UserID/Password will be granted after training. This ID is not to be shared amongst the study staff. All users must have a unique account to enter or review data. The eCRF should be filled out by the site 3 days after each visit. It is not expected that the eCRF will serve as source for any data collected in this trial. If there is a reason for a site to do so, it must be approved by Sponsor and documented in the site files.

Prior to the database being locked, the investigator or designee will review, approve and sign/date each completed eCRF. This signature serves as attestation of the Investigator's responsibility for ensuring that all data entered into the eCRF are complete, accurate and authentic. After the end of the trial, a copy of the data will be provided to the site. This copy will contain the final data, an audit trail of activity on the data, and any queries and answers that were posted for data clarification.

For this study, the End of Trial will be defined as the last visit of the last subject.

10. STATISTICAL PLAN

This is an 8-10 subject non-randomized pilot study. Subjects with homozygous familial hypercholesterolemia will be recruited from approximately 5 sites globally. Subjects that qualify for entry into the study will be enrolled to receive ALN-PCS$_{SC}$. The primary objective of this study is to characterize the effect of 90 and 180 days of subcutaneous ALN-PCS$_{SC}$ on the percent change from Day 1 in low-density lipoprotein cholesterol (LDL-C) in subjects with homozygous familial hypercholesterolemia. A separate Statistical Analysis Plan (SAP) document will provide more detailed specifications in data analysis and presentation.

The primary hypothesis is that ALN-PCS$_{SC}$, when used in combination with maximally tolerated statin therapy with or without ezetimibe, will be well-tolerated, will suppress circulating PCSK9 >70% and will result in reduction of LDL-C, defined as mean percent change from Day 1 following 90 and 180 days of treatment, in subjects with homozygous familial hypercholesterolemia.

10.1. Sample Size

The sample size was chosen based on clinical considerations.

10.2. Randomization

This study is non-randomized.

10.3. General Statistical Considerations and Definitions

10.3.1. General Statistical Methods

All study-collected data will be summarized by treatment group using descriptive statistics, graphs, and/or raw data listings. Descriptive statistics for continuous variables will include number of subjects (n), mean, standard deviation (SD), median, quartiles (Q1 and Q3), minimum (min) and maximum (max) values. Analysis of categorical variables will include frequency and percentage.

Summary statistics for continuous variables will include the number of subjects, mean, median, standard deviation or standard error, minimum and maximum. For categorical variables, the frequency and percentage will be given.

10.3.2. Analysis Populations

The following populations will be used for data analyses and/or presentation.

10.3.2.1. Modified Intent-to-Treat (mITT) Population

All ITT subjects (who receive at least one dose of study drug), who have baseline and at least one post-baseline measurement for the primary endpoint. This will be the primary population for the efficacy analyses; unless specified otherwise, the mITT will be the default analysis set in this study.

10.3.2.2. Safety Population

All subjects who received at least one dose of study drug. Treatment classification will be based on the actual treatment received. This will be the primary population for the safety analyses.

The primary analysis set is the modified Intent-to-Treat (mITT), which will include all enrolled subjects who have received at least one dose of study drug. Unless specified otherwise, the mITT will be the default analysis set in this study.

10.3.3. Analysis Windows and Baseline

The observational period for the study includes the screening period (Day -28 to Day -1), treatment and follow up period (Day 1 to Day 180), additional follow up period (up to Day 300, only for subjects in whom LDL-C reduction is less than 20% of the absolute reduction from baseline to Day 90). Any event occurring beyond the defined observational period, even if collected on the CRF, will not be included in the planned statistical analysis. However, all data, including that reported after the defined observational period, will be included in the subject data listings.

Unless otherwise specified, for evaluations that are collected at multiple occasions prior to initiation of study drug administration, the latest evaluation will be considered the "Baseline" evaluation for analysis.

10.3.4. Missing Data Handling

Missing data will not be imputed.

