Official Title: The Effects of Single Dose Rifampin on Pharmacokinetics of Fluvastatin in Uninduced and Hepatically Induced Healthy Volunteers

NCT number: NCT04029584

Document date: 4/26/2019

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Clinical Research Protocol

The Effects of Single Dose Rifampin on Pharmacokinetics of Fluvastatin in Healthy Volunteers

Protocol Number:	
Version Date:	
Investigational Product:	
IND Number:	
Development Phase:	
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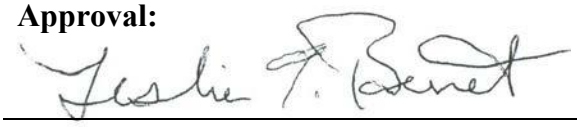

PI or Sponsor Signature (Name and Title)

Dec 27,2018

Date

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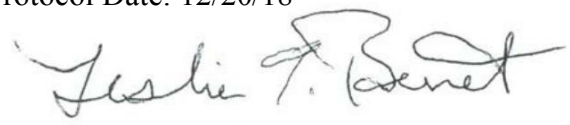
PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Investigator, my duties include ensuring the safety of the study subjects enrolled under my supervision and providing sponsor with complete and timely information, as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site. Furthermore, on behalf of the study staff and myself, I agree to maintain the procedures required to carry out the study in accordance with accepted GCP principles and to abide by the terms of this protocol.

Protocol Number: Number

Protocol Title: The Effects of Single Dose Rifampin on Pharmacokinetics of Fluvastatin in Healthy Volunteers

Protocol Date: 12/20/18



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LIST OF ABBREVIATIONS

AE	adverse event
AUC0-∞	Area under the concentration versus time curve from time = 0 to infinity.
AUC0-12	Area under the concentration versus time curve from time = 0 to t = 12 hours.
BUN	blood urea nitrogen
CFR	Code of Federal Regulations
Cmax	maximum concentration
CRF	case report form
CRP	C-reactive protein
ESR	erythrocyte sedimentation rate
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act of 1996
ICF	informed consent form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IV	intravenous
PI	Principal Investigator
PK	pharmacokinetic
SAE	serious adverse experience
Tmax	time to Cmax

PROTOCOL SYNOPSIS

TITLE	The Effects of Single Dose Rifampin on Pharmacokinetics of Fluvastatin in Healthy Volunteers
NUMBER OF SITES	1
RATIONALE	The effect of OATP1B1 transporter inhibition at clinical doses of fluvastatin, a BDDCS class 1 drug, has not been studied to date. A single dose of IV rifampin can be used as model OATP1b1 inhibitor to evaluate the significance of OATP1B1 transporter effects on fluvastatin disposition.
STUDY DESIGN	*This is* two-period, randomized, unblinded, crossover clinical trial.
PRIMARY OBJECTIVE	The primary objective is to determine whether or not inhibition of OATP1B1-mediated drug transport of fluvastatin will be clinically significant.
NUMBER OF SUBJECTS	10-12
SUBJECT SELECTION CRITERIA	Inclusion Criteria: ● Healthy male or female, ages 18-65 years old, with no current medical conditions or active diagnoses as determined by the study doctor based on history, physical exam, and laboratory evaluations. ● Subjects who take no other medications two weeks prior to the study and during the time course of the study including prescription medications, over-the-counter medications, dietary supplements, or drugs of abuse. ● Subjects able to maintain adequate birth control during the study independent of hormonal contraceptive use (use of condoms). ● Subjects able to abstain from grapefruit, grapefruit juice, oranges, orange juice, caffeinated beverages and/or alcoholic beverages from 7am the day before the study to completion of that study day. ● Participants determined to have normal liver and kidney function as measured at baseline (ALT: ≤ 2x upper level of

	normal (ULN), AST: ≤ 2x ULN, SCr: ≤ 1.5x ULN, T. Bili: 0.1-1.2mg/dL, Albumin: 3.4 – 4.7 mg/dL). ● BMI between 18.0 - 30 kg/m2 o Subjects capable of fasting from food and beverages at least 8 hours prior to medication dosing. ● Be able to read, speak, and understand English. ● Subjects capable of providing informed consent and completing the requirements of the study. <u>Exclusion Criteria</u>: ● Subjects with active medical problems ● Subjects on chronic prescription or OTC medication that cannot be stopped 2 weeks prior to and during the study. ● Subjects incapable of multiple blood draws (HCT < 30mg/dL) ● Subjects with a history of rhabdomyolysis ● Subjects with a history of drug-related myalgias ● Subjects with a history or diagnosis of hemorrhagic tendencies or blood dyscrasias ● Subjects with a history of GI bleed or peptic ulcer disease ● Subjects who smoke tobacco or have ongoing alcohol or illegal drug use ● Subjects who are pregnant, lactating, or trying to conceive during the study period ● Subjects allergic to fluvastatin or rifampin or any known component of the medications ● Anyone who in the opinion of the study investigators is unable to do the study
TEST PRODUCT, DOSE, AND ROUTE OF ADMINISTRATION	fluvastatin 20 mg by mouth as a 20 mg Lescol® capsule Rifampin 600mg IV infusion over 30 minutes (t = -30 minutes)
CONTROL PRODUCT, DOSE AND ROUTE OF ADMINISTRATION	fluvastatin 20 mg by mouth as a 20 mg Lescol® capsule

DURATION OF SUBJECT PARTICIPATION AND DURATION OF STUDY	subject will be on study for up to 2 days Screening: One 2-hour screening visit one week prior to the study Treatment: 13 hours stays in the CRC(separated by at least 1 day) The total time required is 27 hours over about 2 day period.
CONCOMMITANT MEDICATIONS	Allowed: No concomitant medication allowed Prohibited: subject will not allow to take any other medications two weeks prior to the study and during the time course of the study including prescription medications, over-the-counter medications, dietary supplements, or drugs of abuse.
PRIMARY ENDPOINT	The primary outcome will be fluvastatin AUC0-12h and AUC0-∞ (AUC = area under the plasma concentration versus time curve).
SECONDARY ENDPOINTS	Secondary outcomes will include fluvastatin maximum plasma concentration (Cmax) and time to Cmax (Tmax) .
SAFETY EVALUATIONS	Safety evaluation will be determined by the incidence of adverse events. Subjects will be monitored on an ongoing basis while involved with the study by study personnel. Subjects will be screened with a medical history and physical examination by the study physician and have screening hematology, chemistries, liver, renal and coagulation laboratories. Subjects must have normal function of all major organs. At study completion, subjects will have repeat physical examination and repeat hematology, chemistries, liver, renal and coagulation laboratories for safety monitoring.
PLANNED INTERIM ANALYSES	Serious adverse events will be monitored by the committee on an ongoing basis throughout the study.
STATISTICS **Primary Analysis Plan**	AUC0-∞ will be calculated using the trapezoidal rule from time 0 to Clast + Clast/ke. Parameters such as maximum plasma concentration (Cmax) and time to Cmax (Tmax) will be determined from the observed data. The terminal elimination rate constant (ke) will be obtained using linear regression of log transformed concentrations over the terminal log-linear decline phase. Oral clearance (CL/F) will be calculated using Dose/AUC0-∞. Other estimated pharmacokinetic parameters will be calculated using non-compartmental methods with

	WinNonlin Professional software (Pharsight, Inc., Mountain View, CA). Log-transformed pharmacokinetic parameters will be compared across treatments with analysis of variance for repeated measures. Differences will be regarded as statistically significant if $p < 0.05$. Data will be presented as mean values with standard deviations. Each subject will serve as his/her own control. Differences in pharmacokinetic parameters between treatments will be calculated using student's paired t-test. Statistical calculations will be done using STATA 10.0 (StataCorporation, College Station, TX).
Rationale for Number of Subjects	Using a paired t-test and prior data, a sample size of n=10 in each group is needed to detect a 50% difference in AUC0-12 between the two study regimens with a statistical power of 80% (two sided alpha = 0.05) and standard deviation of 50%. Due to the lesser effects of rifampin on fluvastatin AUC, the within subject variability can affect the sample size. We plan to enroll sample size of 12 subjects to maintain an adequate sample size in case of potential attrition.

1 BACKGROUND

Membrane transporters located throughout the body play an important role in drug absorption, disposition, metabolism, and elimination[1].The inhibition of many of these transporters has been linked to significant drug-drug interactions[1]. Located on the sinusoidal membrane of hepatocytes, the Organic Anion Transporter Polypeptide transporter OATP1B1 is responsible for the uptake of substrates from the blood into the liver where they can be metabolized and cleared[1-3]. When OATP1B1-mediated transport is inhibited with known inhibitors such as ritonavir, rifampin, or cyclosporine, drug metabolism and elimination into bile are diminished, resulting in elevated concentrations of the drug in the blood. Another important membrane transporter, Breast Cancer Resistance Protein or BCRP, present in the intestinal epithelia and liver, is also important for drug elimination[1].If BCRP is inhibited, drug concentrations in the liver and blood may also rise.

Statins are commonly prescribed cholesterol-lowering agents whose efficacy and safety is closely linked with the function of the BCRP and OATP1B1 transporters[1-3]. These drugs must enter the liver to exert their efficacy. Compared to other statins like rosuvastatin and pravastatin, fluvastatin is extensively metabolized [4]. It has a more hydrophobic profile making it highly permeable through the membranes and in theory less dependent on OATP1B1-mediated transport[5].

To predict hepatic uptake transporter effects on drug disposition, Wu and Benet developed the Biopharmaceutics Drug Disposition Classification System (BDDCS) system which categorizes drugs based on extent of metabolism and solubility characteristics [6]. According to this classification system, the plasma exposure of Fluvastatin, a BDDCS class I drug should be minimally influenced by transporter effects[6].

1.1 Overview of Non-Clinical Studies

Available in vitro data demonstrates that inhibition of OATP1B1 by rifampin results in reduced rosuvastatin uptake in Human Embroyonic Kidney (HEK293) cells, which implies the drug's significant dependence on the membrane transporter[7]. Animal studies have also confirmed that rifampin significantly alters hepatobiliary transport of rosuvastatin and increases its plasma exposure (AUC) and Cmax[8].

1.2 Overview of Clinical Studies

Contrary to our expectations, one clinical study has demonstrated that co-administration of single-dose rifampin with 2 mg of fluvastatin results in significant increase in exposure of the cholesterol-lowering agent[9]. However this study was conducted with one tenth to one

fortieth of the common dose of fluvastatin[10], and we believe that the effect of rifampin on fluvastatin exposure in this study to be due to the low concentration of fluvastatin.

Clinical studies have demonstrated that co-administration of single-dose rifampin with the BDDCS Class II drug atorvastatin results in marked increases in concentrations of the cholesterol-lowering agent[11,12]. Similar studies with the BDDCS class III drug rosuvastatin and class II drug glyburide showed similar transporter effects which increased plasma concentrations of the two drugs when co-administered with a single dose of rifampin[13,14]. These studies have demonstrated the significant effects of hepatic uptake transporters on the pharmacokinetics of BDDCS class II and III drugs. It is expected that these transporter effects will not be significant for BDDCS class I drugs like fluvastatin.

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2 STUDY RATIONALE

Characterizing the effect of inhibition of hepatic uptake transporters by rifampin, on the pharmacokinetics of fluvastatin will advance our understanding of statin clinical pharmacology. It will give us a better understanding of the effects of hepatic uptake transporters on statin pharmacokinetics, statin drug-drug interaction and will serve as a validation check for the BDDCS classification system.

