AstraZeneca

Revised Clinical Study Protocol

Drug Substance	Ticagrelor
Study Code	D5130C00074
Edition Number	2.0
Date	

A Single-Center, Open-Label, Randomized, 3-Treatment, 3-Period Cross-Over Study to Investigate the Potential Effect of Cyclosporine on the Pharmacokinetics, Safety, and Tolerability of Ticagrelor and the Effect of Ticagrelor on the Pharmacokinetics, Safety, and Tolerability of Cyclosporine Administered Concomitantly in Healthy Male Subjects

Sponsor: AstraZeneca AB, 151 85 Södertälje, Sweden

AstraZeneca Research and Development site representative

_____ _____
Date

The following Amendment(s) and Administrative Changes are included in this revised protocol:

Amendment No.	Date of Amendment	Local Amendment No.	Date of local Amendment
1			

Administrative change No.	Date of Administrative Change	Local Administrative change No.	Date of local Administrative Change

AstraZeneca

PROTOCOL SYNOPSIS

A Single-Center, Open-Label, Randomized, 3-Treatment, 3-Period Cross-Over Study to Investigate the Potential Effect of Cyclosporine on the Pharmacokinetics, Safety, and Tolerability of Ticagrelor and the Effect of Ticagrelor on the Pharmacokinetics, Safety, and Tolerability of Cyclosporine Administered Concomitantly in Healthy Male Subjects

Principal Investigator

Study center and number of subjects planned

This study will be conducted at a single study center,
in the United States of America. Up to 26 healthy male subjects will be enrolled to ensure 18 completed subjects.

Study period	Phase of development
Estimated date of first subject enrolled	Clinical Pharmacology (Phase I)
Estimated date of last subject completed	

Objectives

Primary objectives

- To investigate the effect of cyclosporine on the pharmacokinetics of ticagrelor in healthy male subjects
- To investigate the effect of ticagrelor on the pharmacokinetics of cyclosporine in healthy male subjects

Secondary objective

- To assess the safety and tolerability of co-administration of ticagrelor and cyclosporine in healthy male subjects by assessment of adverse events, safety laboratory variables, physical examination, electrocardiogram, and vital signs

Exploratory objective

- To collect plasma samples for possible biomarker research (these data will not be reported in the Clinical Study Report)

Study design

This is a single-center, open-label, randomized, cross-over study with 3 treatments administered at 3 visits to investigate the potential pharmacokinetic interaction of 600 mg cyclosporine and 180 mg ticagrelor. All subjects will receive 3 treatments, Treatment A (a single oral dose of 600 mg cyclosporine + a single oral dose of 180 mg ticagrelor), Treatment B (a single oral dose of 600 mg cyclosporine), and Treatment C (a single 180 mg ticagrelor dose) in a cross-over design. Healthy male subjects will be randomized to receive the treatments in 1 of 6 sequences, separated by a wash-out period of at least 14 days between doses.

Target subject population

Healthy male subjects aged 18 to 45 years (inclusive) with a body mass index of 18 to 30 kg/m^2 (inclusive).

Investigational product, dosage, and mode of administration

2 x 90 mg oral ticagrelor tablets (single 180 mg dose)

6 x 100 mg oral cyclosporine, Neoral® Soft Gelatine Capsules (single 600 mg dose)

Comparator, dosage, and mode of administration

None

Duration of treatment

The study will comprise 5 visits: Visit 1 (screening) will take place within 28 days of Visit 2; Visits 2, 3, and 4 (separated by a wash-out period of at least 14 days between doses) will each consist of admission on Day -1, investigational product administration on Day 1, and discharge on Day 3 (48 hours post-dose); and Visit 5 (follow-up) will take place 7 to 10 days after discharge from Visit 4.

Outcome variable(s):

- Pharmacokinetics

  The following pharmacokinetic parameters will be calculated for ticagrelor and its metabolite AR-C124910XX in plasma:

  - Observed maximum plasma concentration (C_{max})
  - Time to maximum concentration (t_{max})

- Area under plasma concentration-time curve from zero to the last measurable concentration [$AUC_{(0-t)}$]
- Area under plasma concentration-time curve from zero to infinity (AUC)
- Terminal half-life ($t_{1/2}$)
- AR-C124910XX : ticagrelor ratios for C_{max}, $AUC_{(0-t)}$, and AUC

The following pharmacokinetic parameters will be calculated for cyclosporine in whole blood:

- Observed maximum whole blood concentration (C_{max})
- Time to maximum concentration (t_{max})
- Area under whole blood concentration-time curve from zero to the last measurable concentration [$AUC_{(0-t)}$]
- Area under whole blood concentration-time curve from zero to infinity (AUC)Terminal half-life ($t_{1/2}$)

- Safety

  Adverse events, laboratory variables, vital signs, physical examination, and electrocardiogram

Statistical methods

Plasma concentrations of ticagrelor and the metabolite AR-C124910XX and their derived pharmacokinetic parameters will be summarized by treatment by using appropriate descriptive statistics.

The PK parameters for ticagrelor, AR-C124910XX, and cyclosporine will be natural log-transformed prior to analysis. The natural log-transformed AUC and C_{max} data will be separately analyzed using a mixed effects model with terms for treatment, period, and sequence as fixed effects and subject nested within sequence as a random effect. The estimated least squares means and intra-subject variability from the mixed effects model will be used to construct 90% confidence intervals for the difference in means on the log scale between the 2 treatments. The treatment effect and its corresponding 90% confidence intervals will be back-transformed using anti-logarithms to its original scale and reported as ratio of treatments (ticagrelor + cyclosporine / ticagrelor alone or ticagrelor + cyclosporine / cyclosporine alone).

Safety variables will be summarized with descriptive statistics.

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

The following abbreviations and special terms are used in this study Clinical Study Protocol.

Abbreviation or special term	Explanation
%AUCext	Percent of area extrapolated for area under the plasma concentration-time curve from zero to infinity
λ_z	Terminal rate constant
ACS	Acute coronary syndromes
ADP	Adenosine diphosphate
AE	Adverse event (see definition in Section 6.3.1)
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ASA	Acetylsalicylic acid
AST	Aspartate aminotransferase
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 the last measurable concentration
B	Blood
BLQ	Below the lower limit of quantification
BMI	Body mass index
CI	Confidence interval
C_{max}	Maximum plasma concentration
CPA	Clinical Pharmacology Alliance
CSP	Clinical Study Protocol
CSR	Clinical Study Report
CV	Coefficient of variation
CYP	Cytochrome P450
DAE	Discontinuation due to adverse event
DBP	Diastolic blood pressure
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
FDA	Food and Drug Administration
GCP	Good Clinical Practice

Abbreviation or special term	Explanation
GGT	Gamma-glutamyl transferase
GMP	Good Manufacturing Practice
H	High
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
IATA	International Air Transport Association
ICH	International Conference on Harmonization
IRB	Institutional Review Board
L	Low
LLOQ	Lower limit of quantification
MTD	Maximum tolerated dose
MedDRA	Medical Dictionary for Regulatory Activities
NA	Not Applicable
ND	Not Determined
NSTEMI	Non-ST segment elevation myocardial infarction
OAE	Other significant adverse event (see definition in Section 11.1.1)
P	Plasma
P-gp	P-glycoprotein
PD	Pharmacodynamic(s)
PK	Pharmacokinetic(s)
Rsq	Regression coefficient
S	Serum
SAE	Serious adverse event (see definition in Section 6.3.2).
SBP	Systolic blood pressure
SD	Standard deviation
STEMI	ST segment elevation myocardial infarction
SUSAR	Suspected unexpected serious adverse reaction
$t_{1/2}$	Terminal half-life
t_{max}	Time to maximum plasma concentration
U	Urine
USA	United States of America

1. INTRODUCTION

1.1 Background

Atherosclerosis is a progressive disease of the large conduit arteries. It is a worldwide public health concern, primarily due to the death and disability caused through its clinical manifestations as coronary (eg, unstable angina and myocardial infarction) and cerebral (eg, stroke and transient ischemic attack) thrombotic events. The term acute coronary syndromes (ACS) encompasses a range of clinical conditions that includes unstable angina, non-ST segment elevation myocardial infarction (NSTEMI), and ST segment elevation myocardial infarction (STEMI). The process central to ACS is disruption or erosion of an atherosclerotic plaque, ultimately leading to the promotion of platelet aggregation and a thrombus.

Adenosine diphosphate (ADP) is one of the primary mediators of platelet aggregation and inhibition of ADP mediated platelet aggregation by clopidogrel in combination with acetylsalicylic acid (ASA) has been shown to provide improved efficacy over ASA therapy alone in ACS, with a favorable bleeding profile. However, clopidogrel has a slow onset of action, irreversibly binds to its receptor, resulting in a slow offset of effect, and often has incomplete and variable inhibition of platelet aggregation from patient to patient.

Ticagrelor has been developed for the prevention of thrombotic events in patients with ACS and the goal of the ticagrelor Phase III program is to demonstrate substantial improvements in clinical efficacy (ie, clinical thrombotic event reduction) with an acceptable safety profile compared with anti-platelet therapies available at the time of this Clinical Study Protocol (CSP) (ie, ASA and clopidogrel). Most patients use concomitant ASA with ticagrelor.

The focus of the clinical pharmacology program for ticagrelor, which includes 41 studies in approximately 1000 subjects at the time of this CSP, has been to examine the exposure-response relationship, investigate the safety, and characterize drug interactions. The dose range of ticagrelor administered during these studies was 0.1 to 1260 mg. A dose level of 900 mg was established as the maximum tolerated dose (MTD) in healthy subjects.

Inhibition of platelet aggregation mediated by ticagrelor increases with increasing plasma concentrations of ticagrelor and its active metabolite AR-C124910XX until almost complete inhibition is obtained. Ticagrelor has a more rapid onset, higher and less variable inter-patient inhibition of platelet aggregation, and faster offset rate of inhibition of platelet aggregation compared to clinical doses of clopidogrel. Ticagrelor has a number of drug-drug interactions that are of clinical relevance since it is a substrate and inhibitor of cytochrome P450 (CYP) 3A, a potential activator of CYP3A4, and a substrate and inhibitor of the P-glycoprotein (P-gp) transporter.

Refer to the Investigator's Brochure for further details on ticagrelor exposure, pharmacokinetics (PK), and safety findings.

