AstraZeneca

Amended Clinical Pharmacology Study Protocol

Drug Substance	Rosuvastatin Calcium
Study Code	D3560C00059
Edition No.	1
Date	

A Phase I, Open Label, Parallel Group, Single and Multiple Dose Study in Taiwanese Subjects Identified as CYP2C19 Poor Metabolizers or Extensive Metabolizers Receiving 20 Milligrams of Rosuvastatin Calcium

Sponsor: AstraZeneca Taiwan, 21st Fl., 207, Tun-Hwa South Rd., Sec. 2, Taipei Metro

AstraZeneca Research and Development
Site Representative

The following Amendment(s) and Administrative Changes have been made to this protocol since the date of preparation:

Amendment No.	Date of Amendment
1	
Administrative Change No.	**Date of Administrative Change**

ASTRAZENECA PROCEDURES IN CASE OF EMERGENCY, OVERDOSE OR PREGNANCY

In the case of a medical emergency you may contact the Study Delivery Team Leader. If the Study Delivery Team Leader is not available, contact the Study Delivery Team Physician at the AstraZeneca Research and Development site shown below.

Role in the study	**Name**	**Address and Telephone number**
Study Delivery Team Leader		
Study Delivery Team Physician		
Product Physician		

For further clarifications regarding:

- Procedures in case of medical emergency see Section 8.2
- Procedures in case of overdose see Section 8.3.
- Procedures in case of pregnancy see Section 8.4

PROTOCOL SYNOPSIS

A Phase I, Open Label, Parallel Group, Single and Multiple Dose Study in Taiwanese Subjects Identified as CYP2C19 Poor Metabolizers or Extensive Metabolizers Receiving 20 Milligrams of Rosuvastatin Calcium

Investigator

Study center, type and number of subjects planned

This will be a single center study conducted in Taipei, Taiwan.

Approximately 25 healthy Taiwanese subjects that have been genotyped as poor metabolizers (PMs) of CYP2C19 and 25 healthy Taiwanese subjects that have been genotyped as extensive metabolizers (EMs) of CYP2C19 will be recruited to obtain at least 40 evaluable subjects (20 for each group).

An evaluable subject is defined as a subject satisfying the inclusion and exclusion criteria, completing all study procedures from the screening period to the final blood sampling for plasma levels of rosuvastatin, and had no major protocol deviation or violation.

Study period	**Phase of development**
Estimated date of first subject enrolled	I
Estimated date of last subject completed	

Objectives

Primary Objectives

The primary objectives of this study are to:

- Explore the exposure of rosuvastatin calcium in Taiwanese subjects who have been identified as CYP2C19 PMs to exposure in Taiwanese subjects who have been identified as CYP2C19 EMs by examining the pharmacokinetic profile of rosuvastatin and its metabolites after single and multiple dosing of 20 milligrams (mg) rosuvastatin calcium

- Measure the effect of rosuvastatin calcium on lipid parameters as compared to baseline after 2 weeks of daily dosing of 20 mg rosuvastatin calcium.

Secondary Objective

The secondary objective of this study is to assess safety and tolerability.

Study design

This is an open label, parallel group, single and multiple dose study.

Investigational product, dosage and mode of administration

Rosuvastatin calcium will be administered as a 20 mg oral dose.

Duration of treatment

Approximately 25 subjects identified as PMs of CYP2C19 and 25 subjects identified as EMs of CYP2C19 (to obtain 20 subjects each) will participate for approximately 53 days including a 35-day screening period, 6 inpatient days and 12 outpatient days.

Variables

- **Pharmacokinetic**

The primary variables to be assessed for rosuvastatin after a single dose are:

- C_{max} maximum plasma concentration
- AUC area under the plasma concentration-time curve from zero to infinity
- $AUC_{(0-t)}$ area under the plasma concentration-time curve from zero to time of last quantifiable concentration
- AUMC area under the first moment curve
- t_{max} time to maximum concentration
- λ_z terminal elimination rate constant
- $t_{1/2,\lambda z}$ half-life associated with the terminal slope (λ_z) of a semi logarithmic plasma concentration-time curve
- MRT mean residence time
- CL/F apparent oral clearance
- Vd/F apparent volume of distribution

The primary variables to be assessed for rosuvastatin after multiple dosing are:

- $C_{ss,max}$ maximum plasma concentration at steady state
- $C_{ss,min}$ minimum plasma concentration at steady state
- $C_{ss,av}$ average plasma concentration at steady state
- $t_{ss,max,}$ time to maximum concentration at steady state
- $t_{ss,1/2}$ the apparent terminal half-life at steady-state
- AUC_{ss} area under the concentration-time curve at steady state
- DF degree of fluctuation (at steady state)
- AR Accumulation ratio (at steady state)

The secondary variables to be assessed for rosuvastatin metabolites N-desmethyl rosuvastatin and rosuvastatin lactone after a single dose are:

- C_{max} maximum plasma concentration
- AUC area under the plasma concentration-time curve from zero to infinity
- $AUC_{(0-t)}$ area under the plasma concentration-time curve from zero to time of last quantifiable concentration
- t_{max} time to maximum concentration
- λ_z terminal elimination rate constant
- $t_{1/2,\lambda z}$ half-life associated with the terminal slope (λ_z) of a semi logarithmic plasma concentration-time curve

The secondary variables to be assessed for rosuvastatin metabolites N-desmethyl rosuvastatin and rosuvastatin lactone after multiple dosing are:

- $C_{ss,max}$ maximum plasma concentration at steady state
- $C_{ss,min}$ minimum plasma concentration at steady state
- $C_{ss,av}$ average plasma concentration at steady state
- $t_{ss,max}$ time to maximum concentration at steady state
- AUC_{ss} area under the concentration-time curve at steady state
- DF degree of fluctuation (at steady state)

- AR Accumulation ratio (at steady state)

Maximum concentration (C_{max}) and area under the concentration curve from zero to infinity (AUC) of rosuvastatin will be the primary variables for this study to summarize exposure in each subject. If AUC data cannot be determined in all subjects completing the study, area under the curve of plasma concentration against time from zero to last quantifiable concentration ($AUC_{(0-t)}$) will replace AUC as the primary variable.

- Pharmacodynamic

Blood samples (10 mL) will be collected for assessment of pharmacodynamic (lipid) parameters on the mornings of Days –1 and 18.

Pharmacodynamic variables will include the following:

- tChol total cholesterol
- LDL-C low-density lipoprotein cholesterol
- HDL-C high-density lipoprotein cholesterol
- TG triglycerides

- Safety

Safety and tolerability of rosuvastatin calcium will be evaluated by physical examination, clinical laboratory tests, vital signs, and collection of adverse events.

- Genetics

After signing informed consent, prospective subjects will have whole blood collected and genotyped to determine if they are PMs (CYP2C19 *2/*2, *2/*3, or *3/*3 alleles) or EMs (CYP2C19 *1/*1, *1/*2, or *1/*3 alleles) of CYP2C19.

To control for other possible genetic factors that may contribute to increased exposure of rosuvastatin, all prospective subjects coding for OATP-C 1B1 *5 and *15, and/or BCRP 421C>A will be excluded from the study. Additionally, prospective subjects must code for wild-type CYP2C9 (*1 homozygous or heterozygous). There will be no other genetic research completed on the blood samples collected.

- Statistical methods

Demographics and baseline endpoints will be analyzed using descriptive statistics (number [n], mean, standard deviation, median, minimum and maximum) for continuous variables and frequency counts and percentages for categorical variables.

Except for t_{max}, descriptive statistics will be used to summarize pharmacokinetic data for each treatment group (n, mean, standard deviation, minimum, median, maximum, geometric mean,

and coefficient of variation [CV] percentages); t_{max} will be summarized using n, minimum, median, and maximum.

Geometric means (including 95% confidence intervals [CIs]) and CV will be calculated and listed. In addition, the geometric mean ratios, (PMs vs. EMs) of AUC, $AUC_{(0-t)}$, $C_{max,}$ (for rosuvastatin, N-desmethyl rosuvastatin, and rosuvastatin lactone) and metabolite to parent ratios and their 90% CIs will also be calculated.

Half-lives for the two groups will be summarized using least squares means and the comparison between PMs and EMs will be by least squares mean difference and 90% CI.

To evaluate whether steady state has been reached, the trough values of individual subjects will be plotted against time.

Descriptive statistics will be presented for tChol, LDL-C, HDL-C, and TG, and their changes from baseline. Baseline is defined as the lipid profile measurements before dose administration.

This study is exploratory and not powered to obtain a formal statistical comparison between PMs and EMs of CYP2C19 and exposure to rosuvastatin calcium.

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

The following abbreviations and special terms are used in this study protocol.

