Study Title: Pharmacodynamic Biomarkers to Support Biosimilar Development: Clinical Study 2 (PCSK9 Inhibitors – Alirocumab and Evolocumab)

Document Title: Clinical Study Protocol – Study No. SCR-007

Document Date: 18 May 2020

NCT Number: NCT04189484

CLINICAL STUDY PROTOCOL

Pharmacodynamic Biomarkers to Support Biosimilar Development: Clinical Study 2

(PCSK9 Inhibitors – Alirocumab and Evolocumab)

PROTOCOL NO. SCR-007

Sponsor: U.S. Food and Drug Administration
White Oak Building #64, Room 2072
10903 New Hampshire Avenue
Silver Spring, MD 20993

Sponsor Study Lead: David Strauss, MD, PhD
Director, Division of Applied Regulatory Science
U.S. Food and Drug Administration
Telephone: 301-796-6323
Email: david.strauss@fda.hhs.gov

Sponsor Medical Monitor: Keith Burkhart, MD
U.S. Food and Drug Administration
Telephone: 301-796-2226
Email: keith.burkhart@fda.hhs.gov

Project Manager: Jeffry Florian, PhD
U.S. Food and Drug Administration

Study Monitor: Jill Brown
FDA IRB Project Manager
U.S. Food and Drug Administration

Version of Protocol: 5.0

Date of Protocol: 18 May 2020

CONFIDENTIAL

Sponsor Signature Page

This study will be conducted with the highest respect for the individual participants in accordance with the requirements of this clinical study protocol and also in accordance with the following:

- The ethical principles that have their origin in the Declaration of Helsinki;
- International Council for Harmonisation (ICH) harmonised tripartite guideline E6 (R2): Good Clinical Practice; and
- All applicable laws and regulations, including without limitation, data privacy laws and compliance with appropriate regulations, including human subject research requirements set forth by the Institutional Review Board (IRB).

Digitally signed by David Strauss -S
DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=David Strauss -S, 0.9.2342.19200300.100.1.1=2000507494
Date: 2020.06.07 14:58:51 -04'00'

David Strauss, MD, PhD
Director, Division of Applied
Regulatory Science
U.S. Food and Drug Administration

Date

Investigator Signature Page

I confirm that I have read and that I understand this protocol, the investigator brochure, and other product information provided by the sponsor. I agree to conduct this study in accordance with the requirements of this protocol and also protect the rights, safety, privacy, and well-being of study subjects in accordance with the following:

- The ethical principles that have their origin in the Declaration of Helsinki;
- ICH harmonised tripartite guideline E6 (R2): Good Clinical Practice;
- All applicable laws and regulations, including without limitation data privacy laws and regulations;
- Human subject research requirements set forth by the IRB;
- Regulatory requirements for reporting of serious adverse events (SAEs) defined in Section 5.7.4.1 of this protocol; and
- Terms outlined in the Clinical Study Site Agreement.

I further authorize that my personal information may be processed and transferred in accordance with the uses contemplated in Section 7 of this protocol.

Colleen Nalepinski PA-C

Colleen Nalepinski MPAS, PA-C
Principal Investigator

22 MAY 2020
Date

Table of Contents

List of Tables

Protocol Synopsis

Protocol Number:	SCR-007
Title:	Pharmacodynamic Biomarkers to Support Biosimilar Development: Clinical Study 2 (PCSK9 Inhibitors – Alirocumab and Evolocumab)
Investigators:	Principal Investigator: Colleen Nalepinski MPAS, PA-C Study Physician: Carlos Sanabria, MD
Study Phase:	1
Study Period:	The duration of study participation will be up to 84 days (excluding the screening period).
Study Site:	Spaulding Clinical Research Unit, West Bend, Wisconsin
Background and Motivation:	Per statute, biosimilarity is defined when the biologic product "is highly similar to the reference product notwithstanding minor differences in clinically inactive components" and "there are no clinically meaningful differences between the biological product and the reference product in terms of the safety, purity, and potency of the product" (Public Health Service Act, Section 351(i)(2)). Biosimilars approved to date have generally included comparative clinical outcome studies. These typically require large sample sizes and long study length. However, FDA guidance documents outline that biosimilars may be approved based on clinical pharmacology pharmacokinetic (PK)/pharmacodynamic (PD) biomarker data without a comparative clinical study that contains an efficacy endpoint. FDA guidance outlines general principles for a PD biomarker; however, an evidentiary framework for acceptance and evaluation of PD biomarkers to support biosimilar approval does not exist. To support a determination of biosimilarity based on clinical pharmacology studies, FDA guidance states that clinical PK and PD studies should be conducted in the most sensitive and informative population. This should generally be healthy subjects if the product can be safely administered to them and a PD response is observed, as there is generally less variability. In addition, the most sensitive dose should be selected to detect and evaluate differences in the PK and PD profiles between products, which will usually be a dose on the steep part of the dose-response curve. Of note, for many biologics the approved dose is a super-saturating dose on the flat part of the dose-response curve. This highlights the potential need for studying doses that may be substantially lower than the approved clinical dose. Thus, FDA guidance recommends performing a pilot study with the reference product to determine an optimally informative dose.

Furthermore, FDA's guidance states that a small pilot study can evaluate the PK/PD relationship at multiple dose levels (e.g. low, intermediate, and clinical (i.e., high) doses) to obtain dose-/exposure-response data. If multiple dose levels are studied, PK/PD parameters such as the maximum PD response (Emax), the concentration that gives-half maximal response (EC50) and the slope of the concentration-effect relationship should be evaluated for similarity to further reduce uncertainty, though the evidentiary framework for comparing model-based assessments of PD similarity still needs to be developed.

PCSK9 Inhibitors

Two monoclonal antibodies (evolocumab and alirocumab) that inhibit proprotein convertase subtilisin/kexin type 9 (PCSK9) were approved that can reduce low-density lipoprotein cholesterol (LDL-C) concentration beyond statin therapy alone. PCSK9 promotes LDL-receptor degradation, which can lead to increased LDL-C. The PCSK9 inhibitors bind to PCSK9 next to the region that is required for LDL-receptor interaction, thus more LDL-receptors remain in circulation, which decrease circulating LDL-C. PCSK9 drugs also decrease apolipoprotein B (Apo B).

These drugs were initially approved based on 6-month outcome studies with LDL-C as a surrogate endpoint for longer-term cardiovascular outcomes. While these drugs have a biomarker tied to long-term clinical outcomes, most biologics are not approved based on a surrogate endpoint. However, FDA biosimilars guidance indicates that biomarkers should be tied to the mechanism of action, but not necessarily long-term clinical outcomes. In addition to assessing specific PD biomarkers and their parameters (e.g. area under the effect curve and time of maximal response) at single dose levels, we will also explore applying modeling-based approaches (e.g., Emax, EC50, and slope of concentration-effect relationship) to the PD data.

Summary

This study will obtain data over a range of doses, including information on the steep part of dose-/exposure-response curve, to inform clinical trial operating characteristics of subsequent PK and PD similarity studies for these drugs. This would be similar to and could substitute for what is suggested as a pilot study in the existing FDA biosimilars guidance. The study will collect data on various PD markers to better understand the dose-response relationship and variability in healthy subjects of traditional PD measurement-based approaches at a single dose (e.g. AUEC and

maximal response at a single time point). The study will also evaluate use of model-based approaches for analyzing data from the various dose levels to inform selection of sensitive doses. In addition, as many biologics from other classes do not have biomarkers associated with the known mechanism-of-action, this study will explore the use of proteomics and small RNA transcriptomics, and the analytical approach needed to identify and characterize circulating biomarkers that could be used for a PD similarity assessment.

Objectives and Endpoints:

The objectives of this study are:

Primary Objective(s)

1. Inform clinical trial operating characteristics for future clinical pharmacology PK and PD similarity studies using the different biomarker-based approaches.

Secondary Objective(s)

1. Determine the values and variability of PK and PD parameters at four dose levels (i.e., low, intermediate low, intermediate high, and high doses).
2. Explore PK and PD relationships using appropriate models.

Exploratory Objective(s)

1. To explore the utility of circulating proteins and small RNAs as potential PD biomarkers.
2. To inform on the analytical approach and experimental design required for identifying exploratory proteomic- and small RNA-based PD biomarkers

The endpoints of this study are:

Primary Endpoint

1. The values and variability of standard PD metrics (AUEC and maximal difference at a single time-point) for LDL-C at low, intermediate low, intermediate high, and high doses of evolocumab and alirocumab.

Secondary Endpoints

1. The values and variability of standard PD metrics (AUEC and maximal difference at a single time-point) for Apolipoprotein B (ApoB) at low, intermediate low, intermediate high, and high doses of evolocumab and alirocumab.
2. The values and variability of pharmacokinetic parameters (C_{max} and AUC of free drug concentration) at low, intermediate low, intermediate high, and high doses of evolocumab and alirocumab.

3. Parameters (E_{max}, and EC_{50}) calculated by the model after combining data from low, intermediate low, intermediate high, and high doses of evolocumab or alirocumab with placebo data.

Exploratory Endpoints

1. Plasma proteomics (differential expression of circulating proteins)
2. Plasma small RNA transcriptomics (differential expression of circulating small RNAs)
3. Differential levels will be evaluated in a systemic approach starting with high doses (discovery) of evolocumab and alirocumab, respectively, then progressing through intermediate high, intermediate low, and low doses (replication phases).

Study Design:

This is a pilot randomized, placebo-controlled, single-dose, parallel arm study. Healthy subjects will be randomized to four dose groups (low, intermediate low, intermediate high, and high) of each drug (evolocumab or alirocumab) or placebo.

Subjects will report to the study site for screening assessments from Days -21 to -2 and then will return to the site on Day -1 for baseline assessments. Prior to and following study drug or placebo administration on Day 1, they will undergo assessments as described in the Schedule of Events (Table 9-1). Subjects will stay in house for the first week.

Depending on the treatment arm, subjects will return to the clinic up to 9 times for PK and PD blood draws and additional study procedures as outlined in the schedule of events. For all scheduled outpatient visits (i.e., all visits after 1 week of in-house residency, see **Error! Reference source not found.**), subjects will be required to return to the clinic within a four-hour window.

The planned cohort sizes and investigational product doses are as follows:

Subjects (n)	Treatment Group	Drug
8	A	Evolocumab low (21 mg)
8	B	Evolocumab intermediate low (35 mg)
8	C	Evolocumab intermediate high (70 mg)
8	D	Evolocumab high (140 mg)

8	E	Alirocumab low (15 mg)
8	F	Alirocumab intermediate low (25 mg)
8	G	Alirocumab intermediate high (50 mg)
8	H	Alirocumab high (100 mg)
8	I	Placebo

Subjects in groups A, B, E, and F will follow the schedule of events through day 42. Subjects in groups C and G will follow the schedule of event through day 56. Subjects in groups D, H, and I will follow the schedule of events through day 84. Each treatment group should include equal representation of male and female subjects.

Subjects will be screened for study eligibility from Days –21 to –2. During the screening visit, the inclusion and exclusion criteria will be reviewed to ensure the subject is appropriate for the study. The informed consent form will be reviewed with the subject by a member of the study team and the subject will be encouraged to ask questions to ensure he or she has a good understanding of the study. If the subject is eligible and agrees to participate, the subject will be asked to sign the informed consent form before any study-specific procedure is performed, including randomization.

After the consent process is complete, demographic data, medical history, and concomitant medications will be recorded. A physical examination will be performed by a study team member. Clinical laboratory tests (hematology, serum chemistry, and urinalysis) will be performed. Female subjects must have a negative pregnancy test result. Screening tests will be performed within 21 days of and no later than 2 days before Day 1.

Results of all screening tests will be evaluated by the study clinician/investigator against the inclusion/exclusion criteria to confirm subject eligibility. At check-in, eligibility criteria will be reviewed, any changes in medical history (including concomitant medications) will be documented, vital sign measurements and a 12-lead electrocardiogram(ECG) will be performed, clinical laboratory, drug and alcohol, and pregnancy tests (for females) will be performed, an intravenous (IV) catheter may be inserted into the subject's forearm region for blood collection (if needed), study drug will be administered, and blood samples will be collected per protocol.

U.S. Food and Drug Administration (FDA) will prepare the randomization schedule. Subjects will enter the study clinic for

	check-in procedures (Day -1), the day before study drug administration on Day 1. All study drugs and placebo will be administered subcutaneously (SC). Subjects will be discharged from the study after completion of all study procedures. If a subject discontinues from the study prematurely, all procedures scheduled for the end of the study will be performed. Meal timing and components, activity levels, and general conditions in the study clinic will be as similar as possible on the treatment days.
Subject Population:	Approximately 72 healthy subjects are planned for enrollment. Up to 14 subjects may be qualified as replacements. Thus, a maximum of 86 subjects will be exposed to study drugs and procedures during the study. Every effort will be made to maintain an approximate 50:50 male to female sex distribution. Recruitment materials (e.g., internet, radio, and print advertisements, social media posts) will be approved by the local Institutional Review Board (IRB, i.e., Advarra) before telephone screening. Subjects will be offered payment for Screening and participation in the study, but no special incentives are offered.
Study Drugs, Dosage, and Route of Administration:	Repatha (evolocumab) 140 mg/mL solution in a single-use prefilled syringe will be obtained. For the low (21 mg), intermediate low (35 mg), and intermediate high (70 mg) doses, the drug will be measured and reduced to the target dose (Table 5-1). The target dose will be administered to the upper arm subcutaneously. Praluent (alirocumab) 150 mg/mL in a single-dose prefilled pen will be obtained. For all doses, the drug will be measured and reduced to the target dose (Table 5-1). The target dose will be administered to the upper arm subcutaneously. For all drugs, standard drug doses or lower doses will be used in this research study. A single dose of the assigned study drug will be administered to each subject during the treatment period. **<u>Study Drugs</u>** • Repatha (evolocumab) • Praluent (alirocumab)

Reference Drug, Dosage, and Route of Administration: A placebo subcutaneous injection of equal volume and comparable pH to the diluent for the high dose of each drug will be injected into upper arm subcutaneously.

