Clinical Study Protocol

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A open label, randomized, cross-over and potential parallel, single dose study of ticagrelor 180 mg and acetylsalicylic acid (ASA) in healthy volunteers followed by autologous *in vivo* platelet transfusion to determine the effects of platelet supplementation on the reversibility of platelet inhibition

Sponsor: AstraZeneca AB, 151 85 Södertälje, Sweden

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

Amendment No.	Date of Amendment	Local Amendment No:	Date of Local Amendment

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AstraZeneca

PROTOCOL SYNOPSIS

A open label, randomized, crossover and potential parallel, single dose study of ticagrelor 180 mg and acetylsalicylic acid (ASA) in healthy volunteers followed by autologous *in vivo* platelet transfusion to determine the effects of platelet supplementation on the reversibility of platelet inhibition

Principal Investigator

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Quintiles Early Clinical Development Services

Study center(s) and number of subjects planned

The study will be conducted at the Quintiles Early Clinical Development Services, Overland Park, United States, under the supervision of Dr Phil Leese. Approximately 48 healthy male and female (of non-childbearing potential) volunteers will be randomised.

Study period		Phase of development
Estimated date of first healthy volunteer enrolled	Q4 2012	Clinical Pharmacology (Phase I)
Estimated date of last healthy volunteer completed	Q2 2013	

Objectives

Primary objective

To evaluate the effect of autologous platelet transfusions in healthy volunteers at 24 and 48 hours (and potentially 12 hours)[1], after a loading dose of ticagrelor, on platelet inhibition as measured by light transmission aggregometry (LTA)

[1] If the 24-hour and 48-hour cohorts are both successful, a platelet transfusion at 12 hours after the loading dose of ticagrelor (Day 1) in a single treatment, without crossing over to a non-transfusion period, hence a parallel group, will be performed.

Secondary objectives

- To potentially evaluate the effect of autologous platelet transfusions in healthy volunteers at either 12, 24 or 48 hours after a loading dose of clopidogrel on platelet inhibition as measured by LTA[1]
- To evaluate the effect of autologous platelet transfusions in healthy volunteers at 24 and 48 hours (and potentially 12 hours) after a loading dose of ticagrelor on platelet inhibition as measured by VerifyNow™
- To potentially evaluate the effect of autologous platelet transfusions in healthy volunteers at either 12, 24 or 48 hours after a loading dose of clopidogrel on platelet inhibition as measured by VerifyNow™
- To evaluate the durability of restoration of platelet aggregation following platelet transfusion

Safety objective

To describe the safety and tolerability of ticagrelor when administered as a loading dose prior to an autologous platelet transfusion.

Study design

This is an open label, randomized, cross-over study of 180 mg ticagrelor administered in healthy volunteers followed by autologous in vivo platelet transfusion to determine the effects of platelet supplementation on the reversibility of platelet inhibition. The study will be conducted at a single study center in approximately 48 healthy male and female (of non-childbearing potential) volunteers aged 18 to 50 years (inclusive).

The study will consist of 2 treatments each for cohort 1 (platelet transfusion at 24 hours) and cohort 2 (platelet transfusion at 48 hours):

Treatment A: platelet apheresis on Day -2 followed by a ticagrelor 180 mg loading dose on Day 1 for cohort 1; and platelet apheresis on Day -1 followed by a ticagrelor 180 mg loading dose on Day 1 for cohort 2, respectively. Background ASA treatment (81 mg once daily) will commence immediately after platelet apheresis on the relevant day, based on the randomization scheme and treatment group, up to and including the day prior to the platelet transfusion.

Treatment B: ticagrelor 180 mg loading dose on Day 1. Background ASA treatment (81 mg once daily) will commence on the relevant day, based on the randomization scheme and treatment group, up to and including the day prior to the randomized platelet transfusion from Treatment A.

During each treatment period healthy volunteers will receive a loading dose of 180 mg of ticagrelor with background ASA. The healthy volunteers will have platelet apheresis and

platelet transfusion performed during 1 of the 2 treatment periods based on the randomization scheme:

Cohort 1: will have the platelet transfusion performed 24 hours after receiving the 180 mg ticagrelor loading dose. Healthy volunteers will receive ASA background treatment once on Day -2 immediately after completion of the platelet apheresis, on Day -1, and again on Day 1 when ticagrelor is administered.

Cohort 2: will have the platelet transfusion performed 48 hours after receiving the 180 mg ticagrelor loading dose. Healthy volunteers will receive ASA background treatment once on Day -1 immediately after completion of the platelet apheresis, and again on Day 1 when ticagrelor is administered, as well as once on Day 2.

The 180 mg loading dose administrations of ticagrelor will be separated by a washout period of at least 14 days.

After completion of cohort 1 and cohort 2, a decision will be made regarding the continuation of the study and the subsequent cohort(s) to be initiated according to the results observed from cohort 1 and cohort 2.

Healthy volunteers will be evaluated for eligibility at screening (Visit 1, Days -30 to -3), which will take place no more than 28 days before Visit 2. Eligible healthy volunteers will be admitted to the study center, and randomized to one of the 2 treatment periods for cohort 1 or cohort 2, on Day -2. Healthy volunteers will remain in the study center until 96 hours (Day 5) after the ticagrelor loading dose was administered (Day 1).

Approximately 48 healthy volunteers will be randomized based on the total number of cohorts to be assessed. There is a minimum of 2 cohorts and up to a maximum of 4 cohorts planned for the study. Each cohort will comprise 12 healthy volunteers. The study duration for each healthy volunteer will be approximately 9 weeks, comprising 4 visits.

The screening for the 12 healthy volunteers needed for the relevant clopidogrel cohort will include the assessment of their cytochrome P450 (CYP) 2C19 genotype status to ensure that they carry 2 functional (*1) alleles (2C19 *1/*1). An additional 5 day pre-screening will be performed to allow time for the assessment of the CYP2C19 enzymatic status of the 12 evaluable healthy volunteers to be included in the relevant clopidogrel cohort.

Target subject population

Healthy male and/or female volunteers aged 18 to 50 years, inclusive, with a platelet count >240 $x10^9$/L. Healthy volunteers with adenosine diphosphate (ADP)-induced platelet aggregation <60% prior to platelet apheresis will not be included.

Only healthy volunteers with cytochrome P450 (CYP) 2C19 *1/*1 genotypes (normal/extensive metabolizers) will be included in the relevant clopidogrel cohort.

Investigational product, dosage and mode of administration

Ticagrelor 180 mg (2 x 90 mg tablets) loading dose administered once, orally on Day 1 of each treatment period.

Comparator, dosage and mode of administration

Clopidogrel (PLAVIX®) 600 mg (2 x 300 mg tablets) administered once, orally on Day 1 if randomized to the clopidogrel cohort.

A background treatment of ASA (81 mg chewable tablet) administered from Day -2 (cohort 1) or Day -1 (cohort 2) to the day before the platelet transfusion for each treatment period according to the randomization scheme. The ASA administration will commence immediately after the platelet apheresis.

Each healthy volunteer will have a platelet apheresis either on Day -2 (cohort 1) or Day -1 (cohort 2) and one autologous apheresis platelet unit transfusion during one of the treatment periods at either 24, 48, or 12 hours based upon the cohort assignment.

Duration of treatment

The study duration for each healthy volunteer will be approximately 9 weeks, comprising 4 visits. The 180 mg loading dose ticagrelor administrations will be separated by a washout period of at least 14 days.

Outcome variable(s):

- Pharmacodynamics
  - Percent of inhibition of adenosine phosphatase (ADP)-induced platelet aggregation assessed by light transmission aggregometry (LTA)
  - Platelet reactivity as measured by $P2Y_{12}$ Reaction Units (PRU) using VerifyNow™
- Safety

  Vital signs (blood pressure and pulse rate), electrocardiogram, laboratory safety data (clinical chemistry, hematology, complete blood count, and urinalysis), physical examination and adverse events

Statistical methods

All safety and pharmacodynamic data recorded during the study will be listed and summarized as appropriate. Continuous variables will be summarized using descriptive statistics (n, mean, standard deviation, minimum, median, maximum) by cohort, treatment, and by time points where applicable. Categorical variables will be summarized in frequency tables (frequency and proportion) by cohort and treatment. Graphical presentations will be used as appropriate.

To assess the effect of platelet transfusion on the inhibition of platelet aggregation (IPA) determined by LTA and VerifyNow™, 12-hour post transfusion values will be compared by cohort. LTA IPA will be the primary endpoint and VerifyNow PRU will be the secondary. Where data are available, comparisons between the platelet transfusion treatment (test) and those without platelet transfusion treatment (reference) will be performed by IPA method and cohort. Least squares means and associated 95% confidence interval (CI) will be calculated and presented. Also, the difference between platelet transfusion and without transfusion treatment and associated 95% CI's will be calculated and presented.