2.1 Risk / Benefit Assessment

There is minimum risk involved in this study. Only approved dosage of FDA approved medications are being used in this study. As outlined by the Data and Safety Management Plan for the study all precautions are being considered to uphold the safety and best interest of all participants. The subject may stop the study at any time for any reason.

There is no direct benefit for subjects to participate in the study.

3 STUDY OBJECTIVES

3.1 Primary Objective

The primary objective is to determine whether or not inhibition of OATP1B1-mediated drug transport of fluvastatin will be clinically significant. The study will assess if a IV dose of rifampin (to inhibit hepatic uptake transporters) immediately prior to oral fluvastatin dosing will have any effect on fluvastatin total drug exposure.

4 STUDY DESIGN

4.1 Study Overview

The effect of rifampin on the pharmacokinetics of fluvastatin will be studied in healthy volunteers in a two-period, randomized, unblinded, crossover clinical trial. On period 1 of the study, subjects will be randomized to one of two treatment groups:

(i) one oral dose of fluvastatin (Lescol®) 20mg capsule

(ii) one oral dose of fluvastatin (Lescol®) 20mg capsule immediately following a 30-min intravenous infusion of rifampin 600mg in 10ml Normal Saline.

The two study periods will preferentially be on a Tuesday and Thursday, separated by one day of washout. Subjects will be instructed to fast overnight the day before study periods 1 and 2, and for 3h post-dosing. In both treatments, venous blood samples (~8ml each) will be drawn at 0, 0.33, 0.67, 1,1.5, 2, 2.5, 3, 4, 6, 9, 12h after fluvastatin dosing. Period 2 will begin after at least a 1-day washout when subjects will receive the second treatment, followed by blood draws at the same time points as after fluvastatin dosing alone.

	Period 1	Period 2
Group 1	A	B
Group 2	B	A

A= fluvastatin; B =rifampin +fluvastatin

5 CRITERIA FOR EVALUATION

5.1 Primary Efficacy Endpoint

Primary Outcome: The primary outcome will be fluvastatin AUC0-12h and AUC0-∞.

5.2 Secondary Efficacy Endpoints

Secondary outcomes will include fluvastatin maximum plasma concentration (Cmax) and time to Cmax (Tmax)

5.3 Safety Evaluations

Subjects will be monitored on an ongoing basis while involved with the study by study personnel. Subjects will be screened with a medical history and physical examination by the study physician and have screening hematology, chemistries, liver, renal and coagulation laboratories. Subjects must have normal function of all major organs. At study completion, subjects will have repeat physical examination and repeat hematology, chemistries, liver, renal and coagulation laboratories for safety monitoring. The safety evaluations include change in clinical laboratory finding(Creatinine, phosphokinase) and also the Incidence of adverse events

6 SUBJECT SELECTION

6.1 Study Population

Subjects who meet the inclusion and exclusion criteria will be eligible for participation in this study.

6.2 Inclusion Criteria

1. Healthy male or female, ages 18-65 years old, with no current medical conditions or active diagnoses as determined by the study doctor based on history, physical exam, and laboratory evaluations.
2. Subjects who take no other medications two weeks prior to the study and during the time course of the study including prescription medications, over-the-counter medications, dietary supplements, or drugs of abuse.
3. Subjects able to maintain adequate birth control during the study independent of hormonal contraceptive use (use of condoms).
4. Subjects able to abstain from grapefruit, grapefruit juice, oranges, orange juice, caffeinated beverages and/or alcoholic beverages from 7am the day before the study to completion of that study day.
5. Participants determined to have normal liver and kidney function as measured at baseline (ALT: ≤ 2x upper level of normal (ULN), AST: ≤ 2x ULN, SCr: ≤ 1.5x ULN, T. Bili: 0.1-1.2mg/dL, Albumin: 3.4 – 4.7 mg/dL).
6. BMI between 18.0 - 30 kg/m2 o Subjects capable of fasting from food and beverages at least 8 hours prior to medication dosing.
7. Be able to read, speak, and understand English.
8. Subjects capable of providing informed consent and completing the requirements of the study.

6.3 Exclusion Criteria

1. Subjects with active medical problems
2. Subjects on chronic prescription or OTC medication that cannot be stopped 2 weeks prior to and during the study.
3. Subjects incapable of multiple blood draws (HCT < 30mg/dL)
4. Subjects with a history of rhabdomyolysis
5. Subjects with a history of drug-related myalgias
6. Subjects with a history or diagnosis of hemorrhagic tendencies or blood dyscrasias
7. Subjects with a history of GI bleed or peptic ulcer disease
8. Subjects who smoke tobacco or have ongoing alcohol or illegal drug use
9. Subjects who are pregnant, lactating, or trying to conceive during the study period
10. Subjects allergic to fluvastatin or rifampin or any known component of the medications
11. Anyone who in the opinion of the study investigators is unable to do the study

7 CONCURRENT MEDICATIONS

All subjects should not take any other concurrent medication

7.1 Allowed Medications and Treatments

Subjects will take no other medications two weeks prior to the study and during the time course of the study including prescription medications, over-the-counter medications, dietary supplements, or drugs of abuse.

7.2 Prohibited Medications and Treatments

No other medications besides study medications are allowed.

8 STUDY TREATMENTS

8.1 Method of Assigning Subjects to Treatment Groups.

Once included in the study, patients will be randomized using computer-generated random numbers, corresponding to each treatment. This will be performed by someone other than the investigator and will ensure equal number of patients in each treatment sequence.

8.2 Blinding

The study is not blinded

8.3 Formulation of Test and Control Products

8.3.1 Formulation of Test and Control Product

Fluvastatin capsule, develop by Norvatis Pharmaceutical Corporation, is an HMG-CoA reductase inhibitor to reduce elevated TC, LDL-C, Apo B, and TG, and to increase HDL-C in adult patients with hypercholesterolemia. Fluvastatin sodium is a white to pale yellow, hygroscopic powder soluble in water, ethanol and methanol. LESCOL is supplied as capsules containing fluvastatin sodium, equivalent to 20 mg or 40 mg of fluvastatin.

Trade Drug Name	Lescol
Generic Drug name	Fluvastatin sodium (capsules)
Investigational Drug Name	
Identify the name of the manufacturer or source of investigational drug/biologic:	Novartis Pharmaceuticals Corporation
Active ingredient	fluvastatin sodium
Inactive ingredient	calcium carbonate, gelatin, magnesium stearate, microcrystalline cellulose, pregelatinized starch (corn), red iron oxide, sodium bicarbonate, talc, titanium dioxide, yellow iron oxide, and other ingredients.
PH	4.5

Rifampin is used only to treat or prevent infections that are proven or strongly suspected to be caused by bacteria. Rifampin is a red-brown crystalline powder very slightly soluble in water at neutral pH, freely soluble in chloroform, soluble in ethyl acetate and in methanol.

Trade Drug Name:	RIFADIN IV
Generic Drug Name:	RIFAMPIN

Identify the name of the manufacturer or source of investigational drug/biologic:	Sanofi-Aventis
Active Ingredient	rifampin 600 mg
Inactivate Ingredient	sodium formaldehyde sulfoxylate 10 mg, and sodium hydroxide to adjust pH
PH	7.8–8.8

8.4 Supply of Study Medications at the Site

The investigator will supply study drug to the investigational sites.

Dosage/Dosage Regimen

fluvastatin 20 mg by mouth as a 20 mg Lescol® capsule

30-min intravenous infusion of rifampin (Rifadin®, Sanofi-Aventis, Bridgewater, NJ) 600 mg in 10 ml sterile normal saline

Dispensing

The investigator has authority to dispense the drug

8.4.1 Administration Instructions

During study period 1 or 2, subjects will take fluvastatin 20 mg by mouth as a 20mg lescol capsule. Subjects will also receive 30-min intravenous infusion of rifampin (Rifadin®, Sanofi-Aventis, Bridgewater, NJ) 600 mg in 10 ml sterile normal saline at a rate of 20 mg/min immediately prior to fluvastatin dosing on one of the study periods. All subjects will be asked to abstain from caffeinated beverages, alcoholic beverages, all fruit juices, oranges, and grapefruits from 7am the day before each study period until the study is complete.

Storage

Study drugs should be stored by the study site at controlled room temperature, 15 to 30ºC (59 to 86ºF). If the temperature of study drug storage in the clinic/pharmacy exceeds or falls below this range, this should be reported to the Sponsor or designee and captured as a deviation. It is important store the medication in original packaging (foil pouch and protected from light) at room temperature according to the instructions outlined on the Drug Administration Instructions.

8.5 Study Drug Accountability

An accurate and current accounting of the dispensing and return of study drug for each subject will be maintained on an ongoing basis by a member of the study site staff. The number of study drug dispensed and returned by the subject will be recorded on the Investigational Drug Accountability Record. The study monitor will verify these documents throughout the course of the study.

8.6 Measures of Treatment Compliance

Investigator will monitor the subjects when they take fluvastatin and when they undergo 30-min intravenous infusion of rifampin (Rifadin®, Sanofi-Aventis, Bridgewater, NJ) 600 mg in 10 ml sterile normal saline at a rate of 20 mg/min

9 STUDY PROCEDURES AND GUIDELINES

A Schedule of Events representing the required testing procedures to be performed for the duration of the study is diagrammed in Appendix 1.

Prior to conducting any study-related activities, written informed consent and the Health Insurance Portability and Accountability Act (HIPAA) authorization must be signed and dated by the subject. If appropriate, assent must also be obtained prior to conducting any study-related activities.

9.1 Clinical Assessments

9.1.1 Concomitant Medications

Only subjects who take no other medications two weeks prior to the study and during the time course of the study including prescription medications, over-the-counter medications, dietary supplements, or drugs of abuse will be allowed to participate in the trial.

9.1.2 Demographics

A descriptive analysis of the demographic characteristics of the study subjects will be performed. Demographic characteristics of interest are age, race, height, weight and gender will be recorded at screening.

9.1.3 Medical History

Relevant medical history, including history of current disease, other pertinent respiratory history, and information regarding underlying diseases will be recorded at screening

9.1.4 Physical Examination

A complete physical examination will be performed by either the investigator who is a physician at screening visit. Qualified staff (MD) may complete the abbreviated physical exam at all other visits. New abnormal physical exam findings must be documented and will be followed by a physician or other qualified staff at the next scheduled visit.

9.1.5 Vital Signs

Body temperature, blood pressure, pulse and respirations will be performed after resting for 5 minutes at the screening

9.1.6 Adverse Events

Information regarding occurrence of adverse events will be captured throughout the study. Duration (start and stop dates and times), severity/grade, outcome, treatment and relation to study drug will be recorded on the case report form (CRF).

9.2 Clinical Laboratory Measurements

9.2.1 Hematology

Blood will be obtained and sent to the site's clinical hematology lab for a complete blood count (hemoglobin, hematocrit, red blood cell count, white blood cell count, white blood cell differential, and platelet count), erythrocyte sedimentation rate (ESR), and serum C-reactive protein (CRP) determinations for assessment of systemic evidence for infection and/or inflammation.

9.2.2 Blood Chemistry Profile

Blood will be obtained and sent to each site's clinical chemistry lab forHgb, HCT, RBC, Plt, WBC with differential, Ca++, Na+, K+, Cl-, CO2, glucose, serum creatinine, BUN, phosphorus, AST,ALT, alkaline phosphatase, CPK, total bilirubin, albumin, total protein, cholesterol, and triglycerides.

9.2.3 Pregnancy Test

A urine or serum pregnancy test will be obtained from female subjects who are of childbearing age prior to their participation in the study.

9.2.4 Urinalysis

Urine will be obtained and sent to each site's clinical laboratory for determination of (list as applicable) color, specific gravity, pH, protein, glucose, ketones, and blood.