10.4. Statistical Analyses

10.4.1. Demographic and Background Characteristics

Subject demographics and baseline characteristics will be summarized by treatment group using the mITT and safety populations.

10.4.2. Study Drug and Concomitant Medications

Summary of each prior (pre-Day 1) medication and concomitant (Day 1 or later) medication will be provided by treatment. [Medication will be coded with WHO drug dictionary]. Subjects will be counted only once within each period by medication.

10.4.3. Efficacy Analysis

10.4.3.1. Primary Efficacy Endpoint

The primary endpoint is the percent change in LDL-C following 90 and 180 days of treatment.

Summary statistics and 95% CI of this primary endpoint will be provided. Changes of LDL-C from Day 1 by scheduled visits will also be summarized. Response rate of subjects with 15% or greater reduction in LDL-C from Day 1 following 90 and 180 days of treatment will be calculated.

The analysis for the primary endpoint will be descriptive. Means and confidence intervals will be provided.

10.4.3.2. Secondary Efficacy Endpoints

Analyses of secondary efficacy endpoints will be similar to the primary analysis of the primary endpoint.

10.4.3.3. Tertiary Efficacy Endpoints

Formation of Anti-drug antibodies (ADA) to ALN-PCS$_{SC}$

Response of LDL-C by the causal genetic mutations of HoFH

10.4.4. Safety Analysis

Safety summaries will include the incidence of adverse events, summaries of laboratory parameters (including shift tables), vital signs, ECGs and anti-drug antibodies.

10.4.4.1. Adverse Events

The Medical Dictionary for Regulatory Activities (MedDRA) will be used for coding adverse events (AEs). An AE (classified by preferred term) occurring during the study drug treatment period will be counted as a treatment emergent AE (TEAE) either if it is not present at baseline or if it is present at baseline but increased in severity during the treatment period.

The number (percentage) of subjects reporting TEAEs for each preferred term will be tabulated by system-organ class, by system-organ class and severity, and by system-organ class and relationship to study drug. If more than one event occurred with the same preferred term for the same subject, the subject will be counted only once for that preferred term using the most severe or related occurrence for the summary by severity, or relationship to study drug, respectively.

10.4.4.2. Laboratory Tests

Laboratory values will be summarized by treatment group, including changes and percent changes from Day 1 at each time point. Analyses will also be performed for each lab parameter by treatment group for incidence rates of potentially clinical significant (PCS) values for subjects without PCS value at baseline.

10.4.4.3. Vital Signs

Change and percent change from baseline in vital signs will be summarized descriptively at each scheduled time point by treatment group.

11. RECORDS RETENTION

FDA regulations require all investigators participating in clinical study drug/device trials to maintain detailed clinical data for one of the following periods:

- At least two years following the date on which a New Drug Application is approved by the FDA, or
- Two years after the Sponsor notifies the investigator that no further application is to be filed with the FDA.

Similarly, current EU Directives / Regulations and ICH guidelines collectively require that essential clinical trial documents (including case report forms) other than subject's medical files must be retained for the following time period:

- — for at least 15 years after completion or discontinuation of the trial,
- — or for at least two years after the granting of the last marketing authorisation in the European Community and when there are no pending or contemplated marketing applications in the European Community,
- — or for at least two years after formal discontinuation of clinical development of the study drug/device.

Subject's medical files should be retained in accordance with applicable legislation and in accordance with the maximum period of time permitted by the hospital, institution or private practice. The documents can be retained for a longer period, however, if required by the applicable regulatory requirements or by agreement with the sponsor.

To comply with these requirements, the investigator will not dispose of any records relevant to this study without either (1) written permission from the Sponsor or (2) providing an opportunity for the Sponsor to collect such records. The investigator shall take responsibility for maintaining adequate and accurate hard copy source documents of all observations and data generated during this study, including the hard copy or discs received from the sponsor of the final data. Such documentation is subject to inspection by the Sponsor or its agents, the FDA and/or other regulatory agencies.