Abbreviation or special term	Explanation
λz	Terminal elimination rate constant
Ad libatum	As desired
ADME	Absorption/Distribution/Metabolism/Excretion
AE	Adverse event
Assessment	An observation made on a variable involving a subjective judgment
ALT	Alanine aminotransferase
ApoB	Apolipoprotein B
AR	Accumulation ratio (at steady state)
AST	Aspartate aminotransferase
AUC	Area under the concentration curve from zero to infinity
$AUC_{(0-t)}$	Area under the curve of plasma concentration against time from zero to time of last quantifiable concentration
AUC_{ss}	Area under the concentration-time curve at steady state
AUMC	Area under the first movement curve
BMI	Body mass index
BCRP	Breast cancer resistance protein
BUN	Blood urea nitrogen
°C	Degrees Celsius
CI	Confidence interval
CK	Creatine kinase
CL/F	Apparent oral clearance
C_{last}	Last quantifiable plasma concentration after single dose or last administration
C_{max}	Maximum concentration
CRC	Clinical research center
CRF	Case report form
CSR	Clinical study report
$C_{ss,av}$	Average plasma concentration at steady state
$C_{ss,max}$	Maximum plasma concentration at steady state

Abbreviation or special term	Explanation
$C_{ss,min}$	Minimum plasma concentration at steady state
CV	Coefficient of variance
CYP2A6	Cytochrome P450, family 2, subfamily A, polypeptide 6
CYP2C9	Cytochrome P450, family 2, subfamily C, polypeptide 9
CYP2C19	Cytochrome P450, family 2, subfamily C, polypeptide 19
CYP2D6	Cytochrome P450, family 2, subfamily D, polypeptide 6
DF	Degree of fluctuation (at steady state)
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
EDTA	Ethylenediamine tetra-acetic acid
EM	Extensive metabolizer
G	Relative centrifugal force
GCP	Good Clinical Practice
gmean	Geometric mean
HbsAG	Hepatitis B surface antigen
HCG	Human chorionic gonadotrophin
HDL-C	High density lipoprotein cholesterol
HIV	Human immunodeficiency virus
HMG-CoA	3-hydroxy-3methlglutaryl coenzyme A
ICH	International Conference on Harmonization
Kg	kilogram
LDH	Lactic dehydrogenase
LDL	Low density lipoprotein
LDL-C	Low density lipoprotein cholesterol
LC/MS/MS	Liquid chromatography/mass spectrometry/mass spectrometry
MedDRA	Medical Dictionary for Drug Regulatory Activities
mg	Milligram
mL	Milliliter
MRT	Mean residence time
n	number

Abbreviation or special term	Explanation
OAE	Other Significant Adverse Event (i.e., adverse events of particular clinical importance, other than SAEs and those AEs leading to discontinuation of the subject from study treatment; see definition in Section 4.7.1.1)
OATP-C1B1	Organic anion transporter polypeptide solute carrier family, member 1B1
OTC	Over-the-counter
pCRF	Paper Case Report Form
PM	Poor metabolizer
PP	Per protocol
Parameter	A quantity (usually unknown) that characterizes the distribution of a variable in a population of subjects
SAE	Serious adverse event
SAP	Statistical analysis plan
τ	Dosing interval (hours)
$t_{1/2,\lambda z}$	half-life associated with the terminal slope (λ_z) of a semi logarithmic plasma concentration-time curve
tChol	Total cholesterol
TG	Triglycerides
t_{max}	Time to maximum concentration
$t_{ss,1/2}$	the apparent terminal half-life at steady-state
$t_{ss,max}$	Time to maximum concentration at steady state
ULN	Upper limit of normal
Variable	A characteristic or a property of a subject that may vary e.g., from time to time or between subjects
Vd/F	Apparent volume of distribution
VLDL	Very low density lipoproteins

1. INTRODUCTION

Rosuvastatin is a selective, potent and competitive inhibitor of HMG-CoA reductase, the rate-limiting enzyme that converts 3-hydroxy-3-methylglutaryl coenzyme A to mevalonate, a precursor of cholesterol. Triglycerides (TG) and cholesterol in the liver are incorporated, with apolipoprotein B (ApoB), into very-low-density lipo-proteins (VLDL) and released into the plasma for delivery to peripheral tissues. VLDL particles are TG-rich. Cholesterol-rich low-density-lipoprotein (LDL) is formed from VLDL and is cleared primarily through the high affinity LDL receptor in the liver.

Rosuvastatin produces its lipid-modifying effects in two ways; it increases the number of hepatic LDL receptors on the cell-surface, enhancing uptake and catabolism of LDL and it inhibits the hepatic synthesis of VLDL, thereby reducing the total number of VLDL and LDL particles.

Rosuvastatin undergoes first pass extraction in the liver, which is the primary site of cholesterol synthesis and low-density lipoprotein cholesterol (LDL-C) clearance.

Rosuvastatin is not extensively metabolized; approximately 10% of a radiolabeled dose is recovered as metabolite. The major metabolite is N-desmethyl rosuvastatin, which is formed principally by cytochrome P450 2C9.

In vitro and in vivo data indicate that rosuvastatin clearance is not dependent on metabolism by cytochromes P450 3A4 and 2C9 to a clinically significant extent. Rosuvastatin also does not interact to a clinically significant extent with the p-glycoprotein transporter.

1.1 Background

Previous studies have demonstrated that for the same dose of rosuvastatin, systemic exposure in Japanese subjects living in Japan is approximately two-fold higher than the exposure observed in Caucasian subjects living in Europe. The results of a study in subjects in Singapore found that rosuvastatin plasma exposure in Chinese, Malay, and Indian subjects was approximately 2-fold higher compared to a Caucasian control group living in Singapore. In a United States study comparing plasma exposure in 6 different Asian ethnic groups (Chinese, Filipino, Asian Indian, Korean, Vietnamese, and Japanese) living in the United States to a Caucasian control group, plasma exposure was consistently higher across the Asian ethnic groups, with the exception of the Asian Indian group which was intermediate.

1.2 Rationale

In Asians, approximately 20% of the population can be identified as poor metabolizers (PMs) of CYP2C19. Although CYP2C19 has not been identified as the primary metabolic pathway in the metabolism of rosuvastatin calcium, it has been established that it does play some role. Because it has been established that Asians inherently have a higher exposure to rosuvastatin, it is important to compare the exposure in Asians identified as PMs of CYP2C19 to those

identified as extensive metabolizers (EMs) to determine if there are further significant differences in exposure between these groups.

The pharmacodynamic effect of rosuvastatin calcium in Taiwanese subjects is included in this study as part of the continued licensing requirements of rosuvastatin calcium in Taiwan.

1.2.1 CYP2C19

In the P450 cytochrome enzyme family, CYP2D6, CYP2C9, CYP2C19 and CYP2A6 are functionally polymorphic. Together these isoenzymes represent approximately 40% of human P450 dependent drug metabolism, and therefore are significant due to the wide variations possible in mutated alleles. Currently there are 12 different alleles of the CYP2C19 gene that have been identified. Eleven of these alleles code for inactive versions of the enzyme. These mutated alleles are identified as CYP2C19 *2, *2B to *11. The nomenclature for the wild-type allele is CYP2C19*1. Individuals are considered EMs if at least one wild-type allele is present. Individuals are considered PMs if both alleles code for inactive enzymes. In Asians, the alleles CYP2C19*2 and *3 together account for the majority of defective alleles in PMs.

2. STUDY OBJECTIVES

2.1 Primary objectives

The primary objectives of this study are to:

- Explore the exposure of rosuvastatin calcium in Taiwanese subjects who have been identified as CYP2C19 PMs to exposure in Taiwanese subjects who have been identified as CYP2C19 EMs by examining the pharmacokinetic profile of rosuvastatin and its metabolites after single and multiple dosing of 20 milligrams (mg) rosuvastatin calcium

- Measure the effect of rosuvastatin calcium on lipid parameters of Taiwanese subjects after 2 weeks of daily dosing of 20 mg rosuvastatin calcium

2.2 Secondary objective(s)

The secondary objective of the study is to assess safety and tolerability

3. STUDY PLAN AND PROCEDURES

3.1 Overall study design

This Clinical Study Protocol has been subjected to a peer review according to AstraZeneca standard procedures.

This is an open label, parallel group, single and multiple dose study.

After signing informed consent, prospective subjects will have approximately 9 mL of whole blood collected for genotyping. Approximately 20-25 poor metabolizers of CYP2C19 (CYP2C19 *2/*2, *2/*3, or *3/*3 alleles) and 20-25 EMs of CYP2C19 (CYP2C19 *1/*1, *1/*2, or *1/*3 alleles) will be randomly selected to continue with the study.

To control for other possible genetic factors that may contribute to increased exposure of rosuvastatin, all prospective subjects coding for OATP-C 1B1 *5 and *15, and/or BCRP 421C>A will be excluded from the study. Additionally, prospective subjects must code for wild-type CYP2C9 (*1 homozygous or heterozygous). There will be no other genetic research completed on the blood samples collected.

The study period will start with a screening visit. Subjects will undergo a health examination including physical examination, ECG, vital signs, clinical laboratory tests, and urinalysis.

Subjects will be admitted to the clinical research center (CRC) on Day –1. Day –1 assessments will be completed and blood will be collected for a pre-treatment (baseline) lipid profile. After an overnight fast, subjects will receive a 20 mg rosuvastatin calcium tablet on Day 1. Blood samples will be collected to 72 hours post-dose to assess the pharmacokinetic profile. On the morning of Day 4, (after the 72 hour blood collection) the subjects will again be dosed with 20 mg of rosuvastatin. The subjects will then be discharged from the CRC with study drug and instructions to take one 20 mg tablet of rosuvastatin calcium each morning for the next 2 days (Days 5-6). On the mornings of Days 7, 8, and 9 the subjects will return to the CRC to have blood collected for trough levels of rosuvastatin prior to dosing. After blood collection and dosing on Day 9, the subjects will leave the CRC with study drug and instructions to take one 20 mg tablet of rosuvastatin calcium each morning for the next 7 days (Days 10-16). The subjects will return to the CRC in the afternoon of Day 16 for readmission to the CRC. On the morning of Day 17, subjects will receive the last dose of 20 mg rosuvastatin calcium and blood samples will be collected to 24 hours post dose to assess the pharmacokinetic profile at steady-state. On Day 18, final assessments, including a blood sample to assess post-treatment lipid profiles, will be completed and the subjects discharged from the study.