1.2 Rationale for conducting this study

The ticagrelor drug-drug interaction program has assessed the effect of ticagrelor co-administration on CYP3A4 substrates (midazolam, simvastatin, and atorvastatin), a CYP2C9 substrate (tolbutamide), and a P-gp substrate (digoxin). Cyclosporine is a clinically important drug that is both a substrate of CYP3A4, and a potent inhibitor of P-gp. The aim of this study is to investigate the effect of cyclosporine on the PK of ticagrelor and the impact of ticagrelor on the PK of cyclosporine.

1.3 Benefit/risk and ethical assessment

There are no direct benefits for the subjects participating in the study.

Ticagrelor, like other inhibitors of platelet aggregation, increases the risk of bleeding. Reported events in previous Phase II and Phase III studies have included bleeding in areas considered to be clinically important such as the lung, brain, eye, and joints. In previous studies in healthy subjects, adverse events (AEs) associated with bleeding were infrequent and were generally considered to be mild. In this study, necessary measures will be taken to minimize this risk of bleeding by excluding subjects at high risk of bleeding, such as subjects with history of hemophilia, von Willebrand's disease, lupus anticoagulant, or other diseases/syndromes that can either alter or increase the propensity for bleeding. Also subjects with a history of a clinically significant non-traumatic bleed or clinically significant bleeding risk, as judged by the Investigator, will be excluded.

The risk of ticagrelor exposure to subjects in this study is expected to be equivalent to the safety profile in subjects observed in previous Phase I studies where similar doses have been administered. The most common AEs, with an incidence of at least 2%, reported to date in the Phase I studies with at least 3 days of ticagrelor dosing include headache, somnolence, dizziness, epistaxis, nausea, abdominal pain, back pain, dyspnea, ecchymosis, lethargy, pharyngolaryngeal pain, blurred vision, postural dizziness, pollakiuria (frequent urination), and increased tendency for bruising.

The most common side effects ($\geq 1/100$, $<1/10$) of treatment with cyclosporine in a clinical setting are: hyperlipidemia, anorexia, hyperuricemia, hyperkalemia, hypomagnesemia, tremor, headache, paraesthesia, hypertonia, nausea, vomiting, stomach ache, diarrhea, gingivahyperplasia, decreased liver function, hypertricosis, muscle ache, myalgia, and tiredness. However, any of the aforementioned is unlikely to be observed after a single dose.

2. STUDY OBJECTIVES

2.1 Primary objectives

- To investigate the effect of cyclosporine on the PK of ticagrelor in healthy male subjects

- To investigate the effect of ticagrelor on the PK of cyclosporine in healthy male subjects

2.2 Secondary objective

- To assess the safety and tolerability of co-administration of ticagrelor and cyclosporine in healthy male subjects by assessment of AEs, safety laboratory variables, physical examination, electrocardiogram (ECG), and vital signs

2.3 Exploratory objective

- To collect plasma samples for possible biomarker research (these data will not be reported in the Clinical Study Report [CSR])

3. STUDY PLAN AND PROCEDURES

This CSP has been subject to a peer review according to AstraZeneca standard procedures.

3.1 Overall study design and flow chart

This is a single-center, open-label, randomized, cross-over study with 3 treatments administered at 3 visits to investigate the potential PK interaction of 600 mg cyclosporine and 180 mg ticagrelor.

Up to 26 healthy male subjects will be enrolled at a single study center. Withdrawn subjects will be replaced to ensure 18 completed subjects. This study will comprise 5 visits:

Visit 1 (screening): within 28 days of Visit 2. Screening procedures will only be performed for subjects who give voluntary written informed consent.

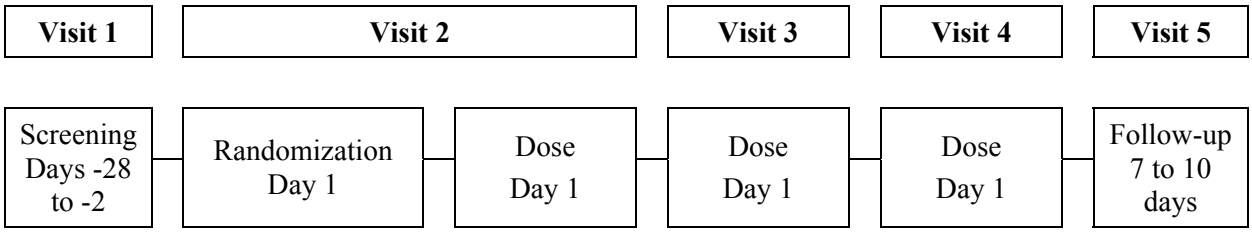
Visits 2, 3, and 4 (treatment): subjects will be admitted to the study center on Day -1. Subjects will be randomized to 1 of 6 sequences. Treatments will be administered on Day 1 with 240 mL water after an overnight fast of at least 8 hours. Subjects will be discharged on Day 3, 48 hours after investigational product administration. Each investigational product administration will be separated by a wash-out period of at least 14 days (calculated from Day 1 of Visit 2 to Day 1 of Visit 3 to Day 1 of Visit 4).

- Treatment A: a single oral dose of 600 mg cyclosporine dose (Neoral®) + a single oral dose of 180 mg ticagrelor
- Treatment B: a single oral dose of 600 mg cyclosporine dose (Neoral®)
- Treatment C: a single oral dose of 180 mg ticagrelor

Visit 5 (follow-up): subjects will return to the study center 7 to 10 days after discharge from Visit 4 for follow-up assessments.

The study design flow chart is presented in Figure 1 and the study assessments in Table 1.

Figure 1 Study design flow chart



	Period 1	Period 2	Period 3
Sequence 1	A	B	C
Sequence 2	A	C	B
Sequence 3	B	A	C
Sequence 4	B	C	A
Sequence 5	C	A	B
Sequence 6	C	B	A

Day 1 of each visit will be separated by a wash-out period of at least 14 days.
Subjects will be admitted to the study center for Visits 2, 3, and 4 on the morning of the day before investigational product administration (Day -1) and will be confined to the study center until 48 hours post-dose (Day 3), after collection of the last PK sample.

Table 1 Study assessments

	Visit 1	**Visits 2, 3, and 4**				**Visit 5**
	Screening	**Treatment visits 1, 2, and 3**				**Follow-up**
Assessment	**Days -28 to -2**	**Day -1**	**Day 1**	**Day 2**	**Day 3**	**7 to 10 days**
Signed informed consent	√					
Optional safety biomarker sampling[a]	√					
Inclusion and exclusion criteria	√	√[b]				
Relevant medical and surgical history	√					
Height, weight, and body mass index	√					
Demographics	√					
Physical examination	√	√[c]			√[c]	√

Table 1 Study assessments

Assessment	Visit 1	Visits 2, 3, and 4				Visit 5
	Screening	Treatment visits 1, 2, and 3				Follow-up
	Days -28 to -2	Day -1	Day 1	Day 2	Day 3	7 to 10 days
Vital signs	√	√	√	√	√	√
12-Lead paper ECG	√	√			√	√
Admission		√				
Randomization			√[b]			
Investigational product administration			√			
Clinical chemistry and hematology	√	√			√	√
Urinalysis	√	√			√	√
Screen for hepatitis and HIV	√					
Screen for drugs of abuse	√	√				
Alcohol breath test	√	√				
Pharmacokinetic sampling[d]			√	√	√	
Concomitant medications	√	√	√	√	√	√
Adverse events		√	√	√	√	√
Serious adverse events	√	√	√	√	√	√
Discharge					√	

a At Visit 1 (screening) or on Day -1 of Visit 2.
b Visit 2 only.
c Only targeted physical examination: general, cardiovascular system, chest, abdomen, and skin.
d Venous blood will be collected pre-dose and at 0.5, 1, 2, 3, 4, 6, 8, 12, 18, 24, 36, and 48 hours post-dose for the determination of cyclosporine, ticagrelor and AR-C124910XX.
ECG: Electrocardiogram; HIV: Human immunodeficiency virus.

3.2 Rationale for study design, doses and control groups

This study design is in line with the Food and Drug Administration (FDA) guidance for Industry for drug-drug interaction studies (FDA Guidance for Industry, Drug Interaction Studies 2006). An open-label design is acceptable as no pharmacodynamic (PD) endpoints are critical to the assessment of the interaction.

The rationale for choosing healthy subjects is to investigate the potential interaction of ticagrelor and cyclosporine without adding additional confounding factors, such as disease state or concomitant medications. The study is restricted to male subjects as there is a possibility for a gender effect during the female menstrual cycle when drugs are metabolized by CYP3A. By including men only, the possible variability factor is removed.

Ticagrelor will be administered as a single 180 mg dose. Ticagrelor (180 mg loading dose; 90 mg twice daily thereafter) is approved in the European Union and in the United States of America (USA) for the prevention of thrombotic events in patients with ACS. The 180 mg single dose was selected for this study as it is the recommended loading dose and also the highest approval dose expected to be administered to patients. The PK of ticagrelor appeared linear and the maximum plasma concentration (C_{max}) and the area under the plasma concentration-time curve from zero to infinity (AUC) of ticagrelor and AR-C124910XX increased approximately in a dose-proportional manner over the dose range of 30 to 1260 mg. Since no time-dependent changes in ticagrelor PK over the course of multiple dosing were observed during its clinical development and mean accumulation ratios at 90 mg twice daily dosing was approximately 1.8 for ticagrelor and AR-C124910XX, a 180 mg single dose would provide the exposure to ticagrelor and AR-C124910XX higher than those following multiple 90 mg twice daily dosing. Thus, a single dose of 180 mg dosing not only results in a simple study design, but also maximizes the potential impact of ticagrelor on the interaction drug (cyclosporine).

Cyclosporine will be administered as a single 600 mg dose. Plasma concentrations of cyclosporine as a P-gp inhibitor range from 1000 to 5000 ng/mL and a single 300 mg dose of cyclosporine produces a mean plasma concentration of approximately 1000 ng/mL. Therefore, a single 600 mg cyclosporine dose will be administered in this study to provide sufficient exposure to investigate the potential effect on P-gp.

The risk of carry-over has been addressed by a wash-out period of at least 14 days between the administrations of the investigational product.

4. SUBJECT SELECTION CRITERIA

The Investigator should keep a record, the subject screening log, of subjects who entered pre-study screening.