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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
ACS	Acute coronary syndrome
ADP	Adenosine diphosphate
AE	Adverse event (see definition in Section 6.4.1)
ALP	Alkaline phophatase
ALT	Alanine aminotransferase
aPTT	Activated partial thrombin time
ASA	Acetylsalicylic acid
AST	Aspartate aminotransferase
AV	Atrioventricular
B	Blood
BMI	Body mass index
bpm	Beats per minute
CABG	Coronary artery bypass graft
CI	Confidence interval
CPA	Clinical Pharmacology Alliance
CSA	Clinical Study Agreement
CSP	Clinical Study Protocol
CSR	Clinical Study Report
CYP	Cytochrome P450
DBP	Diastolic blood pressure
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
FSH	Follicle-stimulating hormone
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
GRand	Global randomization scheme
HBsAg	Hepatitis B surface antigen

Abbreviation or special term	Explanation
HIV	Human immunodeficiency virus
IATA	International Airline Transportation Association
ICH	International Conference on Harmonization
IPA	Inhibition of platelet aggregation
INR	International normalised ratio
IRB	Institutional Review Board
LH	Luteinizing hormone
LIMS	Laboratory Information Management System
LTA	Light transmission aggregometry
max	Maximum
MedDRA	Medical Dictionary for Regulatory Activities
MI	Myocardial infarction
min	Minimum
MTD	Maximum tolerated dose
NSTEMI	Non-ST segment elevation myocardial infarction
PCI	Percutaneous coronary intervention
Platelet apheresis	Any procedure in which blood is withdrawn from a donor, a fluid or solid portion (plasma, leukocytes, platelets, etc) is separated and retained, and the remainder is retransfused into the donor. Also called platelet pheresis
PK	Pharmacokinetics
PRU	$P2Y_{12}$ Reaction Units
PT	Partial prothrombin time
S	Serum
SAE	Serious adverse event (see definition in Section 6.4.2).
SBP	Systolic blood pressure
SD	Standard deviation
SOC	System Organ Class
SOP	Standard Operating Procedure
STEMI	ST segment elevation myocardial infarction
SUSAR	Suspected unexpected serious adverse reactions
TT	Thrombin time
U	Urine

Abbreviation or special term	Explanation
UA	Unstable angina
ULN	Upper limit of normal

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 [UA] and myocardial infarction [MI]) and cerebral (eg, stroke and transient ischemic attack) thrombotic events. The term "acute coronary syndromes" (ACS) encompasses a range of clinical conditions that includes UA, 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.

Ticagrelor (BRILINTA™, BRILIQUE™) is a $P2Y_{12}$ platelet inhibitor indicated to reduce the rate of thrombotic cardiovascular events in subjects with ACS. Ticagrelor has been shown to reduce the rate of a combined endpoint of cardiovascular death, MI or stroke compared to clopidogrel. The difference between treatments was driven by cardiovascular deaths and MIs with no difference in stroke. In subjects treated with percutaneous coronary intervention (PCI), it also reduces the rate of stent thrombosis.

BRILINTA was approved on 20 July 2011 to reduce the rate of thrombotic cardiovascular events in patients with ACS (UA, NSTEMI, or STEMI).

1.2 Summary of relevant preclinical and clinical information

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 (IPA) from patient to patient.

Ticagrelor is an oral, reversible ADP receptor antagonist acting via the $P2Y_{12}$ receptor, which has been developed for the prevention of thrombotic events in patients with ACS. The goal of the ticagrelor phase III programme was to demonstrate substantial improvements in clinical efficacy (ie, clinical thrombotic event reduction) with an acceptable safety profile compared with currently available antiplatelet therapies (ie, ASA and clopidogrel). Most patients took concomitant ASA with ticagrelor. 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 IPA, and faster offset compared to clinical doses of clopidogrel.

The focus of the clinical pharmacology program for ticagrelor, which included 42 studies in approximately 1000 subjects at the time of this Clinical Study Protocol (CSP), has been to

examine the exposure-response relationship, investigate safety, and characterize drug interactions. The pharmacokinetics (PK) of ticagrelor, as well as the metabolism of the compound, has been characterized in healthy subjects and patients with coronary heart disease. The dose range of ticagrelor administered during these studies was 0.1 to 1260 mg, and 900 mg was established as the maximum tolerated dose (MTD) in healthy subjects.

1.3 Rationale for conducting this study

The rationale for this study is to determine the role of autologous in vivo platelet transfusion on the reversibility of platelet inhibition in ticagrelor and ASA treated healthy volunteers.

This study will answer the following question: what is the effect of autologous platelet transfusions performed at 24 and 48 hours (and potentially 12 hours) after a loading dose of ticagrelor on platelet inhibition as measured by light transmission aggregometry (LTA).

Clopidogrel inhibits the platelet aggregation for the life of the platelet, approximately 7 to 10 days. Therefore, the label states that clopidogrel should be stopped 5 days before surgery (PLAVIX PI 2010). The label also states that it may be possible to restore hemostasis by administering exogenous platelets; however, platelet transfusions within 4 hours of the loading dose or 2 hours of the maintenance dose may be less effective. An ex vivo platelet transfusion study using platelet rich plasma has documented the restoration of hemostasis following clopidogrel administration, however rather large quantities of normal platelets were necessary to achieve this effect (Vilahur et al 2007). A recent in vivo study using 2 autologous platelet concentrates was not able to reverse ADP induced aggregation 24 hours after the last dose of clopidogrel (Pruller et al 2011). Therefore, the clopidogrel label is not supported by the current in vivo study data. Ticagrelor, a reversible inhibitor of the $P2Y_{12}$ receptor, has not been studied with respect to in vivo platelet transfusions. The label for ticagrelor currently states, when possible, to discontinue ticagrelor 5 days prior to any surgery and that no data exists with ticagrelor regarding a hemostatic benefit of platelet transfusions. The half life of ticagrelor is 7 hours and that of its active metabolite, AR-C124910XX, is 9 hours. The timing of platelet transfusions is based upon what is known about the platelet aggregation response and the PK of ticagrelor. The question remains what role autologous platelet transfusions may have in restoring platelet reactivity after exposure to ticagrelor, and in order to test this, this clinical study is proposed.

1.4 Benefit/risk and ethical assessment

The study will not provide any direct medical benefits to the healthy volunteers who participate. The major risk to healthy volunteers participating in this study is from potential adverse events (AEs) induced by ticagrelor, clopidogrel, or ASA and the risk of bruising and infection from numerous blood sampling.

The safety of ticagrelor in patients with ACS (UA, NSTEMI and STEMI) was evaluated in a single large phase III study (PLATO, which compared patients treated with ticagrelor [loading dose of 180 mg of ticagrelor and a maintenance treatment of 90 mg twice daily] to patients

treated with clopidogrel [300 to 600 mg loading dose followed by 75 mg once daily maintenance treatment] both given in combination with ASA and other standard therapies).

The most commonly reported AEs in patients treated with ticagrelor were dyspnea, headache, and epistaxis and these events occurred at higher rates than in the clopidogrel treatment group. During the treatment period, the ticagrelor group had a higher incidence of discontinuation due to AEs than clopidogrel (7.4% vs 5.4%).

There was no difference with ticagrelor compared to clopidogrel for fatal non-procedural bleeding. 'Major Fatal/Life-threatening' gastrointestinal bleeding was the same with ticagrelor and clopidogrel, with more fatal events for clopidogrel (5) than for ticagrelor (none). There were more 'Major Fatal/Life-threatening' intracranial nonprocedural bleeding events with ticagrelor (n=27 events in 26 patients, 0.3%) than with clopidogrel (n=14 events, 0.2%), of which 11 bleeding events with ticagrelor and 1 with clopidogrel were fatal.

Despite greater IPA with ticagrelor, results from the PLATO study showed that major bleeding with ticagrelor did not differ from those observed with clopidogrel. Adverse events observed in adult patients administered ticagrelor, other than bleeding, included dyspnea, minimal increases in serum creatinine, increased serum uric acid concentrations, and ventricular pauses that were largely asymptomatic, reflected as no difference in the 2 treatments with respect to incidence of syncope or pacemaker implantation. None of these events is considered to pose a risk, as they can be adequately handled in the clinical situation.

As with other antiplatelet agents, the use of ticagrelor in patients with known increased risks for bleeding should be balanced against the benefit of the treatment in terms of the prevention of thrombotic events. If a patient has to undergo elective surgery and antiplatelet effect is not desired, ticagrelor should be discontinued 5 days prior to surgery. No data exist with ticagrelor regarding a hemostatic benefit of platelet transfusions and circulating ticagrelor may inhibit transfused platelets. Antifibrinolytic therapy (aminocaproic acid or tranexamic acid) and/or recombinant factor VIIa may augment hemostasis. Ticagrelor may be resumed after the cause of bleeding has been identified and controlled.