Inclusion Criteria: Subjects who meet all the following inclusion criteria will be eligible to participate in the study:

1. Subject signs an IRB-approved written informed consent and privacy language as per national regulations (e.g., Health Insurance Portability and Accountability Act authorization) before any study related procedures are performed.
2. Subject is a healthy man or woman, 18 to 55 years of age, inclusive, who has a body mass index of 18.5 to 32.0 kg/m2, inclusive, at Screening.
3. Subject has a LDL-C level >=100 and <=190 mg/dL inclusive, at Screening.
4. Subject has normal medical history findings, clinical laboratory results, vital sign measurements, 12 lead electrocardiogram (ECG) results, and physical examination findings at Screening or, if abnormal, the abnormality is not considered clinically significant (as determined and documented by the investigator or designee).
5. Subject must have a negative test result for alcohol and drugs of abuse at screening and Check-in (Day -1).
6. Female subjects must be of non-childbearing potential or, if they are of childbearing potential, they must: 1) have been strictly abstinent for 1 month before Check in (Day -1) and agree to remain strictly abstinent for the duration of the study and for at least 1 month after the last application of study drug; OR 2) be practicing 2 highly effective methods of birth control (as determined by the investigator or designee; one of the methods must be a barrier technique) from at least 1 month before Check in (Day -1) until at least 1 month after the last application of study drug.
7. Male subjects must agree to practice 1 highly effective method of birth control (as determined by the investigator or designee) from at least 1 month before Check in (Day-1) until at least 1 month after the last application of study drug.
8. Subject is highly likely (as determined by the investigator) to comply with the protocol defined procedures as to complete the study.

Exclusion Criteria:

Subjects who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Subject is taking cholesterol medication (e.g. statins).
2. Subject is anemic (i.e., with Hct or Hgb less than the lower limit of normal) or has any chronic condition(s) that may impact blood sample collection.
3. Subject has had previous exposure to the biologic evolocumab or alirocumab.
4. Subject has a history of asthma.
5. Subject has a history of anaphylaxis from environmental exposures such as peanuts or bee stings.
6. Subject has an allergic history that includes urticaria, angioedema or respiratory coughing or bronchospasm.
7. Subject has a history of severe local reactions or generalized erythema from skin allergen testing.
8. Subject has used any prescription or nonprescription drugs (including aspirin or NSAIDs and excluding oral contraceptives and acetaminophen) within 14 days or 5 half-lives (whichever is longer) or complementary and alternative medicines within 28 days before the first dose of study drug.
9. Subjects are currently participating in another clinical study of an investigational drug or are have been treated with any investigational drug within 30 days or 5 half-lives (whichever is longer) of the compound.
10. Subject has used nicotine-containing products (e.g., cigarettes, cigars, chewing tobacco, snuff) within 6 weeks of Screening.
11. Subject has consumed alcohol, xanthine-containing products (e.g., tea, coffee, chocolate, cola), caffeine, grapefruit, or grapefruit juice within 48 hours of dosing. Subjects must refrain from ingesting these throughout the study.
12. Subject has any underlying disease or surgical or medical condition (e.g., cancer, human immunodeficiency virus [HIV], severe hepatic or renal impairment) that could put the subject at risk or would normally prevent participation in a clinical study. This includes subjects with any underlying medical conditions that put subjects at higher risk for coronavirus disease of 2019 (COVID-19) complications; per current Center for Disease Control and Prevention (CDC) recommendations this includes:

- People with chronic lung disease or moderate to severe asthma
- People who have serious heart conditions
- People who are immunocompromised
- Many conditions can cause a person to be immunocompromised, including cancer treatment, smoking, bone marrow or organ transplantation, immune deficiencies, poorly controlled HIV, and prolonged use of corticosteroids and other immune weakening medications
- People with severe obesity (BMI of 40 or higher)
- People with diabetes
- People with chronic kidney disease undergoing dialysis
- People with liver disease

13. Subject has any signs or symptoms that are consistent with COVID-19. Per current CDC recommendations this includes subjects with the symptoms cough or shortness of breath or difficulty breathing, or at least two of the following symptoms: fever, chills, repeated shaking with chills, muscle pain, headache, sore throat or new loss of taste/smell. In addition, the subject has any other findings suggestive of COVID-19 risk in the opinion of the investigator.
14. Subject tests positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by a molecular diagnostic test performed prior to admission.
15. Subject has known or suspected allergies or sensitivities to any study drug.
16. Subject has clinical laboratory test results (hematology, serum chemistry lipid panel and comprehensive metabolic panel) at Screening that are outside the reference ranges provided by the clinical laboratory and considered clinically significant by the investigator.
17. Subject has a positive test result at Screening for HIV 1 or 2 antibody, hepatitis C virus load, hepatitis C virus antibodies, or hepatitis B surface antigen.
18. Subject is unable or unwilling to undergo multiple venipunctures for blood sample collection because of poor tolerability or poor venous access.
19. Female subjects are pregnant or lactating before enrollment in the study.

Sample Collection

The following samples should be collected and processed as follows. Specific timepoints can be found in Table 9-1 and additional details regarding the assessments are in the following sections.

1. For the PK assessments, approximately **5 mL of whole blood will be collected and processed for serum**. PK assessments will be collected in a fasted state at the following timepoints: 0-hour (predose), +24-hour (Day 2), Day 3, Day 4, Day 5, Day 6, Day 7 and all outpatient visits. All other timepoints will be collected non-fasted. The serum should be aliquoted into two approximately equal aliquots (Aliquot A and B). Samples will be stored at -80° C and will be shipped to the FDA on dry ice at study completion. Drug concentrations will be measured by FDA.

2. For the lipid analysis, **approximately 5 mL of whole blood will be collected and processed for serum**. All lipid analyses will be collected in a fasted state.

3. For proteomic and small RNA transcriptomic analyses, **approximately 5 mL of whole blood will be collected and processed for plasma**. Proteomic and small RNA transcriptomic analyses will be collected in a fasted state at the following timepoints: predose, +24-hour (Day 2), Day 3, Day 4, Day 5, Day 6, Day 7 and all outpatient visits. All other timepoints (including Check-in) will be collected non-fasted. The plasma should be equally aliquoted into separate sterile nuclease-free 1.5 mL safe-lock Eppendorf tubes with approximate volumes of 500 µL. Plasma should be prepared, aliquoted, and placed into the -80° C freezer within 2 hours of collection. Samples will be shipped to the FDA on dry ice at study completion for analysis by DARS research staff.

4. For genomic analyses**, one buffy coat sample per subject will be collected** at time 0 (pre-dose) on Day 1. The buffy coat will be collected from a proteomic and small RNA transcriptomic plasma preparation. After the upper plasma phase is transferred to a sterile tube (conical shape bottom), the buffy coat layer will be transferred to a sterile 2 mL Eppendorf (safe-lock) tube (on wet ice). Buffy coat samples should be immediately frozen in a dry ice/ethanol bath (for ~ 40 seconds) and then transferred to a -80oC freezer. Only sterile (nuclease-free) plastic pipettes, pipette tips and Eppendorf tubes should be used. All barcoded buffy coat Eppendorf tubes should be frozen and stored at -80oC as described above. Samples will be

shipped to the FDA on dry ice at study completion for analysis by DARS research staff.

5. At specified times for urine sample collection, **3-5 mL of urine will be collected and processed** as described in the SOP for preparation, aliquoting, and storage of urine samples. All urine samples will be collected in the fasted state. Approximately 6 processed aliquots will be immediately frozen in a dry ice/ethanol bath (for ~40 second or until frozen) and then immediately transferred to -80oC for long-term storage. Sample analysis will be performed by DARS research staff.

Pharmacokinetic Assessments:

Samples will be collected as described above and in the schedule of events for quantification of evolocumab or alirocumab concentration.

The following PK parameters will be determined for each subject:

- Maximum concentration (observed peak drug concentration) (Cmax)
- Time at which Cmax occurs (Tmax)
- AUC from time 0 to the sampling time corresponding to the last quantifiable concentration (Clast) (AUC0-t)
- Elimination rate constant (Kel)
- Terminal half-life (t1/2)
- AUC from time 0 extrapolated to infinity (AUC0-inf)

Pharmacodynamic Assessments:

Lipid analysis

Whole-blood samples for the lipid analysis will be collected for determination of LDL-C and ApoB as a part of a lipid panel.

Proteomics and Small RNA Transcriptomics:
Whole-blood samples will be collected and processed to provide plasma for proteomic and small RNA analyses.

Additional pharmacodynamic sampling and analyses:

The PK sample that was collected will also be used for determination of PCSK9 concentration.

Primary PD Biomarker:

- Low density lipoprotein cholesterol (LDL-C)

Primary PD Assessments:

- Area under the effect curve (AUEC) change from baseline truncated after return to baseline
- Percentage area under the effect curve (pAUEC) change from baseline truncated after return to baseline
- Maximum change from baseline at single time-point

Secondary PD Assessments:

- Apoliprotein B (Apo B)
- Modeling-based measures using multiple doses
  - Maximum PD response (Emax)
  - Concentration that gives half maximal response (EC50)

Exploratory PD Biomarkers:

- Serum free PCSK9 target concentration
- Plasma proteomics (assessment of circulating proteins including PCSK9 and ApoB levels)
- Plasma small RNA transcriptomics (assessment of circulating small RNAs)

Safety Assessments:

Safety will be evaluated in terms of adverse events (AEs), clinical laboratory results (hematology, serum chemistry, and urinalysis), vital sign measurements (blood pressure, heart rate, respiratory rate, and oral body temperature), safety 12-lead ECG results, and physical examination findings.

Other Assessments:

Buffy coat samples will be collected and processed for genomic analyses. Maintenance of privacy of the genetic information of study subjects is a priority. All DNA samples will be de-identified. DNA may be stored and used for analyses for up to 10 years.

Urine samples will be collected and sample analysis will be performed by DARS research staff.

Sample Size and Threshold Determination:

This sample size (8 subjects per arm) was determined empirically based on general sample size requirements for estimating values and variability of primary PD and PK/PD characteristics that can be used to inform the study design and sample size of future biosimilarity studies.

Statistical Methods:

All data will be presented in data listings. Data from subjects excluded from an analysis population will be presented in the data listings but not included in the calculation of summary statistics. The number of subjects who enroll in the study and the number and percentage of subjects who complete each assessment will be presented. The frequency and percentage of subjects who withdraw or discontinue from the study and the reason for withdrawal or discontinuation will be summarized. Demographic and baseline characteristics will be summarized overall and by treatment for all subjects.

Descriptive statistics will be used to summarize demographic and baseline subject characteristics. For continuous variables, the mean, median, standard deviation (SD), minimum, and maximum values will be reported. For categorical (nominal) variables, the number and percentage of subjects (or observations) will be reported.

PD and PK: The PD and PK population will include all subjects who receive study drug and have at least 1 estimable PK parameter after dosing. PD parameters of evolocumab and alirocumab will be listed and summarized using descriptive statistics (n, geometric mean, coefficient of variation, minimum, median, and maximum).

Primary Analysis

Pharmacodynamics

Peripheral blood LDL-C and ApoB AUEC will be calculated for each subject. For each dose, separate intervals will be used for the AUEC assessment to truncate noise introduced from baseline fluctuations. Maximum decrease from baseline will be determined for each subject at each dose using all sampled timepoints. For both assessments and biomarkers, measures will be log-transformed and utilize an ANCOVA approach for calculating geometric means and 90% confidence intervals for each treatment.

Separate population PD analyses will be conducted using nonlinear mixed effect modeling with NONMEM. AUECs calculated for each subject from all doses for a drug and placebo patient will be combined and evaluated assuming an E_{max} relationship to characterize system parameters such as E_{max} and EC_{50}.

Pharmacokinetics: C_{max} and AUC are the primary PK parameters of interest in PK similarity studies with subcutaneous drug administration. These and additional PK parameters will be determined for each subject using non-compartmental methods. All parameters will be reported with standard descriptive statistics

including the geometric mean and coefficient of variation. Calculation of PK parameters will be performed using actual sampling times.

Safety

The safety population will include all subjects who receive at least 1 dose of any of the study drugs. All AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). The incidence of AEs, organized by system organ class and preferred term, will be summarized with a focus on treatment-emergent AEs. Vital sign measurements will be summarized using descriptive statistics by time point. All values will be evaluated for clinically notable results. Data for additional safety parameters (e.g., physical examination findings) will be listed.