Each subject should meet all of the inclusion criteria and none of the exclusion criteria for this study. Under no circumstances can there be exceptions to this rule.

4.1 Inclusion criteria

For inclusion in the study subjects should fulfill the following criteria:

1. Provision of signed and dated written informed consent prior to any study-specific procedures

2. Healthy male subjects aged 18 to 45 years (inclusive) with suitable veins for cannulation or repeated venipuncture

3. Have a body mass index (BMI) of between 18 and 30 kg/m^2 (inclusive) and a minimum weight of 50 kg, but no more than 100 kg

4. Subjects should use double barrier contraception (ie, condoms and spermicide) and avoid fathering a child while participating in the study and until 4 weeks after the last investigational product administration

In addition, for inclusion in the optional biomarker research, subjects should fulfill the following criterion:

5. Provision of signed and dated written informed consent for sampling for biomarker research

   Subjects who refuse to provide written informed consent for the biomarker research will not be excluded from other aspects of the study described in the CSP, provided they give consent

4.2 Exclusion criteria

Subjects should not enter the study if any of the following exclusion criteria are fulfilled:

1. History of any clinically significant disease or disorder which, in the opinion of the Investigator, may either put the subject at risk because of participation in the study, or influence the results or the subject's ability to participate in the study

2. History or presence of gastrointestinal, hepatic or renal disease, or any other condition known to interfere with absorption, distribution, metabolism, or excretion of drugs

3. Any clinically significant illness, medical/surgical procedure, or trauma within 4 weeks of the first administration of the investigational product

4. A history of hemophilia, von Willebrand's disease, lupus anti-coagulant, or other diseases/syndromes that can either alter or increase the propensity for bleeding

5. A personal history of vascular abnormalities including aneurysms; a personal history of severe hemorrhage, hematemesis, melena, hemoptysis, severe epistaxis, severe thrombocytopenia, intracranial hemorrhage, or rectal bleeding within 1 year (or as judged by the Investigator) prior to Visit 1 or a history suggestive of peptic ulcer disease

6. History of a clinically significant non-traumatic bleed or clinically significant enhanced bleeding risk, as judged by the Investigator

7. History of a previous or ongoing psychiatric disease/condition, including psychosis, affective disorder, suicidality, anxiety disorder, borderline state, or personality disorder

8. Any clinically significant abnormal clinical chemistry, hematology, or urinalysis results as judged by the Investigator

9. Any positive result on screening for serum hepatitis B surface antigen (HBsAg), hepatitis C virus (HCV) antibody, and human immunodeficiency virus (HIV)

10. Abnormal vital signs, after 10 minutes supine rest at screening (may repeat), defined as any of the following:

    - Systolic blood pressure (SBP) >140 mmHg
    - Diastolic blood pressure (DBP) >90 mmHg
    - Pulse rate <40 or >100 bpm

11. Any clinically important abnormalities in rhythm, conduction, or morphology of resting ECG, as judged by the Investigator

12. Prolonged QTcF >450 ms or shortened QTcF <340 ms or family history of long QT syndrome

13. Known or suspected history of drug abuse, as judged by the Investigator

14. Smoking more than 7 cigarettes per week or consumption of more than 3 portions of snuff or the equivalent per week

15. History of alcohol abuse or excessive intake of alcohol, as judged by the Investigator

16. Positive screen for drugs of abuse and/or alcohol breath test at screening or on admission to the study center for both Visits 2 and 3

17. History of severe allergy/hypersensitivity or ongoing clinically significant allergy/hypersensitivity, as judged by the Investigator

18. Excessive intake of caffeine containing drinks, eg, coffee, tea, caffeine-containing energy drinks, and cola (more than 5 cups of coffee or the equivalent per day)

19. Use of drugs with enzyme inducing properties such as St John's Wort within 3 weeks prior to the first administration of the investigational product

20. Use of any prescribed or non-prescribed medication including antacids, analgesics other than paracetamol/acetaminophen, and herbal remedies during the 2 weeks

prior to the first administration of the investigational product or longer if the medication has a long half-life. Occasional use of paracetamol/acetaminophen (up to 2 g per day) is allowed for minor pains and headache. Over-the-counter saline nasal spray is allowed for relief of nasal congestion

21. Any intake of grapefruit, grapefruit juice, Seville oranges, Seville orange marmalade, or other products containing grapefruit or Seville oranges within 7 days before admission to the study center

22. Food containing poppy seeds within 7 days before admission to the study center

23. Plasma donation within 1 month of screening or any blood donation/blood loss of >500 mL during the 3 months prior to screening

24. Has received another new chemical entity (defined as a compound which has not been approved for marketing) or has participated in any other clinical study that included drug treatment within 3 months of the first administration of the investigational product in this study. The period of exclusion begins at the time of the last dose in the prior study. Note: subjects consented and screened, but not dosed in this study or a previous Phase I study, are not excluded

25. Involvement in the planning and/or conduct of the study (applies to AstraZeneca and personnel, and any other personnel involved in the study)

26. Judgment by the Investigator that the subject should not participate in the study if he is considered unlikely to comply with study procedures, restrictions, and requirements

27. Previous randomization to treatment in the present study

For procedures for withdrawal of incorrectly enrolled subjects see Section 5.3.

5. STUDY CONDUCT

5.1 Restrictions during the study

Subjects will be required to:

1. Fast (except for water) for at least 8 hours before investigational product administration on Day 1 of Visits 2, 3, and 4 and remain fasting until 4 hours after investigational product administration. Water is allowed until 1 hour before and 1 hour after dosing. Water (up to 240 mL) needed for the investigational product administration is allowed

2. Eat and drink only the standardized meals and drinks provided (apart from water) while resident in the study center

3. Abstain from consuming any of the following:

   - Alcohol from 72 hours before admission to Visits 2, 3, and 4, while resident in the study center, and for 72 hours before follow-up

   - Energy drinks containing taurine or glucoronolactone, eg, Red Bull, from 72 hours before admission (Day -1), while resident in the study center, and for 72 hours before follow-up

   - Caffeine-containing drinks while resident in the study center, apart from any provided as part of a standardized meal. Excessive intake of caffeine should be avoided during the wash-out period and between discharge from the study center and follow-up

   - Poppy seeds found in speciality bread from 7 days before admission to Visit 2 until discharge from Visit 4

   - Grapefruit, grapefruit juice, Seville oranges, Seville orange marmalade, or other products containing grapefruit or Seville oranges and grapefruit from 7 days before admission to Visit 2 until discharge from Visit 4

   - Refrain from the use of tobacco or nicotine-containing products and drugs of abuse from the time of consent until after the final medical examination at follow-up

   - Refrain from taking any medication (prescribed or over-the-counter products, including St. John's Wort, herbal medications, and medicines purchased via the Internet) during the 2 weeks prior to the first administration of the investigational product (3 weeks for St. John's Wort) until follow-up, unless the Investigator has given prior consent. Vitamins and minerals in doses below the manufacturer's recommendations are allowed until Day -1 but disallowed during Visits 2, 3, and 4. Occasional use of acetaminophen/paracetamol (up to 2 g per day) is allowed for minor pains and headache and over-the-counter saline nasal spray is allowed for relief of nasal congestion

4. Refrain from strenuous physical activity, which is not within the subject's normal daily routine, from 72 hours before admission to Visits 2, 3, and 4, while resident in the study center, and for 72 hours before follow-up

5. Abstain from blood or plasma donation until 3 months after the last investigational product administration

6. Refrain from scheduling surgery, including dental surgery, at any time following screening and throughout the study

7. Stay in the study center from the morning of Day -1 and until Day 3 (48 hours after investigational product administration) of Visits 2, 3, and 4. The stay could be prolonged for safety reasons, if judged necessary by the Investigator

8. Subjects should use a condom to prevent pregnancy and drug exposure of a partner and refrain from donating sperm or fathering a child until 4 weeks after the last administration of investigational product

5.2 Subject enrolment and randomization and initiation of investigational product

The Investigator will:

1. Obtain signed informed consent from the potential subject before any study-specific procedures are performed

2. Assign the potential subject a unique enrollment number, beginning with 'E#'

3. Determine subject eligibility. See Sections 4.1 and 4.2

4. Assign each eligible subject a unique randomization code (subject number) beginning with '101'

If a subject withdraws from participation in the study, then his enrolment/randomization code cannot be re-used.

5.2.1 Procedures for randomization

The randomization scheme will be generated by using the AstraZeneca global randomization system. Subjects will be randomized to 1 of 6 sequences on Day 1 of Visit 2.

	Period 1	Period 2	Period 3
Sequence 1	A	B	C
Sequence 2	A	C	B
Sequence 3	B	A	C
Sequence 4	B	C	A
Sequence 5	C	A	B
Sequence 6	C	B	A

Treatment A: a single oral dose of 600 mg cyclosporine dose (Neoral®) + a single oral dose of 180 mg ticagrelor
Treatment B: a single oral dose of 600 mg cyclosporine dose (Neoral®)
Treatment C: a single oral dose of 180 mg ticagrelor

Randomization codes will be assigned strictly sequentially as subjects become eligible for randomization.

5.3 Procedures for handling subjects incorrectly enrolled or randomized or initiated on investigational product

Subjects who fail to meet the inclusion/exclusion criteria should not, under any circumstances, be enrolled or randomized. There can be no exceptions to this rule.

Where subjects who do not meet the selection criteria are randomized in error or incorrectly started on treatment, or where subjects subsequently fail to meet the study criteria post-initiation, a discussion should occur between the AstraZeneca Clinical Pharmacology Alliance (CPA) Physician and the Investigator regarding whether to continue or discontinue the subject from treatment.

The AstraZeneca CPA Physician is to ensure all such decisions are appropriately documented. In situations where an agreement cannot be reached, further administration of the investigational product should be stopped.

5.4 Blinding and procedures for unblinding the study

This is an open-label study and therefore this section is not applicable.

5.5 Treatments

5.5.1 Identity of investigational product(s)

The investigational products used in this study are presented in Table 2.

Table 2 Identity of investigational products

Investigational product	Dosage form and strength	Manufacturer
Ticagrelor	90 mg tablets	AstraZeneca
Cyclosporine	100 mg capsules	

Ticagrelor 90 mg will be manufactured in accordance with Good Manufacturing Practice (GMP) and provided by AstraZeneca to the study center.

Details of the soft gelatine cyclosporine capsules can be found in the Neoral® package insert.