Apheresis platelets are collected from a single donor and contain a minimum of $3x10^{11}$ platelets (approximately equivalent to 5 or 6 platelet concentration units) suspended in 200 to 300 mL plasma. An approximate 20% decrease in platelets could be expected after apheresis with an increase in platelets of 50,000 to 60,000 $x10^9$/L after platelet transfusion.

Thienopyridines inhibit platelet aggregation for the lifetime of the platelet (7 to 10 days), so withholding a dose will not be useful in managing a bleeding event or the risk of bleeding associated with an invasive procedure. Because the half-life of clopidogrel's active metabolite is short, it may be possible to restore hemostasis by administering exogenous platelets; however, platelet transfusions within 4 hours of the loading dose or 2 hours of the maintenance dose may be less effective.

There is no known antidote to reverse the effects of ticegrelor. The average half-life of ticagrelor is approximately 12 hours, so blood levels of ticagrelor should be low by 48 to

72 hours (ie, 4 to 6 half-lives) after discontinuation. Platelet transfusions may be given. It is not known if new platelets may be inhibited by ticagrelor as long as it is circulating in blood.

Major bleeding events should be managed according to accepted standard of care.

Hazards that apply specifically to components that contain platelets:

Bacterial contamination: Although methods to limit and detect bacterial contamination have been implemented for most platelet components, they remain the most likely blood components to be contaminated with bacteria. Gram-positive skin flora are the most commonly recovered bacteria. Symptoms may include high fever (=2.0 °C or =3.5 °F increase in temperature), severe chills, hypotension, or circulatory collapse during or immediately after transfusion. In some instances, symptoms, especially when associated with contamination by gram-positive organisms, may be delayed for several hours following transfusion. Prompt management should include broadspectrum antibiotic therapy along with cultures from the patient, suspected blood component(s), and administration set. A Gram's stain of suspected contaminated unit(s) should be performed whenever possible. Apheresis platelets are usually tested for bacterial contamination before issue.

Because of the reversible binding of ticagrelor, restoration of platelet aggregation occurs faster with ticagrelor compared to clopidogrel. In the OFFSET study, mean IPA for ticagrelor at 72 hours postdose was comparable to mean IPA for clopidogrel at 120 hours postdose. The more rapid offset of effect may predict a reduced risk of bleeding complications, eg, in settings where antiplatelet therapy must be temporarily discontinued due to surgery or trauma.

In the PLATO study, patients undergoing a coronary artery bypass graft (CABG), ticagrelor had a similar rate of major bleeds compared to clopidogrel at all days after stopping therapy except Day 1 where ticagrelor had a higher rate of major bleeding.

Dyspnea, usually mild to moderate in intensity and often resolving without the need for treatment discontinuation, is reported in patients treated with ticagrelor (approximately 13.8%). The mechanism has not yet been elucidated. If a patient reports new, prolonged or worsened dyspnea this should be investigated fully and if not tolerated, treatment with ticagrelor should be stopped.

Caution is advised in patients with moderate hepatic impairment because ticagrelor has not been studied in these patients. Use of ticagrelor is contraindicated in patients with severe hepatic impairment.

In PLATO, use of BRILINTA with maintenance doses of aspirin above 100 mg decreased the effectiveness of BRILINTA. Therefore, after the initial loading dose of aspirin (325 mg), use BRILINTA with a maintenance dose of aspirin of 75 to 100 mg.

Patients who require discontinuation of ticagrelor are at increased risk for cardiac events. Premature discontinuation of treatment should be avoided. If ticagrelor must be temporarily

stopped due to an AE, it should be re-initiated as soon as possible when the benefits outweigh the risks of the AE or when the AE has come to resolution.

The following are contraindications for ticagrelor:

- Hypersensitivity to ticagrelor or any of the excipients
- Active pathological bleeding
- History of intracranial hemorrhage
- Severe hepatic impairment

Please refer to the Investigator's Brochure for more details regarding the overall risk/benefit, ethical assessment and references of ticagrelor treatment.

2. STUDY OBJECTIVES

2.1 Primary objective

- To evaluate the effect of autologous platelet transfusions in healthy volunteers at 24 and 48 hours (and potentially 12 hours)[2] after a loading dose of ticagrelor on platelet inhibition as measured by LTA

2.2 Secondary objectives

- To potentially evaluate the effect of autologous platelet transfusions in healthy volunteers at either 12, 24 or 48 hours after a loading dose of clopidogrel on platelet inhibition as measured by LTA[2]
- To evaluate the effect of autologous platelet transfusions in healthy volunteers at 24 and 48 hours (and potentially 12 hours) after a loading dose of ticagrelor on platelet inhibition as measured by VerifyNow™
- To potentially evaluate the effect of autologous platelet transfusions in healthy volunteers at either 12, 24 or 48 hours after a loading dose of clopidogrel on platelet inhibition as measured by VerifyNow™
- To evaluate the durability of restoration of platelet aggregation following platelet transfusion

[2] If the 24-hour and 48-hour cohorts are both successful, a platelet transfusion at 12 hours after the loading dose of ticagrelor (Day 1) in a single treatment, without crossing over to a non-transfusion period, hence a parallel group, will be performed.

2.3 Safety objective

To describe the safety and tolerability of ticagrelor administered as a loading dose prior to an autologous platelet transfusion.

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 an open label, randomized, cross-over study of 180 mg ticagrelor administered in healthy volunteers followed by autologous in vivo platelet transfusion to determine the effects of platelet supplementation on the reversibility of platelet inhibition. The study will be conducted at a single study center in approximately 48 healthy male and female (of non-childbearing potential) volunteers aged 18 to 50 years (inclusive).

The study will consist of 2 treatments each for cohort 1 (platelet transfusion at 24 hours) and cohort 2 (platelet transfusion at 48 hours) (see Figure 2).

Treatment A: platelet apheresis on Day -2 followed by a ticagrelor 180 mg loading dose on Day 1 for cohort 1; and platelet apheresis on Day -1 followed by a ticagrelor 180 mg loading dose on Day 1 for cohort 2, respectively. Background ASA treatment (81 mg once daily) will commence immediately after platelet apheresis on the relevant day, based on the randomization scheme and treatment group, up to and including the day prior to the platelet transfusion.

Treatment B: ticagrelor 180 mg loading dose on Day 1. Background ASA treatment (81 mg once daily) will commence on the relevant day, based on the randomization scheme and treatment group, up to and including the day prior to the randomized platelet transfusion from Treatment A.

During each treatment period healthy volunteers will receive a loading dose of 180 mg of ticagrelor with background ASA. The healthy volunteers will have platelet apheresis and platelet transfusion performed during 1 of the 2 treatment periods based on the randomization scheme (see Figure 1).

Cohort 1: will have the platelet transfusion performed 24 hours after receiving the 180 mg ticagrelor loading dose. Healthy volunteers will receive ASA background treatment once on Day -2 immediately after completion of the platelet apheresis, on Day -1, and again on Day 1 when ticagrelor is administered.

Cohort 2: will have the platelet transfusion performed 48 hours after receiving the 180 mg ticagrelor loading dose. Healthy volunteers will receive ASA background treatment once on Day -1 immediately after completion of the platelet apheresis, and again on Day 1 when ticagrelor is administered, as well as once on Day 2.

The cohorts are defined as follows (see Figure 1):

- Cohort 1 = 24-hour postdose platelet transfusion + ticagrelor
- Cohort 2 = 48-hour postdose platelet transfusion + ticagrelor
- Cohort 3a = 12-hour postdose platelet transfusion + ticagrelor
- Cohort 3b = 48-hour postdose platelet transfusion + clopidogrel
- Cohort 4a = 12-hour postdose platelet transfusion + clopidogrel
- Cohort 4b = 24-hour postdose platelet transfusion + clopidogrel

After completion of cohort 1 and cohort 2, a decision will be made regarding the continuation of the study and the subsequent cohorts to be initiated according to the results observed from cohort 1 and cohort 2 (see Figure 1).

The definition of success for each cohort is:

- A decrease (at 5% significance level) in IPA (LTA) measured 12 hours after transfusion between Treatment A and Treatment B

Based on the results of cohort 1 and cohort 2, the following scenarios will apply (see Figure 1):

Both cohort 1 and cohort 2 are successful

Cohort 3a: if cohort 1 and cohort 2 are both successful, screening for cohort 3a will be initiated. Eligible healthy volunteers included in cohort 3a will have a platelet transfusion at 12 hours after the ticagrelor loading dose in the evening (Day 1). These healthy volunteers will receive ASA background treatment on Day -2 immediately after completion of the platelet apheresis, on Day -1, and again on Day 1.