Date of Protocol: 18 May 2020

1. List of Abbreviations

Abbreviation	Definition
AE	adverse event
Ag/Ab	antigen/antibody
ANCOVA	analysis of covariance
ApoB	Apolipoprotein B
AUC	area under the concentration-time curve
AUEC	area under the effect curve
pAUEC	percentage area under the effect curve
CFR	Code of Federal Regulations
C_{max}	maximum observed concentration
COVID-19	coronavirus disease of 2019
DNA	deoxyribonucleic acid
EC_{50}	the concentration that gives-half maximal response
ECG	electrocardiogram
eCRF	electronic case report form
E_{max}	maximum PD response
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HBsAg	hepatitis B surface antigen
HepC	hepatitis C
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human immunodeficiency virus
ICH	International Council for Harmonisation
IRB	Institutional Review Board
IV	intravenous
kg	kilogram
LDL-C	low-density lipoprotein (LDL) cholesterol
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram
NSAID	nonsteroidal anti-inflammatory drug
PBMC	peripheral blood mononuclear cell
PCSK9	Proprotein convertase subtilisin/kexin type 9
PD	pharmacodynamic
PK	pharmacokinetic
QA	quality assurance
RNA	ribonucleic acid
SAE	serious adverse event

SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SC	subcutaneously
SD	standard deviation
TEAE	treatment-emergent adverse event
T_{max}	time of C_{max}

2. Introduction

Per statute, biosimilarity is defined when the biologic product "is highly similar to the reference product notwithstanding minor differences in clinically inactive components" and "there are no clinically meaningful differences between the biological product and the reference product in terms of the safety, purity, and potency of the product" (Public Health Service Act, Section 351(i)(2)). Biosimilars approved to date have generally included comparative clinical outcome studies. These typically require large sample sizes and long study length. However, FDA guidance documents outline that biosimilars may be approved based on clinical pharmacology pharmacokinetic (PK)/pharmacodynamic (PD) biomarker data without a comparative clinical study that contains an efficacy endpoint. FDA guidance outlines general principles for a PD biomarker; however, an evidentiary framework for acceptance and evaluation of PD biomarkers to support biosimilar approval does not exist.

To support a determination of biosimilarity based on clinical pharmacology studies, FDA guidance states that clinical PK and PD studies should be conducted in the most sensitive and informative population. This should generally be healthy subjects if the product can be safely administered to them and a PD response is observed, as there is generally less variability. In addition, the most sensitive dose should be selected to detect and evaluate differences in the PK and PD profiles between products, which will usually be a dose on the steep part of the dose-response curve. Of note, for many biologics the approved dose is a super-saturating dose on the flat part of the dose-response curve. This highlights the potential need for studying doses that may be substantially lower than the approved clinical dose. Thus, FDA guidance recommends performing a pilot study with the reference product to determine an optimally informative dose.

Furthermore, FDA's guidance states that a small pilot study can evaluate the PK/PD relationship at multiple dose levels (e.g. low, intermediate, and clinical (i.e., high) doses) to obtain dose-/exposure-response data. If multiple dose levels are studied, PK/PD parameters such as the maximum PD response (Emax), the concentration that gives-half maximal response (EC50) and the slope of the concentration-effect relationship should be evaluated for similarity to further reduce uncertainty, though the evidentiary framework for comparing model-based assessments of PD similarity still needs to be developed.

2.1. PCSK9 Inhibitors

Two monoclonal antibodies (evolocumab and alirocumab) that inhibit proprotein convertase subtilisin/kexin type 9 (PCSK9) were approved that can reduce low-density lipoprotein cholesterol (LDL-C) concentration beyond statin therapy alone. PCSK9 promotes LDL-receptor degradation, which can lead to increased LDL-C. The PCSK9 inhibitors bind to PCSK9 next to the region that is required for LDL-receptor interaction,

thus more LDL-receptors remain in circulation, which decrease circulating LDL-C. PCSK9 drugs also decrease apolipoprotein B (Apo B).

These drugs were initially approved based on 6-month outcome studies with LDL-C as a surrogate endpoint for longer-term cardiovascular outcomes. While these drugs have a biomarker tied to long-term clinical outcomes, most biologics are not approved based on a surrogate endpoint. However, FDA biosimilars guidance indicates that biomarkers should be tied to the mechanism of action, but not necessarily long-term clinical outcomes. In addition to assessing specific PD biomarkers and their parameters (e.g. area under the effect curve and time of maximal response) at single dose levels, we will also explore applying modeling-based approaches (e.g., Emax, EC50, and slope of concentration-effect relationship) to the PD data.

2.2. Summary

This study will obtain data over a range of doses, including information on the steep part of dose-/exposure-response curve, to inform clinical trial operating characteristics of subsequent PK and PD similarity studies for these drugs. This would be similar to and could substitute for what is suggested as a pilot study in the existing FDA biosimilars guidance. The study will collect data on various PD markers to better understand the dose-response relationship and variability in healthy subjects of traditional PD measurement-based approaches at a single dose (e.g. AUEC and maximal response at a single time point). The study will also evaluate use of model-based approaches for analyzing data from the various dose levels to inform dose selection for similarity studies. In addition, as many biologics from other classes do not have biomarkers associated with the known mechanism-of-action, this study will explore the use of proteomics and small RNA transcriptomics, and the analytical approach needed to identify and characterize circulating biomarkers that could be used for a PD similarity assessment.

3. Study Objectives

3.1. Primary Objective

The primary objective of this study is:

1. To inform clinical trial operating characteristics for future clinical pharmacology PK and PD similarity studies using the different biomarker-based approaches.

3.2. Secondary Objectives

The secondary objectives of this study are:

1. To determine the values and variability of PK and PD parameters at four dose levels (i.e., low, intermediate low, intermediate high, and high doses).
2. Explore PK and PD relationships using appropriate models

3.3. Exploratory Objective

The exploratory objective of this study is:

1. To evaluate the utility of circulating proteins and small RNAs as potential PD biomarkers.
2. To inform on the analytical approach and experimental design needed for identifying exploratory proteomic- and RNA-based PD biomarkers in plasma

4. Study Endpoints

4.1. Primary Endpoint

The primary endpoint of this study is:

1. The values and variability of standard PD metrics (AUEC and maximal difference at a single time-point) for LDL-C at low, intermediate low, intermediate high, and high doses of evolocumab and alirocumab.

4.2. Secondary Endpoints

The secondary endpoints of this study are:

1. The values and variability of standard PD metrics (AUEC and maximal difference at a single time-point) for ApoB at low, intermediate low, intermediate high, and high doses of evolocumab and alirocumab.
2. The values and variability of pharmacokinetic characteristics (Cmax and AUC of free drug concentration) at low, intermediate low, intermediate high, and high doses of evolocumab and alirocumab.
3. Parameters (Emax, and EC50) calculated by the model after combining data from low, intermediate low, intermediate high, and high doses of evolocumab or alirocumab with placebo data.

4.3. Exploratory Endpoints

The exploratory endpoints of this study are:

1. Plasma proteomics (differential expression of circulating proteins)
2. Plasma small RNA transcriptomics (differential expression of circulating small RNAs)
3. Differential levels will be evaluated in a systemic approach starting with high doses (discovery) of evolocumab and alirocumab, respectively, then progressing through intermediate high, intermediate low, and low doses (replication phases).

5. Investigational Plan

5.1. Study Design

This is a randomized, placebo-controlled, single-dose, parallel arm study. Healthy subjects will be randomized to four dose groups (low, intermediate low, intermediate high, and high) of each drug (evolocumab or alirocumab) or placebo (See Table 5-1).

Subjects will report to the study site for screening assessments from days -21 to -2 and then will return to the site on day -1 for baseline assessments. Prior to and following study drug or placebo administration on day 1, they will undergo assessments as described in the Schedule of Events (Table 9-1). Subjects will stay in house for the first week and will return to the clinic for study procedures as identified in the Schedule of Events (Table 9-1).

Depending on the treatment arm, subjects will return to the clinic up to 9 times for PK and PD blood draws and additional study procedures as outlined in the Schedule of Events (Table 9-1).

All subjects will be sampled daily for the first week. Subjects in groups A, B, E, and F will follow the schedule of events through day 42. Subjects in groups C and G will follow the schedule of event through day 56. Subjects in groups D, H, and I will follow the schedule of events through day 84. Each treatment group should include equal representation of male and female subjects.

Table 5-1: Study Treatment Groups

Subjects (n)	Treatment Group	Drug
8	A	Evolocumab low (21 mg)
8	B	Evolocumab intermediate low (35 mg)
8	C	Evolocumab intermediate high (70 mg)
8	D	Evolocumab high (140 mg)
8	E	Alirocumab low (15 mg)
8	F	Alirocumab intermediate low (25 mg)
8	G	Alirocumab intermediate high (50 mg)
8	H	Alirocumab high (100 mg)
8	I	Placebo

5.1.1 Common Procedures

At the study clinic (Spaulding Clinical Research unit in West Bend, Wisconsin), subjects will be screened for study eligibility from Day -21 to Day -2. On Day -1, subjects will enter the study clinic for check-in procedures the day before study drug administration. During the screening visit, the inclusion and exclusion criteria will be reviewed to ensure the subject is appropriate for the study. The informed consent form will be reviewed with

the subject by a member of the study team and the subject will be encouraged to ask questions to ensure the subject has a good understanding of the study. If the subject is eligible and agrees to participate, the subject will be asked to sign the informed consent form before any study-specific procedure is performed, including randomization.

After the consent process is complete, demographic data, medical history, and concomitant medications (including over-the-counter and complimentary/alternative supplements) will be recorded. A physical examination will be performed by a study team member. Clinical laboratory tests (hematology, serum chemistry, and urinalysis) will be performed. Female subjects must have a negative pregnancy test result. Any values outside the reference range will be evaluated for clinical significance. If a value is determined to be clinically significant or the subject has a positive pregnancy test result, the subject will be instructed to follow-up with his or her personal physician. Screening tests will be performed within 21 days of and no later than 1 day before Day -1.

Screening procedures will be performed by clinic staff, and all screening results will be evaluated by the study clinician/investigator against the inclusion/exclusion criteria to confirm subject eligibility. At check-in, eligibility criteria will be reviewed, any changes in medical history (including concomitant medications) will be documented, vital sign measurements and a 12-lead ECG will be performed, clinical laboratory, drug and alcohol, and pregnancy tests (for females) will be performed, an intravenous (IV) catheter may be inserted into the subject's forearm region for blood collection (if needed), study drug will be administered, and blood samples will be collected per protocol.

The FDA project biostatistician will prepare the randomization schedule, and subjects will be randomly assigned to 1 of 9 treatment groups, described in Table 5-1.

Subjects will enter the study clinic for check-in procedures the day before study drug administration of the first period (Day -1). All study drugs will be administered subcutaneously. On Day 1, subjects will receive their assigned treatment according to the randomization schedule.

5.1.2 Dosing Schedule

- Treatment Group A: Evolocumab 21 mg, SC
- Treatment Group B: Evolocumab 35 mg, SC
- Treatment Group C: Evolocumab 70 mg, SC
- Treatment Group D: Evolocumab 140 mg, SC
- Treatment Group E: Alirocumab 15 mg, SC
- Treatment Group F: Alirocumab 25 mg, SC
- Treatment Group G: Alirocumab 50 mg, SC

- Treatment Group H: Alirocumab 100 mg, SC
- Treatment Group I: Placebo SC

Subjects assigned to treatment groups A, B, E, and F will be discharged from the study on Day 42, subjects assigned to treatment groups C and G will be discharged from the study on Day 56, and subjects in treatment groups D, H, and I will be charged from the study on Day 84. If a subject discontinues from the study prematurely, all procedures scheduled for discharge Day (e.g., Day 42 for treatment groups A, B, E, and F) will be performed. Meal timing and components, activity levels, and general conditions in the study clinic will be as similar as possible on the treatment days.

Safety will be evaluated in terms of AEs, clinical laboratory results (hematology, serum chemistry, and urinalysis), vital sign measurements (blood pressure, heart rate, respiratory rate, and oral body temperature), safety 12-lead ECG results, and physical examination findings (see Table 9-1).

5.1.3 Risk/Benefit

Subjects will be informed that participation in a human PK-PD study like the present one cannot be of benefit to healthy volunteers. Nevertheless, the information from the physical examination, vital sign measurements, and ECG results may be shared with the subject's personal physician if this is the subject's choice. Subjects will be informed that it is also their choice to inform their personal physician that they are participating in this research study.

Subjects will be informed that their contribution to the study is of major importance to agencies like the U.S. FDA for helping this agency better evaluate biomarkers. However, since this is a study involving healthy volunteers, subjects will be informed that they have the alternative not to participate.