9.3 Pharmacokinetic Measurements

Blood for determination of serum concentrations of fluvastatin will be collected 0, 0.33, 0.67, 1,1.5, 2, 2.5, 3,4, 6, 9, and 12h after fluvastatin dosing.Plasma will be separated and stored at –80°C until analysis of drug levels.

Plasma concentrations of fluvastatin and rifampin in all collected plasma samples will be measured via a previously validated tandem liquid chromatography mass spectrometry (LC-MS/MS) method. Determined plasma concentrations of fluvastatin will then be modeled using pharmacokinetic modeling software to calculate the corresponding AUC, oral clearance, and oral volume of distribution of fluvastatin in the treatment groups.

9.4 Research Laboratory Measurements

Cell Count and Differential blood sample (30mL) for determination of Hgb, HCT, RBC, Plt, WBC with differential will be collected during screen.

10 EVALUATIONS BY VISIT

10.1 Screening Visit (at least one week prior to study period 1)

1) Written informed consent will be obtained.
2) A medical history and a physical exam including vital signs (oral temperature, heart rate, blood pressure, and respiratory rate), height, and weight will be performed. Subjects will also be asked about prescriptions, dietary supplements, and illegal drug use.
3) Blood (30 ml) and urine tests (Hgb, HCT, RBC, Plt, WBC with differential, Ca++, Na+, K+, Cl-, CO2, glucose, serum creatinine, BUN, phosphorus, AST,ALT, alkaline phosphatase, CPK, total bilirubin, albumin, total protein, cholesterol, triglycerides, urinalysis.
4) For female subjects, a urine pregnancy test will be required.

10.2 Study period 1 (Tuesday of the Week):

1) **Admit to Clinical Research Center (CRC) at UCSF** - subjects will be admitted to the Clinical Research Center (CRC) at UCSF (12th Floor Moffitt Hospital). Subjects will be discharged after the 12 hour blood sample.
2) **Urine Pregnancy Test** - For female subjects, a urine pregnancy test will be performed.
3) **Catheter placement-** For the purpose of administering rifampin and drawing blood samples, two indwelling peripheral venous catheters will be inserted aseptically into the forearm vein by a nurse in the CRC immediately prior to medication administration. The rifampin catheter will be removed immediately after rifampin administration, the second catheter will be removed after the final blood draw. If the catheter malfunctions and needs to be removed prior to the last blood draw, a subject may choose to have remaining blood draws via peripheral venous needle sticks or have a new catheter placed.
4) **Dose study medications**: Subjects will receive one of the following dose of medications:

   a. Dose fluvastatin 20 mg by mouth as a 20 mg Lescol® capsule (t = 0)

   b. Dose rifampin 600mg IV infusion over 30 minutes (t = -30 minutes) followed by a dose of fluvastatin 20 mg by mouth as a 20 mg Lescol® capsule (t = 0)

   The order in which subjects receive the above treatments will be randomized via random number generator.

5) Serial blood sampling- Venous blood samples (8 mL) will be drawn at 0, 0.33, 0.67, 1,1.5, 2, 2.5, 3,4, 6, 9, and 12h after fluvastatin dosing.Plasma will be separated and stored at –80°C until analysis of drug levels.

10.3 Study Period 2 (Thursday of the week)

1) **Admit to Clinical Research Center (CRC) at UCSF** - During each study period, subjects will be admitted to the Clinical Research Center (CRC) at UCSF (12th Floor Moffitt Hospital). Subjects will be discharged after the 12 hour blood sample.

2) **Urine Pregnancy Test** - For female subjects, a urine pregnancy test will be performed.

3) **Catheter placement-** For the purpose of administering rifampin and drawing blood samples, two indwelling peripheral venous catheter will be inserted aseptically into the forearm vein by a nurse in the CRC immediately prior to medication administration. The rifampin catheter will be removed immediately after rifampin administration, the second catheter will be removed after the final blood draw. If the catheter malfunctions and needs to be removed prior to the last blood draw, a subject may choose to have remaining blood draws via peripheral venous needle sticks or have a new catheter placed.

4) **Dose study medications**: Subjects will receive one of the following dose of medications:

a. Dose fluvastatin 20 mg by mouth as a 20 mg Lescol® capsule (t = 0)

b. Dose rifampin 600mg IV infusion over 30 minutes (t = -30 minutes) followed by a dose of fluvastatin 20 mg by mouth as a 20 mg Lescol® capsule (t = 0)

The order in which subjects receive the above treatments will be randomized via random number generator.

5) **Serial blood sampling**- Venous blood samples (8 mL) will be drawn at 0, 0.33, 0.67, 1, 1.5, 2, 2.5, 3, 4, 6, 9 and 12h after fluvastatin dosing.Plasma will be separated and stored at –80°C until analysis of drug levels.

6) Safety Monitoring- At study completion, subjects will have repeat physical examination and repeat hematology, chemistries, liver, renal and coagulation laboratories for safety monitoring.

11 ADVERSE EXPERIENCE REPORTING AND DOCUMENTATION

11.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a clinical investigation of a patient administered a pharmaceutical product and that does not necessarily have a causal

relationship with the treatment. An AE is therefore any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the administration of an investigational product, whether or not related to that investigational product. An unexpected AE is one of a type not identified in nature, severity, or frequency in the current Investigator's Brochure or of greater severity or frequency than expected based on the information in the Investigator's Brochure.

The Investigator will probe, via discussion with the subject, for the occurrence of AEs during each subject visit and record the information in the site's source documents. Adverse events will be recorded in the patient CRF. Adverse events will be described by duration (start and stop dates and times), severity, outcome, treatment and relation to study drug, or if unrelated, the cause.

AE Severity

The National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0 should be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant. The modified criteria can be found in the study manual. If the experience is not covered in the modified criteria, the guidelines shown in Table 1 below should be used to grade severity. It should be pointed out that the term "severe" is a measure of intensity and that a severe AE is not necessarily serious.

Table 1. AE Severity Grading

Severity (Toxicity Grade)	Description
Mild (1)	Transient or mild discomfort; no limitation in activity; no medical intervention or therapy required. The subject may be aware of the sign or symptom but tolerates it reasonably well.
Moderate (2)	Mild to moderate limitation in activity, no or minimal medical intervention/therapy required.
Severe (3)	Marked limitation in activity, medical intervention/therapy required, hospitalizations possible.
Life-threatening (4)	The subject is at risk of death due to the adverse experience as it occurred. This does not refer to an experience that hypothetically might have caused death if it were more severe.

AE Relationship to Study Drug

The relationship of an AE to the study drug should be assessed using the following the guidelines in Table 2.

Table 2. AE Relationship to Study Drug

Relationship to Drug	Comment

Definitely	Previously known toxicity of agent; or an event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is not explained by any other reasonable hypothesis.
Probably	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is unlikely to be explained by the known characteristics of the subject's clinical state or by other interventions.
Possibly	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to that suspected drug; but that could readily have been produced by a number of other factors.
Unrelated	An event that can be determined with certainty to have no relationship to the study drug.

11.2 Serious Adverse Experiences (SAE)

An SAE is defined as any AE occurring at any dose that results in any of the following outcomes:

- death
- a life-threatening adverse experience
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly/birth defect
- Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the subject or require intervention to prevent one of the outcomes listed.

11.2.1 Serious Adverse Experience Reporting

Study sites will document all SAEs that occur (whether or not related to study drug) per UCSF CHR Guidelines. The collection period for all SAEs will begin after informed consent is obtained and end after procedures for the final study visit have been completed.

In accordance with the standard operating procedures and policies of the local Institutional Review Board (IRB)/Independent Ethics Committee (IEC), the site investigator will report SAEs to the IRB/IEC.

12 DISCONTINUATION AND REPLACEMENT OF SUBJECTS

12.1 Early Discontinuation of Study Drug

A subject may be discontinued from study treatment at any time if the subject, the investigator, or the Sponsor feels that it is not in the subject's best interest to continue. The following is a list of possible reasons for study treatment discontinuation:

- Subject withdrawal of consent (or assent)
- Subject is not compliant with study procedures
- Adverse event that in the opinion of the investigator would be in the best interest of the subject to discontinue study treatment
- Protocol violation requiring discontinuation of study treatment
- Lost to follow-up
- Sponsor request for early termination of study
- Positive pregnancy test (females)

If a subject is withdrawn from treatment due to an adverse event, the subject will be followed and treated by the Investigator until the abnormal parameter or symptom has resolved or stabilized.

All subjects who discontinue study treatment should come in for an early discontinuation visit as soon as possible and then should be encouraged to complete all remaining scheduled visits and procedures.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents Refer to Section 10 for early termination procedures.

12.2 Withdrawal of Subjects from the Study

A subject may be withdrawn from the study at any time if the subject, the investigator, or the Sponsor feels that it is not in the subject's best interest to continue.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents. Subjects who withdraw after study period 1 but prior to study period 2 should be encouraged to come in for a final visit (and the procedures to be followed would include those for their next scheduled visit).

12.3 Replacement of Subjects

Subjects who withdraw from the study treatment will be replaced.

Subjects who withdraw from the study will be replaced.

13 PROTOCOL VIOLATIONS

A protocol violation occurs when the subject, investigator, or sponsor fails to adhere to significant protocol requirements affecting the inclusion, exclusion, subject safety and primary endpoint criteria. Protocol violations for this study include, but are not limited to, the following:

- Failure to meet inclusion/exclusion criteria
- Use of a concomitant medication

Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation. The sponsor will determine if a protocol violation will result in withdrawal of a subject.

When a protocol violation occurs, it will be discussed with the investigator and a Protocol Violation Form detailing the violation will be generated. This form will be signed by a Sponsor representative and the Investigator. A copy of the form will be filed in the site's regulatory binder and in the Sponsor's files.

14 DATA SAFETY MONITORING

The study does not required a Data and Safety Monitoring Board

15 STATISTICAL METHODS AND CONSIDERATIONS

Prior to the analysis of the final study data, a detailed Statistical Analysis Plan (SAP) will be written describing all analyses that will be performed. The SAP will contain any modifications to the analysis plan described below.

15.1 Data Sets Analyzed

All eligible patients who are randomized into the study will be included in the data analysis.

15.2 Demographic and Baseline Characteristics

A descriptive analysis of the demographic characteristics of the study subjects will be performed. Demographic characteristics of interest are age, race, height, weight and gender.

15.3 Analysis of Primary Endpoint

AUC0-∞ will be calculated using the trapezoidal rule from time 0 to Clast + Clast/ke. Parameters such as maximum plasma concentration (Cmax) and time to Cmax (Tmax) will be determined from the observed data. The terminal elimination rate constant (ke) will be obtained using linear regression of log transformed concentrations over the terminal log-linear decline phase. Oral clearance (CL/F) will be calculated using Dose/AUC0-∞. Other estimated pharmacokinetic parameters will be calculated using non-

compartmental methods with WinNonlin Professional software (Pharsight, Inc., Mountain View, CA). Log-transformed pharmacokinetic parameters will be compared across treatments with analysis of variance for repeated measures. Differences will be regarded as statistically significant if $p< 0.05$. Data will be presented as mean values with standard deviations. Each subject will serve as his/her own control. Differences in pharmacokinetic parameters between treatments will be calculated using student's paired t-test. Statistical calculations will be done using STATA 10.0 (StataCorporation, College Station, TX).

15.4 Analysis of Secondary Endpoints

Parameters such as maximum plasma concentration (Cmax) and time to Cmax (Tmax) will be determined from the observed data. The terminal elimination rate constant (ke) will be obtained using linear regression of log transformed concentrations over the terminal log-linear decline phase. Oral clearance (CL/F) will be calculated using Dose/AUC0-∞.