3.1.1 Stopping criteria for dose escalation – Not applicable

Figure 1 Study flow chart

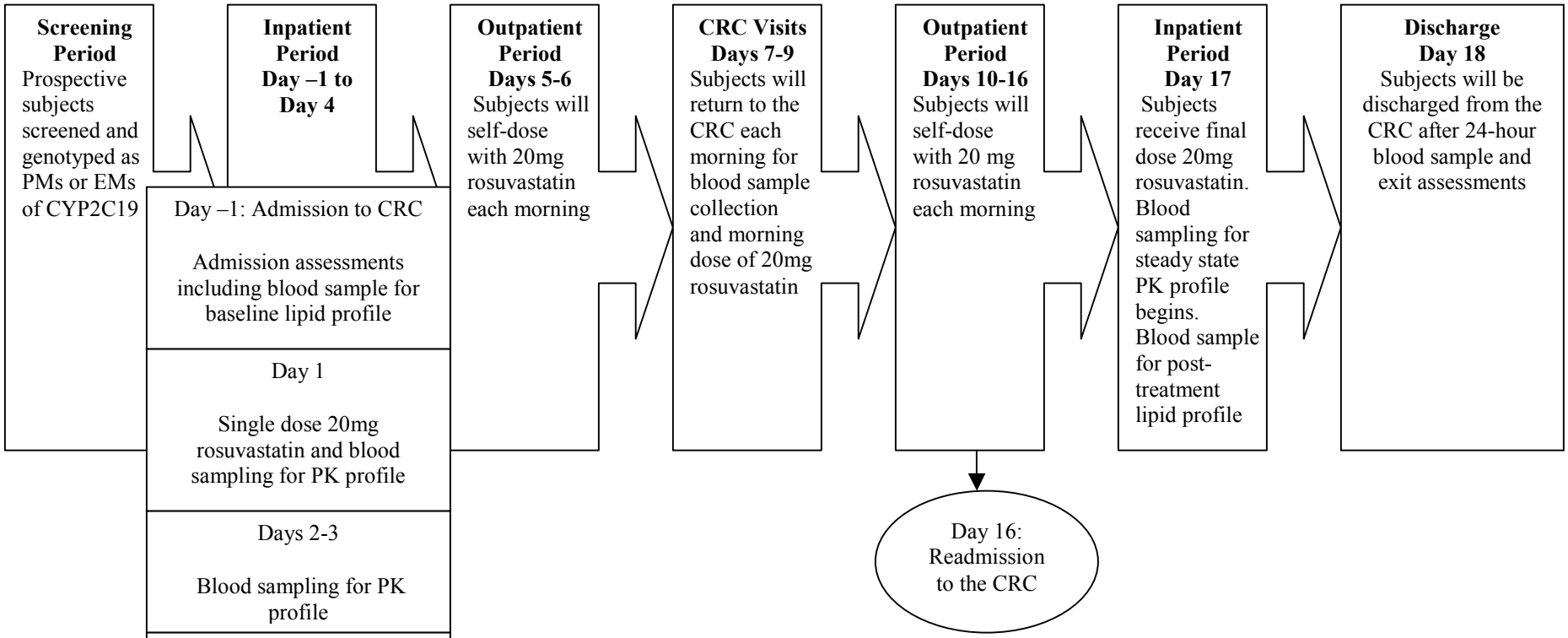

Table 1 Study plan

Study Procedures	Pre-Screening	Screening	Day –1	Days 1-4	Days 5-6	Day 7-9	Days 10-15	Day 16	Day 17	Day 18
Informed Consent	X									
Inclusion/Exclusion Criteria	X	X	X							
Blood sample for genotyping	X									
Demographic Data		X								
Med/Medication/Surg History		X								
Urine Drug Screen		X	X							
HIV/Hepatitis Screening		X								
Serum Pregnancy Test		X	X							X
Height and Weight		X								
Body Temperature		X	X							
12-lead ECG		X								X
Urinalysis		X	X							X
Clinical Chemistry/Hematology		X	X							X
Complete Physical Examination		X								X
Brief Physical Examination			X							
Lipid Profile			X							X
Blood Pressure and Pulse		X	X	X		X			X	X
Administration of 20 mg rosuvastatin calcium				X[a]	X[b]	X[c]	X[b]	X[b]	X[d]	
Blood sampling for PK assessment of rosuvastatin calcium				X[e]		X[f]			X[e]	X
Adverse Events		X[g]	X	X	X	X	X	X	X	X
Concomitant Medications		X	X	X	X	X	X	X	X	X
In-Patient			X	X				X	X	
Out-Patient		X			X	X	X			
Discharge from study										X

a. Rosuvastatin calcium administered on Day 1 and Day 4 only. Day 1: 20 mg rosuvastatin calcium administered by study site staff. Day 4: 20 mg rosuvastatin calcium administered by study staff after collection of 72-hour blood sample.

b. 20 mg rosuvastatin calcium self administered by subjects while out-patient
c. 20 mg rosuvastatin calcium administered by study site staff after collection of blood samples for trough levels
d. 20 mg rosuvastatin administered by study site staff
e. Serial blood sampling for rosuvastatin will be done at the following time points: Day 1 pre-dose (within 30 minutes), and 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 18, 24, 30, 36, 48, 54, 60, and 72 hours post-dose; Day 17 pre-dose (within 30 minutes), and 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 18, and 24 hours post-dose
f. Pre-dose blood samples collected for trough levels
g. Adverse events (AEs) will be collected beginning Day –1 through final assessment on Day 18 Serious adverse events (SAEs) will be collected from the time the informed consent is signed through final assessments on Day 18

3.2 Rationale and risk/benefit assessment

3.2.1 Rationale for study design, doses and control groups

The design of this study is standard for determining the pharmacokinetics of 20 mg rosuvastatin.

A 20 mg dose is sufficient to allow complete characterization of the rosuvastatin plasma concentration-time profile and it is the highest approved dose in Taiwan.

Two-weeks multiple dosing with 20 mg rosuvastatin is sufficient to show pharmacodynamic effect in lipid levels.

3.2.2 Risk/benefit and ethical assessment

Rosuvastatin calcium has been well tolerated in a broad spectrum of patients/subjects independent of gender, age, or race. There is no health benefit for healthy normal subjects, and the risk of participating is low.

3.3 Selection of study population

3.3.1 Study selection record

The Investigator must keep a record of subjects who were considered for enrollment but never enrolled (e.g., subject screening log), according to local procedures. This information is necessary to establish that the subject population was selected without bias.

3.3.2 Inclusion criteria

For inclusion in the study subjects must fulfill all of the following criteria:

1. Provision of signed written informed consent, ability to communicate with the investigator, and to understand and comply with the requirements of the study

2. Males and females aged 20-65, inclusive

3. Body Mass Index (BMI) between 18-29, inclusive, (BMI will be calculated as weight in kilogram (kg)/height in meters^2 [m^2])

4. Women who are surgically sterilized, post-menopausal for at least one year, or not pregnant and/or lactating. Women of childbearing potential must be willing to abstain from sexual activity or use an effective double barrier method of contraception during the study period (e.g., condom and diaphragm, condom and foam, condom and sponge, etc.), or intrauterine devices

5. Subject must have blood drawn for genotyping (determination of EM or PM of CYP2C19, determination of OATP-C1B1, BCRP 421C>A, and CYP2C9 as outlined in Section 3.1)

3.3.3 Exclusion criteria

Any of the following is regarded as a criterion for exclusion from the study:

1. Use of prescription medication for a chronic medical condition

2. Subjects with deoxyribonucleic acid (DNA) that codes for OATP-C 1B1 *5 and *15, BCRP 421C>A and/or non wild-type CYP2C9

3. Acute illness or use of prescription medication for an acute medical condition within 2 weeks of Day –1

4. Any contraindication determined by review of a detailed medical and drug history, complete physical examination, vital signs, blood chemistry, hematology, and electrocardiogram (ECG)

5. Medical history or psychological conditions which, in the opinion of the investigator, would compromise the subject's safety or successful participation in the study

6. History of adverse drug reaction or hypersensitivity to statins or drugs with a similar chemical structure to rosuvastatin

7. History or presence of gastrointestinal, hepatic, or renal disease or other conditions known to interfere with absorption, distribution, metabolism and excretion (ADME) of drugs

8. Positive test results for human immunodeficiency virus (HIV) antibody, hepatitis B surface antigen (HbsAG), or hepatitis C antibody

9. Positive urine drug screen

10. History of alcohol abuse

11. Involvement in the planning and conduct of the study (applies to both AstraZeneca staff or staff at the study site)

12. Participation in a clinical study during the last 30 days

3.3.4 Restrictions

Subjects will be required to:

1. Refrain from alcohol, grapefruit containing products, and apple juice 1 week prior to CRC admission through the final study evaluation

2. Refrain from using over-the-counter (OTC) medications, traditional Chinese medicine, and herbal supplements 2 weeks prior to CRC admission through the final study assessments

3. Refrain from strenuous exercise during the screening period and through final study assessments and discharge from the study

4. Refrain from eating or drinking starting 10 hours before study drug administration and continuing through 4 hours post study drug administration on Day –1 and Day 17.