Subjects will be informed that they may be exposed to risks associated with the pharmacological properties of the investigational product and the study procedures. The following summary of potential AEs for the study drugs will be provided to and discussed with the subjects:

1. Evolocumab (1)
    - Hypersensitivity reactions: Angioedema, rash, and urticaria have occurred.
    - Common adverse reactions (> 5% of patients treated with REPATHA and occurring more frequently than placebo in previous clinical trials): nasopharyngitis, upper respiratory tract infection, influenza, back pain, and injection site reactions, diabetes mellitus.
2. Alirocumab (2)

- Hypersensitivity reactions (e.g., pruritus, rash, urticaria), including some serious events (e.g., hypersensitivity vasculitis and hypersensitivity reactions requiring hospitalization).
- Commonly occurring adverse reactions (≥5% of patients treated with PRALUENT and occurring more frequently than with placebo in previous clinical trials) are nasopharyngitis, injection site reactions, and influenza.

The study drugs will not be administered to anyone who is pregnant. All women must take a pregnancy test before receiving any study drug in this study. All woman of childbearing potential enrolled on this study will be informed that they must use effective birth control methods (abstinence, intrauterine device, and contraceptive foam and a condom [i.e., double-barrier method]) during treatment. Subjects will be informed that they must notify the investigator if they or their female partners become pregnant during the course of the study.

Subjects will be informed that insertion of an IV catheter may be required for blood sample collection and, during insertion of the catheter, soreness, bruising, or infection at the insertion site are possible but unlikely. Subjects will also be informed that dizziness and lightheadedness may occur during direct venipuncture, insertion of the IV catheter, or during blood collection.

Subjects will be informed that they may eat only meals and snacks that are provided during periods of their stay in the study clinic, and that they must consume all of each meal that is served at a reasonable pace (within 25 minutes).

Subjects will be informed that blood samples will be collected for genomic testing to explore how a person's genes or genome may affect the way the body and drug interact.

Subjects will be informed that the confidentiality of their data will be respected at all times according to state law, and the study personnel handling their study data are bound by confidentiality agreements.

Subjects will be informed that extra precautions will be put in place that will limit the risk of COVID-19. Precautions will be documented in a COVID-19 risk management plan. Currently, this includes phone screening to prevent symptomatic participants from entering the clinic; triage of all potential study subjects entering the building at screening and check-in for potential contacts with COVID-19, signs and symptoms, temperature monitoring and potential serology screening for severe acute respiratory syndrome corona virus 2 (SARS CoV-2); SARS CoV-2 molecular testing just prior to or at check-in for admission to the study floor; all study participants and staff wearing masks except when in a private room or for a limited time for a study procedure (e.g. study drug administration); staff wearing personal protective equipment, social distancing during screening and in-house stays including 1 subject per room for overnight stays; extra hand sanitation stations with hand washing and sanitation policies per CDC recommendations;

closing common areas and serving food at subjects' room resulting in subjects spending most of their time in their rooms with the exception of specified times for walking in the halls; daily temperature screening; and separate staff for confined vs. not-confined participants whenever possible. Designated isolation rooms will be set up to segregate any participant(s) that develop any symptoms of concern while housed in the unit and COVID-19 testing will be done when deemed necessary by the Investigator. When subjects return to the site for follow-up, outpatient visits that require entering the building will also have COVID-19 triage. When outpatient visits only require a blood draw, this may be performed in a covered area outside the building. Subjects will be informed that despite the extra precautions there is still a risk of them contracting COVID-19. Any changes to the COVID-19 precautions (e.g. due to updated CDC recommendations or new testing becoming available) will be documented in the COVID-19 risk mitigation plan.

Subjects will be informed that the study drug and all tests, procedures, and visits required by the study are provided at no cost to them. If subjects become ill or physically injured because of participation in this study, they will be informed that costs of treatment will not be covered by the sponsor.

If a subject becomes pregnant, she will be informed that neither Spaulding Clinical Research nor the sponsor will be responsible for the cost of any obstetric or related care, or for the child's care.

5.2. Selection of Study Population

Subjects will be screened, and the data collected will be reviewed by the principal investigator. Only those subjects who meet all of the eligibility criteria will be enrolled. Approximately 72 healthy subjects are planned for enrollment. Up to 14 subjects may be qualified as replacements as described in Section 5.5.2. Thus, a maximum of 86 subjects will be exposed to study drugs and procedures during the study. Every effort will be made to maintain an approximate 50:50 male to female sex distribution.

5.2.1 Inclusion Criteria

Subjects who meet all of the following inclusion criteria will be eligible to participate in the study:

1. Subject signs an IRB approved written informed consent and privacy language as per national regulations (e.g., Health Insurance Portability and Accountability Act authorization) before any study related procedures are performed.
2. Subject is a healthy man or woman, 18 to 55 years of age, inclusive, who has a body mass index of 18.5 to 32.0 kg/m^2, inclusive, at Screening.
3. Subject has a LDL-C level >=100 and <=190 mg/dL inclusive, at Screening.

4. Subject has normal medical history findings, clinical laboratory results, vital sign measurements, 12 lead electrocardiogram (ECG) results, and physical examination findings at Screening or, if abnormal, the abnormality is not considered clinically significant (as determined and documented by the investigator or designee).
5. Subject must have a negative test result for alcohol and drugs of abuse at screening and Check-in (Day -1).
6. Female subjects must be of non-childbearing potential or, if they are of childbearing potential, they must: 1) have been strictly abstinent for 1 month before Check in (Day -1) and agree to remain strictly abstinent for the duration of the study and for at least 1 month after the last application of study drug; OR 2) be practicing 2 highly effective methods of birth control (as determined by the investigator or designee; one of the methods must be a barrier technique) from at least 1 month before Check in (Day -1) until at least 1 month after the last application of study drug.
7. Male subjects must agree to practice 1 highly effective method of birth control (as determined by the investigator or designee) from at least 1 month before Check in (Day-1) until at least 1 month after the last application of study drug.
8. Subject is highly likely (as determined by the investigator) to comply with the protocol defined procedures and to complete the study

5.2.2 Exclusion Criteria

Subjects who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Subject is taking cholesterol medication (e.g. statins).
2. Subject is anemic (i.e., with Hct or Hgb less than the lower limit of normal) or has any chronic condition(s) that may impact blood sample collection.
3. Subject has had previous exposure to the biologic Evolocumab or Alirocumab.
4. Subject has a history of asthma.
5. Subject has a history of anaphylaxis from environmental exposures such as peanuts or bee stings.
6. Subject has an allergic history that includes urticaria, angioedema or respiratory coughing or difficulty breathing.
7. Subject has a history of severe local reactions or generalized erythema from skin allergen testing.
8. Subject has used any prescription or nonprescription drugs (including aspirin or NSAIDs and excluding oral contraceptives and acetaminophen) within 14 days or 5 half-lives (whichever is longer) or complementary and alternative medicines within 28 days before the first dose of study drug.
9. Subjects are currently participating in another clinical study of an investigational drug or are have been treated with any investigational drug within 30 days or 5 half-lives (whichever is longer) of the compound.

10. Subject has used nicotine-containing products (e.g., cigarettes, cigars, chewing tobacco, snuff) within 6 weeks of Screening.
11. Subject has consumed alcohol, xanthine-containing products (e.g., tea, coffee, chocolate, cola), caffeine, grapefruit, or grapefruit juice within 48 hours of dosing. Subjects must refrain from ingesting these throughout the study.
12. Subject has any underlying disease or surgical or medical condition (e.g., cancer, human immunodeficiency virus [HIV], severe hepatic or renal impairment) that could put the subject at risk or would normally prevent participation in a clinical study. This includes subjects with any underlying medical conditions that put subjects at higher risk for coronavirus disease of 2019 (COVID-19) complications; per current Center for Disease Control and Prevention (CDC) recommendations this includes:
    - People with chronic lung disease or moderate to severe asthma
    - People who have serious heart conditions
    - People who are immunocompromised
    - Many conditions can cause a person to be immunocompromised, including cancer treatment, smoking, bone marrow or organ transplantation, immune deficiencies, poorly controlled HIV, and prolonged use of corticosteroids and other immune weakening medications
    - People with severe obesity (body mass index of 40 or higher)
    - People with diabetes
    - People with chronic kidney disease undergoing dialysis
    - People with liver disease
13. Subject has any signs or symptoms that are consistent with COVID-19. Per current CDC recommendations this includes subjects with the symptoms cough or shortness of breath or difficulty breathing, or at least two of the following symptoms: fever, chills, repeated shaking with chills, muscle pain, headache, sore throat or new loss of taste/smell. In addition, the subject has any other findings suggestive of COVID-19 risk in the opinion of the investigator.
14. Subject tests positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by a molecular diagnostic test performed prior to admission.
15. Subject has known or suspected allergies or sensitivities to any study drug.
16. Subject has clinical laboratory test results (hematology, serum chemistry lipid panel and comprehensive metabolic panel) at Screening that are outside the reference ranges provided by the clinical laboratory and considered clinically significant by the investigator.
17. Subject has a positive test result at Screening for HIV 1 or 2 antibody, hepatitis C virus load, hepatitis C virus antibodies, or hepatitis B surface antigen.
18. Subject is unable or unwilling to undergo multiple venipunctures for blood sample collection because of poor tolerability or poor venous access.
19. Female subjects are pregnant or lactating before enrollment in the study.

5.3. Screening Failures

Subjects who sign and date the informed consent form but who fail to meet the inclusion and exclusion criteria are defined as screening failures. A screening log, which documents the subject initials and reason(s) for screening failure, will be maintained by the investigator for all screening failures. A copy of the log should be retained in the investigator's study files.

If a subject fails the screening process because of an abnormal laboratory result, they can receive a copy of the results upon request. The investigator will determine if follow-up for the abnormal laboratory result is needed and will encourage the subject to follow-up with his or her personal physician as appropriate. All subjects will be informed as to the reason(s) they are excluded from study participation, even if follow-up is not required. If a subject fails the screening process because of a positive test result for human immunodeficiency virus or hepatitis, the positive result will be reported to local health authorities as required by law.

5.4. Termination of Study or Investigational Site

5.4.1 Criteria for Termination of the Study

The study will be completed as planned unless one of the following criteria is satisfied that requires early termination of the study.

- New information regarding the safety or efficacy of the study drug(s) that indicates a change in the known risk profile for the study drug(s), such that the risk is no longer acceptable for subjects participating in the study.
- Significant violation of Good Clinical Practice (GCP) that compromises the ability to achieve the primary study objective or compromises subject safety.

5.4.2 Criteria for Termination of Investigational Site

The study site may be terminated if the site (including the investigator) is found in significant violation of GCP, the protocol, the contractual agreement, or is unable to ensure adequate performance of the study.

In the event that the sponsor elects to terminate the study or the investigational site, a study-specific procedure for early termination will be provided by the sponsor; the procedure will be followed by the applicable investigational site during the course of termination.

5.5. Criteria for Subject Withdrawal

Subjects may withdraw from the study at any time at their own request, or they may be withdrawn by the investigator without the approval of the subject based on the

investigator's clinical judgment. A subject is not required to provide a written request to withdraw from the study; however, a written request is required if a subject withdraws consent for his or her personal data to be used for study-related purposes.

A subject may be discontinued for any of the following reasons:

- AE: The subject has experienced an AE that, in the opinion of the investigator, requires early termination. The appropriate electronic case report form (eCRF) must be completed for each AE. If a subject is discontinued from the study due to an AE, the investigator is required to follow-up with the subject until the event resolves or becomes stable. If a subject dies during the study, the cause of death must be reported as a serious AE (SAE), with an outcome of death noted in the eCRF.
- Protocol Violation: The subject failed to meet protocol entry criteria or did not adhere to protocol requirements, and continued participation poses an unnecessary risk to the subject's health.
- Withdrawal by Subject: The subject (or other responsible individual [e.g., caregiver]) wishes to withdraw from the study in the absence of a medical need.

  NOTE: Withdrawal due to an AE should not be recorded in the "voluntary withdrawal" category.
- Study Terminated by Sponsor: The sponsor, IRB, FDA, or other regulatory agency terminates the study.
- Pregnancy: The subject is found to be pregnant.

  NOTE: If the subject is found to be pregnant, the subject must be withdrawn immediately. The pregnancy will be followed-up to term, and the outcome, including any premature termination will be recorded. All live births must be followed for a minimum of 30 days or until the first well-baby visit.
- Other.

  NOTE: This category records withdrawals caused by an accidental or a medical emergency, unblinding, and other rare cases. The specific reason should be recorded in the comment space of the eCRF.

5.5.1 Handling of Withdrawals

The investigator may terminate a subject's study participation at any time during the study when the subject meets the criteria described in Section 5.5. In addition, a subject may discontinue his or her participation without giving a reason at any time during the study. Subjects will be informed that their participation in the study is voluntary, that refusal to participate will involve no penalty or loss of benefits to which the subject is

otherwise entitled, and that the subject may discontinue participation at any time without penalty or loss of benefits to which the subject is otherwise entitled.

Should a subject's participation be discontinued, the primary reason for termination must be recorded. In addition, efforts should be made to perform all procedures scheduled for the early termination visit. Any data and samples collected before subject withdrawal will become the property of the sponsor.

5.5.2 Replacement Subjects

Approximately 72 healthy subjects are planned for enrollment and randomized to 1 of 9 treatment groups. Up to 14 subjects may be qualified as replacements. Thus, a maximum of 86 subjects will be exposed to study drugs and procedures during the study.