12. QUALITY CONTROL AND QUALITY ASSURANCE

12.1. Monitoring

The Sponsor has ethical, legal and scientific obligations to carefully follow this study in accordance with established research principles and applicable regulations. The investigator, as part of his responsibilities, is expected to cooperate with the Sponsor in ensuring that the study adheres to the protocol and GCP requirements.

As part of a concerted effort to fulfill these obligations, the Sponsor's monitor will visit the center(s) during the study in accordance with the Monitoring Plan set forth for this trial. The investigator will permit the Sponsor to monitor the study as frequently as is deemed necessary and provide access to medical records/source documents to ensure that data are being recorded adequately, that data are verifiable and that protocol adherence is satisfactory.

12.2. Auditing

The Sponsor may conduct audits at the study center(s). Audits will include, but not be limited to, study drug supply, presence of required documents, the informed consent process, and comparison of eCRFs with source documents. The investigator agrees to permit audits conducted at a reasonable time in a reasonable manner.

Regulatory authorities worldwide may also inspect the investigator during or after the study. The investigator should contact the Sponsor immediately if this occurs, and must permit regulatory authority inspections.

12.3. Protocol Deviations

This study will be conducted as described in this protocol, except for an emergency situation in which the protection, safety, and well-being of the subject requires immediate intervention, based on the judgment of the investigator (or a responsible, appropriately trained professional designated by the investigator). In the event of a significant deviation from the protocol due to an emergency, accident, or mistake, the investigator or designee must contact the Sponsor, or their agent, at the earliest possible time by telephone. This will allow an early joint decision regarding the subject's continuation in the study. The investigator and the Sponsor will document this decision. The IRB/EC will be informed of all protocol changes by the investigator in accordance with the IRB/EC established procedure. No deviations from the protocol of any type will be made without complying with all the IRB/EC established procedures.

The following Protocol Deviations will require additional information in the eCRF explaining why the deviation occurred and what will be done to prevent it from re-occurring:

- Inclusion criteria violation
- Exclusion criteria violation
- Injection not administered for any reason other than subject safety or withdrawal

- Wrong dose (dose concentration, wrong dose, wrong treatment, wrong regimen, wrong injection site)*
- Missed assessment as per the Schedule of Events/Assessments (Section 6.1) at Baseline, dosing visits and EOS
- Subject not adhering to protocol subject restrictions
- Subject taking any prohibited concomitant medication

*If the mis-dosing was unintended, i.e. a medication error, the error should be reported as per instructions in Section 8.3.3, Procedure for Medication Error Reporting.

13. ETHICS AND RESPONSIBILITY

This study will be conducted in compliance with the protocol, the Sponsor's standard operating procedures and/or guidelines, the United States Food and Drug Administration (FDA) regulations, the International Conference on Harmonization (ICH) GCP guidelines, the Declaration of Helsinki and other local regulations, as applicable.

13.1. Informed Consent

Written informed consent will be obtained from all subjects (or their guardian or legally authorized representative), and whenever possible, verbal assent will be obtained from children 12 years of age or older, or as per IRB guidelines, before any study-related procedures (including any pre-treatment procedures) are performed. The investigator(s) has both ethical and legal responsibility to ensure that each subject (and their guardian or legally authorized representative) being considered for inclusion in this study is given a full explanation of the protocol. This shall be documented on a written informed consent form, which shall be approved by the same IRB or EC responsible for approval of this protocol. Each informed consent form shall include the elements required by ICH, Part E6, Section 4.8 and any applicable local regulations. The investigator agrees to obtain approval from the Sponsor of any written informed consent form used in the study, preferably prior to submission to the IRB or EC.

Once the appropriate essential information has been provided to the subject and fully explained by the investigators (or a qualified designee) and it is felt that the subject understands the implications of participating, the subject and the investigator (or designee) shall sign the IRB- or EC-approved written informed consent form. The subject shall be given a copy of the signed informed consent form, and the original shall be filed appropriately, according to the institution. A second copy may be filed in the subject's medical record, if allowed by the institution.