Water will be allowed ad libatum until 2 hours prior to study drug administration and again 2 hours post study drug administration. Lunch will be served no sooner than 4 hours after the dose of rosuvastatin. At all other times during the in-patient periods, subjects will be served standard meals on a regular basis.

3.3.5 Discontinuation of subjects from treatment or assessment

3.3.5.1 Criteria for discontinuation

Subjects may be discontinued from study treatment and assessments at any time. Specific reasons for discontinuing a subject from this study are:

- Voluntary discontinuation by the subject, who is at any time free to discontinue his/her participation in the study without prejudice to further treatment

- Safety reasons as judged by the investigator and/or AstraZeneca

- Severe non-compliance to protocol as judged by the investigator and/or AstraZeneca.

- Incorrect enrollment i.e., the subject does not to meet the required inclusion/exclusion criteria for the study

- Subject lost to follow-up

3.3.5.2 Procedures for discontinuation

Subjects who discontinue should always be asked about the reason(s) for their discontinuation and the presence of any adverse events. If possible, they should be seen and assessed by an

investigator(s). Adverse events should be followed up and the subject should return any investigational products.

If a subject is being withdrawn due to a suspected infection in WHO risk categories 2, 3, and 4, (See Appendix C) no biological samples from this subject are allowed to be sent to the laboratory. Samples will be destroyed according to normal routines at the study site.

3.3.5.3 Procedures for handling incorrect enrolled subjects

Subjects not meeting the inclusion/exclusion criteria for this study should, under no circumstances, be enrolled - there can be no exceptions to this rule. If subjects not meeting the study criteria are enrolled in error, incorrectly randomized, or if subjects subsequently fail to meet the criteria for the study post enrollment, they shall be discharged from the study after proper exit assessments (physical exam, safety labs, etc.). These subjects will be included in the safety portion of the clinical study report, but will not be considered evaluable subjects for the pharmacokinetic or pharmacodynamic aspects of the study.

3.3.5.4 Procedures for discontinuation from genetic aspects of the study

Subjects will not be participating in optional genetic research. Blood samples will be collected for establishing eligibility status (OATP-C 1B1 *5 and *15, BCRP 421C> A , and wild type CYP2C9) and for metabolizer status (EMs and PMs). Subjects that discontinue from the study should be informed that the genetic sample will be stored for two years after study database lock.

3.4 Treatment(s)

3.4.1 Investigational product(s)

Rosuvastatin calcium 20 mg will be supplied as tablets for oral use.

Table 2 Identity of Investigational product

Investigational product	Dosage form and strength	Manufacturer	Formulation number
Rosuvastatin calcium	20 mg	AstraZeneca Pharmaceuticals, LP	F12673

3.4.1.1 Labeling

The investigational product will be supplied as bulk supply for use in all subjects. This medication will be labeled with the study code, quantity of material (including form and strength), the expiry date, batch number, opening instructions, and storage conditions.

Study site dispensary staff will dispense tablets as required to each subject and keep a dispensing record of the investigational product on a subject-by-subject basis. Individual

dosing records will contain at least the study number, subject number/enrollment code, and study day, description of the medication dispensed (including quantity, form, and strength) and the batch number. The drug product information can be found on the label.

3.4.1.2 Storage

All investigational products must be kept in a secure and locked place under appropriate storage conditions. The storage conditions for the drug can be found on the Clinical Study Label affixed to the packaging.

3.4.1.3 Accountability

The medication provided for this study is for use only as directed in the protocol. All unused drugs will be accounted for and destroyed appropriately by designated personnel. The study personnel will account for all drugs dispensed. Certificates of delivery, destruction and/or return must be signed and kept in the investigator file.

3.4.2 Doses and treatment regimens

On the mornings of Day 1 and Day 17, all subjects will receive a single 20 mg dose of rosuvastatin calcium under fasting conditions (See Section 3.3.4). Tablets will be taken orally with 240 milliliters (mL) of distilled, room temperature water. Tablets are not to be crushed or chewed. Subjects must remain in an upright position (sitting or standing) for 4 hours after dosing.

On the morning of Day 4 subjects will receive a single 20 mg dose of rosuvastatin calcium after the 72- hour blood sample collection.

During the outpatient periods (Days 5-6 and 10-16) subjects will self-administer one tablet of 20 mg rosuvastatin calcium each morning.

On Days 7-9 subjects will return to the CRC to receive the morning dose of rosuvastatin calcium following the collection of a blood sample to measure trough levels.

3.4.3 Method of assigning subjects to treatment groups

Written informed consent will be obtained before enrollment. Each subject will be assigned a unique enrollment number. Enrollment numbers will begin with E00001101 and will continue consecutively as prospective subjects are enrolled.

Subjects fulfilling the eligibility criteria and continuing in the study will be assigned unique subject numbers that will identify the site and the genotype of the subject. Subject numbers will begin with 1101 for subjects identified as EMs and will continue consecutively as subjects enter the study. Subject numbers will begin with 1201 for subjects identified as PMs and will continue consecutively as subjects enters the study

If a subject discontinues from the study, the subject number will not be re-used and the subject will not be allowed to re-enter the study.

3.4.4 Blinding and procedures for unblinding the study –Not applicable

3.4.5 Concomitant medication

During the screening period, subjects will be instructed to consult with the investigative staff prior to taking OTC and prescription medications, dietary supplements, traditional Chinese medicine, and herbal remedies. All medications, (OTC products, dietary supplements, traditional Chinese medicine, and herbal remedies) will be discontinued 2 weeks prior to entry into the CRC. Women with a history of previous oral contraceptive use must have discontinued the regimen at least 3 months prior to Day –1.

Subjects will not be permitted to take any medication, dietary supplements, traditional Chinese medicine, or herbal remedies during the in-patient and out patient periods. Acetaminophen preparations will be allowed for analgesia, if absolutely necessary, for the well being of the subject. If acetaminophen is necessary, administration is not to exceed 2 grams in any 12-hour period.

Any medication which is considered necessary for the subject's safety and well-being may be given at the discretion of the investigator(s). The administration of all medication (including investigational products) must be recorded in the appropriate sections of the case report form (CRF).

3.4.6 Treatment compliance

Compliance will be assured by the supervised administration of the study drug by site personnel, by query of the subject at return from outpatient periods and by drug accountability.

4. MEASUREMENT OF STUDY VARIABLES

The following study measurements will be obtained. The timing of these measurements is detailed in Table 1

4.1 Medical examination and demographic measurements

4.1.1 Enrollment medical examination and demographic measurements

Each subject will undergo an enrollment medical examination and screening in the 35 days prior to Day –1. This will consist of:

- Recording of demographic data
- A standard medical history and a physical examination including the cardiovascular and respiratory systems
- A blood sample for standard clinical chemistry and hematology assessments, a mid-stream urine sample for urinalysis, and drugs of abuse screen

- A blood sample for serology (HIV, HbsAg, Hepatitis C)
- A resting blood pressure and heart rate measurement.
- A serum pregnancy test (for women of child-bearing potential)
- An electrocardiogram (ECG)

4.1.1.1 Demographics

At the screening visit, demographic data will be collected including the following: date of birth, sex, and race.

4.1.1.2 Medical History and Complete Physical Exam

A complete medical and drug history will be recorded for each subject at the initial screening visit and reviewed for any additions or omissions on admission to the CRC (Day –1). Significant medical conditions that have occurred within the past 2 years, or conditions that are ongoing (i.e., headache, backache, indigestion) are to be recorded in the CRF. The drug history must identify any known drug allergies, presence or history of drug abuse, and use of chronic medications.

The complete physical examination will include an assessment of the following:

- General appearance,
- Skin, head, neck, and lymph nodes
- Musculoskeletal/extremities (including spine)
- Cardiovascular
- Lungs
- Abdomen
- Neurological (reflexes)

Physical examination data to be recorded on the CRF will include:

- Normal/abnormal
- Description of any abnormalities.

Height in centimeters (cm) and weight in kg will be measured at screening only and will be recorded on the CRF.

4.1.1.3 Urine drug screen

A urine screen for drugs of abuse will be conducted at the time specified in Table 1.

If a test result is positive for drugs of abuse, the subject will not participate in the study. The following drugs of abuse will be screened:

- cannabinoids
- cocaine
- opiates
- amphetamines
- benzodiazepines
- barbiturates,
- methaqualone
- propoxyphene
- methadone.

4.1.1.4 HIV and hepatitis testing

A 10 mL blood sample to test for HIV antibody, HbsAG, and hepatitis C antibody will be performed on all subjects at screening. If a test result is positive, the subject will not be allowed to proceed in the study. Although the results of the HIV and hepatitis screens have to be documented in the subject's file, they will not be collected on the CRFs and will therefore not be recorded in the study database.

4.1.1.5 Serum pregnancy test

Female subjects of childbearing potential will have a serum pregnancy test conducted at the times specified in Table 1 for human chorionic gonadotrophin (HCG). The serum pregnancy test can be collected with the same sample as the blood chemistry. If the result is positive, the subject will not be allowed to proceed in the study.

4.1.1.6 Electrocardiographic measurements

A standard resting 12-lead ECG will be obtained at screening and at discharge only and will be recorded after the subject has been lying down for 10 minutes.

4.1.2 Post-study medical examination

A complete physical exam will be given at the time of discharge for all subjects.