15.5 Interim analysis

There is no scheduled interim analysis, as the trial is short in duration, but serious adverse events will be monitored by the committee on an ongoing basis throughout the study.

15.6 Sample Size and Randomization

Using a paired t-test and prior data, a sample size of n=10 in each group is needed to detect a 50% difference in AUC0-12 between the two study regimens with a statistical power of 80% (two sided alpha = 0.05) and standard deviation of 50%. Due to the lesser effects of rifampin on fluvastatin AUC, the within subject variability can affect the sample size. We plan to enroll sample size of 12 subjects to maintain an adequate sample size in case of potential attrition.

Once included in the study, patients will be randomized using computer-generated random numbers, corresponding to each treatment. This will be performed by someone other than the investigator and will ensure equal number of patients in each treatment sequence.

16 DATA COLLECTION, RETENTION AND MONITORING

16.1 Data Collection Instruments

The Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each subject treated with the study drug. The investigator will enter data from source documents corresponding to a subject's visit into the protocol-specific electronic Case Report Form (eCRF) when the information corresponding to that visit is available.

The Investigator is responsible for all information collected on subjects enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the Investigator. A copy of the CRF will remain at the Investigator's site at the completion of the study.

16.2 Data Management Procedures

The data will be entered into a validated database. The Data management group will be responsible for data processing, in accordance with procedural documentation. Database lock will occur once quality assurance procedures have been completed.

All procedures for the handling and analysis of data will be conducted using good computing practices meeting FDA guidelines for the handling and analysis of data for clinical trials.

16.3 Data Quality Control and Reporting

After data have been entered into the study database, a system of computerized data validation checks will be implemented and applied to the database on a regular basis. The study database will be updated in accordance with the resolved queries. All changes to the study database will be documented.

16.4 Archival of Data

The database is safeguarded against unauthorized access by established security procedures; appropriate backup copies of the database and related software files will be maintained. Databases are backed up by the database administrator in conjunction with any updates or changes to the database.

At critical junctures of the protocol (e.g., production of interim reports and final reports), data for analysis is locked and cleaned per established procedures.

16.5 Availability and Retention of Investigational Records

The Investigator must make study data accessible to the monitor, other authorized representatives of the Sponsor (or designee), IRB/IEC, and Regulatory Agency (e.g., FDA) inspectors upon request. A file for each subject must be maintained that includes the signed Informed Consent, HIPAA Authorization and Assent Form and copies of all source documentation related to that subject. The Investigator must ensure the reliability and availability of source documents from which the information on the CRF was derived.

All study documents (patient files, signed informed consent forms, copies of CRFs, Study File Notebook, etc.) must be kept secured for a period of two years following marketing of the investigational product or for two years after centers have been notified that the IND has been discontinued. There may be other circumstances for which the Sponsor is required to maintain study records and, therefore, the Sponsor should be contacted prior to removing study records for any reason.

16.6 Monitoring

Monitoring visits will be conducted by representatives of the Sponsor according to the U.S. CFR Title 21 Parts 50, 56, and 312 and ICH Guidelines for GCP (E6). By signing this protocol, the Investigator grants permission to the Sponsor (or designee), and appropriate regulatory authorities to conduct on-site monitoring and/or auditing of all appropriate study documentation.

16.7 Subject Confidentiality

Only the investigators listed on this application and research personnel directly involved in the study will have access to personal information. All personal and medical data will be considered confidential. All subject information will be kept confidential as possible under the law.

17 ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a coded number and initials only. All study records will be kept in a locked file cabinet and code sheets linking a patient's name to a patient identification number will be stored separately in another locked file cabinet. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the FDA. The Investigator must also comply with all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC).

17.1 Protocol Amendments

Any amendment to the protocol will be written by the investigator. Protocol amendments cannot be implemented without prior written IRB/IEC approval except as necessary to eliminate immediate safety hazards to patients. A protocol amendment intended to eliminate an apparent immediate hazard to patients may be implemented immediately, provided the IRBs are notified within five working days.

17.2 Institutional Review Boards and Independent Ethics Committees

The protocol and consent form will be reviewed and approved by the IRB/IEC of each participating center prior to study initiation. Serious adverse experiences regardless of causality will be reported to the IRB/IEC in accordance with the standard operating procedures and policies of the IRB/IEC, and the Investigator will keep the IRB/IEC informed as to the progress of the study. The Investigator will obtain assurance of IRB/IEC compliance with regulations.

Any documents that the IRB/IEC may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB/IEC. The IRB/IECs written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. The IRB/IECs unconditional approval statement will be transmitted by the Investigator prior to the shipment of study supplies to the site. This approval must refer to the study by exact protocol title and number and should identify the documents reviewed and the date of review.

Protocol and/or informed consent modifications or changes may not be initiated without prior written IRB/IEC approval except when necessary to eliminate immediate hazards to the patients or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB/IEC and written verification that the modification was submitted and subsequently approved should be obtained.

The IRB/IEC must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

17.3 Informed Consent Form

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, US Code of Federal Regulations for Protection of Human Subjects (21 CFR 50.25[a,b], CFR 50.27, and CFR Part 56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA, if applicable), and local regulations.

The Investigator will prepare the informed consent form, assent and HIPAA authorization and provide the documents to the Sponsor or designee for approval prior to submission to the IRB/IEC. The consent form generated by the Investigator must be acceptable to the Sponsor and be approved by the IRB/IEC. The written consent document will embody the elements of informed consent as described in the International Conference on Harmonisation and will also comply with local regulations. The Investigator will send an IRB/IEC-approved copy of the Informed Consent Form to the Sponsor (or designee) for the study file.

A properly executed, written, informed consent will be obtained from each subject prior to entering the subject into the trial. Information should be given in both oral and written form and subjects (or their legal representatives) must be given ample opportunity to inquire about details of the study. If appropriate and required by the local IRB/IEC, assent from the subject will also be obtained. If a subject is unable to sign the informed consent form (ICF) and the HIPAA authorization, a legal representative may sign for the subject. A copy of the signed consent form (and assent) will be given to the subject or legal representative of the subject and the original will be maintained with the subject's records.

17.4 Publications

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the study Sponsor and participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

17.5 Investigator Responsibilities

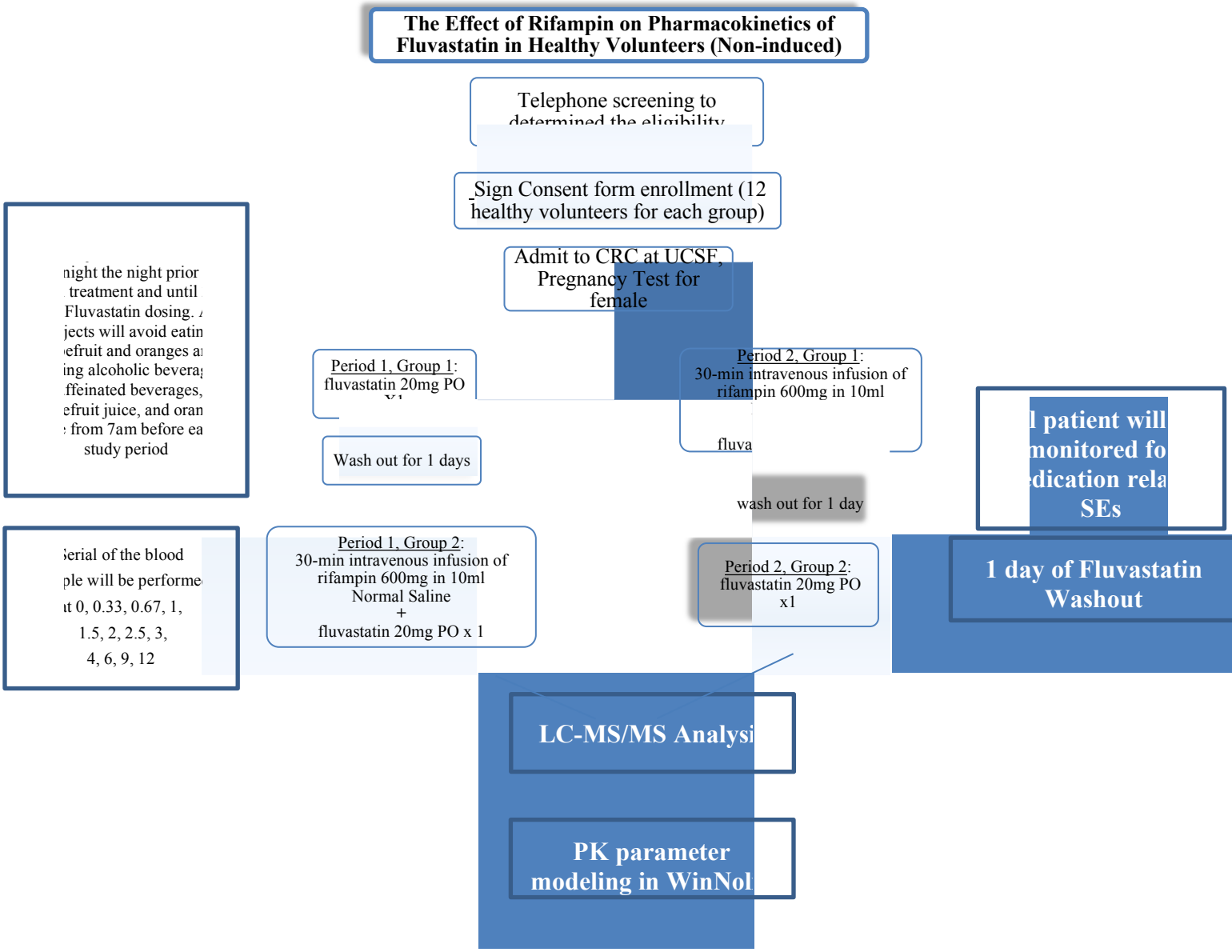
By signing the Agreement of Investigator form, the Investigator agrees to:

1. Conduct the study in accordance with the protocol and only make changes after notifying the Sponsor (or designee), except when to protect the safety, rights or welfare of subjects.
2. Personally conduct or supervise the study (or investigation).
3. Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.
4. Report to the Sponsor or designee any AEs that occur in the course of the study, in accordance with §21 CFR 312.64.
5. Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.
6. Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection with the Sponsor (or designee).
7. Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the clinical study.
8. Promptly report to the IRB and the Sponsor (or designee) all changes in the research activity and all unanticipated problems involving risks to subjects or others (to include amendments and IND safety reports).
9. Seek IRB approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/subjects.
10. Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

APPENDIX 1. SCHEDULE OF STUDY VISITS

	SCREENING VISIT (Day/Week/Month #)[a]	STUDY PERIOD 1 (Day/Week/Month #)[a]	STUDY PERIOD 2 (Day/Week/Month #)[a]
Informed Consent	X		
Medical History	X		
Complete Physical Exam	X		
Abbreviated Physical Exam		X	X
Height	X	X	X
Weight	X	X	X
Vital Signs	X	X	X
Pharmacokinetics		X	
Chemistry	X		
Pregnancy Test (Urine or Serum)	X		
Hematology	X		
ESR	X		
C-Reactive Protein	X		
Urinalysis	X		
Randomization	X		
Dispensing or Administration of Study Drug	X	X	X
Counting of Returned Study Drug		X	X
Initiate Subject Diary	X		
Concomitant Medication Review	X	X	X
Adverse Experiences			