13.2. Institutional Review Board/Ethics Committee

This protocol, the written informed consent form and any materials presented to subjects shall be submitted to the IRB or EC identified with this responsibility. Notification in writing of approval must come from the IRB or EC chairman or secretary, to the investigator, either as a letter or as a copy of the appropriate section of the IRB or EC meeting minutes where this protocol and associated informed consent form were discussed. The investigator will not participate in the decision. If the investigator is an IRB or EC member, the written approval must indicate such non-participation in the voting session. The investigator will submit status reports to the IRB or EC as required by the governing body. The IRB or EC must be notified by the investigator in writing of the interruption and/or completion of the study; the investigator must promptly report to the IRB or EC all changes in research (protocol amendments) and will not make such changes without IRB or EC approval, except where necessary to eliminate apparent immediate hazards to human subjects. In cases where it is necessary to eliminate immediate hazards to subjects, the IRB or EC must then be notified of the change as per local requirements. The investigator is required to maintain an accurate and complete record of all written correspondence to and received from the IRB or EC and must agree to share all such documents and reports with the Sponsor.

14. CONFIDENTIALITY

All information generated in this study must be considered highly confidential and must not be disclosed to any persons not directly concerned with the study without written prior permission from the Sponsor. However, authorized regulatory officials and Sponsor personnel will be allowed full access to the records. All medications provided and subject bodily fluids and/or other materials collected shall be used solely in accordance with this protocol, unless otherwise agreed to in writing by the Sponsor.

Only unique subject numbers in eCRFs will identify subjects. Their full names may, however, be made known to a product regulatory agency or other authorized official if necessary.

With respect to the clinical trial data that is received from countries in the European Economic Area and Switzerland, MDCO has certified adherence to the US-EU and the US-Swiss Safe Harbor Principles.

15. INVESTIGATOR AGREEMENT

I have read and understand the protocol (including the Investigator's Brochure) and agree that it contains all the ethical, legal and scientific information necessary to conduct this study. I will personally conduct the study as described.

I will provide copies of the protocol to all physicians, nurses and other professional personnel responsible to me who will participate in the study. I will discuss the protocol with them to assure myself that they are sufficiently informed regarding the new study drug ALN-PCS$_{SC}$, the concurrent medications, the efficacy and safety parameters and the conduct of the study in general. I am aware that this protocol must be approved by the Institutional Review Board (IRB) or Ethics Committee (EC) responsible for such matters in the Clinical Study Facility where ALN-PCS$_{SC}$ will be tested prior to commencement of this study. I agree to adhere strictly to the attached protocol. I understand that this IRB or EC approved protocol will be submitted to relevant regulatory authorities by the Sponsor, as appropriate. I agree that clinical data entered on case report forms by me and my staff will be utilized by the Sponsor in various ways such as for submission to governmental regulatory authorities and/or in combination with clinical data gathered from other research sites, whenever applicable. I agree to allow Sponsor monitors and auditors full access to all medical records/source documents at the research facility for subjects screened or enrolled in the study.

I agree to provide all subjects with informed consent forms, as required by government and ICH regulations. I further agree to report to the Sponsor any adverse experiences in accordance with the terms of this protocol, ICH guideline, Part E6, Section 4.11 and applicable local regulations.

Principal Investigator (Signature)	Date
Principal Investigator (Printed Name)	Protocol Version: Amendment 1 (07 November 2016)
Institution Name	

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APPENDIX A. NEUROLOGICAL EXAMINATION

MOTOR FUNCTION

When assessing motor function, from a neurological perspective, the assessment should focus on arm and leg movement. You should consider the following:

1. Muscle size
2. Muscle tone
3. Muscle strength
4. Involuntary movements
5. Posture, gait

Symmetry is the most important consideration when identifying focal findings. Compare one side of the body to the other when performing your assessment.