A replacement algorithm will be pre-specified in the subject replacement plan to guide the unblinded Spaulding pharmacist to determine this while the rest of the staff and sponsor remain blinded. A maximum of 14 replacement subjects may be enrolled in the study, and replacement subjects (if needed) must complete the treatment period. A replacement subject will receive the same treatment as the subject being replaced.

5.6. Study Visits

5.6.1 Recruitment

Recruitment materials (e.g., internet, radio, and print advertisements, social media posts) will be approved by the local IRB before telephone screening. The sponsor is responsible for registration of the study on clinicaltrials.gov; however, this may not occur until the local IRB has approved the final study protocol.

5.6.1.1 Compensation

Subjects will be offered payment for Screening; however, if the results of their alcohol and drug screening tests are positive, they will not be compensated. Subjects who complete the entire study will receive payment according to the schedule provided in the informed consent form. No special incentives are offered. Final payment will not be released until all follow-up procedures have been completed and accepted by the investigator.

If a subject chooses to withdraw from the study prematurely, he or she will only be compensated for completed days. If subjects are withdrawn for medical reasons or if the study is halted temporarily or permanently, the subjects will receive compensation proportional to the time spent in the study. No compensation will be provided if a subject is dismissed from the study for noncompliance (e.g., improper conduct, ingesting alcohol and/or drugs [including recreational drugs], tampering with the study drug, consuming any prohibited foods or beverages).

If subjects are required to stay in the clinic for a longer period for safety reasons, they will be compensated at a rate proportional to the entire compensation for the study. If a subject becomes ill or physically injured because of participation in this study, the subject will be referred for treatment.

5.6.2 Screening

The following procedures and assessments will be performed at Screening (Day –21 to Day –2):

- Obtain informed consent/HIPAA authorization. The informed consent process will be performed by a clinical research nurse in a private room. The subject will be given unlimited time to ask questions regarding study participation, and each subject will be questioned to ensure their understanding.

After informed consent is obtained:

- Review inclusion/exclusion criteria to confirm subject eligibility
- Record demographic information
- Measure height, weight, and calculate body mass index
- Perform serology screening (HIV antigen/antibody [Ag/Ab] Combo 1/2, HepC antibody, HBsAg)
- Record medical history
- Perform alcohol and drug screening (amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, alcohol, opiates, phencyclidine, propoxyphene, and methadone)
- Perform a serum pregnancy test (female subjects only)
- Perform FSH measuring (postmenopausal [i.e., without menses for two years] female subjects only)
- Record prior medications
- Monitor for AEs
- Perform clinical laboratory tests (hematology, serum chemistry, and urinalysis)
- Measure vital signs (blood pressure, heart rate, respiratory rate, and oral body temperature)
- Perform a safety 12-lead ECG
- Perform a complete physical examination
- Collect whole blood for LDL-C and ApoB quantitation (5 mL)

5.6.3 Study Periods

This is a randomized, placebo-controlled, single-dose, parallel arm study. Healthy subjects will be randomized to four dose groups (low, intermediate low, intermediate high, and high) of each drug (evolocumab and alirocumab) or placebo. Subjects assigned to Groups A, B, E, and F will be kept in the study for 42 days. Subjects assigned to Groups C and G will be kept in the study for 56 days, and the remaining subjects (Groups D, H, I) will remain in the study for 84 days.

5.6.3.1 Check-in

The following procedures and assessments will be performed at Check-in (Day -1):

- Perform/review results from SARS-CoV-2 molecular diagnostic test
- Review inclusion/exclusion criteria to confirm subject eligibility
- Review medical history
- Perform alcohol and drug screening (amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, alcohol, opiates, phencyclidine, propoxyphene, and methadone)
- Perform a serum pregnancy test (female subjects only)
- Admit subject to the study clinic
- Randomization (after completion of check-in procedures on Day -1 or just before dosing on Day 1)
- Record concomitant medications
- Monitor for AEs
- Perform clinical laboratory tests (hematology, serum chemistry, and urinalysis)
- Measure vital signs (blood pressure, heart rate, respiratory rate, and oral body temperature)
- Perform a safety 12-lead ECG
- Perform a comprehensive physical examination
- Collect whole blood for LDL-C and ApoB quantitation (5 mL)
- Collect whole blood (5 mL) and process for plasma for proteomic and small-RNA transcriptomic analyses
- Collect urine (3-5 mL) for omic analysis (e.g., transcriptomics and/or metabolomics)

5.6.3.2 Treatment

The following procedures and assessments will be performed during the treatment period according to the Schedules of Events (Table 9-1):

- Record concomitant medications
- Monitor for AEs
- Perform clinical laboratory tests
- Administer study drug according to the randomization schedule following all other pre-dose examinations and specimen collection
- Perform a complete or targeted physical examination
- Measure vital signs (blood pressure, heart rate, respiratory rate, and oral body temperature).
- Perform safety 12-lead ECG. If scheduled for the same time, the safety 12-lead ECG will always be performed before vital sign measurement and blood sample collection
- Perform alcohol and drug screening (amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, alcohol, opiates, phencyclidine, propoxyphene, and methadone)
- Perform a serum pregnancy test (female subjects only)
- Collect PK blood samples (5 mL). There are no washout periods for this study.
- Collect blood samples for LDL-C and ApoB (5 mL)
- Collect whole blood (5 mL) for plasma proteomic and small RNA transcriptomic analyses
- Collect urine (3-5 mL) for omic analysis (e.g., transcriptomics and/or metabolomics)
- Collect buffy coat for genomic analysis

5.6.4 Discharge (or Early Termination)

The following procedures and assessments will be performed before the subject is discharged from the study (Day 42, Day 56, or Day 84, depending on treatment arm) or at early termination according to Table 9-1:

- Perform a serum pregnancy test (female subjects only)
- Record concomitant medications
- Monitor for AEs
- Perform clinical laboratory tests (hematology, serum chemistry, and urinalysis)

- Measure vital signs (blood pressure, heart rate, respiratory rate, and oral body temperature)
- Perform a safety 12-lead ECG
- Perform a complete physical examination
- Measure height, weight, and calculate body mass index
- Perform alcohol and drug screening (amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, alcohol, opiates, phencyclidine, propoxyphene, and methadone)
- Collect a single PK blood sample (5 mL)
- Collect whole blood samples (5 mL) for LDL-C and ApoB
- Collect whole blood (5 mL) and process for plasma for proteomic and small RNA transcriptomic analyses
- Collect urine (3-5 mL) for omic analysis (e.g., transcriptomics and/or metabolomics)
- Remove IV catheter (if applicable)
- Discharge subject from the study clinic after completion of all study procedures

5.7. Study Procedures

5.7.1 Pharmacokinetic Assessments

5.7.1.1 Pharmacokinetic Sample Collection

Pharmacokinetic blood samples (5 mL) for determination of evolocumab or alirocumab concentration will be collected at the following time points:

- Day 1: 0 (pre-dose), 0.5, 1, 2, 3, 4, 6, 8, 12, 24 h
- Day 2, 3, 4, 5, 6, 7, 10, 14, 21, 28, 35, 42, 56, 70, 84

PK blood samples will be collected in a fasted state at the following timepoints: 0-hour (predose), +24-hour (Day 2), Day 3, Day 4, Day 5, Day 6, Day 7 and all outpatient visits. All other timepoints will be collected non-fasted.

Blood samples will be collected by direct venipuncture or by inserting an IV catheter into the subject's forearm region. Each blood sample will be labeled with subject number, study number, study day, time point, event, and a barcode that matches that belonging to the subject.

5.7.1.2 Pharmacokinetic Specimen Handling

The PK blood samples (5 mL each) will be collected into vacutainer tubes and allowed to clot at room temperature for at least 30 min (not exceeding 60 mins). Within 60 minutes of collection, the samples will be centrifuged for 15 minutes, at 2000 g, at 4°C, by a study team member.

The serum will be separated using a disposable plastic pipette and approximately equal volume of serum will be aliquoted into two aliquots Aliquot A (primary) and Aliquot B (backup). The serum samples will be appropriately labeled and stored frozen at –80°C or below until shipment. Temperature monitoring logs should be maintained and accessible for review by the study monitor.

The Aliquot A samples (primary) will be shipped first, on dry ice, to the bioanalytical laboratory at FDA for processing when requested by the sponsor. The backup aliquot sample will be held for a further shipment which will be communicated by the sponsor. None of the PK blood samples will be stored for other analyses not specified in this protocol.

5.7.1.3 Pharmacokinetic Parameters

The following PK parameters will be determined for each subject:

- Maximum concentration (observed peak drug concentration) (Cmax)
- Time at which Cmax occurs (Tmax)
- AUC from time 0 to the sampling time corresponding to the last quantifiable concentration (Clast) (AUC0-t)
- Elimination rate constant (Kel)
- Terminal half-life (t1/2)
- AUC from time 0 extrapolated to infinity (AUC0-inf)

5.7.2 Pharmacodynamic Assessments

5.7.2.1 Pharmacodynamic Sample Collection

5.7.2.1.1 Primary and Secondary PD Biomarker

Blood samples (5 mL) for primary pharmacodynamic biomarker (peripheral blood LDL-C) and secondary pharmacodynamic biomarker (peripheral blood ApoB) assessment will be collected at the following time points:

- Day -1 (check-in)
- Day 1: 0 (Pre-dose), 24 h
- Day 2, 3, 4, 5, 7, 10, 14, 21, 28, 35, 42, 56, 70, 84

All blood samples for primary and secondary pharmacodynamic biomarker assessments will be collected in the fasted state.

Blood samples will be collected by direct venipuncture or by inserting an IV catheter into the subject's forearm region. Each blood sample will be labeled with subject number, study number, study day, time point, event, and a barcode that matches that belonging to the subject.

5.7.2.1.2 Exploratory PD Biomarkers

Exploratory PD biomarkers will be evaluated using plasma proteomics and small RNA transcriptomics. Whole blood samples (5 mL) will be collected and processed for plasma at the following time points:

- Day -1 (check-in)
- Day 1: 0 (pre-dose), 0.5, 1, 2, 3, 4, 6, 8, 12, 24 h
- Day 2, 3, 4, 5, 6, 7, 7, 10, 14, 21, 28, 35, 42, 56, 70, 84

Blood samples for proteomic and small RNA transcriptomic analyses will be collected in a fasted state at the following timepoints: predose, +24-hour (Day 2), Day 3, Day 4, Day 5, Day 6, Day 7 and all outpatient visits. All other timepoints (including Check-in) will be collected non-fasted.

Blood samples will be collected by direct venipuncture or by inserting an IV catheter into the subject's forearm region. Each blood sample will be labeled with subject number, study number, study day, time point, event, and a barcode that matches that belonging to the subject. All blood samples will be processed for preparation of plasma.

5.7.2.2 Pharmacodynamic Specimen Handling

5.7.2.2.1 Primary and Secondary PD Biomarker

The PD blood samples for primary and secondary biomarkers (5 mL each) will be collected into tubes containing K_2EDTA, inverted several times to mix the blood with the anticoagulant, and placed in an ice bath. Within 30 minutes of collection, the samples will be centrifuged for 10 minutes, at 3000 revolutions per minute, at 4°C, by a study team member.

5.7.2.2.2 Exploratory PD Biomarkers

Whole-blood samples will be collected at each time-point specified (Table 9-1). Approximately 5 mL per patient will be collected and processed to provide plasma for proteomics and small-RNA transcriptomics.

Whole blood should be collected in BD Vacutainer® Venous Blood Collection Tubes containing EDTA (or any other primary blood collection tube containing EDTA as

anticoagulant) and stored at room temperature (15–25°C) or 4°C before processing within 1 hour. Heparin-containing blood collection tubes should not be used as this anticoagulant can interfere with downstream analyses.

Plasma samples should be processed, aliquoted (approximately 500 µL volumes), and placed into the -80C freezer within 2 hours of collection.

5.7.2.3 Pharmacodynamic Parameters

5.7.2.3.1 Primary and Secondary PD Biomarker

The following PD parameters will be determined for each subject:

- Area under the effect curve (AUEC) change from baseline truncated after return to baseline
- Percentage area under the effect curve (pAUEC) change from baseline truncated after return to baseline
- Maximum change from baseline at single time-point (Emax)

5.7.2.3.2 Exploratory PD Biomarkers

The following PD parameters of the exploratory biomarkers will be assessed for all subjects:

- Serum free PCSK9 target concentration
- Plasma proteome and small RNA transcriptome: Circulating proteins and small RNAs across ten or more specific time-points (to allow modeling of AUEC) and multiple drug doses will be evaluated using a systematic approach to discovery and replication of PD biomarkers and/or hypothesis-generation. Differential expression analysis (as well as candidate pathway analyses) of proteomic and small RNA data will be performed, respectively, at specified time points in the higher dose groups (discovery phase). Any promising PD biomarkers or patterns identified in this phase will then be independently and technically evaluated for differential expression in the remaining intermediate and low dose groups (replication and technical validation phase).

5.7.3 Additional Assessments

5.7.3.1 Sample Collection

Samples for genomic or other omic biomarker assessment will be collected at the following time points:

- Buffy coat: Day 1 (Pre-dose)
- Urine Sample Collection: Screening, Day -1 (check-in), Day 1 (pre-dose and 24 hours), and Days 7, 28, 56, and 84

All of these samples will be collected in the fasted state.