APPENDIX 2. DIAGRAM OF STUDY PLAN


The Effect of Rifampin on Pharmacokinetics of Fluvastatin in Healthy Volunteers (Non-induced)
Telephone screening to
Sign Consent form enrollment (12 healthy volunteers for each group)
Admit to CRC at UCSF, Pregnancy Test for female
night the night prior
treatment and until
Fluvastatin dosing.
jects will avoid eatin
efruit and oranges a
ing alcoholic bevera
ffeinated beverages,
efruit juice, and oran
from 7am before ea
study period
Period 1, Group 1:
fluvastatin 20mg PO
Wash out for 1 days
Period 2, Group 1:
30-min intravenous infusion of rifampin 600mg in 10ml
fluva
wash out for 1 day
patient will
monitored fo
dication rela
SEs
erial of the blood
ple will be performe
t 0, 0.33, 0.67, 1,
1.5, 2, 2.5, 3,
4, 6, 9, 12
Period 1, Group 2:
30-min intravenous infusion of rifampin 600mg in 10ml Normal Saline
+
fluvastatin 20mg PO x 1
Period 2, Group 2:
fluvastatin 20mg PO x1
1 day of Fluvastatin Washout
LC-MS/MS Analysi
PK parameter modeling in WinNol

LESLIE Z. BENET, PHD

Clinical Research Protocol

The Effects of Rifampin on Pharmacokinetics of Fluvastatin in hepatically induced Healthy Volunteers

Protocol Number:	
Version Date:	
Investigational Product:	
IND Number:	
Development Phase:	
Sponsor:	Leslie Z. Benet, PhD
Funding Organization:	
Principal Investigator:	Name: Leslie Z. Benet, PhD Telephone:(415)476-3853 Fax: (415)476-8887 E-mail:leslie.benet@ucsf.edu
Medical Monitor:	Name: Lynda A. Frassetto Telephone: (415)476-6143 Fax:(415) 885-7396 E-mail:Lynda.Frassetto@ucsf.edu
Coordinating Center:	If applicable

Approval:

PI or Sponsor Signature (Name and Title)

Dec 27, 2018

Date

This confidential information about an investigational product is provided for the exclusive use of investigators of this product and is subject to recall at any time. The information in this document may not be disclosed unless federal or state law or regulations require such disclosure. Subject to the foregoing, this information may be disclosed only to those persons involved in the study who have a need to know, with the obligation not to further disseminate this information.

PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Investigator, my duties include ensuring the safety of the study subjects enrolled under my supervision and providing with complete and timely information, as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site. Furthermore, on behalf of the study staff and myself, I agree to maintain the procedures required to carry out the study in accordance with accepted GCP principles and to abide by the terms of this protocol.

Protocol Number: Number

Protocol Title: The Effects of Rifampin on Pharmacokinetics of Fluvastatin in hepatically induced Healthy Volunteers

Protocol Date: 12/20/18

12/27/18

Investigator Signature *Date*

Leslie Z. Benet, PhD, Professor

Print Name and Title

Site # _____

Site Name _____

Address 533 Parnassus Ave, U-68

UCSF Box 0912

San Francisco, CA 94143

Phone Number 14154763853

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AE	adverse event
AUC0-∞	Area under the concentration versus time curve from time = 0 to infinity.
AUC0-12	Area under the concentration versus time curve from time = 0 to t = 12 hours.
BUN	blood urea nitrogen
CFR	Code of Federal Regulations
Cmax	maximum concentration
CRF	case report form
ESR	erythrocyte sedimentation rate
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act of 1996
ICF	informed consent form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IV	intravenous
PI	Principal Investigator
PK	pharmacokinetic
SAE	serious adverse experience
Tmax	time to Cmax

PROTOCOL SYNOPSIS

TITLE	The Effects of Rifampin on Pharmacokinetics of Fluvastatin in in hepatically induced Healthy Volunteers
NUMBER OF SITES	1
RATIONALE	The effect of OATP1B1 transporter inhibition after a rifampin pre-induction regimen on fluvastatin, a BDDCS class 1 drug, has not been studied to date. A preinduction regimen of rifampin followed by a single IV infusion of rifampin can be used to evaluate the combined effects of enzyme induction and OATP1B1 transporter inhibition on fluvastatin disposition.
STUDY DESIGN	This is a two-period, randomized, unblinded, crossover clinical trial.
PRIMARY OBJECTIVE	The primary objective is to determine whether or not inhibition of OATP1B1-mediated drug transport of fluvastatin will be clinically significant when hepatic enzymes are induced.
NUMBER OF SUBJECTS	10-12
SUBJECT SELECTION CRITERIA	Inclusion Criteria: ● Healthy male or female, ages 18-65 years old, with no current medical conditions or active diagnoses as determined by the study doctor based on history, physical exam, and laboratory evaluations. ● Subjects who take no other medications two weeks prior to the study and during the time course of the study including prescription medications, over-the-counter medications, dietary supplements, or drugs of abuse. ● Subjects able to maintain adequate birth control during the study independent of hormonal contraceptive use (use of condoms). ● Subjects able to abstain from grapefruit, grapefruit juice, oranges, orange juice, caffeinated beverages and/or alcoholic

	beverages from 7am the day before the study to completion of that study day. ● Participants determined to have normal liver and kidney function as measured at baseline (ALT: ≤ 2x upper level of normal (ULN), AST: ≤ 2x ULN, SCr: ≤ 1.5x ULN, T. Bili: 0.1-1.2mg/dL, Albumin: 3.4 – 4.7 mg/dL). ● BMI between 18.0 - 30 kg/m2 o Subjects capable of fasting from food and beverages at least 8 hours prior to medication dosing. ● Be able to read, speak, and understand English. ● Subjects capable of providing informed consent and completing the requirements of the study. <u>Exclusion Criteria</u>: ● Subjects with active medical problems ● Subjects on chronic prescription or OTC medication that cannot be stopped 2 weeks prior to and during the study. ● Subjects incapable of multiple blood draws (HCT < 30mg/dL) ● Subjects with a history of rhabdomyolysis ● Subjects with a history of drug-related myalgias ● Subjects with a history or diagnosis of hemorrhagic tendencies or blood dyscrasias ● Subjects with a history of GI bleed or peptic ulcer disease ● Subjects who smoke tobacco or have ongoing alcohol or illegal drug use ● Subjects who are pregnant, lactating, or trying to conceive during the study period ● Subjects allergic to fluvastatin or rifampin or any known component of the medications ● Anyone who in the opinion of the study investigators is unable to do the study
TEST PRODUCT, DOSE, AND ROUTE OF ADMINISTRATION	Fluvastatin 20 mg by mouth as a 20 mg Lescol® capsule Rifampin 600mg IV infusion over 30 minutes (t = -30 minutes) Rifadin 600mg by mouth as two 300mg rifadin capsules
CONTROL PRODUCT, DOSE AND ROUTE OF ADMINISTRATION	Fluvastatin 20 mg by mouth as a 20 mg Lescol® capsule

DURATION OF SUBJECT PARTICIPATION AND DURATION OF STUDY	The time required for this study will include approximately: 5 minutes each day taking the rifampin capsule for 6 days, one 2-hour screening visit, and two 13 hour stays in the CRC (separated by at least 1 day). The total time required is 27 ½ hours over about a 8 day period. Additional time maybe required if study is prolonged for any reasons.
CONCOMMITANT MEDICATIONS	Allowed: No concomitant medications allowed Prohibited: subjects will not be allowed to take any other medications two weeks prior to the study and during the time course of the study including prescription medications, over-the-counter medications, dietary supplements, or drugs of abuse
PRIMARY ENDPOINT	The primary outcome will be fluvastatin AUC0-12h and AUC0-∞ (AUC = area under the plasma concentration versus time curve).
SECONDARY ENDPOINTS	Secondary outcomes will include fluvastatin maximum plasma concentration (Cmax) and time to Cmax (Tmax) .
SAFETY EVALUATIONS	Safety evaluation will be determined by the incidence of adverse events. Subjects will be monitored on an ongoing basis while involved with the study by study personnel. Subjects will be screened with a medical history and physical examination by the study physician and have screening hematology, chemistries, liver, renal and coagulation laboratories. Subjects must have normal function of all major organs. At study completion, subjects will have repeat physical examination and repeat hematology, chemistries, liver, renal and coagulation laboratories for safety monitoring
PLANNED INTERIM ANALYSES	Serious adverse events will be monitored by the committee on an ongoing basis throughout the study.
STATISTICS **Primary Analysis Plan**	AUC0-∞ will be calculated using the trapezoidal rule from time 0 to Clast + Clast/ke. Parameters such as maximum plasma concentration (Cmax) and time to Cmax (Tmax) will be determined from the observed data. The terminal elimination rate constant (ke) will be obtained using linear regression of log transformed concentrations over the terminal log-linear decline phase. Oral clearance (CL/F) will

	be calculated using Dose/AUC0-∞. Other estimated pharmacokinetic parameters will be calculated using non-compartmental methods with WinNonlin Professional software (Pharsight, Inc., Mountain View, CA). Log-transformed pharmacokinetic parameters will be compared across treatments with analysis of variance for repeated measures. Differences will be regarded as statistically significant if $p< 0.05$. Data will be presented as mean values with standard deviations. Each subject will serve as his/her own control. Differences in pharmacokinetic parameters between treatments will be calculated using student's paired t-test. Statistical calculations will be done using STATA 10.0 (StataCorporation, College Station, TX).
Rationale for Number of Subjects	Using a paired t-test and prior data, a sample size of n=10 in each group is needed to detect a 50% difference in AUC(0-12) between the two study regimens with a statistical power of 80% (two sided alpha = 0.05) and standard deviation of 50%. Due to the lesser effects of rifampin on fluvastatin AUC, the within subject variability can affect the sample size. We plan to enroll sample size of 12 subjects to maintain an adequate sample size in case of potential attrition.

1 BACKGROUND

Membrane transporters located throughout the body play an important role in drug absorption, disposition, metabolism, and elimination[1].The inhibition of many of these transporters has been linked to significant drug-drug interactions[1]. Located on the sinusoidal membrane of hepatocytes, the Organic Anion Transporter Polypeptide transporter OATP1B1 is responsible for the uptake of substrates from the blood into the liver where they can be metabolized and cleared[1-3]. When OATP1B1-mediated transport is inhibited with known inhibitors such as ritonavir, rifampin, or cyclosporine, drug metabolism and elimination into bile are diminished, resulting in elevated concentrations of the drug in the blood. Another important membrane transporter, Breast Cancer Resistance Protein or BCRP, present in the intestinal epithelia and liver, is also important for drug elimination[1].If BCRP is inhibited, drug concentrations in the liver and blood may also rise.

Statins are commonly prescribed cholesterol-lowering agents whose efficacy and safety is closely linked with the function of the BCRP and OATP1B1 transporters[1-3]. These drugs must enter the liver to exert their efficacy. Compared to other statins like rosuvastatin and pravastatin, fluvastatin is extensively metabolized[4]. It has a

more hydrophobic profile making it highly permeable through the membranes and in theory less dependent on OATP1B1-mediated transport[5].

To predict hepatic uptake transporter effects on drug disposition, Wu and Benet developed the Biopharmaceutics Drug Disposition Classification System (BDDCS) system which categorizes drugs based on extent of metabolism and solubility characteristics [6]. According to this classification system, the plasma exposure of Fluvastatin, a BDDCS class I drug should be minimally influenced by transporter effects[6].

1.1 Overview of Non-Clinical Studies

Available in vitro data demonstrates that inhibition of OATP1B1 by rifampin results in reduced rosuvastatin uptake in Human Embroyonic Kidney (HEK293) cells, which implies the drug's significant dependence on the membrane transporter[7]. Animal studies have also confirmed that rifampin significantly alters hepatobiliary transport of rosuvastatin and increases its plasma exposure (AUC) and Cmax[8].