Limb assessment of a conscious subject usually involves a grading of strength.

Grade Strength

Grade strength Description
5 Full range of motion against gravity and resistance; normal muscle strength
4 Full range of motion against gravity and a moderate amount of resistance; slight weakness
3 Full range of motion against gravity only, moderate muscle weakness
2 Full range of motion when gravity is eliminated, severe weakness
1 A weak muscle contraction is palpated, but no movement is noted, very severe weakness
0 Complete paralysis

NB: In a conscious subject, the single best test to quickly identify motor weakness is the “drift test”. Have the subject hold their arms outward at 90 degrees from the body. With palms up, have the subject close their eyes and hold the arms for a couple of minutes. “Drifting” will occur if one side is weak.

Lower Extremities

Assess the subject in a supine position. Ask him/her to separate both legs to test for hip abduction. Then ask the subject to bring the legs back together to test for hip adduction. Sit the subject on the side of the bed to assess knee flexion and extension. Ask the subject to flex and extend the knee. If able to do this, apply resistance as these movements are repeated. Test plantar and dorsiflexion by having the subject push down against your hand with their foot and then pull up against your hand with their foot. Remember to compare the left side to the right side.

Upper Extremities

Assess ability to flex elbow (biceps) and straighten (triceps). Assess ability to raise shoulders and return to a resting position. Assess wrist flexion and extension. Test each function with

resistance. For focused upper extremity assessment, assess each digit for flexion, extension and lateral movement.

SENSORY FUNCTION

When assessing sensory function remember that there are three main pathways for sensation and they should be compared bilaterally:

1. Pain and temperature sensation

2. Position sense (proprioception)

3. Light touch

Pain can be assessed using a sterile pin. Light touch can be assessed with a cotton wisp. To test proprioception, grasp the subject's index finger from the middle joint and move it side to side and up and down. Have the subject identify the direction of movement. Repeat this using the great toe.

Sensory Tests:

A number of tests for lesions of the sensory cortex can be done. Examples include the following:

- **Stereognosis**: The ability to recognize an object by feel. Place a common object in the persons hand and ask them to identify the object.

- **Graphesthesis:** "Draw" a number in the palm of the person's hand and ask them to identify the number.

- **Two-Point Discrimination:** Simultaneously apply two pin pricks to the skin surface. Continually repeat the test while bringing the two pins closer together, until the individual can no longer identify two separate stimuli. The finger tips are the most sensitive location for recognizing two point differences while the upper arms, thighs and back are the least sensitive.

- **Extinction:** Touch the same spot on both sides of the body at the same time (eg, the left and right forearms). Ask the individual to describe how many spots are being touched. Normally, both sides are felt; with sensory lesions the individual will sense only one.

- **Point Locations:** Touch the surface of the skin and remove the stimulus quickly. Ask the individual to touch the spot where the sensation was felt. Sensory lesions can impair accurate identification, even if they retain their sensation of light touch.

TONE and REFLEXES

Upper motor neuron problems (brain and spinal cord) are associated with increased tone. Lower motor neuron problems are associated with decreased tone.

Look at the muscles on each side of the body in pairs. Assess for symmetry of bulk.

Evaluation of the stretch reflexes assesses the intactness of the spinal reflex arc at various spinal cord levels. The limb should be relaxed while applying a short and snappy blow with a reflex hammer. Hold the hammer loosely in a relaxed manner, making a wrist action. Allow the hammer to bounce.

Reflex responses:
0 No response
1+ Diminished, low normal
2+ Average, normal
3+ Brisker than normal
4+ Very brisk, hyperactive

Lower motor neuron disease is associated with 0 or 1+, upper motor neuron disease is associated with 3+ or 4+.

Biceps Reflex (C5 – C6)

Support the forearm on the examiners forearm. Place your thumb on the bicep tendon (located in the front of the bend of the elbow; midline to the anticubital fossa). Tap on your thumb to stimulate a response.