Once cohort 3a has been completed and all the results are available, a second interim meeting will be convened to decide which of cohorts 4a or 4b will be initiated (see Figure 1).

The definition of success for cohort 3a is:

- A decrease (at 5% significance level) in IPA (LTA) of Cohort 3a 12-hour posttransfusion values as compared to the 24-hour post-treatment values (without platelet transfusion treatment group only) from both cohorts 1 and 2

Based on the results of cohort 3a (platelet transfusion at 12 hours), the following will occur (see Figure 1):

- Cohort 3a successful

Cohort 4a: if cohort 3a is successful, screening for cohort 4a will be initiated. Eligible healthy volunteers included in cohort 4a will receive a 600 mg clopidogrel loading dose and a platelet transfusion at 12 hours.

- Cohort 3a unsuccessful

Cohort 4b: if cohort 3a is unsuccessful, screening for cohort 4b will be initiated. Eligible healthy volunteers in cohort 4b will receive a 600 mg clopidogrel loading dose and a platelet transfusion at 24 hours.

Cohort 1 unsuccessful and cohort 2 is successful

Cohort 3b: if cohort 1 fails and cohort 2 is successful, screening for cohort 3b will be initiated. Eligible healthy volunteers in cohort 3b will receive a 600 mg clopidogrel loading dose and a platelet transfusion at 48 hours.

Cohort 1 successful and cohort 2 unsuccessful

If the cohort 1 is successful and the cohort 2 fails, addition of subsequent cohorts will be assessed and discussed by the Sponsor.

Both cohort 1 and cohort 2 are unsuccessful

If both cohort 1 and cohort 2 fail no additional cohorts will be assessed.

In addition to the loading dose of ticagrelor, the healthy volunteers will also receive background ASA (81 mg once daily). The administration and duration of the ASA background treatment will be dependent on the transfusion cohort the healthy volunteer is allocated to.

The 180 mg loading dose administrations of ticagrelor will be separated by a washout period of at least 14 days.

The ASA background treatment for the clopidogrel cohorts (cohort 4b, cohort 3b, and cohort 4a) will be identical to those for the ticagrelor administration for the same transfusion timing, ie, 24, 48, or 12 hours (cohort 1, cohort 2, and cohort 3a) (see Figure 1 and Figure 2).

Samples for LTA and VerifyNow™ P2Y12 are collected prior to the platelet transfusion in each cohort. All healthy volunteers will have blood samples collected for the determination of IPA (LTA and VerifyNow™ $P2Y_{12}$) at -24, 0, 2, 6, 12, 24, 36, 48, 60, 72, 84 and 96 hour time points relative to the ticagrelor administration for each treatment period. In addition, blood samples for the determination of IPA will be collected at 0.25, 1, 2, 6 and 12 hours following the end of the platelet transfusion. The platelet transfusion will be approximately 30 minutes in duration (see Table 1 to Table 5).

Healthy volunteers will be evaluated for eligibility at screening (Visit 1, Days -30 to -3), which will take place no more than 28 days before Visit 2. Eligible healthy volunteers will be admitted to the study center, and randomized to one of the 2 treatment periods for cohort 1 or

cohort 2, on Day -2. Healthy volunteers will remain in the study center until 96 hours (Day 5) after the ticagrelor loading dose was administered (Day 1).

Approximately 48 healthy volunteers will be randomized based on the total number of cohorts to be assessed. There is a minimum of 2 cohorts and up to a maximum of 4 cohorts planned for the study. Each cohort will comprise 12 healthy volunteers. The study duration for each healthy volunteer will be approximately 9 weeks, comprising 4 visits.

The screening for the 12 healthy volunteers needed for the relevant clopidogrel cohort will include an assessment of their cytochrome P450 (CYP) 2C19 genotype status to ensure that they carry 2 functional (*1) alleles (2C19 *1/*1). An additional 5 day pre-screening will be performed to allow time for the assessment of the CYP2C19 enzymatic status of the healthy volunteers to be included in the relevant clopidogrel cohort (see Table 1). Due to the incidence of CYP2C19 *1/*1 genotype in the American population, the number of healthy volunteers to be screened for the relevant clopidogrel cohort will be higher than for the other cohorts.

Figure 1 Determination of transfusion continuation

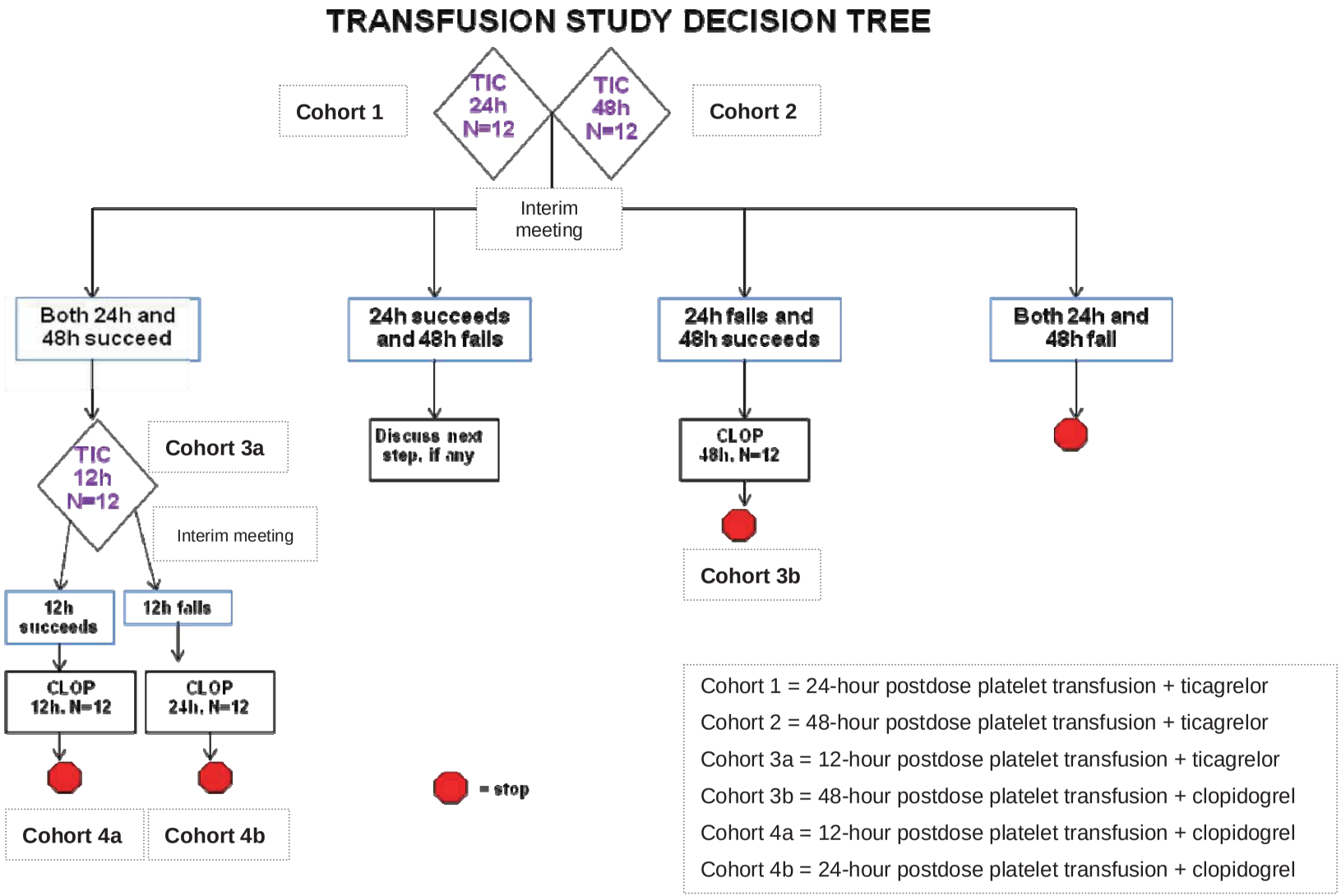
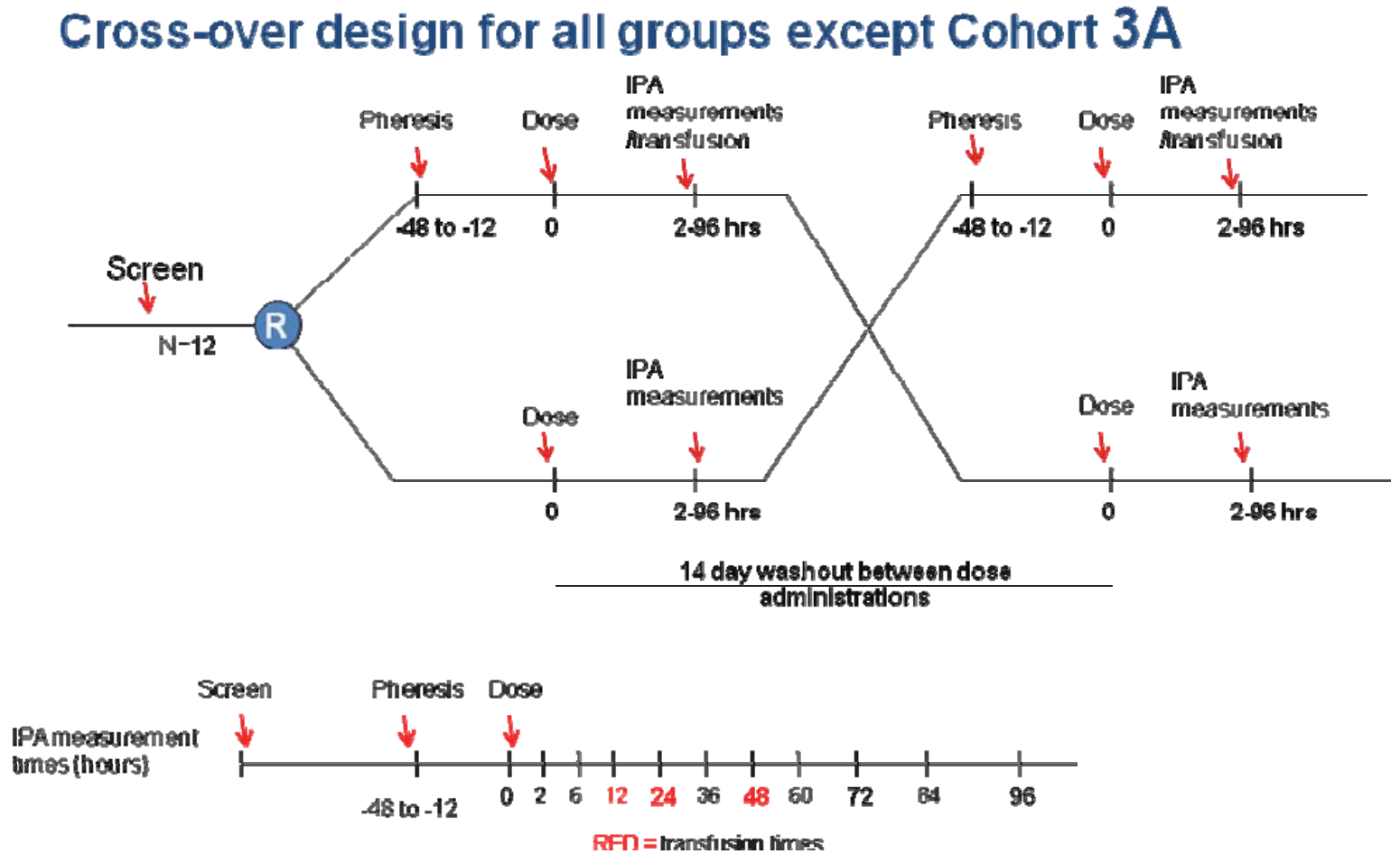