Similar results to those observed in clinical studies have been observed in in vivo and in vitro rat studies where the effects of inhibition of hepatic uptake transporters offset the effect of the hepatic enzyme induction for the BDDCS class IV drug digoxin[9]. In this study, hepatic enzyme induction by dexamethasone decreased AUC values relative to control, but when hepatic uptake transporters were inhibited by rifampin, the AUC values matched those of the control. Unfortunately, a direct comparison to the human glyburide trial cannot be made because no analogous uninduced, rifampin treatment was conducted in this rat study. Interestingly, similar AUC ratios for the induced treatment (1.99(in vitro), 2.15 (in vivo)) were observed, which may indicate a similar effect of hepatic uptake transporters in rats and humans[9].

1.2 Overview of Clinical Studies

Clinical studies have demonstrated that co-administration of single-dose rifampin with the BDDCS Class II drug atorvastatin results in marked increases in concentrations of the cholesterol-lowering agent[10,11]. Similar studies with the BDDCS class III drug rosuvastatin and class II drug glyburide showed similar transporter effects which increased plasma concentrations of the two drugs when co-administered with a single IV dose of rifampin[12,13]. These studies have demonstrated the significant effects of hepatic uptake transporters on the

pharmacokinetics of BDDCS class II and III drugs. It is expected that these transporter effects will not be significant for BDDCS class I drugs like fluvastatin.

Contrary to this expectation, one clinical study has demonstrated that co-administration of single-dose rifampin with 2 mg of fluvastatin results in significant increase in exposure of the cholesterol-lowering agent[14]. However this study was conducted with one tenth to one fortieth of the common dose of fluvastatin[15], and we believe that the effect of rifampin on fluvastatin exposure in this study to be due to the low concentration of fluvastatin.

Clinical studies have demonstrated that the effects of hepatic OATP1B1 transporters on the BDDCS Class II drug glyburide are the same under induced and non-induced conditions[13]. Concomitant rifampin treatment under the the induced condition yielded a glyburide AUC_{IVrif}:$AUC_{noIVrif}$ ratio of 2.06, which is comparable to the AUC ratio of 2.18 under the non-induced condition, despite the lower absolute AUC values in the CYP enzyme induced cohort[13]. For fluvastatin (at clinical doses), it is expected that this AUC_{IVrif}:$AUC_{noIVrif}$ will be closer to 1, and it is desired to compare these ratios between induced and uninduced states as has previously been done for glyburide.

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2. STUDY RATIONALE

To our knowledge, the effect of OATP1B1 transporter inhibition after a rifampin pre-induction regimen on fluvastatin, a BDDCS class 1 drug, has not been studied to date. A preinduction regimen of rifampin followed by a single IV infusion of

rifampin can be used to evaluate the combined effects of enzyme induction and OATP1B1 transporter inhibition on fluvastatin disposition.

2.1 Risk / Benefit Assessment

There is minimum risk involved in this study. Only approved doses of FDA approved medications are being used in this study. As outlined by the Data and Safety Management Plan for the study all precautions are being considered to uphold the safety and best interest of all participants. The subject may stop the study at any time for any reason.

There is no direct benefit for subjects to participate in the study. .

3. STUDY OBJECTIVES

3.1 Primary Objective

The primary objective is to determine whether or not inhibition of OATP1B1-mediated drug transport of fluvastatin will be clinically significant when hepatic enzymes are induced.

4. STUDY DESIGN

4.1 Study Overview

The effect of rifampin on the pharmacokinetics of fluvastatin under a hepatic enzyme induced state will be studied in healthy volunteers in a two-period, randomized, unblinded, crossover clinical trial. For the first 5 days of the trial, the patients will be pretreated with 600mg oral rifampin (two 300mg rifadin capsule) once daily to induce hepatic enzymes (and transporters). For study period 1, subjects will be randomized to one of two treatment groups:

(i) one oral dose of fluvastatin (Lescol®) 20mg capsule

(ii) one oral dose of fluvastatin (Lescol®) 20mg capsule immediately following a 30-min intravenous infusion of rifampin 600mg in 10ml Normal Saline

The two study periods will ideally be on a Tuesday and Thursday, separated by one day of fluvastatin washout.The oral food intake will be standardized and subjects will be instructed to fast overnight the day before study period 1 and period 2, and for 3h post-dosing. In both treatments, venous blood samples (8ml each) will be drawn at 0, 0.33, 0.67, 1,1.5, 2, 2.5, 3, 4, 6, 9, 12h after fluvastatin dosing. During the 1 day of fluvastatin washout, patients will continue pretreatment with one 600mg dose of oral rifampin. Period 2 will begin after 1 day of fluvastatin wash out, when subjects will receive the second treatment, followed by blood draws at the same time points after fluvastatin dosing. If study period two does not follow one day after study period one, it will most likely be conducted on Tuesday or

Thursday of the following week. In this case, the subjects would be maintained in a state of induction of hepatic enzymes by the same preinduction regimen with 600 mg of oral rifampin for 5 days before study period 2.

	Period 1	Period 2
Group 1	A	B
Group 2	B	A

A= fluvastatin; B =rifampin +fluvastatin

5. CRITERIA FOR EVALUATION

5.1 Primary Efficacy Endpoint

Primary Outcome: The primary outcome will be fluvastatin $AUC_{(0\text{-}12h)}$ and $AUC_{(0\text{-}\infty)}$.

5.2 Secondary Efficacy Endpoints

Secondary outcomes will include fluvastatin maximum plasma concentration (C_{max}) and time to C_{max} (T_{max})

5.3 Safety Evaluations

Subjects will be monitored on an ongoing basis while involved with the study by study personnel. Subjects will be screened with a medical history and physical examination by the study physician and have screening hematology, chemistries, liver, renal and coagulation laboratories. Subjects must have normal function of all major organs. At study completion, subjects will have repeat physical examination and repeat hematology, chemistries, liver, renal and coagulation laboratories for safety monitoring. The safety evaluations include change in clinical laboratory finding(Creatinine, phosphokinase) and also the Incidence of adverse events

6. SUBJECT SELECTION

6.1 Study Population

Subjects who meet the inclusion and exclusion criteria will be eligible for participation in this study.

6.2 Inclusion Criteria

1. Healthy male or female, ages 18-65 years old, with no current medical conditions or active diagnoses as determined by the study doctor based on history, physical exam, and laboratory evaluations.
2. Subjects who take no other medications two weeks prior to the study and during the time course of the study including prescription medications, over-the-counter medications, dietary supplements, or drugs of abuse.
3. Subjects able to maintain adequate birth control during the study independent of hormonal contraceptive use (use of condoms).
4. Subjects able to abstain from grapefruit, grapefruit juice, oranges, orange juice, caffeinated beverages and/or alcoholic beverages from 7am the day before the study to completion of that study day.
5. Participants determined to have normal liver and kidney function as measured at baseline (ALT: ≤ 2x upper level of normal (ULN), AST: ≤ 2x ULN, SCr: ≤ 1.5x ULN, T. Bili: 0.1-1.2mg/dL, Albumin: 3.4 – 4.7 mg/dL).
6. BMI between 18.0 - 30 kg/m2 o Subjects capable of fasting from food and beverages at least 8 hours prior to medication dosing.
7. Be able to read, speak, and understand English.
8. Subjects capable of providing informed consent and completing the requirements of the study.

6.3 Exclusion Criteria

1. Subjects with active medical problems
2. Subjects on chronic prescription or OTC medication that cannot be stopped 2 weeks prior to and during the study.
3. Subjects incapable of multiple blood draws (HCT < 30mg/dL)
4. Subjects with a history of rhabdomyolysis
5. Subjects with a history of drug-related myalgias
6. Subjects with a history or diagnosis of hemorrhagic tendencies or blood dyscrasias
7. Subjects with a history of GI bleed or peptic ulcer disease
8. Subjects who smoke tobacco or have ongoing alcohol or illegal drug use
9. Subjects who are pregnant, lactating, or trying to conceive during the study period
10. Subjects allergic to fluvastatin or rifampin or any known component of the medications
11. Anyone who in the opinion of the study investigators is unable to do the study

7. CONCURRENT MEDICATIONS

All subjects should not take any other concurrent medication

7.1 Allowed Medications and Treatments

Subjects will take no other medications two weeks prior to the study and during the time course of the study including prescription medications, over-the-counter medications, dietary supplements, or drugs of abuse.

7.2 Prohibited Medications and Treatments

No other medications besides study medications are allowed.

8. STUDY TREATMENTS

8.1 Method of Assigning Subjects to Treatment Groups

Once included in the study, patients will be randomized using computer-generated random numbers, corresponding to each treatment. This will be performed by someone other than the investigator and will ensure equal number of patients in each treatment sequence.

8.2 Blinding

The study is not blinded

8.3 Formulation of Test and Control Products

Formulation of Test and Control Product

Fluvastatin sodium is a white to pale yellow, hygroscopic powder soluble in water, ethanol and methanol. LESCOL is supplied as capsules containing fluvastatin sodium, equivalent to 20 mg or 40 mg of fluvastatin.

Trade Drug Name	Lescol
Generic Drug name	Fluvastatin sodium (capsules)
Investigational Drug Name	
Identify the name of the manufacturer or source of investigational drug/biologic:	Novartis Pharmaceuticals Corporation
Active ingredient	fluvastatin sodium
Inactive ingredient	calcium carbonate, gelatin,

	magnesium stearate, microcrystalline cellulose, pregelatinized starch (corn), red iron oxide, sodium bicarbonate, talc, titanium dioxide, yellow iron oxide, and other ingredients.
PH	4.5

Rifampin is used only to treat or prevent infections that are proven or strongly suspected to be caused by bacteria. Rifampin is a red-brown crystalline powder very slightly soluble in water at neutral pH, freely soluble in chloroform, soluble in ethyl acetate and in methanol.

Trade Drug Name:	RIFADIN IV
Generic Drug Name:	RIFAMPIN
Identify the name of the manufacturer or source of investigational drug/biologic:	Sanofi-Aventis
Active Ingredient	rifampin 600 mg
Inactivate Ingredient	sodium formaldehyde sulfoxylate 10 mg, and sodium hydroxide to adjust pH
PH	7.8–8.8

Trade Drug Name:	RIFADIN capsules
Generic Drug Name:	RIFAMPIN
Identify the name of the manufacturer or source of investigational drug/biologic:	Sanofi-Aventis
Active Ingredient	rifampin 300 mg
Inactivate Ingredient	corn starch, D&C Red No. 28, FD&C Blue No. 1, FD&C Red No. 40, gelatin, magnesium stearate, and

	titanium dioxide.
PH	7.8–8.8

8.4 Supply of Study Drug at the Site

The Sponsor (or designee) will supplies Study Drug to the investigational sites through hospital pharmacy.

Dosage/Dosage Regimen

- fluvastatin 20 mg by mouth as a 20 mg Lescol® capsule
- 30-min intravenous infusion of rifampin (Rifadin®, Sanofi-Aventis, Bridgewater, NJ) 600 mg in 10 ml sterile normal saline
- rifampin 600 mg oral by mouth as two 300mg rifadin capsules for 5 days in the morning

Dispensing

The investigator has authority to dispense the drug

8.41 Administration Instructions

Patient will take two 300 mg rifampin capsules by mouth for the 5 days prior to the study period 1 and another two 300 mg rifampin capsules during fluvastatin washout day (between study periods 1 and 2). During study periods 1 and 2, subjects will take fluvastatin 20 mg by mouth as a 20mg lescol capsule. Subjects will also receive 30-min intravenous infusion of rifampin (Rifadin®, Sanofi-Aventis, Bridgewater, NJ) 600 mg in 10 ml sterile normal saline at a rate of 20 mg/min, administered immediately prior to oral fluvastatin dosing on one of the study periods. All subjects will be asked to abstain from caffeinated beverages, alcoholic beverages, all fruit juices, oranges, and grapefruits from 7am the day before each study period until the study is complete

8.5 Supply of Study Drug at the Site

The investigator will supply study drug to the investigational sites.