Triceps Reflex (C7-C8)

Have the individual bend their elbow while pointing their arm downward at 90 degrees. Support the upper arm so that the arm hangs loosely and "goes dead". Tap on the triceps tendon located just above the elbow bend (funny bone).

Brachioradialis Reflex (C5-C6):

Hold the person's thumb so that the forearm relaxes. Strike the forearm about 2-3 cm above the radial styloid process (located along the thumb side of the wrist, about 2-3 cm above the round bone at the bend of the wrist). Normally, the forearm with flex and supinate.

Quadriceps Reflex (Knee jerk) L2 – L4

Allow the lower legs to dangle freely. Place one hand on the quadriceps. Strike just below the knee cap. The lower leg normally will extend and the quadriceps will contract.

If the subject is supine: Stand on one side of the bed. Place the examiners forearm under the thigh closest to the examiner, lifting the leg up. Reach under the thigh and place the hand on the thigh of the opposite leg, just above the knee cap. Tap the knee closest to the examiner, (the one that has been lifted up with the examiners forearm).

Achilles Reflex (ankle jerks) L5 – S2:

Flex the knee and externally rotate the hip. Dorsiflex the foot and strike the Achilles tendon of the heel. In conscious subjects, kneeling on a chair can help to relax the foot.

Heel Lift

While the subject is supine, bend the knee and support the leg under the thigh. Have the leg "go dead". Briskly jerk the leg to lift the heel of the bed. Normally, the leg will remain relaxed and the heel will slide upward; increased tone will cause the heel and leg to stiffen and lift off the bed.

Babinski Response:

Dorsiflexion of the great toe with fanning of remaining toes is a positive Babinski response. This indicates upper motor neuron disease. It is normal in infants.

CEREBELLAR FUNCTION

The cerebellum is responsible for muscle coordination and balance on the same side. To test cerebellar function use the following tests:

1. Finger to finger test: have the subject touch their index finger to your index finger (repeat several times).

2. Finger to nose test: perform with eyes open and then eyes closed.

3. Tandem walking: heel to toe on a straight line.

4. Romberg test: stand with feet together and arms at their sides. Have subject close his/her eyes and maintain this position for 10 seconds. If the subject begins to sway, have them open their eyes. If swaying continues, the test is "positive" or suggestive of cerebellum problems.

Dizziness that occurs in response to position changes is usually blood pressure initiated. If the subject sways during a Romberg test, but stops when the eyes are opened, the problem is probably visual or CN VIII (vestibular).

APPENDIX B. SAMPSON CRITERIA FOR DIAGNOSING ANAPHYLAXIS

Anaphylaxis is highly likely when any one of the following three criteria is fulfilled:

1. Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (eg, generalized hives, pruritus or flushing, swollen lips-tongue-uvula)

AND AT LEAST ONE OF THE FOLLOWING:

a. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced peak expiratory flow [PEF], hypoxemia)

b. Reduced blood pressure or associated symptoms of end-organ dysfunction (eg, hypotonia [collapse], syncope, incontinence)

2. Two or more of the following that occur rapidly after exposure to a likely allergen for that subject (minutes to several hours):

a. Involvement of the skin-mucosal tissue (eg, generalized hives, itch-flush, swollen lips-tongue-uvula)

b. Respiratory compromise (eg, dyspnea, wheeze, bronchospasm, stridor, reduced PEF, hypoxemia

c. Reduced BP or associated symptoms (eg, hypotonia [collapse], syncope, incontinence)

d. Persistent gastrointestinal symptoms (eg, painful abdominal cramps, vomiting)

3. Reduced blood pressure after exposure to a known allergen for that subject (minutes to several hours):

a. Infants and children: low systolic blood pressure (age specific) or > 30% decrease in systolic blood pressure*

b. Adults: systolic blood pressure <90 mmHg or >30% decrease from that person's baseline

*Low systolic blood pressure for children is age specific and defined as: <70 mmHg for age 1 month to 1 year; <70 mmHg + [2 x age] for age 1 to 10 years; <90 mmHg for age 11 to 17 years.