These samples will be used for analyses that will be considered hypothesis-generating and exploratory. All sample analysis will be performed by DARS staff.

5.7.3.2 Specimen Handling

A buffy coat from each study participant will be used for exploratory genomic analyses. On the specified collection day/time (Day 1, pre-dose), 5 mL of whole blood sample will be processed for plasma and the upper plasma phase will be transferred to a new sterile Falcon tube without disturbing the intermediate buffy coat layer. The buffy coat layer will then be transferred to a sterile 2 mL Eppendorf (safe-lock) tube (on wet ice). Buffy coat samples should be immediately frozen in a dry ice/ethanol bath (for ~ 40 seconds) and then transferred to a -80°C freezer. Only sterile (nuclease-free) plastic pipettes, pipette tips and tubes should be used. All barcoded buffy coat Eppendorf tubes should be frozen and stored at -80°C as described above until appropriately transported to the FDA for long-term storage. Sample analysis will be performed by DARS research staff.

At specified times for urine sample collection, 3-5 mL of urine will be processed according to the SOP, separated into 6 approximately equal aliquots, immediately frozen in a dry ice/ethanol bath (for ~40 second or until frozen), and then immediately transferred to -80°C for long-term storage. Sample analysis will be performed by DARS staff.

5.7.4 Safety Assessments

Safety will be evaluated in terms of AEs, clinical laboratory results (hematology, serum chemistry, and urinalysis), vital sign measurements (blood pressure, heart rate, respiratory rate, and oral body temperature), safety 12-lead ECG results, and physical examination findings.

5.7.4.1 Adverse Events

5.7.4.1.1 Adverse Event Definitions

An AE is defined as any untoward and/or unintended sign, including an abnormal clinical laboratory finding, symptom, or disease temporally associated with the use of a study drug, whether or not considered related to the study drug. Events or conditions that increase in frequency or severity during or as a consequence of use of a drug in human clinical trials will also be considered AEs.

A treatment-emergent adverse event (TEAE) is defined as an AE that begins after study drug administration.

An unexpected AE is any AE having a specificity or severity not consistent with the current investigator's brochure for the study drug(s).

A serious adverse event (SAE) is defined as any AE occurring at any dose that meets the following criteria:

- Results in death,
- Is life threatening,
- Requires hospitalization or prolongation of existing hospitalization,
- Results in persistent or significant disability or incapacity,
- Results in a congenital anomaly/birth defect due to exposure prior to conception or during pregnancy, or
- Is an important medical event that may not meet the previous criteria but, based upon appropriate medical judgment, jeopardizes the subject or requires medical or surgical intervention to prevent one of the outcomes listed previously.

5.7.4.1.2 Adverse Event Reporting

The recording of AEs will begin after the subject signs the informed consent form and will continue until discharge (or early termination). All AEs, whether serious or nonserious and whether or not related to the study drug, must be recorded in the eCRF. Study subjects will be instructed to warn study staff if he or she has any unexpected symptoms. In addition, all subjects will receive a reminder telephone call approximately 24 hours before Check-in.

Any SAE (whether expected or unexpected) must be entered into the eCRF system and reported by facsimile to the medical monitor or designee using the SAE Reporting Form within 24 hours of the investigator or study clinic staff becoming aware of the event. It is the responsibility of the investigator to report all SAEs to the medical monitor, to provide the most complete report possible, and to assess each SAE for its relationship to the study drug. The investigator is responsible for obtaining follow-up information on all SAEs and submitting follow-up SAE data. Any unexpected SAEs must be reported promptly to the investigator's IRB as per the IRB's requirements.

In the event of a fatal or life-threatening SAE, the sponsor will notify the appropriate FDA authorities by telephone or facsimile within 7 calendar days of receipt of the report. The sponsor will follow all 7-day alert reports with a written report within 10 working days of receipt of the case. Serious AE cases that concern nonfatal, nonlife-threatening events that are unexpected and at least possibly related to the study drug will be submitted in writing to the FDA within 10 working days of receipt.

Furthermore, any AEs that are not expected, occur at a higher frequency, or would require modification of the study protocol and/or informed consent must be reported to the FDA within 10 working days.

Adverse events that are assessed by the investigator as possibly or probably related to the study drug will be followed until they resolve or stabilize. All SAEs will be followed until resolution.

5.7.4.1.3 Assessment of Severity

The investigator will assess the severity of each AE using the following scale:

- Mild: The subject is aware of the AE but is still able to perform all activities; minimal or no medical intervention or therapy is required.
- Moderate: The subject has to discontinue some activities due to the AE; minimal or no medical intervention or therapy is required.
- Severe: The subject is incapacitated by the AE and is unable to perform normal activities; significant medical intervention or therapy is required, and hospitalization is possible.

5.7.4.1.4 Assessment of Causality

The investigator will assess the causal relationship/relatedness of each AE to the study drug using the following scale:

- Not Related: Onset of the AE has no reasonable temporal relationship to administration of the study drug, a causal relationship to administration of the study drug is biologically implausible, or the event is attributed to an alternative etiology.
- Unlikely Related: Onset of the AE has a reasonable temporal relationship to study drug administration and although a causal relationship is unlikely, it is biologically plausible.
- Possibly Related: Onset of the AE has a strong temporal relationship to administration of the study drug, cannot be explained by the subject's clinical state or other factors, and a causal relationship is biologically plausible.
- Probably Related: Onset of the AE shows a distinct temporal relationship to administration of the study drug that cannot be explained by the subject's clinical state or other factors, the AE is a known reaction to the product or chemical group, or can be predicted by the product's pharmacology.

5.7.4.1.5 Pregnancy

A serum pregnancy test will be performed for female subjects at the time points presented in the Schedules of Events (Table 9-1). If a subject becomes pregnant while on the study, this should be reported immediately to the investigator, the subject will be withdrawn from the study and the medical monitor and the subject will be instructed to follow-up

with his or her personal physician. All pregnancies are to be reported as an AE and followed for outcome.

5.7.4.2 Clinical Laboratory Tests

Clinical laboratory and diagnostic screening tests will be performed at the time points presented in the Schedules of Events (Table 9-1) and will be collected in accordance with acceptable laboratory procedures. Clinical laboratory testing will be performed by the clinical study contractor. The clinical laboratory tests that will be performed are presented in Table 5-2. Unused clinical laboratory test samples will not be stored for future use.

Table 5-2: Clinical Laboratory Tests and Diagnostic Screening Tests

Hematology	Serum Chemistry	Urinalysis	Lipid Panel
Hematocrit Hemoglobin Platelet count Red blood cell count White blood cell count (with automated differential)	Alanine aminotransferase Albumin Alkaline phosphatase Aspartate aminotransferase Bicarbonate Bilirubin (total, direct, and indirect) Blood urea nitrogen Calcium Chloride Creatinine (including calculated creatinine clearance) Glucose Lactate dehydrogenase Magnesium Phosphorus Potassium Sodium Total protein Uric acid	Appearance Bilirubin Blood Color Glucose Ketones Leukocyte esterase Microscopic examination: red blood cells, white blood cells, epithelial cells, bacteria, crystals, and casts (if present) Nitrite pH Protein Specific gravity Urobilinogen	Total cholesterol High-density lipoprotein cholesterol (HDL-C) Low-density lipoprotein cholesterol (LDL-C) Triglycerides
Diagnostic Screening Tests:			
Serum	**Urine**		**Other**
Serology (human immunodeficiency virus Ag/Ab Combo 1/2, hepatitis C virus antibody, and hepatitis B surface antigen) **Female Subjects Only** Human chorionic gonadotropin (for pregnancy)	Drug screen including: amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, alcohol, opiates, phencyclidine, propoxyphene, and methadone		SARS-CoV2 molecular test

Clinical laboratory results will be reviewed by the investigator or designee together with data in the eCRF. Any values outside the reference range will be evaluated for clinical significance. If a value is determined to be clinically significant, the subject will be instructed to follow-up with his or her personal physician. The investigator or designee may repeat the clinical laboratory tests if deemed appropriate. The investigator will maintain a copy of the laboratory accreditation and the reference ranges for the laboratory used.

5.7.4.3 Vital Sign Measurements

Vital signs (blood pressure, heart rate, respiratory rate, and oral body temperature) will be measured using an automated device at the time points presented in the Schedules of Events (Table 9-1). The subject should be in a supine position, if possible, for a minimum of 5 minutes before vital signs are measured.

5.7.4.4 Safety 12-lead Electrocardiograms

12-lead ECGs will be obtained with the subjects in the supine position for a minimum of 5 minutes before recording. ECGs will be overread by a physician. If an abnormality is observed, the subject will be instructed to follow-up with his or her personal physician.

5.7.4.5 Physical Examinations

A complete physical examination will be performed at the time points presented in the Schedules of Events (Table 9-1).

The complete physical examination will include, but not be limited to, assessments of the head, eyes, ears, nose, throat, skin, thyroid, nervous system, respiratory system, cardiovascular system, abdomen (liver and spleen), lymph nodes, and extremities. Height, weight (without shoes and wearing the lightest possible clothing), and calculation of body mass index will be performed at Screening only.

If a clinically significant abnormality is observed upon physical examination, the subject will be instructed to follow-up with his or her personal physician.

5.7.5 Demographics and Medical History

Demographic data (date of birth, gender, race, and ethnicity) will be collected at Screening.

Each subject will provide a complete medical history at Screening that will be reviewed at Check-in. Specific information relating to any prior or existing medical conditions/surgical procedures will be recorded in the subject's eCRF.

5.8. Study Treatments

For each of the study drugs, doses were selected based on the doses studied in prior PK and PD studies.

5.8.1 Treatments Administered

On Day 1, subjects will receive 1 of the following 9 treatments according to the randomization schedule:

- Treatment Group A: Evolocumab 21 mg SC
- Treatment Group B: Evolocumab 35 mg SC
- Treatment Group C: Evolocumab 70 mg SC
- Treatment Group D: Evolocumab 140 mg SC
- Treatment Group E: Alirocumab 15 mg SC
- Treatment Group F: Alirocumab 25 mg SC
- Treatment Group G: Alirocumab 50 mg SC
- Treatment Group H: Alirocumab 100 mg SC
- Treatment Group I: Placebo

Study drugs will be administered by a clinical research nurse on the study clinic floor at the subject's bedside. The pharmacist and investigator will be available if needed during study drug administration.

5.8.2 Dose Selection

5.8.2.1 Alirocumab

The doses selected for alirocumab are 15mg, 25mg, 50mg, and 100mg single dose SC injection. Alirocumab is eliminated through a target-specific mechanism at low doses and a nonspecific IgG elimination at high doses. The selected dose range will cover the target-specific elimination pathway and thus allow adequate determination of the sensitive dose range for PD similarity comparison based on the dose/area-under-the effect curve (AUEC) relationship of LDL-C lowering effect. In addition, Emax (nadir) will be determined for all doses. These doses have been safely administered to healthy subjects.

5.8.2.2 Evolocumab

The doses selected for Evolocumab are 21mg, 35mg, 70mg, and 140mg single dose SC injection. Evolocumab is eliminated through a target-specific mechanism at low doses and a nonspecific IgG elimination at high doses. The selected dose range will cover the target-specific elimination pathway and thus allow adequate determination of the

sensitive dose range for PD similarity comparison based on the dose/area-under-the effect curve (AUEC) relationship of LDL-C lowering effect. In addition, Emax (nadir) will be determined for all doses. These doses have been safely administered to healthy subjects.

5.8.3 Method of Assigning Subjects to Treatment Sequence

5.8.3.1 Randomization Process

The project biostatistician will create the specifications that will be used to generate the randomization schedule. The specifications will be based on the protocol requirements and appropriate statistical programming with consideration for study design, number of treatments, number of subjects planned for enrollment, stratification, and blocking.

Based on these specifications, the project biostatistician (or designee) will generate a dummy randomization schedule. The schedule is generated using R.

The project biostatistician (or designee) distributes the 'dummy' randomization schedule to specified personnel for review. Any change (e.g., change in block size, change in stratification levels) that requires an update to the specifications will reset this process. Minor changes (e.g., display formatting) will not require a change to the specifications.

After the approval of the 'dummy' randomization schedule, the project biostatistician generates the final randomization schedule. The output is sent only to designated unblinded recipients at the site, who will maintain a secured digital and printed copy for their use.

Archival of the programs and output is accomplished by the creation of an encrypted, password-protected ZIP file containing the program and output file(s). The ZIP file is copied to a secure storage drive at the sponsor's site.

Randomization will occur after informed consent is obtained, either after completion of check-in procedures on Day -1 or just before dosing on Day 1. Approximately 72 healthy male and female subjects are planned for enrollment. Up to 14 subjects may be qualified as replacements as described in Section 5.5.2. Thus, a maximum of 86 subjects will be exposed to study drugs and procedures during the study. Unique subject numbers will be used in sequential order based on each subject's order of qualification.

Enrolled subjects will be randomly assigned to 1 of 9 different treatment groups.

The treatment groups are presented in Table 5-3.