Storage

Study drugs should be stored by the study site at controlled room temperature, 15 to 30ºC (59 to 86ºF). If the temperature of study drug storage in the clinic/pharmacy exceeds or falls below this range, this should be reported to the Sponsor or designee and captured as a deviation. It is important store the medication in original packaging (foil pouch and protected from light) at room temperature.

8.5 Study Drug Accountability

An accurate and current accounting of the dispensing and return of study drug for each subject will be maintained on an ongoing basis by a member of the study site

staff. The number of study drugs dispensed and returned by the subject will be recorded on the Investigational Drug Accountability Record. The study monitor will verify these documents throughout the course of the study.

8.6 Measures of Treatment Compliance

Subject's daily rifampin dose will be verified by online video chat every morning during the 5 days prior to study, and on the one day washout period.

During study periods 1 and 2, the investigator will monitor the subjects when they take fluvastatin and when they undergo the 30-min intravenous infusion of rifampin (Rifadin®, Sanofi-Aventis, Bridgewater, NJ) 600 mg in 10 ml sterile normal saline at a rate of 20 mg/min.

9. STUDY PROCEDURES AND GUIDELINES

A Schedule of Events representing the required testing procedures to be performed for the duration of the study is diagrammed in Appendix 1.

Prior to conducting any study-related activities, written informed consent and the Health Insurance Portability and Accountability Act (HIPAA) authorization must be signed and dated by the subject. If appropriate, consent must also be obtained prior to conducting any study-related activities.

9.1 Clinical Assessments:

Concomitant Medications

Only subjects who take no other medications two weeks prior to the study and during the time course of the study including prescription medications, over-the-counter medications, dietary supplements, or drugs of abuse will be allowed to participate in the trial.

Demographics

A descriptive analysis of the demographic characteristics of the study subjects will be performed. Demographic characteristics of interest are age, race, height, weight and gender will be recorded at screening.

Medical History

Relevant medical history, including history of current disease, other pertinent respiratory history, and information regarding underlying diseases will be recorded at screening

Physical Examination

A complete physical examination will be performed by either the investigator who is a physician at screening visit. Qualified staff (MD) may complete the

abbreviated physical exam at all other visits. New abnormal physical exam findings must be documented and will be followed by a physician or other qualified staff at the next scheduled visit.

Vital Signs
Body temperature, blood pressure, pulse and respirations will be performed after resting for 5 minutes at the screening.

Adverse Events
Information regarding occurrence of adverse events will be captured throughout the study. Duration (start and stop dates and times), severity/grade, outcome, treatment and relation to study drug will be recorded on the case report form (CRF).

9.2 Clinical Laboratory Measurements

Hematology
Blood will be obtained and sent to each site's clinical hematology lab for a complete blood count (hemoglobin, hematocrit, red blood cell count, white blood cell count, white blood cell differential, and platelet count), erythrocyte sedimentation rate (ESR), and serum C-reactive protein (CRP) determinations for assessment of systemic evidence for infection and/or inflammation.

Blood Chemistry Profile
Blood will be obtained and sent to site's clinical chemistry lab forHgb, HCT, RBC, Plt, WBC with differential, Ca++, Na+, K+, Cl-, CO2, glucose, serum creatinine, BUN, phosphorus, AST,ALT, alkaline phosphatase, CPK, total bilirubin, albumin, total protein, cholesterol, and triglycerides.

Pregnancy Test
A urine or serum pregnancy test will be obtained from female subjects who are of childbearing age prior to their participation in the study.

Urinalysis
Urine will be obtained and sent to each site's clinical laboratory for determination of (list as applicable) color, specific gravity, pH, protein, glucose, ketones, and blood.

9.3 Pharmacokinetic Measurements
Blood for determination of serum concentrations of fluvastatin will be collected 0, 0.33, 0.67, 1,1.5, 2, 2.5, 3,4, 6, 9, and 12h after fluvastatin dosing.Plasma will be separated and stored at –80°C until analysis of drug levels.

Plasma concentrations of fluvastatin and rifampin in all collected plasma samples will be measured via a previously validated tandem liquid chromatography mass spectrometry (LC-MS/MS) method. Determined plasma concentrations of fluvastatin will then be modeled using pharmacokinetic modeling software to calculate the corresponding AUC, oral clearance, and oral volume of distribution of fluvastatin in the treatment groups.

9.4 Research Laboratory Measurements

Cell Count and Differential blood sample (30mL) for determination of Hgb, HCT, RBC, Plt, WBC with differential will be collected during the screening visit.

10 EVALUATIONS BY VISIT

10.1 Screening Visit (at least one week prior to study period 1)

1. Written informed consent will be obtained.
2. A medical history and a physical exam including vital signs (oral temperature, heart rate, blood pressure, and respiratory rate), height, and weight will be performed. Subjects will also be asked about prescriptions, dietary supplements, and illegal drug use.
3. Blood (30 ml) and urine tests (Hgb, HCT, RBC, Plt, WBC with differential, Ca++, Na+, K+, Cl-, CO2, glucose, serum creatinine, BUN, phosphorus, AST,ALT, alkaline phosphatase, CPK, total bilirubin, albumin, total protein, cholesterol, triglycerides, urinalysis.
4. For female subjects, a urine pregnancy test will be obtained.

10.2 Study period 1 (Tuesday of the Week):

1. For female subjects, a urine pregnancy test will be performed.
2. For the purpose of administering rifampin and drawing blood samples, two indwelling peripheral venous catheters will be inserted aseptically into the forearm vein by a nurse in the CRC immediately prior to medication administration. The rifampin catheter will be removed immediately after rifampin administration, the second catheter will be removed after the final blood draw. If the catheter malfunctions and needs to be removed prior to the last blood draw, a subject may choose to have remaining blood draws via peripheral venous needle sticks or have a new catheter placed.
3. **Dose study medications**- Subjects will receive the pre-treatment dose two 300mg Rifampin Capsules as 300mg Rifadin® caspsules for the 5 days prior to the study period 1. Subjects will receive one of the following dose of medications:

   a. Dose fluvastatin 20 mg by mouth as a 20 mg Lescol® capsule
   b. Dose rifampin 600mg IV infusion over 30 minutes (t = -30 minutes) followed by a dose fluvastatin 20 mg by mouth as a 20 mg Lescol® capsule (t = 0 minutes)

   The order in which subjects receive the above treatments will be randomized via random number generator.

4. **Serial blood sampling**- Venous blood samples (8 mL) will be drawn at 0, 0.33, 0.67, 1,1.5, 2, 2.5, 3,4, 6, 9, and 12h after fluvastatin dosing.Plasma will be separated and stored at –80°C until analysis of drug levels.

10.3 Visit 3 (Thursday of the week)

1. Admit to Clinical Research Center (CRC) at UCSF -

During each study period, subjects will be admitted to the Clinical Research Center (CRC) at UCSF (12th Floor Moffitt Hospital). Subjects will be discharged after the 12 hour blood sample.

2. **Urine Pregnancy Test -** For female subjects, a urine pregnancy test will be performed.

3. **Catheter placement-** For the purpose of administering rifampin and drawing blood samples, two indwelling peripheral venous catheter will be inserted aseptically into the forearm vein by a nurse in the CRC immediately prior to medication administration. The rifampin catheter will be removed immediately after rifampin administration, the second catheter will be removed after the final blood draw. If the catheter malfunctions and needs to be removed prior to the last blood draw, a subject may choose to have remaining blood draws via peripheral venous needle sticks or have a new catheter placed.

4. **Dose study medications**- Subjects will receive the pre-treatment dose two 300mg Rifampin Capsules as 300mg Rifadin® caspsules during one day washout of fluvastatin. Subjects will receive one of the following dose of medications:
a. Dose fluvastatin 20 mg by mouth as a 20 mg Lescol® capsule
b. Dose rifampin 600mg IV infusion over 30 minutes (t = -30 minutes) followed by a dose fluvastatin 20 mg by mouth as a 20 mg Lescol® capsule (t = 0 minutes)
The order in which subjects receive the above treatments will be randomized via random number generators.

5. Serial blood sampling- Venous blood samples (8 mL) will be drawn at 0, 0.33, 0.67, 1, 1.5, 2, 2.5, 3, 4, 6, 9 and 12h after fluvastatin dosing.Plasma will be separated and stored at –80°C until analysis of drug levels.

6. Safety Monitoring- At study completion, subjects will have repeat physical examination and repeat hematology, chemistries, liver, renal and coagulation laboratories for safety monitoring.

11. ADVERSE EXPERIENCE REPORTING AND DOCUMENTATION

11.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a clinical investigation of a patient administered a pharmaceutical product and that does not necessarily have a causal relationship with the treatment. An AE is therefore any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the administration of an investigational product, whether or not related to that investigational product. An unexpected AE is one of a type not identified in nature, severity, or frequency in the current Investigator's Brochure or of greater severity or frequency than expected based on the information in the Investigator's Brochure.

The Investigator will probe, via discussion with the subject, for the occurrence of AEs during each subject visit and record the information in the site's source documents. Adverse events will be recorded in the patient CRF. Adverse events will be described by duration (start and stop dates and times), severity, outcome, treatment and relation to study drug, or if unrelated, the cause.

AE Severity

The National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0 should be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant. The modified criteria can be found in the study manual. If the experience is not covered in the modified criteria, the guidelines shown in Table 1 below should be used to grade severity. It should be pointed out that the term "severe" is a measure of intensity and that a severe AE is not necessarily serious.

Table 1. AE Severity Grading

Severity (Toxicity Grade)	Description
Mild (1)	Transient or mild discomfort; no limitation in activity; no medical intervention or therapy required. The subject may be aware of the sign or symptom but tolerates it reasonably well.
Moderate (2)	Mild to moderate limitation in activity, no or minimal medical intervention/therapy required.
Severe (3)	Marked limitation in activity, medical intervention/therapy required, hospitalizations possible.
Life-threatening (4)	The subject is at risk of death due to the adverse experience as it occurred. This does not refer to an experience that hypothetically might have caused death if it were more severe.

AE Relationship to Study Drug

The relationship of an AE to the study drug should be assessed using the following the guidelines in Table 2.

Table 2. AE Relationship to Study Drug

Relationship to Drug	Comment
Definitely	Previously known toxicity of agent; or an event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is not explained by any other reasonable hypothesis.
Probably	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is unlikely to be explained by the known characteristics of the subject's clinical state or by other interventions.
Possibly	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to that suspected drug; but that could readily have been produced by a number of other factors.
Unrelated	An event that can be determined with certainty to have no relationship to the study drug.

11.2 Serious Adverse Experiences (SAE)

An SAE is defined as any AE occurring at any dose that results in any of the following outcomes:

- death
- a life-threatening adverse experience
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly/birth defect
- Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the subject or require intervention to prevent one of the outcomes listed.

Serious Adverse Experience Reporting

Study sites will document all SAEs that occur (whether or not related to study drug) per UCSF CHR Guidelines. The collection period for all SAEs will begin after

informed consent is obtained and end after procedures for the final study visit have been completed.

In accordance with the standard operating procedures and policies of the local Institutional Review Board (IRB)/Independent Ethics Committee (IEC), the site investigator will report SAEs to the IRB/IEC.