Source: Sampson et al, 2005; Sampson et al, 2006.

APPENDIX C. TLC DIET

Definition

Although there are several diets that will result in lowered LDL cholesterol, the National Cholesterol Education Program (NCEP) set forth guidelines for medical professionals to follow when instructing patients on a medical nutrition option for lowering cholesterol. Termed the TLC diet or the Therapeutic Lifestyle Changes Diet it emphasizes heart healthy lifestyle choices.

The Therapeutic Lifestyle Changes diet (TLC) is a cholesterol lowering diet that refers to a cholesterol-lowering treatment that lowers a person's low-density lipoprotein (LDL) level and raises their high-density lipoprotein (HDL) level enough to reduce their risk of a heart attack or other chronic disease caused by hardening of the arteries.

The TLC diet follows these dietary guidelines

- Less than 7% of the day's total calories from saturated fat.
- 25-35% of the day's total calories from fat.
- Less than 200 milligrams of dietary cholesterol a day.

TLC diet tips

Meat, Poultry, Fish, Dry Beans, Eggs, and Nuts

- Limit the total amount of meat to 5 ounces or less per day
- Choose chicken and turkey without skin or remove skin before eating
- Eat fish, like cod, that has less saturated fat than either chicken or meat
- Dry peas and beans and tofu (bean curd) are great meat substitutes
- Limit egg yolks to no more than 2 yolks per week, including egg yolks in baked goods
- Substitute egg whites for whole eggs

Milk, Yogurt, and Cheese

- Eat 2 to 3 servings per day of low-fat or nonfat dairy products
- Choose varieties that have 3 grams of fat or less per ounce, including low-fat (1%) or nonfat cottage cheese
- Buy frozen desserts that are lower in saturated fat, like ice milk, low-fat frozen yogurt, sorbet
- Try low-fat or nonfat sour cream or cream cheese blends

Fats and Oils

- Replace saturated fats with unsaturated fat and limit the total amount of fats or oils
- Use liquid vegetable oils that are high in unsaturated fats (canola, corn, olive, peanut, safflower, sesame, soybean, sunflower oils)
- Use margarine made with unsaturated liquid vegetable oils as the first ingredient
- Limit butter, lard, fatback, and solid shortenings
- Buy light or nonfat mayonnaise and salad dressing

Fruits and Vegetables

- Eat at least 3 to 5 servings of fruits and vegetables each day
- Buy fruits and vegetables to eat as snacks, desserts, salads, side dishes, and main dishes
- Add a variety of vegetables to meat stews or casseroles or make a vegetarian main dish
- Snack on raw vegetables (carrots, broccoli, cauliflower, lettuce)
- Season with herbs, spices, lemon juice, vinegar, fat free or low-fat mayonnaise or salad dressing

Breads, Cereals, Rice, Pasta, and Other Grains

- Eat 6 to 11 servings of foods from this group each day
- Choose whole grain breads and rolls
- Buy dry cereals, most are low in fat, and limit high fat granola, muesli, and oat bran types made with coconut or coconut oil and nuts
- Buy pasta and rice to use as entrees and eliminate the high fat sauces (butter, cheese, cream)
- Limit sweet baked goods that are made with lots of saturated fat

Sweets and Snacks

- Choose sweets and snacks only every now-and-then
- Buy snack foods low in fat
- Some sweets and snacks may be low in fat, but most are not low in calories
- To reduce sodium intake, look for low sodium or unsalted varieties

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- Limit sodium intake to 2400 milligrams or less per day.
- Just enough calories to achieve or maintain a healthy weight and reduce your blood cholesterol level.

Reference: http://www.diet.com/store/facts/tlc-diet