4.2 Pharmacokinetic measurements

4.2.1 Determination of rosuvastatin, N-desmethyl rosuvastatin, and rosuvastatin lactone concentrations in biological samples

Plasma samples for measurement of rosuvastatin, N-desmethyl rosuvastatin, and rosuvastatin lactone concentrations will be analyzed by liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS). Full details of the methodology will be included in the clinical study report (CSR).

4.2.2 Collection of biological samples

Blood samples are expected to be collected at the precise time indicated and must be recorded in real time on the source documents immediately at time of collection.

Samples will be destroyed after the clinical study report has been finalized.

4.2.2.1 Blood sampling for determination of rosuvastatin, n-desmethyl rosuvastatin, and rosuvastatin lactone in plasma

Venous blood samples (4mL) will be collected into tubes containing lithium heparin anticoagulant at the times indicated in Table 3 below.

Blood samples must be protected from light, cooled to 4 degrees Celsius (°C), and centrifuged at 1500 G (relative centrifugal force) for 10 minutes at 4 °C within 30 minutes of blood sampling. Following centrifugation, a 1.5 mL aliquot of plasma will be transferred to a clean polypropylene tube and an equal volume (1.5 mL) of 0.1 M acetate buffer, pH 4.0 will be added and mixed thoroughly with a vortex mixer to provide plasma for analysis of rosuvastatin. See Appendix D for preparation of the buffer solution for plasma samples.

After ensuring that the 3 mL buffered plasma sample (1.5 mL plasma, 1.5 mL buffer solution) has been thoroughly mixed by vortexing, the sample will be divided into 2 aliquots. These 2 aliquots of 1.5 mL each will be transferred to clean 2 mL polypropylene tubes. The samples will be protected from light, frozen, and stored at -70°C until analysis.

One sample will be used for analysis of rosuvastatin, N-desmthyl rosuvastatin, and rosuvastain lactone and the second sample will be kept at the CRC as a spare in the event of loss or damage of the other sample.

Table 3 Schedule of blood sampling and tube numbers for rosuvastatin

Day	Scheduled time	Tube number
1	0.5 hour pre-dose	1
1	0.5 hour post-dose	2
1	1 hour post-dose	3
1	2 hours post-dose	4

Day	Scheduled time	Tube number
1	3 hours post-dose	5
1	4 hours post-dose	6
1	5 hours post-dose	7
1	6 hours post-dose	8
1	8 hours post-dose	9
1	10 hours post-dose	10
1	12 hours post-dose	11
2	18 hours post-dose	12
2	24 hours post-dose	13
2	30 hours post-dose	14
2	36 hours post-dose	15
3	48 hours post-dose	16
3	54 hours post-dose	17
3	60 hours post-dose	18
4	72 hours post-dose	19
7	0.5 hour pre-dose	20
8	0.5 hour pre-dose	21
9	0.5 hour pre-dose	22
17	0.5 hour pre-dose	23
17	0.5 hour post-dose	24
17	1 hour post-dose	25
17	2 hours post-dose	26
17	3 hours post-dose	27
17	4 hours post-dose	28
17	5 hours post-dose	29
17	6 hours post-dose	30
17	8 hours post-dose	31
17	10 hours post-dose	32
17	12 hours post-dose	33
18	18 hours post-dose	34
18	24 hours post-dose	35

4.2.2.2 Labeling of biological samples

AstraZeneca will supply labels for transfer tubes and plasma sample tubes. The labels **must** be applied to the transfer tube and to the plasma sample tubes the day prior to the start of blood sample collections. The plasma sample labels will include at least the following information:

- Study number: (D3560C00059)
- Randomization/Subject number
- Assay: (rosuvastatin)
- Scheduled time
- Matrix: (Plasma-rosuva, or Plasma-rosuva spare)
- Tube number

The transfer tube (tube used to vortex buffer and plasma before splitting sample) will include at least the following information:

- Study number: (D3560C00059)
- Randomization/Subject number
- Scheduled time
- Matrix: (buffered plasma for vortex mixer)
- Tube number

4.2.2.3 Shipping of biological samples

All plasma samples for rosuvastatin assays will be shipped to . The samples must be shipped frozen in dry ice via expedited overnight delivery. The samples must be packed securely to avoid damage during transit, should be double bagged to contain leaks, and should be packed with a sufficient quantity of dry ice to ensure they remain frozen for a minimum of 72 hours. Allowance should be made for possible delay of shipment. All applicable shipping regulations must be followed. Documentation sufficient to identify each sample must be included in the shipment. The contact (see below) must be notified by phone or fax when the samples are shipped and all shipping details must be provided.

It is preferable to ship samples on Mondays-Wednesdays. Do not ship on or the day before a legal holiday.

Samples should be shipped to:

4.3 Pharmacodynamic measurements

Blood samples (10 mL) will be collected for assessment of pharmacodynamic (lipid) parameters on the mornings of Days –1 and 18.

Pharmacodynamic variables will include the following:

- tChol total cholesterol
- LDL-C low-density lipoprotein cholesterol
- HDL-C high density lipoprotein cholesterol
- TG triglycerides

4.4 Safety measurements

4.4.1 Laboratory safety measurements

Blood and urine samples for determination of clinical chemistry, hematology and urinalysis parameters will be taken at the times specified in Table 1. The date and time of collection will be recorded on the appropriate CRF.

4.4.1.1 Urinalysis

A 10 mL midstream urine sample will be collected for urinalysis at the times specified in Table 1. If a sample is positive for protein or blood, a microscopic examination will be performed. Further investigation may be undertaken at the discretion of the investigator.

4.4.1.2 Clinical chemistry, hematology, serology, urinalysis

Samples will be collected in the following volumes specified:

- Clinical chemistry/serum pregnancy - 10 mL

- Hematology - 10 mL
- HIV/HbsAG/HepC - 10 mL
- Urinalysis - 10 mL

The following laboratory variables will be measured:

Clinical chemistry

Calcium	Phosphate
Blood glucose	Total bilirubin
Alkaline phosphatase	Urea nitrogen (BUN)
Uric Acid	Chloride
Carbon dioxide	Creatinine
Total protein	Albumin
Aspartate aminotransferase (AST)	Alanine aminotransferase (ALT)
Sodium	Potassium
Creatine kinase (CK)	Lactic dehydrogenase (LDH)

Hematology

Hemoglobin	Platelet count
Hematocrit	Red blood cell count
White blood cell count with differentials	

Urinalysis

PH	Ketones
Glucose	Proteins
Blood	Bilirubin
Specific gravity	

Microscopic analysis (if applicable)

WBC	Crystals
RBC	Epithelial cells
Casts	Bacteria
Mucous	

4.4.2 Vital signs

For timing of individual measurements refer to Table 1.

Vital signs consist of a sitting heart rate and sitting blood pressure.

Oral temperature will be measured in °C.

4.4.2.1 Blood pressure and heart rate

For timing of individual measurements refer to Table 1.

Heart rate and blood pressure will be measured after the subject has been seated for at least 5 minutes. Heart rate will be determined by palpation of the radial pulse for a period of 30 seconds and then multiplied by two. Blood pressure will be measured using a blood pressure device with an appropriate cuff size. The same arm will be used for each measurement.

4.5 Genetic measurements and co-variables

4.5.1 Genetic assessments and analysis

The pharmacokinetic profile of rosuvastatin calcium will be assessed in relation to CYP2C19 genotype (i.e., PM versus EM see Section 3.1) and subject eligibility will also be assessed according to the inclusion/exclusion criteria (See Sections 3.3.2 and 3.3.3); therefore, genotyping of all prospective subjects is mandatory.

A report will be sent to the clinical site identifying those subjects that are eligible for entry into the study. (See Section 3.1) The phenotype of patients (PM/EM) will be provided to the study site for randomization purposes.

4.5.2 Collection of samples for geneotyping

A single venous blood sample (9 mL) will be collected into a polypropylene tube containing ethylenediamine tetra-acetic acid (EDTA) and gently inverted a minimum of 5 times to mix thoroughly. Tubes will be labeled with the protocol study number, center number, enrollment code and date of sample collection. No personal identifiers (patient name, initials or date of birth) will be placed on the tube or accompanying documentation. A record of the date of the

patient consent to the genetic research and the date of the blood sample collection will be recorded in the appropriate section of the CRF.

4.5.2.1 Sample processing and shipping

The samples for genotyping will be sent directly from the clinical site to the DNA extraction laboratory on wet ice or equivalent at 4°C or below for determination of study eligibility and CYP2C19 metabolizer status. A requisition sheet detailing the protocol study number, center number, enrollment code, and date of sample collection will accompany the blood samples obtained for eligibility and CYP2C19 genotyping. The methods used for genotyping will be documented in the CSR.

4.5.2.2 Storage and coding of DNA samples

The DNA samples will be retained by the extraction laboratory. The DNA samples will be stored for 2 years after database lock and then destroyed. The blood sample for CYP2C19 genotyping will be coded with enrollment code. No personal details identifying the individual will be available to any employee working with the DNA.

4.5.2.3 CYP2C19 genotyping

The CYP2C19 metabolizer status will be derived from the CYP2C19 genotype using the Amplichip CYP450 Test or another agreed and validated test used by the agreed extraction laboratory vendor. Additional methods, such as direct sequencing and electrophoresis, may also be used to determine, or further refine, the CYP2C19 genotype. These analyses will be conducted for randomization purposes only.