Figure 2 Study flow chart



Aspirin administration (81 mg): Day -1 through to the day prior to randomized transfusion cohort for each treatment period
IPA: induced platelet aggregation

Table 1 Study Plan

			Treatment Periods 1 and 2[b]													
Study day	**Screen**	**Day -2**	**Day -1**	**Day 1**					**Day 2**		**Day 3**		**Day 4**		**Day 5**	**Follow-up[c]**
Hour relative to administration	**Days -30 to -3[a]**		**-24 h**	**Pre-dose**	**0 h**	**2 h**	**6 h**	**12 h**	**24 h**	**36 h**	**48 h**	**60 h**	**72 h**	**84 h**	**96 h**	**7-10 days**
Informed consent	X															
Informed consent for genotyping for relevant clopidogrel cohort	X[a]															
Review eligibility	X	X														
Medical/medication history	X															
Physical examination	X	X[d]														X
Alcohol/cotinine/drugs of abuse	X	X														
HIV, HBsAg, Hepatitis C	X															
Clinical Laboratory assessments, including coagulation[e]	X	X		X										X		X
Stool guaiac (fecal occult blood test)	X															
Complete blood count								X[f]	X[f]		X[f]					X
12-lead ECG	X[g]															

Table 1 Study Plan

			Treatment Periods 1 and 2[b]													
Study day	**Screen**	**Day -2**	**Day -1**	**Day 1**					**Day 2**		**Day 3**		**Day 4**		**Day 5**	**Follow-up[c]**
Hour relative to administration	**Days -30 to -3[a]**		**-24 h**	**Pre-dose**	**0 h**	**2 h**	**6 h**	**12 h**	**24 h**	**36 h**	**48 h**	**60 h**	**72 h**	**84 h**	**96 h**	**7-10 days**
Vital signs	X	X		X					X		X		X			X
Genotype status[h]	X															
Randomization[i]		X														
Platelet apheresis[j]		X	X													
Ticagrelor/Clopidogrel administration[k]					X											
ASA administration[l]		X	X	X					X							
ADP-induced platelet aggregation		X	X	X	X	X	X	X	X	X	X	X	X	X	X	
VerifyNow™ $P2Y_{12}$			X	X	X	X	X	X	X	X	X	X	X	X	X	
Platelet transfusion[m]								X^{m}	X^{m}		X^{m}					
Concomitant medication	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
SAEs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
AEs		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Discharge from study center															X	

a An additional 5 day pre-screening will be performed to allow time for the assessment of the CYP2C19 enzymatic status of the healthy volunteers to be included in the relevant clopidogrel cohort. These healthy volunteers will give signed and dated written informed consent for the genetic screening before the blood sample is collected
b Treatment periods will be separated by a washout period of at least 14 days between the 180 mg loading dose administrations of ticagrelor
c The Follow-up visit will take place 7 to 10 days after the last discharge (Day 5 of Treatment Period 2) of each healthy volunteer
d Only a brief history and physical examination will be performed on Day -2
e Clinical laboratory assessments including coagulation, will only be performed to ensure that only healthy volunteers are included in the study
f CBC only will be performed prior to the platelet transfusion, dependent on when the patient received the platelet transfusion. In addition, platelet counts will be determined at 1, 6 and 23.5 hours after the end of the transfusion
g Volunteers should have at least 10 minutes rest before ECG is performed
h Genotype status will only be determined for healthy volunteers to be included in the relevant clopidogrel cohort (12 healthy volunteers with CYP2C19 *1/*1 enzymatic status to be included)
i Randomization will take place on Day -2 of Period 1
j Platelet apheresis will be on Day -2 for cohort 1 (platelet transfusion at 24 hours) and cohort 3a (platelet transfusion at 12 hours), and on Day -1 for cohort 2 (platelet transfusion at 48 hours), based on the randomization scheme and treatment period
k Healthy volunteers included in cohorts will receive either ticagrelor or clopidogrel based on the results of the platelet transfusions at 24 and 48 hours (cohorts 1 and 2) as depicted in Figure 1. For the 12-hour (cohort 3a) platelet transfusion, ticagrelor will be administered in the evening (Table 4)
l Healthy volunteers will receive ASA on Day -2, Day -1 and Day 1 if in cohort 1 (platelet transfusion at 24 hours); healthy volunteers in cohort 2 (platelet transfusion at 48 hours) will receive ASA on Day -1, Day 1, and Day 2, and healthy volunteers in cohort 3a (platelet transfusion at 12 hours) will receive ASA on Day -2, Day -1, and Day 1
m The platelet transfusion will be performed based on the randomization scheme; the transfusion will occur at 24, 48 or potentially 12 hours from administration of ticagrelor or clopidogrel (see Table 2, Table 3 and Table 4). For cohort 3a (platelet transfusion at 12 hours) ticagrelor will be administered in the evening (Table 4)

ADP: adenosine diphosphate; AE: adverse event; ASA: acetylsalicylic acid; CBC: complete blood count; ECG: electrocardiogram; HBsAg: hepatitis B surface antigen; HIV: human immunodeficiency virus; SAE: serious adverse event

Table 2 Timing of platelet function samples during cohort 1 (platelet transfusion at 24 hours)

Protocol time	Ticagrelor administration	ADP platelet aggregation by LTA	VerifyNow™ $P2Y_{12}$	Complete blood count	Platelet apheresis	Platelet counts	ASA administration	Clinical Laboratory assessments	Vital signs
Day -2		X			X		X		
Day -1		X	X				X		
Predose		X	X				X		X
(t=0 min)	X								
2 h		X	X						
6 h		X	X						
12 h		X	X						
24 h		X (pre-transfusion)	X (pre-transfusion)	X (pre-transfusion)					X
24.75 h		X	X						
25 h									
25.5 h		X	X			X			
26.5 h		X	X						
30 h									
30.5 h		X	X			X			
36 h		X	X						
47.5 h									
48 h		X	X			X			X
60 h		X	X						
72 h		X	X						X
84 h		X	X					X	
96 h		X	X						

Postdose sampling time points are independent of the transfusion duration

Table 3 Timing of platelet function samples during cohort 2 (platelet transfusion at 48 hours)

Protocol time	Ticagrelor administration	ADP platelet aggregation by LTA	VerifyNow™ $P2Y_{12}$	Complete blood count	Platelet apheresis	Platelet counts	ASA administration	Clinical laboratory assessments	Vital signs
Day -2		X							
Day -1		X	X		X		X		
Predose		X	X				X		X
(t=0 min)	X								
2 h		X	X						
6 h		X	X						
12 h		X	X						
24 h		X	X				X		X
36 h		X	X						
48 h		X (pre-transfusion)	X (pre-transfusion)	X (pre-transfusion)					X
48.75 h		X	X						
49 h									
49.5 h		X	X			X			
50.5 h		X	X						
54 h									
54.5 h		X	X			X			
60 h		X	X						
71.5 h									
72 h		X	X			X			X
84 h		X	X					X	
96 h		X	X						

Postdose sampling time points are independent of the transfusion duration.