Table 5-3: Study Treatment Groups

Subjects (n)	Treatment Group	Drug
8	A	Evolocumab low (21 mg)
8	B	Evolocumab intermediate low (35 mg)
8	C	Evolocumab intermediate high (70 mg)
8	D	Evolocumab high (140 mg)
8	E	Alirocumab low (15 mg)
8	F	Alirocumab intermediate low (25 mg)
8	G	Alirocumab intermediate high (50 mg)
8	H	Alirocumab high (100 mg)
8	I	Placebo

All randomization information will be secured and housed in a locked storage area, accessible only by the randomization personnel and the assigned pharmacist and his or her verifier.

5.8.4 Identity of Study Drugs

®Alirocumab (PRALUENT®) is a PCSK9 (Proprotein Convertase Subtilisin Kexin Type 9) inhibitor antibody indicated as adjunct to diet and maximally tolerated statin therapy for the treatment of adults with heterozygous familial hypercholesterolemia or clinical atherosclerotic cardiovascular disease, who require additional lowering of LDL-C. Alirocumab has a molecular weight of 146 kg/mol (146 kDa) and the molecular formula $C_{6472}H_{9996}N_{1736}O_{2032}S_{42}$. The physical form is available as 75 mg/ml or 150 mg/ml solution in a single-dose pre-filled pen/syringe.

Evolocumab (REPATHA®) is a PCSK9 inhibitor antibody indicated as an adjunct to diet and maximally tolerated statin therapy for treatment of adults with heterozygous familial hypercholesterolemia (HeFH), clinical atherosclerotic cardiovascular disease (CVD), or other LDL-lowing therapies in patients with homozygous familial hypercholesterolemia (HoFH), who require additional lowering of LDL-C. Evolocumab has a molecular weight of 141.8 kg/mol (144 kDa) and the molecular formula $C_{6242}H_{9648}N_{1668}O_{1996}S_{56}$. The physical form is available as 140 mg/mL solution in a single-dose pre-filled syringe/SureClick® autoinjector.

Placebo of equal volume and comparable pH for the high dose of each drug will be used as placebo control in this study and administered subcutaneously.

5.8.5 Management of Clinical Supplies

5.8.5.1 Study Drug Packaging and Storage

The active study drugs will be obtained from commercial sources. Storage instructions for the active study drugs are as follows:

- Evolocumab (REPATHA®) should be stored in the refrigerator at 2° to 8°C (36° to 46°F) in the original carton to protect from light. Do not freeze. Do not shake. Prior to use, allow evolocumab to warm to room temperature for at least 30 minutes. Do not warm in any other way. Alternatively, for patients and caregivers, evolocumab can be kept at room temperature (up to 25°C (77°F)) in the original carton. However, under these conditions, evolocumab must be used within 30 days.
- Alirocumab (PRALUENT) should be stored in a refrigerator at 36°F to 46°F (2°C to 8°C) in the outer carton in order to protect from light. Do not freeze. Do not expose to extreme heat. Do not shake. Alirocumab should be allowed to warm to room temperature 30 to 40 minutes before use. Do not keep PRALUENT at room temperature for more than 24 hours.
- Placebo should be stored below 25 °C (77 °F). Do not freeze. Vials should be protected from the light until time of use.

5.8.5.2 Study Drug Accountability

Good clinical documentation practices will be employed to record the receipt, storage conditions, accountability, and use or return of the study drug. The study drug will be stored in a secure location with access to the study personnel who will be managing the storage, dispensing, and accountability of the study drug.

Upon completion or termination of the study, final accountability review by the study monitor, and written authorization from the sponsor, all unused and/or partially used study drug should be returned or destroyed at the study clinic. It is the investigator's responsibility to ensure that the sponsor has provided written authorization for study drug disposal, the disposal process follows the study clinic's standard operating procedures, and appropriate records of the disposal are documented and maintained. No unused study drug may be disposed until fully accounted for by the study monitor (or designee). Documentation of unused study drug should include subject number, medication identity (medication #, period #), date, and quantity of study drug used.

5.8.6 Blinding

The pharmacist (and designated staff member responsible for confirmation of study drug dose) will be unblinded to subject treatment assignment; however, the pharmacist will not perform any study procedures other than study drug preparation and dispensing.

Subjects and staff will be blinded to treatment assignment during confinement. The blind will be maintained through a randomization schedule held by the dispensing pharmacist. Subjects and staff will be informed of a subject's end of study day when discharged from confinement. Subjects and staff will not be informed of the specific treatment arm

assignment. The clinical research nurse will administer the subcutaneous study drug in unit dose containers that are not transparent.

5.8.6.1 Breaking the Blind

The study drug blind will not be broken by the investigator or designee unless information concerning the study drug is necessary for the medical treatment of the subject. For unblinding a subject, the randomization information for unblinding can be obtained by contacting the dispensing pharmacist. The sponsor or medical monitor must be notified immediately if the study drug blind is broken. The date, time, and reason that the blind was broken will be recorded in the source documents. If the blind is broken by the investigator or designee, the study drug must be stopped immediately, and the subject must be withdrawn from the study. Data or specimens already collected from subjects who discontinue prematurely and for whom the blind is broken will be made available for analysis if needed.

5.8.7 Treatment Compliance

At Screening, as part of the inclusion criteria, it will be confirmed that subjects are able to comply with the protocol-defined procedure of injecting subcutaneous study drug. All doses of the study drug will be administered in the study clinic either under direct observation of or administered by clinic personnel and recorded in the eCRF. If a subject vomits after dosing, the event will be documented as an AE. The decision to replace any subject who vomits after dosing will be made as described in Section 5.5.2.

5.8.8 Prior and Concomitant Medications

Subjects are prohibited from using any prescription or nonprescription drugs (including aspirin or non-steroidal anti-inflammatory drugs [NSAIDs] and excluding oral contraceptives and acetaminophen) within 14 days or 5 half-lives (whichever is longer), or complementary and alternative medicines within 28 days before the first dose of study drug.

Subjects are also prohibited from currently participating in another clinical study of an investigational drug and may not have been treated with any investigational drug within 30 days or 5 half-lives (whichever is longer) of the compound.

Subjects must be instructed not to take any medications, including over-the-counter products, without first consulting with the investigator.

5.8.9 Subject Restrictions

Subjects are not allowed to use nicotine-containing products (e.g., cigarettes, cigars, chewing tobacco, snuff) within 6 weeks before Screening. In addition, subjects are not allowed to ingest alcohol, xanthine-containing products (e.g., tea, coffee, chocolate, cola),

caffeine, grapefruit, or grapefruit juice for 48 hours before dosing and throughout the study. Subjects are not allowed to use aspirin or NSAIDs within 14 days before the first dose of study drug. Subjects will be asked if they have used any of these substances and their responses will be recorded on the eCRF.

Subjects must be able to tolerate a controlled, quiet study conduct environment, including avoidance of music, television, movies, games, and activities that may cause excitement, emotional tension, or arousal during prespecified times (e.g., before and during ECG extraction windows) throughout the duration of the study.

Subjects must be willing to comply with study rules, including the meal schedule, attempting to void at specified times (e.g., before ECG extraction windows), remaining quiet, awake, undistracted, motionless, and supine during specified times, and avoiding vigorous exercise as directed throughout the duration of the study. Subjects will not be allowed to sleep during any ECG extraction periods.

All subjects will fast overnight for a minimum of 8 hours (no food or fluid except water) before blood collection for tests at check-in, during confinement, and for out-patient visits. Standardized meals will be served at consistent times relative to dosing, and no food or fluids will be served containing caffeine. Outside of meal times, the subjects will only be allowed to intake water, which will be available ad libitum.

5.9. Statistical Methods

5.9.1 Sample Size

Up to 86 healthy subjects will be enrolled (including 14 potential replacement subjects). Subjects will be randomized to one of 9 different active treatment arms (i.e. 8 per treatment arm) and 8 will be randomized to placebo. This sample size was determined empirically based on general sample size requirements for estimating values and variability of primary PD and PK/PD characteristics that can be used to inform the study design and sample size of future biosimilarity studies.

5.9.2 Analysis Populations

The PD and PK populations will include all subjects who receive study drug and have at least 1 estimable PD or PK parameter, respectively, after dosing.

5.9.3 General Statistical Considerations

All data will be presented in data listings. Data from subjects excluded from an analysis population will be presented in the data listings, but not included in the calculation of summary statistics. Demographic and baseline characteristics will be summarized overall and by treatment for all subjects.

5.9.4 Subject Disposition

The number of subjects who enroll in the study and the number and percentage of subjects who complete each assessment will be presented. The frequency and percentage of subjects who withdraw or discontinue from the study and the reason for withdrawal or discontinuation will be summarized.

5.9.5 Demographics and Baseline Characteristics

Descriptive statistics will be used to summarize demographic and baseline subject characteristics. For continuous variables, the mean, median, standard deviation (SD), minimum, and maximum values will be reported. For categorical (nominal) variables, the number and percentage of subjects (or observations) will be reported.

5.9.6 Primary Analysis

5.9.6.1 Pharmacodynamics

Peripheral Blood LDL-C and ApoB_AUEC will be calculated for each subject. For each dose, separate intervals will be used for the AUEC assessment to truncate noise introduced from baseline fluctuations. Maximum decrease from baseline will be determined from all sampled timepoints. For both assessments and biomarkers, measures will be log-transformed and utilize an ANCOVA approach for calculating geometric means and 90% confidence intervals for each treatment. PD parameters of evolocumab and alirocumab will be listed and summarized using descriptive statistics (n, geometric mean, coefficient of variation, minimum, median, and maximum).

Separate population PD analyses will be conducted using nonlinear mixed effect modeling with NONMEM. AUECs calculated for each subject from all doses for a drug and placebo patient will be combined and evaluated assuming a saturating E_{max} relationship to characterize system parameters such as E_{max} and EC_{50}.

5.9.6.2 Pharmacokinetics

C_{max} and AUC are the primary PK parameters of interest in PK similarity studies with a subcutaneous drug administration. These and additional PK parameters will be determined for each subject using non-compartmental methods. All parameters will be reported with standard descriptive statistics including the geometric mean and coefficient

of variation. Calculation of PK parameters will be performed using actual sampling times.

5.9.7 Additional Analyses

5.9.7.1 Proteomics and small RNA Transcriptomics Analysis

A combination of differential expression testing of signals analysis, and clustering techniques will be used demonstrate subject response to each product. Differentially expressed plasma proteins and small RNAs will be identified using a 10% false discovery rate. Following technical and independent validation of the most promising or top 1% biomarker(s), dose- and exposure-response analysis will be performed as described above for the primary biomarker(s). The AUEC change along with maximal effect at a single time point for a promising biomarker(s) will also be calculated. The values, variability and characteristics of the proteomic-array-based biomarkers will be compared to the traditional biomarker analysis outlined above. Based on proteomic and small RNA transcriptomic findings, the potential for a composite of relevant biomarkers to demonstrate PD similarity will also be evaluated. Additional details regarding the statistical methods for the proteomic and small RNA transcriptomic analyses will be described in a separate protocol.

5.9.7.2 Genomic Analysis

Genetic variation may contribute to the PK or PD response to a drug. Analyses of buffy coat DNA may include genome-wide studies, including (but not limited to) to the generation of variant data and/or genomic sequencing in hypothesis-generating studies. Additional details regarding the statistical methods for the genomic analyses will be described in a separate protocol.

5.9.7.3 Other Omic Analyses

Epigenetic variation such as DNA methylation has the capacity to contribute to the PD or PK response to a drug. Moreover, profiling metabolites in biofluids may have utility in the identification of circulating PD biomarkers. The generation of additional omics profiles (e.g., metabolomics and methylomics) from an alternative biospecimen such as urine can inform on the utility of these technologies for PD biomarker identification and the analytical framework required for future studies involving this biospecimen. These analyses may also inform on biofluid specificity. These analyses will be considered hypothesis-generating. Additional details regarding the statistical methods for the omics analyses will be described in a separate protocol.

5.9.8 Safety Analyses

5.9.8.1 Adverse Events

All AEs will be coded using the latest version of the Medical Dictionary for Regulatory Activities. The incidence of TEAEs, organized by system organ class and frequency, will be summarized by seriousness, severity, relationship to treatment, and by treatment at onset of the TEAE. A detailed listing of serious AEs and TEAEs leading to withdrawal will also be provided.

5.9.8.2 Clinical Laboratory Tests

Clinical laboratory results (hematology, serum chemistry, and urinalysis) will be summarized using descriptive statistics (number of subjects, mean, SD, minimum, median, and maximum). Clinical laboratory results will be classified as normal or abnormal, according to the reference ranges of the individual parameter. The number and percentage of subjects with abnormal laboratory results will be provided. No statistical testing will be performed on clinical laboratory data.

5.9.8.3 Vital Sign Measurements

Vital sign measurements and changes from baseline will be summarized using descriptive statistics (number of subjects, mean, SD, minimum, median, and maximum) by treatment and time point.

5.9.8.4 Safety 12-lead Electrocardiograms

Safety 12-lead ECG data and changes from baseline will be summarized using descriptive statistics (number of subjects, mean, SD, minimum, median, and maximum) by treatment and time point. The extent of change in each of the treatments will also be compared. The incidence of pathological ECG interpretive statements at Baseline and during treatment will be assessed among the treatments.