4.6 Volume of blood sampling

The total volume of blood that will be drawn from each subject in this study is as follows:

Table 4 Volume of blood to be drawn from each subject

Assessment		Sample volume (mL)	n of samples	Total volume (mL)
Blood sampling for rosuvastain		4 mL	35	140 mL
Genetic sample		9 mL	1	9 mL
Lipid profile		10 mL	2	20 mL
Safety	HIV/HbsAG/ Hep C	10 mL	1	10 mL
	Clinical chemistry	10 mL	3	30 mL
	Hematology	10 mL	3	30 mL
Total			**45**	**239 mL**

4.7 Adverse Events

The methods for collecting adverse events are described below.

4.7.1 Adverse Events

4.7.1.1 Definitions

The definitions of adverse events (AEs), serious adverse events (SAEs) and other significant adverse events (OAEs) are given below. It is of the utmost importance that all staff involved in the study is familiar with the content of this section. The principal investigator is responsible for ensuring this.

Adverse event

An AE is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product. An undesirable medical condition can be symptoms (eg, nausea, chest pain), signs (eg, tachycardia, enlarged liver) or the abnormal results of an investigation (e.g., laboratory findings, electrocardiogram). In clinical studies, an AE can include an undesirable medical condition occurring at any time, including run-in or washout periods, even if no study treatment has been administered.

Serious adverse event

A SAE is an AE occurring during any study phase (i.e., run-in, treatment, washout, follow-up), and at any dose of the investigational product, comparator or placebo, that fulfills one or more of the following criteria:

- Results in death
- Is immediately life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital abnormality or birth defect
- Is an important medical event that may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above.

The causality of SAEs (ie, their relationship to study treatment) will be assessed by the investigator(s), who, in completing the relevant case report form, must answer “yes” or “no” to the question “Do you consider that there is a reasonable possibility that the event may have been caused by any of the following – study medication – other medication?”. For further guidance on the definition of a SAE and a guide to the interpretation of the causality question, see Appendix B to the Clinical Pharmacology Study Protocol.

Note that SAEs that could be associated with any study procedure should also be reported. For such events the causal relationship is implied as "yes".

Other Significant Adverse Events (OAE)

OAEs will be identified by the Study Delivery Team Physician in consultation with the appropriate Global Drug Safety Physician during the evaluation of safety data for the Clinical Study Report. Significant adverse events of particular clinical importance, other than SAEs and those AEs leading to discontinuation of the subject from study treatment, will be classified as OAEs. Examples of these are marked hematological and other laboratory abnormalities, and certain events that lead to intervention (other than those already classified as serious), dose reduction or significant additional treatment. For each OAE, a narrative may be written and included in the Clinical Study Report.

4.7.1.2 Recording of adverse events

The following variables will be recorded for each AE:

Start date, stop date, maximum intensity, action taken, outcome, causality (yes or no) and whether it constitutes an SAE or not.

The intensity rating is defined as:

- mild (awareness of sign or symptom, but easily tolerated)
- moderate (discomfort sufficient to cause interference with normal activities)
- severe (incapacitating, with inability to perform normal activities)

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria in Section 4.7.1.1. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not a SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke but would be a SAE.

Should an overdose (accidental or deliberate) occur, it must be reported in accordance with the procedures described in Section 8.3, regardless of whether the overdose was associated with any symptom or not. All symptoms associated with the overdose should be reported as AEs.

Should a pregnancy occur, it must be reported in accordance with the procedures described in Section 8.4. Pregnancy in itself is not regarded as an AE unless there is a suspicion that an investigational product may have interfered with the effectiveness of a contraceptive medication.

4.7.1.3 Reporting of serious adverse events

Investigators and other site personnel must inform appropriate AstraZeneca representatives of any SAE that occurs in the course of the study within 1 day (i.e., immediately but no later than the end of the next business day) of when he or she becomes aware of it.

The AstraZeneca representative will work with the investigator to compile all the necessary information and ensure that the appropriate AstraZeneca Drug Safety Department receives a report by day one for all fatal and life-threatening cases and by day five for all other SAEs.

Follow-up information on SAEs must also be reported by the investigator within the same time frames.

If a non-serious AE becomes serious, this and other relevant follow-up information must also be provided to AstraZeneca within 1 day as described above. For a non-serious AE that become serious but which is not fatal or life-threatening a report should be received within 5 days.

All SAEs have to be reported, whether or not considered causally related to the investigational product or to the study procedure(s). All SAEs will be recorded in the CRF. The investigator and/or Sponsor are responsible for informing the Ethics Committee and/or the Regulatory Authority of the SAE as per local requirements.

5. STUDY MANAGEMENT

5.1 Monitoring

5.1.1 Study monitoring

The monitoring of this study will be performed in accordance with the principles of Good Clinical Practice (GCP) as laid out in the International Conference on Harmonization (ICH) document “Good Clinical Practice: Consolidated Guideline”.

The specific requirements of the genetic part of the study will be discussed with the investigator and other personnel involved with the study.

5.1.2 Data verification

It is a prerequisite of this study that the study monitor has direct access to source data for data verification. This will be done by comparing data from the CRFs with those in the subject’s medical notes (permission from the subject will be sought as part of the consent process). Such verification is an essential element of quality control, as it allows the rectification of transcription errors and omissions.

Monitoring including source data verification should routinely be performed prior to the transfer of data to Data Management.

Source verification of the study/genetic informed consent of participating subjects will be performed and to make sure that the investigational team is adhering to the specific requirements of the genetics aspects of the study.

5.2 Audits and inspections

Authorized representatives of AstraZeneca, a regulatory authority, an Ethics Committee may visit the center to perform audits or inspections, including source data verification. The purpose of an AstraZeneca audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, GCP guidelines of the ICH and any applicable regulatory requirements. The investigator should contact AstraZeneca immediately if contacted by a regulatory agency about an inspection at his or her center.

5.3 Training of staff

The principal investigator will maintain a record of all individuals involved in the study (medical, nursing and other staff). He or she will ensure that appropriate training relevant to the study is given to all of these staff, and that any new information of relevance to the performance of this study is forwarded to the staff involved.

Before the first subject is entered into the study the investigational staff will have an opportunity to discuss the procedures associated with the collection of blood samples, extraction of DNA and genetic testing with AstraZeneca personnel. The ethical considerations specific to genotyping and the importance of the informed consent process will be made clear. The requirements for the collections of the subjects' samples will also be made clear.

5.4 Changes to the protocol

Study procedures will not be changed without the mutual agreement of the principal investigator and AstraZeneca.

If it is necessary for the study protocol to be amended, the amendment and/or a new version of the study protocol must be notified to or approved by each Ethics Committee, and in many countries also the local regulatory authority, before implementation. Local requirements must be followed.

If an administrative change is required, such a change must be notified to or approved by each Ethics Committee according to local requirements.

If a protocol amendment requires a change to a particular center's Informed Consent Form, then AstraZeneca and the center's Ethics Committee must be notified. Approval of the revised Master Informed Consent Form by AstraZeneca and by the Ethics Committee is required before the revised form is used.

AstraZeneca will distribute amendments and new versions of the protocol to each principal investigator(s) who in turn is responsible for the distribution of these documents to his or her Ethics Committee, and to the staff at his or her center. The distribution of these documents to the regulatory authority will be handled according to local practice.

5.5 Study agreements

The principal investigator must comply with all the terms, conditions, and obligations of the study agreement for this study. In the event of any inconsistency between this protocol and the study agreement, this protocol shall prevail.

5.6 Study timetable and end of study

The study is expected to start in and to be completed by .

5.7 Data management

5.7.1 Case report forms

Paper CRFs (pCRFs) or Electronic (eCRFs) will be used to record all data. If using pCRFs, data should be recorded legibly onto the pCRFs in blue or black ballpoint pen. Correction fluid or covering labels must not be used.

The monitor will check data at the monitoring visits to the study site. The Investigator will ensure that the data in the pCRFs or entered in the eCRF system are accurate, complete and legible.

Data from the completed pCRFs will be entered into the clinical study database and validated under the direction of the Data Manager. If data are entered directly into an Electronic CRF system the data will be monitored against the source verification documents. Any missing, impossible or inconsistent recordings in the pCRFs/eCRFs will be referred back to the Investigator using a data query form (paper or electronic) and be documented for each individual subject before clean file status is declared.

5.7.2 Genetic data

In the case of genotypic data, the date the subject gave consent to participate in the study and the date the blood sample was taken from the subject will be recorded in the pCRF and database.

5.8 Reporting of genotypic results

The CYP2C19 genotype (PM or EM) will be reported back to the investigator to be used in randomization of the subjects and will not be routinely reported to the participating subjects.

Individual subjects will not be identified in any report or publication resulting from this work. The data and results of this study may be reviewed with collaborators and published, but neither the subject's name nor any other personal identifiers will appear in any publication or report.

6. PHARMACOKINETIC, PHARMACODYNAMIC, SAFETY, GENETIC AND STATISTICAL METHODOLOGY

6.1 Pharmacokinetic / pharmacodynamic evaluation

6.1.1 Calculation or derivation of pharmacokinetic variables

The Clinical Pharmacokinetics Group, AstraZeneca, Wilmington, Delaware will carry out the pharmacokinetic analyses.