5.5.2 Doses and treatment regimens

All subjects will receive all 3 treatments:

- Treatment A: a single oral dose of 600 mg cyclosporine dose (Neoral®) + a single oral dose of 180 mg ticagrelor
- Treatment B: a single oral dose of 600 mg cyclosporine dose (Neoral®)
- Treatment C: a single oral dose of 180 mg ticagrelor

Subjects will be resident in the study center from Day -1 to Day 3 of Visits 2, 3, and 4. The investigational product will be administered orally with 240 mL water on the morning of Day 1 after an overnight fast of at least 8 hours until 4 hours post-dose. Water will be allowed as needed until 1 hour before and from 1 hour after the investigational product administration, with the exception of the water needed for administration. Subjects will receive standardized meals while resident in the study center. There will be a wash-out period of at least 14 days between the administrations of the investigational product.

5.5.3 Labeling

Labels will be prepared in accordance with GMP and local regulatory guidelines. Label text will be in English.

5.5.4 Storage

All investigational products should be kept in a secure place under appropriate storage conditions. The investigational product label on the packs provided specifies the appropriate storage conditions.

5.6 Concomitant and post-study treatment(s)

No concomitant medication or therapy will be allowed except for paracetamol/acetaminophen for pain relief and over-the-counter saline nasal spray for relief of nasal congestion. No other prescription or over-the-counter drugs are allowed as specified in the exclusion criteria and restrictions. The subjects must be instructed that no additional medication will be allowed without the prior consent of the Investigator.

Medication, which is considered necessary for the subject's safety and well-being, may be given at the discretion of the Investigator during the residential period. When any medication is required, it should be prescribed by the Investigator who should inform the AstraZeneca CPA Physician. Following consultation with the CPA Physician, the Investigator should determine whether or not the subject should continue in the study.

Any concomitant medication used should be recorded in the appropriate sections of the electronic Case Report Form (eCRF).

5.7 Treatment compliance

The administration of all investigational products should be recorded in the appropriate sections of the eCRF.

5.7.1 Accountability

The investigational product provided for this study will be used only as directed in this CSP.

The study center personnel will account for all investigational product administered to the subjects.

The study center personnel will account for all investigational products received at the study center, unused investigational products, and for appropriate destruction. Certificates of delivery and destruction should be signed.

5.8 Discontinuation of investigational product

Subjects may be discontinued from treatment and assessments at any time for the following reasons:

- Voluntary discontinuation by the subject who is at any time free to discontinue his participation in the study, without prejudice to further treatment
- Safety reasons, as judged by the Investigator and/or AstraZeneca
- Severe non-compliance to the CSP as judged by the Investigator and/or AstraZeneca
- Incorrectly enrolled subject (the subject does not meet the required inclusion/exclusion criteria for the study) or the incorrect randomization of a subject (the subject is not allocated investigational product as described in the CSP)
- Healthy subject lost to follow-up

5.8.1 Procedures for discontinuation of a subject from investigational product

A subject who decides to discontinue investigational product will always be asked about the reason(s) and the presence of any AEs. If possible, they will be seen and assessed by the Investigator. Adverse events will be followed up (see Sections 6.3.3 and 6.3.4).

If a subject is withdrawn from the study, see Section 5.9.

5.9 Withdrawal from study

Subjects are at any time free to withdraw from the study (investigational product and assessments), without prejudice to further treatment (withdrawal of consent). Such subjects will always be asked about the reason(s) and the presence of any AEs. If possible, they will be seen and assessed by the Investigator. Adverse events will be followed up (see Sections 6.3.3 and 6.3.4).

Withdrawn subjects will be replaced to ensure 18 completed subjects.

6. COLLECTION OF STUDY VARIABLES

Refer to Table 1 for the study assessments. When more than 1 assessment is required at a particular time point, PK samples should be prioritized.

6.1 Recording of data

The Investigator will ensure that data are recorded in the eCRF as specified in the CSP and in accordance with the instructions provided.

The Investigator ensures the accuracy, completeness, and timeliness of the data recorded and of the provision of answers to data queries according to the Clinical Study Agreement. The Investigator will sign the completed eCRFs. A copy of the completed eCRFs will be archived at the study center.

Procedures for data editing, entry, and handling of the data query process will be described in the Data Management Plan.

6.2 Data collection and enrolment

Each subject will undergo screening to confirm eligibility. This will consist of the following:

- Obtaining written informed consent before starting any study-specific procedures
- Review of the inclusion/exclusion criteria with the subject
- Recording of demographic data (date of birth, gender, and race)
- A standard recording of relevant medical and surgical history
- A complete physical examination
- Height, weight, and calculation of BMI
- Vital signs
- Blood sampling for routine clinical chemistry and hematology measurements and a HBsAg, HCV antibodies, and HIV screen
- Urine sampling for routine urinalysis
- Alcohol breath test and drugs of abuse screen in urine
- 12-Lead paper ECG
- Serious adverse event (SAE) questioning
- Concomitant medication recording

6.2.1 Follow-up procedures

Follow-up procedures will be performed 7 to 10 days after discharge from Visit 4. These assessments will include a complete physical examination, vital signs, 12-lead paper ECG,

laboratory variables (clinical chemistry, hematology, and urinalysis), concomitant medication, and AE and SAE recording.

6.3 Safety

The Investigator is responsible for ensuring that all personnel involved in the study are familiar with the content of this section.

6.3.1 Definition of adverse events

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 (eg, laboratory findings, ECG). In clinical studies, an AE can include an undesirable medical condition occurring at any time, including run-in or wash-out periods, even if no investigational product has been administered.

The term AE is used to include both serious and non-serious AEs.

If the intensity of a reported AE changes during the study it should be reported as a new AE in the eCRF.

6.3.2 Definitions of serious adverse event

An SAE is an AE occurring during any study phase (ie, run-in, treatment, wash-out, follow-up), that fulfills 1 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 incapacity or substantial disruption of the ability to conduct normal life functions
- 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

For further guidance on the definition of an SAE, see Appendix B to this CSP.

6.3.3 Recording of adverse events

Time period for collection of adverse events

All AEs will be collected from Day -1 and throughout the study and all SAEs will be collected from the time of signing the informed consent and throughout the study.

Follow-up of unresolved adverse events

Any AEs that are unresolved at follow-up will be monitored by the Investigator for as long as medically indicated, but without further recording in the eCRF. AstraZeneca retains the right to request additional information for any subject with ongoing AE(s)/SAE(s) at the end of the study, if judged necessary.

Variables

The following variables will be collected for each AE:

- AE (verbatim)
- Date and time when the AE started and stopped
- Intensity, rated according to the following scale:
  - 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)
- Whether the AE is serious or not
- Investigator causality rating against the investigational product (yes or no)
- Action taken with regard to the investigational product
- Whether the AE caused the subject's withdrawal from study (yes or no)
- Outcome

In addition, the following variables will be collected for SAEs:

- Date the AE met the SAE criteria
- Date the Investigator became aware of the SAE
- AE is serious due to
- Date of hospitalization

- Date of discharge
- Probable cause of death
- Date of death
- Autopsy performed
- Causality assessment in relation to the study procedure(s)
- Causality assessment in relation to other medication
- Causality assessment in relation to additional investigational product
- Description of the AE

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 6.3.2. 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 an 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 an SAE.

Causality collection

The Investigator will assess the causal relationship between the investigational product and each AE, and answer 'yes' or 'no' to the question 'Do you consider that there is a reasonable possibility that the event may have been caused by the investigational product?'.

For SAEs, the causal relationship will also be assessed for other medication and study procedures and additional investigational product. Note that for SAEs that could be associated with any study procedure, the causal relationship is implied as 'yes'.

A guide to the interpretation of the causality question is found in Appendix B to this CSP.

Adverse Events based on signs and symptoms

All AEs spontaneously reported by the subject or reported in response to the open question from the study center personnel: 'Have you had any health problems since the previous visit/you were last asked?', or revealed by observation will be collected and recorded in the eCRF. When collecting AEs, the recording of diagnoses is preferred (when possible) to recording a list of signs and symptoms. However, if a diagnosis is known and there are other signs or symptoms that are not generally part of the diagnosis, the diagnosis and each sign or symptom will be recorded separately.

Adverse Events based on examinations and tests

The results from the CSP-mandated laboratory tests and vital signs will be summarized in the CSR. Deterioration as compared to baseline in the CSP-mandated laboratory values, vital signs, or ECG should therefore only be reported as AEs if they fulfill any of the SAE criteria or are the reason for discontinuation of treatment with the investigational product.

If deterioration in a laboratory/vital signs value is associated with clinical signs and symptoms, the sign or symptom will be reported as an AE and the associated laboratory/vital signs result will be considered as additional information. Wherever possible the Investigator uses the clinical, rather than the laboratory term (eg, anemia versus low hemoglobin value). In the absence of clinical signs or symptoms, clinically relevant deteriorations in non-mandated parameters should be reported as AE(s).

Any new or aggravated clinically relevant abnormal medical finding at a physical examination as compared with the baseline assessment will be reported as an AE.

6.3.4 Reporting of serious adverse events

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 eCRF.

If any SAE occurs during the course of the study, then the Investigator or other study center personnel will inform the appropriate AstraZeneca representatives within 1 day ie, immediately but **no later than the end of the next business day** of when he or she becomes aware of it.

The designated AstraZeneca representative works with the Investigator to ensure that all the necessary information is provided to the AstraZeneca Patient Safety data entry site **within 1 calendar day** of initial receipt for fatal and life-threatening events **and within 5 calendar days** of initial receipt for all other SAEs.

For fatal or life-threatening AEs where important or relevant information is missing, active follow-up is undertaken immediately. The Investigator or other study center personnel will inform the AstraZeneca representatives of any follow-up information on a previously reported SAE within 1 calendar day, ie, immediately but **no later than the end of the next business day** of when he or she becomes aware of it.

6.3.5 Laboratory safety assessment

Blood and urine samples for determination of clinical chemistry, hematology, and urinalysis will be collected at the times indicated in Table 1.

The safety laboratory variables are presented in Table 3.