Table 4 Timing of platelet function samples during cohort 3a (platelet transfusion at 12 hours)

Protocol time	Ticagrelor administration	ADP platelet aggregation by LTA	VerifyNow™ $P2Y_{12}$	Complete blood count	Platelet apheresis	Platelet counts	ASA administration	Clinical laboratory assessments	Vital signs
Day -2		X			X		X		
Day -1		X	X				X		
Predose		X	X				X		X
(t=0 min)	X (in the evening)								
2 h		X	X						
6 h		X	X						
12 h		X (pre-transfusion)	X (pre-transfusion)	X (pre-transfusion)					
12.75 h		X	X						
13 h									
13.5 h		X	X			X			
14.5 h		X	X						
18 h									
18.5 h		X	X			X			
24 h		X	X						X
35.5 h									
36 h		X	X			X			
48 h		X	X						X
60 h		X	X						
72 h		X	X						X
84 h		X	X					X	
96 h		X	X						

Postdose sampling time points are independent of the transfusion duration.

If the 24-hour and 48-hour cohorts are both successful, cohort 3a will have a platelet transfusion at 12 hours after the loading dose of ticagrelor (Day 1) in a single treatment, without crossing over to a non-transfusion period, hence a parallel group (see Figure 1). These healthy volunteers receive ASA background treatment only on Day -2 immediately after completion of the platelet apheresis, on Day -1, and again on Day 1.

Table 5 Timing of platelet function samples without platelet transfusion

Protocol time	Ticagrelor administration	ADP platelet aggregation by LTA	VerifyNow™ $P2Y_{12}$	ASA administration	Clinical laboratory assessments	Vital signs
Day -2		X		X		
Day -1		X	X	X		
Predose		X	X	X		X
(t=0 min)	X					
2 h		X	X			
6 h		X	X			
12 h		X	X			
24 h		X	X	X		X
36 h		X	X			
48 h		X	X			X
60 h		X	X			
72 h		X	X			X
84 h		X	X		X	
96 h		X	X			

During each treatment period healthy volunteers will receive a loading dose of 180 mg of ticagrelor with background ASA.
Healthy volunteers will receive ASA on Day -2, Day -1, and Day 1 if in cohort 1 (platelet transfusion at 24 hours); healthy volunteers in cohort 2 (platelet transfusion at 48 hours) will receive ASA on Day -1, Day 1, and Day 2, and healthy volunteers in cohort 3a (platelet transfusion at 12 hours) will receive ASA on Day -2, Day -1, and Day 1.

3.2 Rationale for study design, doses and control groups

Ticagrelor is marketed for use in the United States and the study will be conducted at a single study center in the United States.

The study will be conducted in male and female (of non-childbearing potential) healthy volunteers, aged 18 to 50 years, inclusive to avoid interference from disease processes or other drugs. The inclusion and exclusion criteria are defined such that healthy volunteers selected for participation in the study are known to be free from any significant illness.

Ticagrelor has a faster rate of offset of IPA as compared to clopidogrel treatment and resulted in consistently higher IPA compared with clopidogrel. The clinical evidence for the efficacy of ticagrelor is derived from the PLATO study, a comparison of ticagrelor to clopidogrel, both given in combination with ASA and other standard therapy.

Additional analyses suggest that the efficacy of ticagrelor relative to clopidogrel is associated with ASA dose during maintenance therapy. The data showed greater efficacy of ticagrelor compared to clopidogrel when used in conjunction with low maintenance dose ASA (75 to 150 mg). The relative efficacy of ticagrelor versus clopidogrel when used with high doses of ASA (>300 mg) is less certain. Based on this observed relationship between maintenance ASA dose and relative efficacy of ticagrelor compared to clopidogrel, it is recommended that ticagrelor is used with a low maintenance dose of ASA 81 mg.

Ticagrelor is orally active. Unlike clopidogrel, it does not require CYP450 enzyme activity to inhibit platelet aggregation. Polymorphisms in the gene coding for CYP2C19 may impact clopidogrel efficacy. Polymorphism in the gene coding for P-glycoprotein transport (ABCB1) may impact efficacy of both clopidogrel and ticagrelor.

The superiority of ticagrelor over clopidogrel is not significantly affected by the CYP2C19 genotype of the patient. Ticagrelor reduced major cardiovascular events compared to clopidogrel independently of CYP2C19 genotype.

The 180 mg loading dose administrations of ticagrelor will be separated by a washout period of at least 14 days to ensure no carry-over between the treatment periods.

3.3 Interim Review

After completion of cohort 1 and cohort 2, all the data from the 2 cohorts will be evaluated to determine the next steps as presented in Figure 1.

A decision regarding which scenario should be followed after reviewing all the data from cohort 1 and cohort 2 will be made by representatives of AstraZeneca and Quintiles.

Similarly all the data from cohorts 3a, 1 and 2 will be reviewed once cohort 3a is completed to determine which cohort will be done next (see Figure 1).

4. SUBJECT SELECTION CRITERIA

Investigator(s) should keep a record, the healthy volunteer screening log, of healthy volunteers who entered pre-study screening.

Each healthy volunteer 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 healthy volunteers should fulfil the following criteria:

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

2. Healthy male and/or female volunteers aged 18 to 50 years, inclusive, with suitable veins for cannulation or repeated venipuncture. (Healthy as determined by medical history and physical examination, laboratory parameters, electrocardiogram (ECG) performed before first dose administration)

3. Females must have a negative pregnancy test at screening and on admission to the study center, must not be lactating and must be of non-childbearing potential, confirmed at screening by fulfilling one of the following criteria:

   – Post-menopausal defined as amenorrhea for at least 12 months following cessation of all exogenous hormonal treatments and with luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels in the laboratory defined post-menopausal range

   – Documentation of irreversible surgical sterilization by hysterectomy, bilateral oophorectomy or bilateral salpingectomy but not tubal ligation

4. Male healthy volunteers should be willing to use barrier contraception, ie, condoms with spermicide, from the first day of investigational product administration until 3 months after the last investigational product administration

5. Have a body mass index (BMI) between 18 and 35 kg/m^2 (inclusive) and weigh at least 50 kg and no more than 120 kg

6. Have a platelet count $>240x10^9/L$ at screening

7. Provision of signed and dated, written informed consent for genetic/biomarker research (applies for the relevant clopidogrel cohort only)

4.2 Exclusion criteria

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

1. ADP induced platelet aggregation <60% prior to platelet apheresis

2. History of peptic ulcer disease

3. Healthy volunteers with a propensity to bleed (eg, due to recent trauma, recent surgery, active or recent gastrointestinal bleeding or moderate hepatic impairment)

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

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

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

7. Any clinically significant abnormalities in clinical chemistry, hematology or urinalysis results as judged by the Investigator

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

9. Abnormal vital signs, after 10 minutes supine rest, defined as any of the following:

   – Systolic blood pressure (SBP) >140 mm Hg

   – Diastolic blood pressure (DBP) >90 mm Hg

   – Heart rate <45 or >85 beats per minute (bpm)

10. Any clinically important abnormalities in rhythm, conduction or morphology of resting ECG that may interfere with the interpretation of the QTc interval changes

11. Prolonged QTcF >450 ms or shortened QTcF <340 ms; pause >3 s; or family history of long QT syndrome

12. PR(PQ) interval prolongation (>240 ms) intermittent second or third degree atrioventricular (AV) block, or AV dissociation

13. Incomplete, full or intermittent bundle branch block (QRS <110 ms with normal QRS and T wave morphology is acceptable if there is no evidence of left ventricular hypertrophy)