The pharmacokinetic variables of rosuvastatin, N-desmethyl rosuvastatin, and rosuvastatin lactone defined below will be calculated by non-compartmental methods using WinNonlin Enterprise version 4.1, Pharsight Corp., Mountain View, CA. The actual sampling times will be used in the pharmacokinetic calculations. The following parameters will be derived:

The primary variables to be assessed for rosuvastatin after a single dose are:

- C_{max} - the observed maximum plasma concentration following a single dose
- AUC - area under the plasma concentration time curve, calculated by $AUC_{(0-t)}$ and then extrapolated to infinity by the addition of (C_{last} / λ_z)
- $AUC_{(0-t)}$ - area under the plasma concentration time curve from time zero to the last quantifiable plasma concentration (C_{last}), calculated using the linear trapezoidal rule
- AUMC area under the first moment curve; AUMC will be calculated by the linear trapezoidal rule
- t_{max} - the time to reach the observed maximum plasma concentration following a single dose
- λ_z - terminal elimination rate constant, calculated by log-linear regression of the terminal portion of the concentration time profile where there are sufficient data (a minimum of 3 plasma concentration values in the terminal log-linear phase, spanning an interval of at least 2 half-lives)
- $t_{1/2,\lambda z}$ - half-life associated with the terminal slope (λ_z) of a semi logarithmic plasma concentration-time curve. Calculated as $\ln(2)/\lambda_z$
- MRT - mean residence time; calculated as AUMC/AUC
- CL/F - apparent oral clearance, calculated as Dose/AUC (rosuvastatin only). If for an individual, AUC cannot be calculated CL/F will not be calculated.
- Vd/F - volume of distribution (apparent) during terminal (λ_z) phase; calculated as Dose/AUC*λ_z; (rosuvastatin only)

The primary variables to be assessed for rosuvastatin after multiple dosing are:

- $C_{ss,max}$ - maximum (peak) steady state drug concentration in plasma during steady state dosing interval
- $C_{ss,min}$ - minimum (trough) steady state drug concentration in plasma during steady state dosing interval
- $C_{ss,av}$ - average drug concentration in plasma during a dosing interval at steady station on administering a fixed dose at equal dosing intervals; calculated as AUC_{ss}/τ
- $t_{ss,max,}$ - the time to reach the observed maximum plasma concentration at steady-state
- $t_{ss,1/2}$ - the apparent terminal half-life at steady-state
- AUC_{ss} - area under the plasma concentration time curve during any dosing interval at steady state; calculated using the linear trapezoidal rule
- DF - degree of fluctuation; calculated as $(C_{ss,max}- C_{ss,min})/C_{ss,av}*100\%$
- AR - accumulation ratio; calculated as $AUC_{(0-24)}$ (Day 17)/$AUC_{(0-24)}$ (Day 1)

The secondary variables to be assessed for rosuvastatin metabolites N-desmethyl rosuvastatin and rosuvastatin lactone after a single dose are:

- C_{max} - the observed maximum plasma concentration following a single dose
- AUC - area under the plasma concentration time curve, calculated by $AUC_{(0-t)}$ and then extrapolated to infinity by the addition of (C_{last} / λ_z)
- $AUC_{(0-t)}$ - area under the plasma concentration time curve from time zero to the last quantifiable plasma concentration (C_{last}), calculated using the linear trapezoidal rule
- t_{max} - the time to reach the observed maximum plasma concentration following a single dose
- λ_z - terminal elimination rate constant, calculated by log-linear regression of the terminal portion of the concentration time profile where there are sufficient data (a minimum of 3 plasma concentration values in the terminal log-linear phase, spanning an interval of at least 2 half-lives)
- $t_{1/2,\lambda z}$ - half-life associated with the terminal slope (λ_z) of a semi logarithmic plasma concentration-time curve. Calculated as $\ln(2)/\lambda_z$

The secondary variables to be assessed for rosuvastatin metabolites N-desmethyl rosuvastatin and rosuvastatin lactone after multiple dosing are:

- $C_{ss,max}$ - maximum (peak) steady state drug concentration in plasma during steady state dosing interval
- $C_{ss,min}$ - minimum (trough) steady state drug concentration in plasma during steady state dosing interval
- $C_{ss,av}$ - average drug concentration in plasma during a dosing interval at steady station on administering a fixed dose at equal dosing intervals; calculated as AUC_{ss}/τ
- $t_{ss,max,}$ - the time to reach the observed maximum plasma concentration at steady-state
- AUC_{ss} - area under the plasma concentration time curve during any dosing interval at steady state; calculated using the linear trapezoidal rule
- DF - degree of fluctuation; calculated as $(C_{ss,max}- C_{ss,min})/C_{ss,av}*100\%$
- AR - accumulation ratio; calculated as $AUC_{(0-24)}$ (Day 17)/$AUC_{(0-24)}$ (Day 1)

Maximum concentration (C_{max}) and area under the concentration curve from zero to infinity (AUC) of rosuvastatin will be the primary variables for this study to summarize exposure in each subject. If AUC data cannot be determined in all subjects completing the study, area under the curve of plasma concentration against time from zero to last quantifiable concentration ($AUC_{(0-t)}$) will replace AUC as the primary variable.
CL/F

6.1.2 Calculation or derivation of pharmacodynamic variables

Blood samples (10 mL) will be collected for assessment of pharmacodynamic (lipid) parameters on the mornings of Days –1 and 18.

Pharmacodynamic variables will include the following:

tChol	total cholesterol
LDL-C	low-density lipoprotein cholesterol
HDL-C	high density lipoprotein cholesterol
TG	triglycerides

6.1.3 Population analyses – Not applicable

6.2 Safety evaluation

Safety and tolerability will be assessed by the incidence and severity of AEs, clinical laboratory parameters (hematology, clinical chemistry, urinalysis), vital signs measurements, and physical examination findings

6.2.1 Calculation or derivation of safety variables

6.2.1.1 Laboratory data

All laboratory safety data, incorporating hematology, clinical chemistry, and urinalysis data will be listed, with deviations from the normal range explicitly noted on the listings.

Continuous laboratory data will be summarized using standard summary statistics. Both absolute values and change from pre-dose baseline will be summarized.

6.2.1.2 12-lead ECG data

Twelve-lead ECG data will be summarized and listed using standard summary statistics.

6.2.1.3 Vital signs

Vital signs data will be summarized and listed using standard summary statistics. Both absolute values and change from pre-dose baseline will be summarized. In addition, mean plots showing change within treatment group across time will be produced for absolute values.

6.2.1.4 Physical examination

Physical examination abnormalities will be listed.

6.3 Genetics as a co-variate

6.3.1 Calculation or derivation of genetic variables

A 9mL blood sample will be collected and genotyped to determine if subjects are PMs or EMs of CYP2C19.

Subjects coding for CYP2C19 *2/*2, *2/*3, or *3/*3 alleles will be considered PMs. Subjects coding for CYP2C19 *1/*1, *1/*2, or *1/*3 alleles will be considered EMs.

To control for other possible genetic factors that may contribute to increased exposure of rosuvastatin, all prospective subjects coding for OATP-C 1B1 *5 and *15, and/or BCRP 421C>A will be excluded from the study. Additionally, prospective subjects must code for wild-type CYP2C9 (*1 homozygous or heterozygous). There will be no other genetic research completed on the blood samples collected.

There will be no additional exploratory genetic research on the blood samples collected.

6.4 Statistical methods and determination of sample size

6.4.1 Statistical evaluation

A comprehensive SAP will be prepared and finalized before database lock.

6.4.2 Description of analysis sets

Statistical analyses for pharmacokinetic parameters will be performed on the evaluable population. An evaluable subject is defined as a subject satisfying the inclusion and exclusion criteria, completing all study procedures from the screening period to the final blood sampling for plasma levels of rosuvastatin, and had no major protocol deviation or violation.

All volunteers who received at least one dose of study medication will be included in the safety analysis

6.4.3 Methods of statistical analyses

Statistical summaries will be carried out by the biostatistical group at AstraZeneca, Wilmington, Delaware using the SAS system Version 8.

Where standard summary statistics are referenced below, this will include the mean, standard deviation, median, minimum and maximum for continuous variables, and counts and percentages for discrete variables.

6.4.3.1 Demographic and baseline data

All demographic and baseline data, including medications, will be listed and summarized using standard summary statistics. No hypothesis test comparing the subjects that are EMs and the subjects that are PMs will be made.

6.4.3.2 Adverse Events

Adverse events will be summarized by System Organ Class and Preferred Term, using Medical Dictionary for Drug Regulatory Activities (MedDRA). All adverse event data will be listed for all subjects. Separate listings of all serious adverse events, deaths or other significant adverse events will be presented.

6.4.3.3 Pharmacokinetic data

Pharmacokinetic data will be summarized for each group, using descriptive statistics. Geometric means (including 95% CIs) and CV, will be calculated and listed. In addition, the geometric mean ratios (PMs vs. EMs) of AUC, $AUC_{(0\text{-}t)}$, and C_{max}, (for rosuvastatin, N-desmethyl rosuvastatin, and rosuvastatin lactone) and metabolite to parent ratios, and their 90% CIs will be calculated. Half-lives for the two groups will be summarized using least squares means and the comparison between PMs and EMs will be by least squares mean difference and 90% CI.

6.4.3.4 Pharmacodynamic data

Descriptive statistics will be presented for tChol, LDL-C, HDL-C, and TG, and their changes from baseline. Baseline is defined as the lipid profile measurements before dose administration.

6.4.4 Determination of sample size

The sample size has not been decided based upon statistical considerations. This is an exploratory study and thus power calculations based upon statistical hypothesis testing is not applicable.

6.5 Interim analyses – Not applicable

6.6 Data monitoring committee – Not applicable

7. ETHICS

7.1 Ethics review

The final study protocol, including the final version of the Informed Consent Form, must be approved or given a favorable opinion in writing by an Ethics Committee as appropriate. The investigator must submit written approval to AstraZeneca before he or she can enroll any subject into the study.