Table 3 Safety laboratory variables

Clinical chemistry	Hematology
Serum (S)/Plasma (P)-alkaline phosphatase (ALP)	Blood (B)-hemoglobin
S/P-aspartate aminotransferase (AST)	B-hematocrit
S/P-alanine aminotransferase (ALT)	B-absolute leukocyte differential count
S/P-total bilirubin	B-platelet count
S/P-uric acid	Erythrocytes (red blood cell count)
P-glucose	
S/P-albumin	
S/P-calcium, total	
S/P-creatinine	**Urinalysis**
S/P-potassium	Urine (U)-glucose
S/P-sodium	U-hemoglobin
Total protein	U-protein
Blood urea nitrogen	
Gamma-glutamyl transferase (GGT)	

Proteinuria and hematuria will be measured with a urine dipstick.

All subjects will be tested for HBsAg, HCV antibodies, and HIV at screening.

Urine will be tested for drugs of abuse at screening and at admission to Visits 2, 3, and 4. Drugs of abuse include amphetamines, barbiturates, tricyclic anti-depressants, cocaine, methadone, phencyclidine, tetrahydrocannabinol, and opiates. If a subject tests positive to any of these screening tests, he will be excluded from the study.

An alcohol breath test will be performed at screening and at admission to Visits 2, 3, and 4. Laboratory values outside the reference limit suspected to be of any clinical significance will be repeated. Subjects in whom suspected clinical significance is confirmed, will either not be included in the study or if already enrolled, will be monitored until normalization or for as long as the Investigator considers necessary. Additional laboratory variables may be performed for safety reasons if judged necessary by the Investigator.

Samples will be collected in tubes according to standard routines. The safety laboratory samples will be analyzed using routine methods at the Physicians Reference Laboratory.

For blood volume see Section 7.1.

6.3.6 Physical examination

A full physical examination will be performed by medically qualified individuals at screening and at follow-up. A targeted physical examination will be performed on Day -1 and before discharge on Day 3 of Visits 2, 3, and 4 and will include: general, cardiovascular system, chest, abdomen, and skin. Results will be recorded as an overall normal or abnormal interpretation with a listing of abnormalities.

6.3.7 ECG

A 12-lead paper ECG for safety review by the Investigator will be performed after 10 minutes in the supine position and the outcome of the overall evaluation is to be recorded as normal/abnormal in the eCRF, with any abnormalities specified. The print-out of the ECG is to be signed, dated, and filed in the Investigator's Study File. The ECG measurements will be performed at the time points presented in Table 1.

6.3.8 Vital signs

Blood pressure and pulse rate will be measured using non-invasive equipment after 10 minutes supine rest on a bed. The vital signs measurements will be performed at the time points presented in Table 1 (pre-dose on Day 1 of Visits 2, 3, and 4).

6.4 Pharmacokinetics

6.4.1 Collection of samples

Blood samples (2 mL) for determination of ticagrelor and its active metabolite (AR-C124910XX) in plasma will be collected at times presented in the study plan table (Table 1).

Blood samples (1mL) for determination of cyclosporine in blood will be collected at times presented in the study plan table (Table 1).

Samples will be collected, labeled, stored, and shipped as detailed in the Laboratory Manual.

For blood volume see Section 7.1.

6.4.2 Determination of drug concentration

Samples for determination of ticagrelor and its metabolite concentrations in plasma will be analyzed by on behalf of AstraZeneca, using appropriate bioanalytical methods which will be detailed in the Bioanalytical Report appended to the CSR. The lower limits of quantification (LLOQ) of ticagrelor and its metabolite AR-C124910XX in plasma are 1 ng/mL and 2.5 ng/mL, respectively.

Samples for determination of cyclosporine drug concentrations in blood will be analyzed by on behalf of AstraZeneca, using appropriate bioanalytical methods which will be detailed in the Bioanalytical Report appended to the CSR.

6.5 Collection of biomarker samples

Study participants not on any concomitant medications will be offered the option to participate in the biomarker research. After giving written consent for the optional biomarker research, a plasma sample will be collected in accordance with the inclusion criteria and study schedule.

The plasma sample must be collected before administration of any investigational product, according to the study schedule. Only 1 sample should be collected per subject for biomarker research during the study. A 10 mL plasma sample will be collected in a lithium-heparin tube. Samples will be collected, labeled, stored, and shipped as detailed in the Safety Biomarker Laboratory Manual.

Some of the dataset (eg, age, gender, race, body, weight, height, BMI, and fasting state) from the main study may be duplicated within AstraZeneca for exploratory analyses in combination with the optional biomarker data. This information will be provided to AstraZeneca with the biomarker sample shipment. Neither the subject's name nor any other personal identifiers will be part of this dataset. Optional biomarker data will not be reported in the CSR.

For blood volume see Section 7.1.

7. BIOLOGICAL SAMPLING PROCEDURES

7.1 Volume of blood

The approximate total volume of blood that will be collected from each subject in this study is presented in Table 4.

Table 4 Volume of blood to be drawn from each subject

Assessment		Sample volume (mL)	No. of samples	Total volume (mL)
Safety	Clinical chemistry	8.5	8	68
	Hematology	4	8	32
	Serology	8.5	1	8.5
Pharmacokinetic	Ticagrelor and AR-C124910XX	2	26 (13 x 2)	52
	Cyclosporine	1	26 (13 x 2)	26
	Indwelling catheter[a]	2	26	52
Biomarkers (optional)		10	1	10
Total				**248.5**

a If an indwelling catheter is used, 2 mL of blood will be removed prior to the pharmacokinetic sample collections.

The number of samples collected and the volume required for each analysis may be changed during the study (ie, if additional samples are collected for repeated safety assessments). However, the maximum volume to be collected from each subject will not exceed 450 mL, the same volume as would be collected during a regular blood donation.

7.2 Handling, storage, and destruction of biological samples

The samples will be used up or disposed of after analyses or retained for further use as described below.

7.2.1 Safety samples

Blood samples for safety assessments will be disposed of after analysis.

7.2.2 Pharmacokinetic samples

Samples will be disposed of upon instruction from AstraZeneca, after the CSR has been finalized.

7.2.3 Biomarker samples

Biological samples for biomarker research can be retained on behalf of AstraZeneca for a maximum of 15 years following the last subject's last visit in the study. The results from future analyses will not be reported in the CSR, but separately in a Scientific Report.

7.3 Labeling and shipment of biohazard samples

The Investigator ensures that samples are labeled and shipped in accordance with the Laboratory Manual and the Biological Substance, Category B Regulations (materials containing or suspected to contain infectious substances that do not meet Category A criteria), see Appendix C International Air Transport Association (IATA) 6.2 Guidance Document.

Any samples identified as Infectious Category A materials are not shipped and no further samples will be taken from the subject unless agreed with AstraZeneca and appropriate labeling, shipment, and containment provisions are approved.

7.4 Chain of custody of biological samples

A full chain of custody is maintained for all samples throughout their life cycle.

The Investigator keeps full traceability of collected biological samples from the subjects while in storage at the study center until shipment or disposal (where appropriate) and keeps documentation of receipt of arrival.

The sample receiver keeps full traceability of the samples while in storage and during use until used or disposed of or until further shipment and keeps documentation of receipt of arrival.

AstraZeneca keeps oversight of the entire life cycle through internal procedures, monitoring of the study center, and auditing of external laboratory providers.

Samples retained for further use are registered in the AstraZeneca biobank system during the entire life cycle.

7.5 Withdrawal of informed consent for donated biological samples

If a subject withdraws consent to the use of donated biological samples, the samples will be disposed of or destroyed, and the action documented. If samples are already analyzed, AstraZeneca is not obliged to destroy the results of this research.

As collection of the biomarker sample is an optional part of the study, the subject may continue in the study even if consent is withdrawn for collection of this sample.

The Investigator:

- Ensures subjects' withdrawal of informed consent to the use of donated samples is notified immediately to AstraZeneca
- Ensures that biological samples from that subject, if stored at the study center, are immediately identified, disposed of or destroyed, and the action documented
- Ensures the laboratory(ies) holding the samples is/are informed about the withdrawn consent immediately and that samples are disposed of or destroyed, the action documented, and the signed document returned to the study center
- Ensures that the subject and AstraZeneca are informed about the sample disposal

AstraZeneca ensures the laboratory(ies) holding the samples is/are informed about the withdrawn consent immediately and that samples are disposed of or destroyed and the action documented and returned to the study center.

8. ETHICAL AND REGULATORY REQUIREMENTS

8.1 Ethical conduct of the study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with International Conference on Harmonization (ICH)/Good Clinical Practice (GCP), applicable regulatory requirements and the AstraZeneca policy on Bioethics and Human Biological Samples.

8.2 Subject data protection

The 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.

8.3 Ethics and regulatory review

An Institutional Review Board (IRB) should approve the final CSP, including the final version of the Informed Consent Form and any other written information and/or materials to be provided to the subjects. The Investigator will ensure the distribution of these documents to the applicable IRB, and to the study center personnel.

The opinion of the IRB should be given in writing. The Investigator should submit the written approval to AstraZeneca before enrollment of any subject into the study.

The IRB should approve all advertising used to recruit subjects for the study.

AstraZeneca should approve any modifications to the Informed Consent Form that are needed to meet local requirements.

If required by local regulations, the CSP should be re-approved by the IRB annually.

Before enrollment of any subject into the study, the final CSP, including the final version of the Informed Consent Form, is approved by the national regulatory authority or a notification to the national regulatory authority is done, according to local regulations.

AstraZeneca will handle the distribution of any of these documents to the national regulatory authorities.

AstraZeneca will provide regulatory authorities, the IRB, and the Investigator with safety updates/reports according to local requirements, including suspected unexpected serious adverse reactions (SUSARs), where relevant.

The Investigator is responsible for providing the IRB with reports of any serious and unexpected adverse drug reactions from any other study conducted with the investigational product. AstraZeneca will provide this information to the Investigator so that he/she can meet these reporting requirements.

8.4 Informed consent

The Investigator will:

- Ensure each subject is given full and adequate oral and written information about the nature, purpose, possible risk, and benefit of the study
- Ensure each subject is notified that they are free to discontinue from the study at any time
- Ensure that each subject is given the opportunity to ask questions and allowed time to consider the information provided

- Ensure each subject provides signed and dated informed consent before conducting any procedure specifically for the study
- Ensure the original, signed Informed Consent Form(s) is/are stored in the Investigator's Study File
- Ensure a copy of the signed Informed Consent Form is given to the subject
- Ensure that any incentives for subjects who participate in the study as well as any provisions for subjects harmed as a consequence of study participation are described in the Informed Consent Form that is approved by the IRB

8.5 Changes to the protocol and informed consent form

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

If there are any substantial changes to the CSP, then these changes will be documented in an Amendment and where required in a Revised CSP.