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

15. Current smokers, those who have smoked or used nicotine products within the previous 3 months and those who tested positive for cotinine at screening or at admission to the study center

16. History of severe allergy/hypersensitivity or ongoing clinically important allergy/hypersensitivity, as judged by the Investigator or history of hypersensitivity to drugs with a similar chemical structure or class to ticagrelor, clopidogrel or ASA

17. Excessive intake of caffeine-containing drinks eg, coffee, tea, caffeine-containing energy drinks and cola (more than 6 cups of coffee or equivalent per day)

18. Use of any prescribed or non-prescribed medication including antacids, analgesics other than paracetamol/acetaminophen, herbal remedies, vitamins and minerals during the 3 weeks prior to the first administration of investigational product or longer if the medication has a long half-life. Occasional use of paracetamol/ acetaminophen is allowed for minor pains and headache. Hormone (estrogen) replacement therapy for older women may also be allowed, enhancing the potential for postmenopausal women to be included

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

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

21. Any intake of grapefruit, grapefruit juice, Seville oranges, Seville orange marmalade or other products containing grapefruit or Seville oranges within 7 days of the first administration of investigational product

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

23. ASA within 10 days of the platelet apheresis

24. Healthy volunteers requiring ASA treatment

25. Positive screen for drugs of abuse at screening or on admission to the study center or positive screen for alcohol on admission to the unit prior to the first administration of investigational product

26. 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 investigational product in this study. The period of exclusion begins 3 months after the final dose or 1 month after the last visit whichever is the longest. Note: healthy volunteers consented and screened, but not randomized in this study or a previous phase I study, are not excluded

27. Previous randomization to treatment in the present study

28. Involvement of any Quintiles/third party contractor or AstraZeneca personnel and their close relatives regardless of their role in accordance with their internal procedures

29. Judgment by the Investigator that the healthy volunteer should not participate in the study if they have any ongoing or recent (ie, during the screening period) minor medical complaints that may interfere with the interpretation of the study data or are considered unlikely to comply with study procedures, restrictions and requirements

30. Healthy volunteers who are vegans or have medical dietary restrictions

31. Healthy volunteers who cannot communicate reliably with the Investigator

Exclusion criterion specifically for healthy volunteers participating in the relevant clopidogrel cohort only:

32. Healthy volunteers with CYP2C19 genotypes other than CYP2C19 *1/*1 (normal/extensive metabolizers)

Procedures for withdrawal of incorrectly enrolled healthy volunteers see Section 5.3.

5. STUDY CONDUCT

5.1 Restrictions during the study

1. Fast from at least 10 hours before the planned start of investigational product administration. A moderate amount of water is allowed up to 1 hour prior to investigational product administration and may be resumed 1 hour after administration of the investigational product. A meal can be consumed 4 hours after administration of the investigational product.

   Healthy volunteers randomized to either cohort 3a or cohort 4a need only to fast from 4 hours before the planned start of investigational product administration.

2. Eat and drink only the standardized meals and drinks provided (apart from water) during the residential period in the study center.

3. Abstain from consuming any of the following:

   – Alcohol from 72 hours before admission, during the residential period

   – Energy drinks containing taurine or glucuronolactone eg, Red Bull from 72 hours before admission, during the residential period

   – Caffeine-containing drinks during the residential period apart from any provided as part of a standardized meal.

   – Poppy seeds found in speciality bread from time of consent until after the final medical examination

   – Grapefruit, grapefruit juice, Seville oranges, Seville orange marmalade or other products containing grapefruit or Seville oranges from 7 days before admission until after the final medical examination

4. Abstain from nicotine use, smoking and drugs of abuse from the time of informed consent until after the final medical examination

5. Abstain from taking any medication (prescribed or over the counter products), other than paracetamol/acetaminophen and if applicable, hormone replacement therapy, from 2 weeks prior to the first administration of the investigational product until after the final medical examination. However, this should not obviate necessary medical treatment. If any medication is necessary during the residential period, it should be prescribed by the Investigator and the AstraZeneca Clinical Pharmacology Alliance (CPA) Physician should be informed.

6. Healthy volunteers should refrain from strenuous physical activity, which is not within the healthy volunteer's normal daily routine, from 7 days prior to admission to the study center until after the final medical examination.

7. Abstain from blood or plasma donation until 3 months after the final medical examination.

8. Male healthy volunteers should use a condom and spermicide to prevent pregnancy and drug exposure of a partner and refrain from donating sperm or fathering a child from the date of investigational product administration until 3 months after administration with the investigational product

5.2 Subject enrolment, randomization and initiation of investigational product

The Principal Investigator will:

1. Obtain signed informed consent from each potential healthy volunteer before any study specific procedures are performed

2. Assign each potential healthy volunteer a unique enrolment number, beginning with 'E#0001001'

3. Determine the eligibility of each healthy volunteer, see Sections 4.1 and 4.2

4. Assign each eligible healthy volunteer an unique randomization number as described below

If a healthy volunteer withdraws from participation in the study, then his/her enrolment/randomization code cannot be reused.

5.2.1 Procedures for randomization

Healthy volunteers will be enrolled into cohorts sequentially and randomization codes will be assigned sequentially as healthy volunteers become eligible for randomization (ie, the first 12 eligible healthy volunteers will be enrolled into cohort 1 and be randomized sequentially; the next 12 eligible healthy volunteers will be enrolled into cohort 2 and be randomized sequentially and so on). A randomization scheme will be produced by Quintiles using the global randomization system (GRand). The randomization will be done for each cohort using consecutive randomization codes (Volunteer numbers).

Within each cohort except for cohort 3a, healthy volunteers will be randomized in a cross-over fashion. Each healthy volunteer will be randomized to one of these 2 treatment sequences:

1. Platelet apheresis and transfusion in Period 1 → no platelet apheresis and transfusion in Period 2

2. No platelet apheresis and transfusion in Period 1 → platelet apheresis and transfusion in Period 2

The following volunteer numbers will be used for each cohort:

Cohort	Randomization	Condition to run cohort
1	1001 to 1012	Not applicable
2	2001 to 2012	Not applicable
3a	3001 to 3012	If both cohorts 1 & 2 are successful
3b	3001 to 3012	If cohort 1 unsuccessful and cohort 2 is
4a	4001 to 4012	If cohort 3a is successful
4b	4001 to 4012	If cohort 3a is unsuccessful

5.3 Procedures for handling subjects incorrectly randomized

Healthy volunteers who fail to meet the inclusion/exclusion criteria should not, under any circumstances, be enrolled or receive investigational product. There can be no exceptions to this rule.

Where a healthy volunteer, who does not meet the inclusion and/or exclusion criteria, is randomized in error and this is identified before investigational product administration, the

healthy volunteer should be withdrawn from the study. A discussion should occur between the AstraZeneca CPA Physician and the Investigator regarding whether a replacement may be considered.

Where a healthy volunteer, who does not meet the selection criteria, is randomized in error and investigational product administration has been started, or where a healthy volunteer subsequently fails to meet the study criteria post initiation, the Investigator should inform the AstraZeneca CPA Physician immediately. Although investigational product administration should be discontinued, the healthy volunteer should be advised to continue assessments to ensure his/her safety.

The AstraZeneca CPA Physician is to ensure all decisions are appropriately documented.

5.4 Blinding and procedures for unblinding the study (Not applicable)

5.5 Treatments

5.5.1 Identity of investigational product(s)

Please refer to Table 6 for details regarding the investigational product.

Table 6 Identity of the investigational product

Investigational product	Dosage form, strength, and route of administration,	Manufacturer
Ticagrelor	180 mg (2 x 90 mg) tablet, orally	AstraZeneca

The pharmacist at the study center will dispense 180 mg (2 x 90 mg tablets) on Day 1 of each treatment period if enrolled to the ticagrelor cohorts.

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

5.5.2 Additional study drug

Table 7 Identity of the additional study drugs

Investigational product	Dosage form, strength, and route of administration,	Manufacturer
Acetylsalicylic acid (ASA; aspirin)	81 mg chewable tablet	Bayer
Clopidogrel (PLAVIX®)	300 mg (2 x 300 mg) tablets, orally	Bristol-Myers Squibb/Sanofi Pharmaceutical Partnership

The clopidogrel and ASA will be purchased in the United States by the study center.

The pharmacist at the study center will dispense 600 mg (2 x 300 mg tablets) on Day 1 if enrolled to the clopidogrel cohorts.

A background treatment of ASA (81 mg) will be administered from Day -1 for each treatment period according to the randomization scheme. The ASA administration will occur after the platelet apheresis.