The Principal Investigator is responsible for informing the Ethics Committee of any amendment to the protocol in accordance with local requirements. In addition, the Ethics Committee must approve all advertising used to recruit subjects for the study. The protocol must be re-approved by the Ethics Committee annually, as local regulations require.

7.2 Ethical conduct of the study

The study will be performed in accordance with the ethical principles that have their origin in the Declaration of Helsinki and are consistent with Good Clinical Practice, applicable regulatory requirements and the AstraZeneca policy on Bioethics.

7.3 Informed Consent

The principal investigator will ensure that the subject is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study. Subjects must also be notified that they are free to discontinue from the study at any time. The subject should be given the opportunity to ask questions and allowed time to consider the information provided.

The subject's signed and dated informed consent must be obtained before conducting any procedure specifically for the study.

The principal investigator must store the original, signed Informed Consent Form. A copy of the Informed Consent Form must be given to the subject.

If modifications are made according to local requirements, the new version has to be approved by AstraZeneca.

7.4 Subject data protection

The Master Informed Consent Form will incorporate (or, in some cases, be accompanied by a separate document incorporating) wording that complies with relevant data protection and privacy legislation. Pursuant to this wording, subjects will authorize the collection, use and disclosure of their study data by the Investigator and by those persons who need that information for the purposes of the study.

The Master Informed Consent Form will explain that study data will be stored in a computer database, maintaining confidentiality in accordance with national data legislation. All data computer processed by AstraZeneca will be identified by randomization code/study code/and/or initials.

The Master Informed Consent Form will also explain that for data verification purposes, authorized representatives of AstraZeneca, a regulatory authority, an Ethics Committee may require direct access to parts of the hospital or practice records relevant to the study, including subjects' medical history.

Reference to participation in this genetic research should not be recorded into the subjects' general medical records. All notes should be kept within the clinical study records.

AstraZeneca will not provide individual genotype results to any insurance company, any employer, their family members, general physician or any other third party, unless required to do so by law, nor should subjects discuss individual status of this genetic aspect with other study participants.

8. PROCEDURES IN CASE OF EMERGENCY, OVERDOSE OR PREGNANCY

8.1 AstraZeneca emergency contact procedure

In the case of a medical emergency, contact AstraZeneca personnel shown below.

For Serious Adverse event reporting

8.2 Procedures in case of medical emergency

The principal investigator(s) is responsible for ensuring that procedures and expertise are available to cope with medical emergencies during the study. **A medical emergency usually constitutes an SAE and should be reported as such, see Section 4.7.1.3.**

8.3 Procedures in case of overdose

Use of study medication in doses in excess of that specified in the protocol should not be recorded in the CRF as an AE of 'Overdose' unless there are associated symptoms or signs.

An Overdose with associated SAEs should be recorded as the SAE diagnosis/symptoms on the relevant AE forms in the CRF.

An Overdose with associated non-serious AEs should be recorded as the AE diagnosis/symptoms on the relevant AE forms in the CRF. In addition, the Overdose should be reported on the separate AZ "Clinical Study Overdose Report Form."

An Overdose without associated symptoms should not be recorded as an AE in the CRF. The Overdose should be reported on the separate AZ "Clinical Study Overdose Report Form".

8.4 Procedures in case of pregnancy

Pregnancy itself is not regarded as an adverse event unless there is a suspicion that the investigational product under study may have interfered with the effectiveness of a contraceptive medication. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth or congenital abnormality) must be followed up and documented even if the subject was discontinued from the study.

All reports of congenital abnormalities/birth defects are SAEs. Spontaneous miscarriages should also be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. All outcomes of pregnancy must be reported to AstraZeneca on the pregnancy outcomes report form.

9. REFERENCES –NOT APPLICABLE

AstraZeneca

Clinical Pharmacology Study Protocol: Appendix B

Drug Substance	Rosuvastatin calcium
Study Code	D3560C00059
Appendix Edition Number	1
Appendix Date	

Appendix B
Additional Safety Information

FURTHER GUIDANCE ON THE DEFINITION OF A SERIOUS ADVERSE EVENT (SAE)

Life threatening

'Life-threatening' means that the subject was at immediate risk of death from the AE as it occurred or it is suspected that use or continued use of the product would result in the subject's death. 'Life-threatening' does not mean that had an AE occurred in a more severe form it might have caused death (eg, hepatitis that resolved without hepatic failure).

Hospitalisation

Out-patient treatment in an emergency room is not in itself a serious AE, although the reasons for it may be (eg, bronchospasm, laryngeal oedema). Hospital admissions and/or surgical operations planned before or during a study are not considered AEs if the illness or disease existed before the subject was enrolled in the study, provided that it did not deteriorate in an unexpected way during the study.

Important medical event or medical intervention

Medical and scientific judgement should be exercised in deciding whether a case is serious in situations where important medical events may not be immediately life-threatening or result in death, hospitalisation, disability or incapacity but may jeopardize the subject or may require medical intervention to prevent one or more outcomes listed in the definition of serious. These should usually be considered as serious.

Simply stopping the suspect drug does not mean that it is an important medical event; medical judgement must be used.

Examples of such events are:

- Angioedema not severe enough to require intubation but requiring iv hydrocortisone treatment
- Hepatotoxicity caused by paracetamol (acetaminophen) overdose requiring treatment with N-acetylcysteine
- Intensive treatment in an emergency room or at home for allergic bronchospasm
- Blood dyscrasias (eg, neutropenia or anaemia requiring blood transfusion, etc) or convulsions that do not result in hospitalisation
- Development of drug dependency or drug abuse.

A GUIDE TO INTERPRETING THE CAUSALITY QUESTION

The following factors should be considered when deciding if there is a "reasonable possibility" that an AE may have been caused by the drug.

- Time Course. Exposure to suspect drug. Has the subject actually received the suspect drug? Did the AE occur in a reasonable temporal relationship to the administration of the suspect drug?

- Consistency with known drug profile. Was the AE consistent with the previous knowledge of the suspect drug (pharmacology and toxicology) or drugs of the same pharmacological class? OR could the AE be anticipated from its pharmacological properties?

- Dechallenge experience. Did the AE resolve or improve on stopping or reducing the dose of the suspect drug?

- No alternative cause. The AE cannot be reasonably explained by another aetiology such as the underlying disease, other drugs, other host or environmental factors.

- Rechallenge experience. Did the AE reoccur if the suspected drug was reintroduced after having been stopped? AstraZeneca would not normally recommend or support a rechallenge.

- Laboratory tests. A specific laboratory investigation (if performed) has confirmed the relationship?

A "reasonable possibility" could be considered to exist for an AE where one or more of these factors exist.

In contrast, there would not be a "reasonable possibility" of causality if none of the above criteria apply or where there is evidence of exposure and a reasonable time course but any dechallenge (if performed) is negative or ambiguous or there is another more likely cause of the AE.

In difficult cases, other factors could be considered such as:

- Is this a recognised feature of overdose of the drug?

- Is there a known mechanism?

Ambiguous cases should be considered as being a "reasonable possibility" of a causal relationship unless further evidence becomes available to refute this. Causal relationship in cases where the disease under study has deteriorated due to lack of effect should be classified as no reasonable possibility.

Clinical Pharmacology Protocol: Appendix C

Drug Substance	Rosuvastatin calcium
Study Code	D3560C00059
Appendix Edition Number	1
Appendix Date	

Appendix C
WHO Risk Categories

Risk group	Shipping Requirement	Pathogen	Risk to individuals	Risk to the community	Examples of Pathogens and their Risk groups
1	Standard Diagnostic (IATA PI650)	A micro-organism that is unlikely to cause human disease.	NONE OR VERY LOW	NONE OR VERY LOW	Most bacteria, fungi and viruses
2	Standard Diagnostic (IATA PI650)	A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.	MODERATE	LOW	Legionella pneumophila E. Coli 0157
3	Standard Diagnostic (IATA PI650)	A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.	HIGH	LOW	HIV Hepatitis B Hepatitis C
4	High risk(IATA PI602)	A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.	HIGH	HIGH	Lassa Fever Ebola Virus

If a subject is being withdrawn due to a suspected infection in WHO risk categories 2, 3 and 4 no biological samples from this subject are allowed to be sent to the laboratory. Samples will be destroyed according to normal routines at the study site.

AstraZeneca

Clinical Pharmacology Study Protocol Appendix D	
Drug Substance	Rosuvastatin calcium
Study Code	D3560C00059
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Appendix D
Preparation of Buffer Solution for Rosuvastatin Plasma Samples

1. STEP 1 – PREPARE 0.1 M ACETIC ACID

To a 1 liter volumetric flask containing approximately 500 mLs of HPLC water, add 5.75 mLs of concentrated glacial acetic acid. Mix thoroughly and fill to volume with HPLC water.

2. STEP 2 – PREPARE 0.1 M SODIUM ACETATE

To a 500 mL volumetric flask containing approximately 200 mLs of HPLC water, add 6.8 grams of sodium acetate trihydrate. Mix to dissolve and fill to volume with HPLC water

3. STEP 3 – PREPARE BUFFER

In a large beaker, combine 820 mLs of 0.1 M acetic acid and 180 mLs of 0.1 M sodium acetate. Adjust to pH 4.0 with 0.1 M sodium acetate.

Store final buffers in a 1-liter plastic screw cap container. Label with a 45-day expiration, store at room temperature.