The Amendment is to be approved by the relevant IRB and if applicable, also the national regulatory authority, before implementation. Local requirements are to be followed for Revised CSPs.

AstraZeneca will distribute any subsequent Amendments and new versions of the CSP to the Investigator. For distribution to the IRB see Section 8.3.

If an Amendment requires a change to the Informed Consent Form, AstraZeneca, and the IRB are to approve the revised Informed Consent Form before the revised form is used.

If local regulations require, any administrative change will be communicated to or approved by the IRB.

8.6 Audits and inspections

Authorized representatives of AstraZeneca, a regulatory authority, or the IRB may perform audits or inspections at the study center, including source data verification. The purpose of an 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 CSP, ICH/GCP guidelines, and any applicable regulatory requirements. The Investigator will contact AstraZeneca immediately if contacted by a regulatory agency about an inspection at the study center.

9. STUDY MANAGEMENT BY

Study management will be performed by on behalf of AstraZeneca.

9.1 Pre-study activities

Before the first subject is entered into the study, it is necessary for to:

- Determine the adequacy of the facilities
- Determine availability of appropriate subjects for the study
- Discuss with the Investigator (and other study center personnel involved with the study) their responsibilities with regard to CSP adherence, and the responsibilities of and AstraZeneca. This will be documented in a Clinical Study Agreement

9.2 Training of study center personnel

Before the first subject is entered into the study, will review and discuss the requirements of the CSP and related documents with the study center personnel and also train them in any study-specific procedures and system(s) utilized.

The Investigator will ensure that appropriate training relevant to the study is given to all of these personnel, and that any new information relevant to the performance of this study is forwarded to the personnel involved.

The Investigator will maintain a record of all individuals involved in the study (medical, nursing, and other personnel).

9.3 Monitoring of the study

During the study, a monitor will have regular contact with the study center, including visits to:

- Provide information and support to the Investigator
- Confirm that facilities remain acceptable
- Confirm that the study center personnel are adhering to the CSP, that data are being accurately and timely recorded in the eCRFs, that biological samples are handled in accordance with the Laboratory Manual, and that investigational product accountability checks are being performed
- Perform source data verification (a comparison of the data in the eCRFs with the subject's medical records at the hospital or practice, and other records relevant to the study) including verification of informed consent of participating subjects. This will require direct access to all original records for each subject (eg, clinic charts)

- Ensure withdrawal of informed consent to the use of the subject's biological samples is reported and biological samples are identified and disposed of or destroyed accordingly, and the action is documented and reported to the subject

The monitor will be available between visits if the Investigator or other study center personnel needs information and advice about the study conduct.

9.3.1 Source data

Refer to the Clinical Study Agreement for the location of the source data.

9.4 Study agreements

The Investigator at the study center should comply with all the terms, conditions, and obligations of the Clinical Study Agreement, or equivalent, for this study. In the event of any inconsistency between this CSP and the Clinical Study Agreement, the terms of the CSP shall prevail with respect to the conduct of the study and the treatment of subjects. In all other respects not relating to study conduct or treatment of subjects the terms of the Clinical Study Agreement shall prevail.

Agreements between AstraZeneca, and the Investigator should be in place before any study-related procedures can take place, or subjects are enrolled.

9.4.1 Archiving of study documents

The Investigator follows the principles outlined in the Clinical Study Agreement.

9.5 Study timetable and end of study

The end of the study is defined as 'the last visit of the last subject undergoing the study'.

The clinical conduct of this study is expected to start in and to end by

The study may be terminated at the study center if the study procedures are not being performed according to GCP, or if recruitment is slow. AstraZeneca may also terminate the entire study prematurely if concerns for safety arise within this study or in any other study with ticagrelor.

10. DATA MANAGEMENT BY

Data management will be performed by

The data collected through third party sources will be obtained and reconciled against study data.

Adverse events and medical/surgical history will be classified according to the terminology of the latest version the Medical Dictionary for Regulatory Activities (MedDRA). Medications

will be classified according to the AstraZeneca Drug Dictionary. All coding will be performed by Data Management.

Data queries will be raised for inconsistent, impossible, or missing data. All entries to the study database will be available in an audit trail.

The data will be validated as defined in the Data Management Plan. Quality control procedures will be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly.

When all data have been coded, validated, and locked, clean file will be declared. Any treatment revealing data may thereafter be added and the final database will be locked.

Data associated with biological samples will be transferred to laboratories internal or external to AstraZeneca.

11. EVALUATION AND CALCULATION OF VARIABLES BY

11.1 Calculation or derivation of safety variable(s)

Where appropriate, change from baseline variables will be calculated for continuous variables as the post-treatment value minus the value at baseline. Baseline for the statistical analyses will be defined as:

- Clinical laboratory tests: Day -1
- Vital signs: Day 1 pre-dose

If a subject is missing the baseline collection, the previous non-missing evaluation in the period will become the baseline value. If no baseline or previous to baseline evaluations exist, then the baseline value will be treated as missing.

11.1.1 Other significant adverse events

During the evaluation of the AE data, an AstraZeneca medically qualified expert will review the list of AEs that were not reported as SAEs and discontinuation due to AEs. Based on the expert's judgment, significant AEs of particular clinical importance may, after consultation with the Global Patient Safety Physician, be considered other significant adverse events (OAEs) and reported as such in the CSR. A similar review of other safety data will be performed for identification of 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.

11.2 Calculation or derivation of pharmacokinetic variables

The PK analysis of ticagrelor and its metabolite AR-C124910XX will be the responsibility of the Pharmacokineticist at . Standard Operating Procedures and Work Instructions will be used as the default methodology if not otherwise specified.
Actual sampling times will be used for the computation of PK parameters.

Pharmacokinetic parameters will be derived using standard non-compartmental methods with WinNonlin® Professional Version 5.2, or higher
All PK computations will be performed using WinNonlin® Professional Version 5.2 (or higher) or SAS® Version 9.2 or higher
Graphics will be prepared using SAS® Version 9.2 and Sigmaplot® 9.0

Subjects who withdraw from the study following dosing, but prior to study completion, will be included in the PK analysis provided they have evaluable concentrations over the planned collection period. Subjects with partial data will be evaluated on a case-by-case basis to determine if sufficient data are available for meaningful analysis.

If data permits, the following plasma PK parameters will be calculated for ticagrelor and its metabolite AR-C124910XX:

- Observed maximum plasma concentration (C_{max})
- Time to maximum concentration (t_{max})
- Area under plasma concentration-time curve from zero to the last measurable concentration [$AUC_{(0-t)}$]
- Area under plasma concentration-time curve from zero to infinity (AUC)
- Terminal half-life ($t_{1/2}$)

Additionally, the following will be computed for Treatments A and C:

- AR-C124910XX: ticagrelor ratios for $C_{max,}$ $AUC_{(0-t)}$, and AUC

Similarly, if data permits the following pharmacokinetic parameters will be calculated for cyclosporine in whole blood:

  - Observed maximum whole blood concentration (C_{max})
  - Time to maximum concentration (t_{max})
  - Area under whole blood concentration-time curve from zero to the last measurable concentration [$AUC_{(0-t)}$]

- Area under whole blood concentration-time curve from zero to infinity (AUC)
- Terminal half-life ($t_{1/2}$)

The following PK diagnostic parameters will be calculated for all analytes, as appropriate, and will be listed, but not summarized:

- The time interval ($t_{1/2}$, interval) of the log-linear regression to determine $t_{1/2}$
- Number of data points ($t_{1/2}$, N) included in the log-linear regression analysis to determine $t_{1/2}$ (a minimum of 3 points will be used)
- Regression coefficient (Rsq), a goodness-of-fit statistic for calculation of λ_z. If Rsq is less than 0.80, then $t_{1/2}$ and related parameters will not be reported
- Percent AUC that is extrapolated (%AUCext)

If %AUCext is greater than 20% then AUC will not be reported.

12. STATISTICAL METHODS AND SAMPLE SIZE DETERMINATION BY

12.1 Description of analysis sets

12.1.1 Safety analysis set

All subjects who received at least 1 dose of ticagrelor and for whom any post-dose data are available will be included in the safety analysis set.

12.1.2 PK analysis set

The PK analysis set will be a subset of the safety analysis set and will include only subjects who receive at least 1 administration of investigational product and have at least 1 post-dose plasma concentration measurement without important protocol deviations/violations or events thought to significantly affect the PK (eg, subject vomited at or before 2 times median t_{max}; wrong dose administered; prohibited concomitant medication; etc).

12.2 Methods of statistical analyses

12.2.1 General principles

The statistical analyses will be performed using SAS®, Version 9.2 or higher.
Standard Operating Procedures and Work Instructions will be used as the default methodology if not otherwise specified.

Missing data will result in a reduced sample size for that parameter. No action will be taken to handle missing data.

No adjustment for multiplicity will be made.

A subject who withdraws prior to the last planned observation in a study period will be included in the analyses up to the time of discontinuation.

All data will be included in the data listings. Any data excluded from the summaries and statistical analyses will be flagged accordingly.

12.2.2 Subject characteristics

Continuous variables will be summarized using descriptive statistics (n, mean, standard deviation [SD], minimum, median, and maximum) in total. Categorical variables will be summarized in frequency tables (frequency and proportion) in total.

12.2.3 Safety and tolerability

All safety data will be listed in subject listings. Continuous variables (hematology, clinical chemistry, and vital signs) will be summarized using descriptive statistics (n, mean, SD, minimum, median, and maximum) by treatment and/or each scheduled assessment point, both as absolute values and as change from baseline. Categorical variables (urinalysis, ECG interpretation) will be summarized in frequency tables (frequency and proportion) by scheduled assessment point. Laboratory values and vital signs outside reference limits will be marked high (H) and low (L) where appropriate.

For laboratory data and vital signs assessments all repeat values will be included in the data listings. For calculation of descriptive statistics the following rules will be applied: for measurement time points before the first dose the last valid repeat value will be used; for measurement time points after the first dose the first valid value will be used; retake of a pre-dose value will not be used if taken after dose. Laboratory values below the detectable limit (< X) will be replaced by X when summarizing mean, SD etc.

Abnormalities found in physical examinations will be listed.