5.9.8.5 Physical Examinations

Physical examination findings will be presented in a data listing, and abnormal physical examination findings will be recorded as AEs.

5.9.8.6 Other Safety Data

All concomitant medication usage and medications that changed in daily dose, frequency, or both since the subject provided informed consent will be summarized for each subject.

5.9.9 Interim Analyses

No interim analyses are planned.

5.9.10 Missing Data

Missing data will not be imputed. Data that are excluded from the descriptive or inferential analyses will be included in the subject data listings. This will include data from subjects not in the particular analysis population, measurements from unscheduled visits, or extra measurements that may arise from 2 or more analyses of the biofluid sample at the same time point.

5.10. Data Quality Assurance

Completed eCRFs are required for each subject randomly assigned to study drug. Electronic data entry will be accomplished through the ClinSpark® remote electronic data capture system, which allows for on-site data entry and data management. This system provides immediate, direct data transfer to the database, as well as immediate detection of discrepancies, enabling site coordinators to resolve and manage discrepancies in a timely manner. Each person involved with the study will have an individual identification code and password that allows for record traceability. Thus, the system, and subsequently any investigative reviews, can identify coordinators, investigators, and individuals who have entered or modified records.

Furthermore, the investigator retains full responsibility for the accuracy and authenticity of all data entered into the electronic data capture system. The completed dataset and their associated files are the sole property of the sponsor and should not be made available in any form to third parties, except for appropriate governmental health or regulatory authorities, without written permission of the sponsor.

6. Ethical Considerations

6.1. Ethical Conduct of the Study

This study will be performed in accordance with the recommendations guiding physicians in biomedical research involving human subjects adopted by the 18th World Medical Association General Assembly, Helsinki, Finland, 1964 and later revisions, as well as, United States Title 45 Code of Federal Regulations (CFR) Part 46 GCP, and International Council for Harmonisation (ICH) guidelines describing technical requirements for registration of pharmaceuticals for human use.

6.2. Institutional Review Board (IRB)

After the protocol is finalized and approved by FDA, FDA staff with primary responsibility for the FDA's involvement with the project (i.e., the FDA Project Lead) will submit the protocol and associated documentation to FDA's Office of the Chief Scientist to facilitate an Institutional Review Board Authorization Agreement (IAA) with FDA as the relying institution. The FDA Project Lead or investigator will provide the

local IRB (i.e., Advarra) with all required documents, including the study protocol and informed consent form. The study will not be initiated until appropriate IRB approval is obtained from the local IRB. The investigator will provide the FDA Project Lead with copies of the approval documents for the protocol, informed consent form, and all recruiting materials. The sponsor will provide a copy of the local IRB approval letter to the investigator or designee before the study is initiated. The local IRB will also receive copies of any original or amended information sheets or pamphlets given to the study subject in support of the informed consent process and any advertisements or other recruitment material. Such materials will not be employed in the study before approval by the local IRB.

Subjects will be informed that they have the right to contact the local IRB or Office for Human Research Protections if they have any questions, concerns, complaints, or believe they have been harmed by the participation in this research study as a result of investigator negligence. Subjects will be given the address and phone number of the local IRB.

7. Administrative Procedures

7.1. Responsibilities of the Investigator

The following administrative items are meant to guide the investigator in the conduct of the study but may be subject to change based on industry and government standard operating procedures, working practice documents, or guidelines. Changes may be reported to the IRB but will not result in protocol amendments.

7.1.1 Form FDA 1572

The investigator will complete and sign the Form FDA 1572.

7.1.2 Adherence to Protocol

The investigator agrees to conduct the study as outlined in this protocol in accordance with the ICH E6(R2) and all applicable guidelines and regulations.

7.1.3 Reporting Requirements

By participating in this study, the investigator agrees to submit reports of SAEs according to the time line and method outlined in the protocol (Section 5.7.4.1.2). In addition, the investigator agrees to submit reports to the IRB as appropriate. The investigator also agrees to provide the sponsor with an adequate report shortly after completion of the investigator's participation in the study.

7.1.4 Source Documentation

By participating in this study, the investigator agrees to maintain adequate case histories for the subjects treated as part of the research under this protocol. The investigator agrees to maintain accurate eCRFs and source documentation as part of the case histories.

7.1.5 Retention of Records

The investigator agrees to keep the records stipulated in this protocol and those documents that include (but are not limited to) the study-specific documents, identification log of all participating subjects, medical records, source worksheets, all original signed and dated informed consent forms, subject authorization forms regarding the use of personal health information (if separate from the informed consent form), copies of all eCRFs, query responses, and detailed records of drug disposition, to enable evaluations or audits from regulatory authorities, the sponsor, or its designees.

Furthermore, ICH 4.9.5 requires the investigator to retain essential documents specified in ICH E6(R2) (Section 8) until at least 2 years after the last approval of a marketing application for a specified drug indication being investigated or, if an application is not approved, until at least 2 years after the investigation is discontinued and regulatory authorities are notified. In addition, ICH 4.9.5 states that the study records should be retained until an amount of time specified by applicable regulatory requirements or for a time specified in the clinical study site agreement between the investigator and sponsor.

Refer to the clinical study site agreement for the sponsor's requirements on record retention. The investigator should contact and receive written approval from the sponsor before disposing of any such documents.

7.1.6 Financial Disclosure and Obligations

The investigator is required to provide financial disclosure information to allow the sponsor to submit the complete and accurate certification or disclosure statements required under 45 CFR 46. In addition, the investigator must provide to the sponsor a commitment to update this information promptly if any relevant changes occur during the course of the investigation and for 1 year after the completion of the study.

Neither the sponsor nor the study clinic is financially responsible for further testing or treatment of any medical condition that may be detected during the screening process.

7.2. Confidentiality and Disclosure of Data

All subjects will sign a HIPAA-compliant authorization form containing the mandated core elements and requirements before participation in this clinical study. The sponsor and designees affirm and uphold the principle of the subject's right to protection against invasion of privacy. Throughout this study, a subject's source data will only be linked to

the sponsor's electronic data capture system database or documentation via a unique identification number. As permitted by all applicable laws and regulations, limited subject attributes such as gender, age or date of birth, and subject initials may be used to verify the subject and accuracy of the subject's unique identification number.

To comply with ICH Guidelines for GCP and to verify compliance with this protocol, the sponsor requires that the investigator allow review of the subject's original medical records (source data or documents) by the study monitor, representatives from any regulatory authority (e.g., FDA), the sponsor's designated auditors, and the appropriate IRB. These medical records will include, but will not be limited to, clinical laboratory test result reports, ECG reports, admission and discharge summaries for hospital admissions occurring during a subject's study participation, and autopsy reports. Access to a subject's original medical records requires the specific authorization of the subject as part of the informed consent process.

Copies of any subject source documents that are provided to the sponsor must have certain personally identifiable information removed (i.e., subject name, address, and other identifier fields not collected in the subject's eCRF).

Data will be maintained and backed up in the electronic data capture system. All access to the data is protected by username and password, and each staff member and all sponsor staff will have separate access that requires a separate username and password. Access is only given to site staff and requested sponsor staff who have completed the appropriate training.

7.3. Subject Consent

Written informed consent in compliance with 45 CFR 46 will be obtained from each subject before entering the study or performing any unusual or nonroutine procedure that involves risk to the subject. An informed consent template may be provided by the sponsor to the study clinic. If any institution-specific modifications to study-related procedures are proposed or made by the study clinic, the consent should be reviewed by the sponsor or its designee or both before IRB submission. Once reviewed, the consent will be submitted by the investigator to the IRB for review and approval before the start of the study. If the informed consent form is revised during the course of the study, all active participating subjects must sign the revised form.

Before enrollment, each prospective subject will be given a full explanation of the study and be allowed to read the approved informed consent form. The informed consent process will be performed by a clinical research nurse in a private room. The subject will be given unlimited time to ask questions regarding study participation, and each subject will be questioned to ensure their understanding. Once the investigator is assured that the subject understands the implications of participating in the study, the subject will be asked to give consent to participate in the study by signing the informed consent form.

The investigator will provide a copy of the signed informed consent form to the subject. The original form will be maintained in the subject's medical records at the site.

7.4. Data Collection

Full details of procedures for data collection and handling will be documented in the data management plan, which is initiated with the final protocol receipt. The data management plan is a changing document that evolves over the course of the study and is finalized by database lock.

7.5. Publications

No information related to or generated by this study will be released to the public until it has been reviewed by the sponsor. The sponsor shall own intellectual rights for the data . Authorship on publications will be determined by standard journal requirements.

8. Study Management

8.1. Release of Study Drug to the Study Clinic

Before the study drug can be released to the study clinic, the following documents will be collected from the study clinic by the clinical research organization, retained in the trial master file, and a study drug shipment approval form will be completed by the clinical research organization:

- Protocol signature page signed by the investigator
- IRB approval of the protocol and informed consent form and IRB membership list
- Completed Form FDA 1572, curriculum vitae, and medical licenses from each investigator
- Financial disclosure and debarment certification from each investigator
- Executed contract with investigator and study clinic

8.2. Monitoring

The sponsor or its designee will monitor the study to ensure that it is being conducted according to the protocol, GCP standards, and applicable region-specific requirements, and to ensure that study initiation, conduct, and closure are adequate. The investigators and the study clinic staff will be expected to cooperate fully with the study monitors and personnel or agents of the sponsor and be available during monitoring visits to answer questions sufficiently and to provide any missing information. The investigators and their institutions will permit direct access to source data/documents for study-related monitoring activities, audits, IRB reviews, and regulatory inspections.

During any on-site visits, the study monitor will:

- Check and assess the progress of the study
- Review all informed consent forms
- Review study data collected
- Conduct source document verification
- Identify any issues and address their resolution
- Verify that the facility remains acceptable
- Conduct study drug accountability

These monitoring activities will be done in order to verify that the:

- Data are authentic, accurate, and complete.
- The safety and rights of the subjects are being protected.
- The study is being conducted in accordance with the currently approved protocol (including any amendments), GCP, and all applicable regulatory requirements.

In addition, the sponsor, designated auditors, and government inspectors must be allowed access to eCRFs, source documents, and other study files that may be required to evaluate the conduct of the study.

8.3. Management of Protocol Amendments and Deviations

8.3.1 Modification of the Protocol

Any changes in this research activity, except those necessary to remove an apparent immediate hazard to the subject, must be submitted to the sponsor or designee and reviewed and approved by the local IRB before implementation. Amendments to the protocol must be submitted in writing to the investigator's IRB for approval before subjects are enrolled into an amended protocol.

8.3.2 Protocol Violations and Deviations

Any significant protocol deviations that the investigator or study clinic staff believes are of major importance (e.g., incorrect randomizations, subject enrolled but not eligible) should be reported to the sponsor and the investigator's IRB as soon as possible. Significant protocol deviations may include the following:

- Deviations from the inclusion/exclusion criteria that may affect subject safety
- Deviations (omission or delay) of safety monitoring procedures
- Deviations in the administration of the study drug
- Deviations in obtaining informed consent

All subjects who are enrolled and receive the study drug, regardless of whether they have a major protocol violation, must continue to be followed for safety for all follow-up study visits.

9. Appendix

Table 9-1: Schedule of Events by Time Point

Clinical Study 2 - PCSK9 Drugs Schedule of Events																											
	Screening	Checkin / Baseline	Treatment Period																								End of Study
Study Week			1															2		3	4	5	6	7	9	11	Day 42, 56, or 84
Study Day	-21 to -2	-1	Pre	1	1	1	1	1	1	1	1	1	2	3	4	5	6	7	10	14	21	28	35	42	56	70	
Hour			0	0.5	1	2	3	4	6	8	12	24															
Visit	1	2																	3	4	5	6	7	8	9	10	8, 9, 11
Outpatient visit	X																	X	X	X	X	X	X	X	X	X	X
In House Residency		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X										
Informed Consent	X																										
Medical History	X	X																									
Targeted Physical Exam			X		X		X			X																	
Comprehensive Physical Examination	X	X										X						X				X					X
Vital signs	X	X			X							X						X				X					X
12-Lead ECG [1]	X	X										X						X				X					X
Clinical Chemistry and Hematology [1]	X	X										X						X				X					X
Urinalysis	X	X																									X
HIV Ab, HbsAg, HepC Ab	X																										
SARS CoV2 Molecular Test		X																									
Urine Drug and Alcohol Screen	X	X																X									X
Pregnancy Test (females only) [2]	X	X																									X
FSH (females only)	X																										
Drug Administration			X																								
PK Concentration/PCSK9 Serum sample			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urine Sample Collection (PD)		X	X									X						X				X					X
Complete Lipid Profile and ApoB	X	X	X									X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Genomics (buffy coat)			X																								
Proteomics & Small RNA Transcriptomics (plasma)		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications	X	X	X									X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Events	X	X	X									X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

1 Clinically notable results are repeated
2 Serum test

10.Reference List

1. Repatha Package Insert dated 08/27/2015. Found at https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/125522s000lbl.pdf
2. Praluent Package Insert dated 07/24/2015. Found at https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/125559Orig1s000lbledt.pdf