12. DISCONTINUATION AND REPLACEMENT OF SUBJECTS

12.1 Early Discontinuation of Study Drug

A subject may be discontinued from study treatment at any time if the subject, the investigator, or the Sponsor feels that it is not in the subject's best interest to continue. The following is a list of possible reasons for study treatment discontinuation:

- Subject withdrawal of consent (or assent)
- Subject is not compliant with study procedures
- Adverse event that in the opinion of the investigator would be in the best interest of the subject to discontinue study treatment
- Protocol violation requiring discontinuation of study treatment
- Lost to follow-up
- Sponsor request for early termination of study
- Positive pregnancy test (females)

If a subject is withdrawn from treatment due to an adverse event, the subject will be followed and treated by the Investigator until the abnormal parameter or symptom has resolved or stabilized.

All subjects who discontinue study treatment should come in for an early discontinuation visit as soon as possible and then should be encouraged to complete all remaining scheduled visits and procedures.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents Refer to Section 10 for early termination procedures.

12.2 Withdrawal of Subjects from the Study

A subject may be withdrawn from the study at any time if the subject, the investigator, or the Sponsor feels that it is not in the subject's best interest to continue.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents. Subjects who withdraw after study period 1 but prior to study period 2 should be encouraged to come in for a final visit (and the procedures to be followed would include those for their next scheduled visit).

12.3 Replacement of Subjects

Subjects who withdraw from the study treatment will be replaced.

Subjects who withdraw from the study will be replaced.

13. PROTOCOL VIOLATIONS

A protocol violation occurs when the subject, investigator, or sponsor fails to adhere to significant protocol requirements affecting the inclusion, exclusion, subject safety and primary endpoint criteria. Protocol violations for this study include, but are not limited to, the following:
Failure to meet inclusion/exclusion criteria
Use of a concomitant medication
Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation. The sponsor will determine if a protocol violation will result in withdrawal of a subject.
When a protocol violation occurs, it will be discussed with the investigator and a Protocol Violation Form detailing the violation will be generated. This form will be signed by a Sponsor representative and the Investigator. A copy of the form will be filed in the site's regulatory binder and in the Sponsor's files.

14. DATA SAFETY MONITORING

The study does not required a Data and Safety Monitoring Board

15. STATISTICAL METHODS AND CONSIDERATIONS

Prior to the analysis of the final study data, a detailed Statistical Analysis Plan (SAP) will be written describing all analyses that will be performed. The SAP will contain any modifications to the analysis plan described below.

15.1 Data Sets Analyzed

All eligible patients who are randomized into the study will be included in the data analysis.

15.2 Demographic and Baseline Characteristics

A descriptive analysis of the demographic characteristics of the study subjects will be performed. Demographic characteristics of interest are age, race, height, weight and gender.

15.3 Analysis of Primary Endpoint

AUC0-∞ will be calculated using the trapezoidal rule from time 0 to Clast + Clast/ke. Log-transformed pharmacokinetic parameters will be compared across treatments with analysis of variance for repeated measures. Differences will be regarded as statistically significant if $p< 0.05$. Data will be presented as mean values with standard deviations. Each subject will serve as his/her own control. Differences in pharmacokinetic parameters between treatments will be calculated using student's paired t-test. Statistical calculations will be done using STATA 10.0 (StataCorporation, College Station, TX).

15.4 Analysis of Secondary Endpoints

Parameters such as maximum plasma concentration (Cmax) and time to Cmax (Tmax) will be determined from the observed data. The terminal elimination rate constant (ke) will be obtained using linear regression of log transformed concentrations over the terminal log-linear decline phase. Oral clearance (CL/F) will be calculated using Dose/AUC0-∞. Other estimated pharmacokinetic parameters will be calculated using non-compartmental methods with WinNonlin Professional software (Pharsight, Inc., Mountain View, CA).

15.5 Interim analysis

There is no scheduled interim analysis, as the trial is short in duration, but serious adverse events will be monitored by the committee on an ongoing basis throughout the study.

15.6 Sample Size and Randomization

Using a paired t-test and prior data, a sample size of n=10 in each group is needed to detect a 50% difference in AUC0-12 between the two study regimens with a statistical power of 80% (two sided alpha = 0.05) and standard deviation of 50%. Due to the lesser effects of rifampin on fluvastatin AUC, the within subject variability can affect the sample size. We plan to enroll sample size of 12 subjects to maintain an adequate sample size in case of potential attrition.

Once included in the study, patients will be randomized using computer-generated random numbers, corresponding to each treatment. This will be performed by someone other than the investigator and will ensure equal number of patients in each treatment sequence.

16. DATA COLLECTION, RETENTION AND MONITORING

16.1 Data Collection Instruments

The Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each subject treated with the study drug. The investigator will enter data from source documents corresponding to a subject's visit into the protocol-specific electronic Case Report Form (eCRF) when the information corresponding to that visit is available.

The Investigator is responsible for all information collected on subjects enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the Investigator. A copy of the CRF will remain at the Investigator's site at the completion of the study.

16.2 Data Management Procedures

The data will be entered into a validated database. The Data Management group will be responsible for data processing, in accordance with procedural documentation. Database lock will occur once quality assurance procedures have been completed.

All procedures for the handling and analysis of data will be conducted using good computing practices meeting FDA guidelines for the handling and analysis of data for clinical trials.

16.3 Data Quality Control and Reporting

After data have been entered into the study database, a system of computerized data validation checks will be implemented and applied to the database on a regular basis. The study database will be updated in accordance with the resolved queries. All changes to the study database will be documented.

16.4 Archival of Data

The database is safeguarded against unauthorized access by established security procedures; appropriate backup copies of the database and related software files will be maintained. Databases are backed up by the database administrator in conjunction with any updates or changes to the database.

At critical junctures of the protocol (e.g., production of interim reports and final reports), data for analysis is locked and cleaned per established procedures.

16.5 Availability and Retention of Investigational Records

The Investigator must make study data accessible to the monitor, other authorized representatives of the Sponsor (or designee), IRB/IEC, and Regulatory Agency (e.g., FDA) inspectors upon request. A file for each subject must be maintained that includes the signed Informed Consent, HIPAA Authorization and Assent Form and copies of all source documentation related to that subject. The Investigator

must ensure the reliability and availability of source documents from which the information on the CRF was derived.

All study documents (patient files, signed informed consent forms, copies of CRFs, Study File Notebook, etc.) must be kept secured for a period of two years following marketing of the investigational product or for two years after centers have been notified that the IND has been discontinued. There may be other circumstances for which the Sponsor is required to maintain study records and, therefore, the Sponsor should be contacted prior to removing study records for any reason.

16.6 Monitoring

Monitoring visits will be conducted by representatives of the Sponsor according to the U.S. CFR Title 21 Parts 50, 56, and 312 and ICH Guidelines for GCP (E6). By signing this protocol, the Investigator grants permission to the Sponsor (or designee), and appropriate regulatory authorities to conduct on-site monitoring and/or auditing of all appropriate study documentation.

16.7 Subject Confidentiality

Only the investigators listed on this application and research personnel directly involved in the study will have access to personal information. All personal and medical data will be considered confidential. All subject information will be kept confidential as possible under the law.

17. ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a coded number and initials only. All study records will be kept in a locked file cabinet and code sheets linking a patient's name to a patient identification number will be stored separately in another locked file cabinet. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the FDA. The Investigator must also comply with all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC).The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).

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comply with all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC).

17.1 Protocol Amendments

Any amendment to the protocol will be written by investigator. Protocol amendments cannot be implemented without prior written IRB/IEC approval except as necessary to eliminate immediate safety hazards to patients. A protocol amendment intended to eliminate an apparent immediate hazard to patients may be implemented immediately, provided the IRBs are notified within five working days.

17.2 Institutional Review Boards and Independent Ethics Committees

The protocol and consent form will be reviewed and approved by the IRB/IEC of each participating center prior to study initiation. Serious adverse experiences regardless of causality will be reported to the IRB/IEC in accordance with the standard operating procedures and policies of the IRB/IEC, and the Investigator will keep the IRB/IEC informed as to the progress of the study. The Investigator will obtain assurance of IRB/IEC compliance with regulations.

Any documents that the IRB/IEC may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB/IEC. The IRB/IECs written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. The IRB/IECs unconditional approval statement will be transmitted by the Investigator prior to the shipment of study supplies to the site. This approval must refer to the study by exact protocol title and number and should identify the documents reviewed and the date of review.

Protocol and/or informed consent modifications or changes may not be initiated without prior written IRB/IEC approval except when necessary to eliminate immediate hazards to the patients or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB/IEC and written verification that the modification was submitted and subsequently approved should be obtained.

The IRB/IEC must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

17.3 Informed Consent Form

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, US Code of Federal Regulations for Protection of Human Subjects (21

CFR 50.25[a,b], CFR 50.27, and CFR Part 56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA, if applicable), and local regulations.

The Investigator will prepare the informed consent form, assent and HIPAA authorization and provide the documents to the Sponsor or designee for approval prior to submission to the IRB/IEC. The consent form generated by the Investigator must be acceptable to the Sponsor and be approved by the IRB/IEC. The written consent document will embody the elements of informed consent as described in the International Conference on Harmonisation and will also comply with local regulations. The Investigator will send an IRB/IEC-approved copy of the Informed Consent Form to the Sponsor (or designee) for the study file.

A properly executed, written, informed consent will be obtained from each subject prior to entering the subject into the trial. Information should be given in both oral and written form and subjects (or their legal representatives) must be given ample opportunity to inquire about details of the study. If appropriate and required by the local IRB/IEC, assent from the subject will also be obtained. If a subject is unable to sign the informed consent form (ICF) and the HIPAA authorization, a legal representative may sign for the subject. A copy of the signed consent form (and assent) will be given to the subject or legal representative of the subject and the original will be maintained with the subject's records.

17.4 Publications

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the study Sponsor and participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

17.5 Investigator Responsibilities

By signing the Agreement of Investigator form, the Investigator agrees to:

1. Conduct the study in accordance with the protocol and only make changes after notifying the Sponsor (or designee), except when to protect the safety, rights or welfare of subjects.
2. Personally conduct or supervise the study (or investigation).
3. Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.
4. Report to the Sponsor or designee any AEs that occur in the course of the study, in accordance with §21 CFR 312.64.
5. Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.

6. Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection with the Sponsor (or designee).
7. Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the clinical study.
8. Promptly report to the IRB and the Sponsor (or designee) all changes in the research activity and all unanticipated problems involving risks to subjects or others (to include amendments and IND safety reports).
9. Seek IRB approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/subjects.
10. Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

APPENDIX 1. SCHEDULE OF STUDY VISITS

	SCREENING VISIT	STUDY PERIOD 1	STUDY PERIOD
Informed Consent	**X**		
Medical History	**X**		
Complete Physical Exam	**X**		
Abbreviated Physical Exam		**X**	**X**
Height	**X**	**X**	**X**
Weight	**X**	**X**	**X**
Vital Signs	**X**	**X**	**X**
Pharmacokinetics		**X**	
Chemistry	**X**		
Pregnancy Test (Urine or Serum)	**X**		
Hematology	**X**		
ESR	**X**		
C-Reactive Protein	**X**		
Urinalysis	**X**		
Randomization	**X**		
Dispensing or Administration of Study Drug	**X**	**X**	**X**
Counting of Returned Study Drug		**X**	**X**
Initiate Subject Diary	**X**		
Concomitant Medication Review	**X**	**X**	**X**
Adverse Experiences			

[a] ±2 days

APPENDIX 2. DIAGRAM OF STUDY PLAN