Adverse events will be summarized by Preferred Term and System Organ Class using the MedDRA.

The number of subjects who had any AEs, SAEs, discontinuation due to AEs (DAEs), OAEs, AEs with severe intensity, and AEs judged causally related to the investigational product by the Investigator will be summarized. Any SAEs and DAEs will be listed separately.

12.2.4 Pharmacokinetics

A listing of PK blood sample collection times as well as derived sampling time deviations will be provided. Plasma concentrations and PK parameters for ticagrelor and its metabolite will be summarized using appropriate descriptive statistics (ie, n, mean, SD, geometric mean, geometric coefficient of variation [CV], minimum, median, maximum) by analyte and treatment. The geometric mean is calculated as the exponential of the mean calculated from

data on a log scale. The CV% is calculated as $100 \cdot \sqrt{\left(\exp\left(s^2\right)-1\right)}$ where s is the SD of the data on a log scale.

For descriptive statistics, plasma concentrations that are below LLOQ will be handled as follows:

- At a time point where less than or equal to 50% of the values are below the LLOQ (BLQ), all BLQ values will be set to LLOQ, and all descriptive statistics will be calculated
- At a time point where more than half of the values are BLQ, the mean, SD, geometric mean and CV% will be set to Not Determined (ND). The maximum value will be reported from the individual data, and the minimum and median will be set to BLQ
- If all values are BLQ at a time point, no descriptive statistics will be calculated for that time point. Not Applicable (NA) will be written in the field for SD and CV% and BLQ will be written in fields for mean, geometric mean, minimum, median, and maximum
- The number of BLQ values (n below LLOQ) will be reported for each time point

Graphical presentations will include mean (±SD) plasma concentration-time curves by treatment and individual subject plasma concentration-time curves over the PK sampling times. The PK parameters will be presented graphically by scatter plots comparing individual and mean PK parameters between treatments.

The PK parameters for ticagrelor, AR-C124910XX, and cyclosporine will be natural log-transformed prior to analysis. The natural log-transformed AUC and C_{max} data will be separately analyzed using a mixed effects model with terms for treatment, period, and sequence as fixed effects and subject nested within sequence as a random effect. The estimated least squares means and intra-subject variability from the mixed effects model will be used to construct 90% confidence intervals (CIs) for the difference in means on the log scale between the 2 treatments. The treatment effect and its corresponding 90% CIs will be back-transformed using anti-logarithms to its original scale and reported as ratio of treatments (ticagrelor + cyclosporine / ticagrelor alone or ticagrelor + cyclosporine / cyclosporine alone).

- If the 90% CIs for both AUC and C_{max} for the effect of cyclosporine on ticagrelor are entirely contained within the pre-specified range of 0.80 to 1.25 then it will be conclude that a single 600 mg dose of cyclosporine has no effect on the PK of ticagrelor
- Also, if the 90% CIs for both AUC and C_{max} for the effect of ticagrelor on cyclosporine are entirely contained within the pre-specified range of 0.80 to 1.25, then it will be conclude that a single 180 mg dose of ticagrelor has no effect on the PK of cyclosporine

12.3 Determination of sample size

Based on a previous study (D5130C00047), the intra-subject CV for AUC and C_{max} of ticagrelor and AR-C124910XX are estimated to be less than or equal to 19%. Using an intra-subject variability of 19%, a sample size of 18 subjects would provide 90% power that the 2-sided 90% CI for the ratios of interest (AUC and C_{max}) of ticagrelor to that of ticagrelor administered with cyclosporine would be completely contained within the pre-specified equivalence range of 0.80 to 1.25. Twenty-six subjects would be enrolled to allow for drop-outs. These calculations were based on two 1-sided tests at an alpha level of 0.05, assuming a true ratio of 1.0.

13. IMPORTANT MEDICAL PROCEDURES TO BE FOLLOWED BY THE INVESTIGATOR

13.1 Medical emergencies and AstraZeneca contacts

The Investigator is responsible for ensuring that procedures and expertise are available to handle medical emergencies during the study. **A medical emergency usually constitutes an SAE and is to be reported as such, see Section 6.3.4.**

In the case of a medical emergency the Investigator may contact the CPA Physician. If the CPA Physician is not available, contact the CPA Program Director at AstraZeneca Research and Development.

Name	Role in the study	Address & telephone number
	AstraZeneca CPA Program Director	
	AstraZeneca CPA Physician	
Serious adverse event reporting	24-hour emergency cover at central R&D site	
	Principal Investigator	
	Project Manager	

13.2 Overdose

There is currently no known antidote to reverse the effects of ticagrelor, and ticagrelor is not expected to be dialyzable. Treatment of an overdose should follow local standard medical

practice. Bleeding is the expected pharmacologic effect of excessive ticagrelor dosing, if bleeding occurs, appropriate supportive measures should be taken.

Ticagrelor is well tolerated in single doses up to 900 mg. Gastrointestinal toxicity was dose-limiting in a single ascending dose study. Other clinically meaningful adverse effects which may occur with overdose include dyspnea and ventricular pauses.

In the event of overdose, observe for these potential adverse effects and consider ECG monitoring.

- An overdose with associated AEs is recorded as the AE diagnosis/symptoms on the relevant AE modules in the eCRF and on the Overdose eCRF module
- An overdose without associated symptoms is only reported on the Overdose eCRF module

If an overdose on an AstraZeneca investigational product occurs during the course of the study, then the Investigator or other study center personnel will inform appropriate AstraZeneca representatives **within 1 day**, ie, immediately but no later than **the end of the next business day** of when he/she becomes aware of it.

The designated AstraZeneca representative works with the Investigator to ensure that all relevant information is provided to the AstraZeneca Patient Safety data entry site.

For overdoses associated with SAEs, standard reporting timelines apply, see Section 6.3.4. For other overdoses, reporting should be done within 30 days.

13.3 Pregnancy

All outcomes of pregnancy should be reported to AstraZeneca.

13.3.1 Maternal exposure

Women will not be included in this study.

13.3.2 Paternal exposure

Subjects should refrain from fathering a child or donating sperm during the study and 4 weeks following the last dose.

Pregnancy of a subject's partner will not be considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be monitored and documented, if possible.

The outcome of any conception occurring from the date of the first dose until 4 weeks after the last dose should be followed up and documented.

14. LIST OF REFERENCES

FDA Guidance for Industry, Drug Interaction Studies 2006
Food and Drug Administration. Guidance for Industry. Drug interaction studies – study design, data analysis, and implications for dosing and labeling. (Draft guidance.) United States Department of Health and Human Services, Food and Drug Administration Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research.

Clinical Study Protocol Appendix B

Drug Substance	Ticagrelor
Study Code	D5130C00074
Edition Number	1
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).

Hospitalization

Outpatient treatment in an emergency room is not in itself a serious AE, although the reasons for it may be (eg, bronchospasm, laryngeal edema). 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 Study Protocol Appendix C

Drug Substance	Ticagrelor
Study Code	D5130C00074
Edition Number	1
Date	

Appendix C
International Airline Transportation Association (IATA) 6.2 Guidance Document

LABELING AND SHIPMENT OF BIOHAZARD SAMPLES

International Airline Transportation Association (IATA) classifies biohazardous agents into 3 categories **(http://www.iata.org/whatwedo/cargo/dangerous_goods/infectious_substances.htm).** For transport purposes the classification of infectious substances according to risk groups was removed from the Dangerous Goods Regulations (DGR) in the 46th edition (2005). Infectious substances are now classified either as Category A, Category B or Exempt. There is no direct relationship between Risk Groups and categories A and B.

Category A Infectious Substances are infectious substances in a form that, when exposure to it occurs, is capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals. Category A pathogens are eg, Ebola, Lassa fever virus:

- are to be packed and shipped in accordance with IATA Instruction 602.

Category B Infectious Substances are infectious Substances that do not meet the criteria for inclusion in Category A. Category B pathogens are eg, Hepatitis A, B, C, D, and E viruses, Human immunodeficiency virus (HIV) types 1 and 2. They are assigned the following UN number and proper shipping name:

- UN 3373 – Biological Substance, Category B
- are to be packed in accordance with UN3373 and IATA 650

Exempt - all other materials with minimal risk of containing pathogens

- Clinical trial samples will fall into Category B or exempt under IATA regulations
- Clinical trial samples will routinely be packed and transported at ambient temperature in IATA 650 compliant packaging **(http://www.iata.org/whatwedo/cargo/dangerous_goods/infectious_substances.htm)**
- **Biological samples transported in dry ice require additional dangerous goods specification for the dry-ice content**
- IATA compliant courier and packaging materials should be used for packing and transportation and packing should be done by an IATA certified person, as applicable

- Samples routinely transported by road or rail are subject to local regulations which require that they are also packed and transported in a safe and appropriate way to contain any risk of infection or contamination by using approved couriers and packaging / containment materials at all times. The IATA 650 biological sample containment standards are encouraged wherever possible when road or rail transport is used.

Clinical Study Protocol Appendix D

Drug Substance	Ticagrelor
Study Code	D5130C00074
Appendix Edition Number	1
Appendix Date	

Appendix D
Optional Biomarker Research Samples

OPTIONAL BIOMARKER RESEARCH SYNOPSIS

A Single-Center, Open-Label, Randomized, 2-Treatment, 2-Period Cross-Over Study to Investigate the Effect of Cyclosporine on the Pharmacokinetics of Ticagrelor in Healthy Male Subjects

The research activities described in this appendix (including the collection and storage of body fluid samples), are optional for study centres as well as for individual healthy volunteers. These research activities will hereafter be referred to as "this research." The clinical study protocol to which this document is appended will be referred to as "the main study." The term "sample" means:

Plasma, Serum or Urine

This research will be performed only after the appropriate Ethics Committee/IRB has approved it. Informed consent for this research will be obtained using a separate Informed Consent Form from that used for the main study. All sections of the protocol for the main study also apply to this research.

Study center and number of patients who may be enrolled in this biomarker research

It is the intent of AstraZeneca to collect serum, plasma or urine samples from all Clinical Pharmacology studies conducted by the Clinical Pharmacology Alliance (CPA) to further the goal of improving biochemical markers that can be used to monitor or predict drug-induced organ damage. The goal will be to collect approximately 3000 such samples.

Objectives

Objective	Outcome variables
To analyze biological samples (eg, human plasma) for circulating biomarkers from consenting volunteers prior to drug treatment.	

Study design

It is proposed to collect a single serum, plasma or urine from each subject/healthy volunteer enrolled in the trial as optional samples for biomarker analysis. The type of sample to be collected will be determined at the outset of the trial. Provision of these samples for analysis will be optional for all healthy volunteers entering the study, and acceptance of this procedure will not be a requirement for participation in the main study.

The samples and data for optional biomarker analysis in this research will be coded. Each sample will be labelled with the study number and patient enrolment number (E-code). Only the investigator will be able to link the sample to the individual healthy volunteer. The samples and data will not be labelled with personal details.

Target population

All consenting volunteers in all centres participating in the main study.

Statistical methods

The number of volunteers who will agree to participate in this research is unknown. It is therefore not possible to establish whether sufficient data will be generated. A statistical analysis plan will be prepared where appropriate.

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation or special term	Explanation
eCRF	electronic Case Report Form
CSR	Clinical study report
DNA	Deoxyribonucleic acid
EDTA	Ethylenediamine tetra-acetic acid
ICH	International Conference on Harmonisation
pCRF	paper Case Record Form
PD	Pharmacodynamic
PGx	Pharmacogenetics
PK	Pharmacokinetic
RNA	Ribonucleic acid
UK	United Kingdom

1. BACKGROUND

As part of collaborative efforts with other pharmaceutical companies, diagnostic companies and academic institutions) AstraZeneca is collecting samples to perform general research for variations in "safety" biomarker profiles. These biomarkers may be derived from proteins and/or metabolites. By using this information, the aim is to better understand drug effect on major organs in the human body and how circulating biomarkers can be used to better monitor organ function and thus improve safety of drugs.

To achieve this goal, a systematic collection of biological samples (urine, serum and/or blood plasma) will be undertaken as specified where appropriate.

1.1 Rationale for research

AstraZeneca may perform optional sampling determination for biomarker research in some of the studies for the clinical programs of new chemical entities under development. The objective of this research is to explore normal variations in biomarkers (protein or small molecule based) that occur in individuals enrolled in this trial **prior to drug treatment**. In particular, developing better biochemical markers to help assess potential deleterious drug is the primary goal of this research. A key aspect to understanding how to use these new markers is to assess the normal variation in these markers in healthy individuals so we will appropriately interpret how changes in these new biochemical markers are affected by drug treatment. Understanding this normal variation is part of a process known as qualification which attempts to establish sufficient evidence of changes in these biomarkers in relationship to organ damage that they are suitable for monitoring safety for clinical trials. Other recent studies have suggested that using proteomic and metabolomic platforms may help identify other new predictive biomarkers that help explain ALT elevation (Andersson et al 2009).

The ability to acquire appropriate consent to collect biological samples to establish an archive and allow future meta-analysis of data derived from a number of studies is of the utmost importance. This research forms part of this strategy.

The benefits of being able to explore associations between biomarker variations and clinical outcomes are potentially many including the possibility to identify volunteers early who may be at risk of adverse drug reaction or to explain potential adverse reactions related to drug exposure.

2. RESEARCH OBJECTIVES

Biomarker technologies enable the measurement of many different molecules, including proteins and metabolites, within a sample. The objective of this research is to determine if correlations exist between traditional biomarkers used to monitor organ function (such as ALT and bilirubin for liver) and new biomarkers that may be more sensitive and/or specific indicators of drug induced organ damage Research plan and procedures

2.1 Research plan

The healthy volunteer will be asked to participate in this optional biomarker research during their enrolment or screening visit. If the volunteer agrees to participate the following samples will be requested:

A single 10 mL blood sample or a single urine sample of up to 15 mL.

2.2 Selection of optional biomarker research population

2.2.1 Study selection record

All healthy volunteers who take part in the study will be asked to participate in this optional biomarker research. Participation is voluntary and if a volunteer declines to participate in this optional biomarker research they will not be excluded from any aspect of the main study.

2.2.2 Withdrawal of healthy volunteers from this optional biomarker research

2.2.2.1 Criteria for withdrawal

Specific reasons for withdrawing a volunteer from this optional biomarker research are:

- Withdrawal of consent for optional biomarker research. Volunteers may withdraw from this optional biomarker research at any time, independent of any decision concerning participation in other aspects of the main study. Voluntary withdrawal will not prejudice further treatment.

2.2.2.2 Procedures for withdrawal

Volunteers who withdraw from the main study should always be asked specifically whether they are withdrawing or continuing their consent for this optional biomarker research. It must be established whether the volunteer:

- Agrees to the optional biomarker samples and any preparations derived from the sample being kept for research in the future

- Withdraws consent for the samples to be kept for optional biomarker research in the future and wishes the samples to be destroyed. Destruction of the samples (or the preparations derived from the samples) will only be possible so long as the particular samples are traceable. In the event that optional biomarker research has already been performed, AstraZeneca will retain the results and associated data for regulatory reasons but these will not be used in any subsequent analyses.

The principal investigator is responsible for providing written notification to AstraZeneca of any volunteer who has withdrawn consent for the use of the sample taken for optional biomarker research. AstraZeneca will provide written confirmation to the investigator of the actions taken with the sample, which must be filed in the investigator study file.

3. MEASUREMENTS AND CO-VARIABLES

3.1 Summary of objectives and analysis

The purpose of this research is to generate data that will help interpret results from future clinical trials. The results of this research will not form part of the clinical study report (CSR) for the main study. The results may be pooled with data from other studies generate hypotheses to be tested in future studies.

3.2 Collection of samples for Optional Biomarker Research

AstraZeneca or its designee will act as the central laboratory for sample logistics. Details of sample collection, processing, shipping and storage will be described in the laboratory manual.

The samples and data for analysis in this research will be coded and will not be labelled with any personal details. Each sample will be identified with the study number and patient enrolment number. In this way biomarker data may be correlated with clinical data, samples destroyed in the case of withdrawal of consent and regulatory audit enabled. However, only the investigator will be able to link the biomarker sample to the individual volunteer.

The coded samples may be made available to groups or organisations working with AstraZeneca on this research or as part of the development drug project. However, the samples and any results will remain the property of AstraZeneca at all times. AstraZeneca will not give samples, sample derivatives or data derived from the samples to any other parties, except as required by law.

3.2.1 Biomarker Analysis

The precise details of the biomarker analysis will be established by AstraZeneca scientists. However, in some cases, samples may be sent to commercial or academic partners for specialized analyses.

In addition to studies to identify new candidate biomarkers, samples may also be used to measure existing candidate biomarkers by methods that will depend on the specific biomarker.

4. MANAGEMENT OF RESEARCH DATA

Some of the dataset from the main study may be duplicated within AstraZeneca for exploratory analyses in combination with the optional biomarker data. Neither the volunteer's name nor any other personal identifiers will be part of this dataset. Optional biomarker data will not be reported in the CSR. Only the date the volunteer gave consent to participation in the research and the date and time the biological sample(s) (if applicable) was taken from the patient will be recorded in the electronic Case Record Form (eCRF) and database.

AstraZeneca will not provide optional biomarker research results to volunteers, their family members, any insurance company, an employer, clinical study investigator, general physician or any other third party, unless required to do so by law. The volunteer's samples will not be used for any purpose other than optional biomarker research.

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

5. STATISTICAL METHODS

One of the primary goals of these exploratory studies is to establish a large sample cohort that will help us understand the variability of new biomarkers in a general population. The samples that compose this cohort will come from multiple different studies and a statistical analysis plan will be prepared for analyses of each new biomarker. This analysis will help us determine how many subjects will be needed for future trials. However, neither the results of this biomarker work nor the statistical analysis will be included in CSR for the trials from which these samples have been collected.

5.1 Monitoring

During the study, monitors will have regular contacts with the investigational centres. One of the purposes of these visits will be to perform source verification of the informed consent of participating volunteers and to ensure that the investigational team are adhering to the specific requirements of this optional biomarker research.

5.2 Training of staff

Before the first volunteer is entered into the study the investigational staff will have an opportunity to discuss the procedures associated with the collection of samples and optional biomarker research with a representative of AstraZeneca. The requirements for the collections of the volunteers' sample will also be made clear.

5.3 Changes to the protocol

Any changes to the optional biomarker research will comply with the principles described in Section 8.5 of the main body of the protocol.

5.4 Study agreements

The principal investigator at each study centre must comply with all the terms, conditions, and obligations of the Clinical Study Agreement for this research. In the event of any inconsistency between this Clinical Study Protocol (CSP) and the Clinical Study Agreement, the CSP shall prevail. Specific reference to requirements relating to this optional biomarker research will be included in the study agreement(s).

6. ETHICS

6.1 Ethics review

In addition to documenting Ethics Committee/IRB approval of the main study, approval must be obtained for this optional biomarker research and the associated informed consent from the relevant Ethics Committee. It must be clearly stated in the approval that this optional biomarker research is approved. The investigator must submit written approval to AstraZeneca before any patient participates in this optional biomarker research.

6.2 Ethical conduct of the study

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

6.3 Informed consent

The biomarker component of this research is optional and the volunteer may participate in other components of the study without participating in the optional biomarker component. To participate in the optional biomarker component of the study, the volunteer must sign and date both the consent form for the main study and the consent form for the optional biomarker component of the study. Copies of both signed and dated consent forms must be given to the volunteer and the originals filed at the study centre in the investigator's study file. The principal investigator is responsible for ensuring that consent is given freely and that the volunteer understands that they may freely discontinue from the optional biomarker aspect of the study at any time.

6.4 Volunteer data protection

All data protection and confidentiality principles, described in the main study protocol, are applicable to this optional biomarker research.

Due to the exploratory nature of this optional biomarker research, there will be no routine communication of results to volunteers. AstraZeneca will not provide individual results to volunteers, any insurance company, any employer, their family members, general physician or any other third party, unless required to do so by law.

7. REFERENCES

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Andersson U, Lindberg J, Wang S, Balasubramanian R, Marcusson-Ståhl M, Hannula M. A systems biology approach to understanding elevated serum alanine transaminase levels in a clinical trial with ximelagatran. Biomarkers. 2009 Sep 25. Biomarkers 2009;14(8):572-586

Ozer et al 2008
Ozer J, Ratner M, Shaw M, Bailey W, Schomaker S The current state of serum biomarkers of hepatotoxicity . Toxicology. 2008 Mar 20;245(3):194-205.