5.5.3 Doses and treatment regimens

Each healthy volunteer will be administered 180 mg ticagrelor or 600 mg clopidogrel, orally, as a single administration on Day 1 of each treatment period, based on the randomization scheme and cohort assignment. The investigational product will be administered after an overnight fast of at least 10 hours and at least 4 hours for cohorts 3a and 4a. For cohort 3a (platelet transfusion at 12 hours) ticagrelor will be administered in the evening. The 180 mg loading dose ticagrelor administrations will be separated by a washout period of at least 14 days.

Background ASA (81 mg once daily) will be administered from either Day -2 (cohort 1) or Day -1 (cohort 2). The ASA administration will commence immediately after the platelet apheresis. The administration and duration of the ASA background treatment will be dependent on the transfusion cohort the healthy volunteer is allocated to (Table 1).

Each healthy volunteer will have a platelet apheresis either on Day -2 for cohort 1, or on Day -1 for cohort 2, and one autologous apheresis platelet unit transfusion during one of the treatment periods at either 24, 48, or 12 hours based upon the cohort assignment.

5.5.4 Labelling

Labels will be prepared in accordance with GMP and local regulatory guidelines. The labels will fulfil GMP Annex 13 requirements for labelling.

5.5.5 Storage

All investigational products and additional study drug should be kept in a secure place under appropriate storage as specified on the respective labels. Instructions regarding the storage of the platelets will be provided in the Laboratory Manual.

5.6 Concomitant and post-study treatment(s)

If clinically indicated, ticagrelor should be used with caution in the following instances:

- Healthy volunteers with a propensity to bleed (eg, due to recent trauma, recent surgery, active or recent gastrointestinal bleeding or moderate hepatic impairment). The use of ticagrelor is contraindicated in patients with active pathological bleeding and in those with history of intracranial haemorrhage, and severe hepatic impairment

- Healthy volunteers with concomitant administration of medicinal products that may increase the risk of bleeding (eg, non-steroidal anti-inflammatory), oral anticoagulants, and/or fibrinolytics within 24 hours of ticagrelor dosing).

- Since co-administration of ticagrelor with desmopressin did not decrease template bleeding time, desmopressin is unlikely to be effective in managing clinical bleeding events.

- Antifibrinolytic therapy (aminocaproic acid or tranexamic acid) and/or recombinant factor VIIa may augment hemostasis.

- Co-administration of ticagrelor with strong CYP3A4 inhibitors (eg, ketoconazole, clarithromycin, nefazadone, ritonavir, and atanazavir) should be avoided as co-administration may lead to a substantial increase in exposure to ticagrelor.

Other medication, which is considered necessary for the healthy volunteer's safety and well-being, may be given at the discretion of the Investigator and recorded in the appropriate sections of the electronic Case Report Form (eCRF).

5.7 Treatment compliance

The date and time of administration of the investigational product and additional study drugs should be recorded in the appropriate sections of the eCRF.

Treatment compliance will be assured by the supervised administration of the investigational product and additional study drugs by the Investigator or delegate.

5.7.1 Accountability

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

The study personnel will account for all investigational product and additional study drugs administered to the healthy volunteers.

The study center personnel will account for all investigational product and additional study drugs received at the study center, unused investigational product and additional study drugs, as well as for the appropriate destruction.

Certificates of delivery and return should be signed.

5.8 Discontinuation of investigational product

Healthy volunteers may be discontinued from investigational product administration in the following situations:

- Healthy volunteer decision. The healthy volunteer is at any time free to discontinue his or her participation in the study, without prejudice to further treatment

- Adverse Event
- Severe non-compliance to the CSP as judged by the Investigator and/or AstraZeneca
- Development of a condition or an acute event (eg, vascular event, MI, or stroke) that necessitates treatment with one or more prohibited medications, or disables the healthy volunteer from following the study procedures
- Safety reasons as judged by the Investigator, AstraZeneca or their delegate
- Incorrect enrolment (ie, the healthy volunteer does not meet the required inclusion/exclusion criteria for the study)
- Pregnancy

Once randomized into the study, all healthy volunteers will be assessed unless they have withdrawn their informed consent for study participation.

Healthy volunteers are at any time free to withdraw from the study (investigational product, additional study drug, and assessments), without prejudice to further treatment (withdrawal of consent). Such healthy volunteers will always be asked about the reason(s) and the presence of any AEs. If possible, they will be seen and assessed by an Investigator. Adverse events will be followed up as applicable.

5.8.1 Procedures for discontinuation of a subject from investigational product administration

A healthy volunteer that decides to discontinue the investigational product administration will always be asked about the reason(s) and the presence of any AEs. If possible, they will be seen and assessed by an Investigator. Adverse events will be followed up (See Sections 6.4.3 and 6.4.4); and all investigational product and additional study drugs should be returned by the healthy volunteer.

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

5.9 Withdrawal from study

Healthy volunteers are at any time free to withdraw from the study (investigational product, additional study drugs, and assessments), without prejudice to further treatment (withdrawal of consent). Such healthy volunteers will always be asked about the reason(s) and the presence of any AEs. If possible, they will be seen and assessed by an Investigator. Adverse events will be followed up (See Sections 6.4.3 and 6.4.4).

Withdrawn healthy volunteers will be replaced at the discretion of the Sponsor. Healthy volunteers prematurely withdrawn from the study for any reason may be replaced to ensure that 12 healthy volunteers complete each cohort.

6. COLLECTION OF STUDY VARIABLES

The study assessments are described in the sections below and the timing of these assessments are detailed in the study plan (Table 1).

It is important that blood sampling occurs as close as possible to the scheduled time points. In order to achieve this, other assessments scheduled at the same time point may be initiated prior to the time point. The sequence at a particular time point is:

1. Vital signs measurements

2. Blood sampling for ADP-induced platelet aggregation and VerifyNOW™ $P2Y_{12}$

6.1 Recording of data

The Investigator will ensure that data are recorded on the eCRFs 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 (CSA). 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 at enrolment and follow-up

6.2.1 Enrollment procedures

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

- Obtaining signed and dated written informed consent (and informed consent for pharmacogenetic sample collection) before starting any study-specific procedures
- Review of the inclusion/exclusion criteria with the healthy volunteer
- Recording of demographic data (date of birth, gender, and race)
- A standard recording of medical/surgical history
- A complete physical examination
- Height, weight, and calculation of BMI
- Vital signs (resting supine SBP, DBP, pulse rate, and body temperature)

- Blood sampling for routine clinical chemistry and hematology measurements and a hepatitis B, hepatitis C, HIV screen, FSH, and LH
- Urine sampling for routine urinalysis
- Stool sample for fecal occult blood
- Drugs of abuse and alcohol screen in urine
- Cotinine as per the exclusion criteria
- Pregnancy test (if applicable)
- 12-lead ECG
- AE questioning
- Concomitant medication use

Blood samples for the determination of the CYP2C19 genotype status, only for healthy volunteers to be included in the relevant clopidogrel cohort, will be collected on Days -35 to -30 after they have signed and dated the additional informed consent form.

6.2.2 Follow-up procedures

Follow-up procedures will be performed 7 to 10 days after the last PD sampling. These assessments will include a brief history and physical examination, vital signs, safety laboratory measurements (clinical chemistry, hematology and urinalysis), complete blood count, concomitant medication, and AE questioning.

6.3 Pharmacodynamics

Pharmacodynamic Assessment

- Healthy volunteers will be assigned to receive an autologous platelet transfusion at 24, 48, or 12 hours after the dose of ticagrelor or clopidogrel as illustrated in Figure 1 and noted in Table 1.
- IPA will be determined pre- and posttransfusion by LTA and VerifyNow at the time points noted in Table 2, Table 3, Table 4, and Table 5.

6.4 Safety

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

6.4.1 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 screening or washout periods, even if no investigational product and additional study drug have been administered.

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

6.4.2 Definitions of serious adverse event

A serious adverse event (SAE) is an AE occurring during any study phase (ie, pre-screening, screening, treatment periods, washout, and follow-up), that fulfils one or more of the following criteria:

- Results in death
- Is immediately life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/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 healthy volunteer or may require medical intervention to prevent one of the outcomes listed above

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

6.4.3 Recording of adverse events

Time period for collection of adverse events

Adverse events will be collected from Day -2 up to and including the Follow-up visit.

All SAEs will be recorded from the time of informed consent.

Follow-up of unresolved adverse events

Any AEs that are unresolved at the Follow-up visit in the study are followed up 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 healthy volunteer with ongoing AE(s)/SAE(s) at the end of the study, if judged necessary.

Variables

The following variables will be collect for each AE;

- AE (verbatim)
- The date and time when the AE started and stopped
- Maximum 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 and additional study drugs (yes or no)
- Action taken with regard to the investigational product and additional study drugs
- Whether the AE caused the healthy volunteer's withdrawal from the study (yes or no)
- Outcome

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

- Date AE met criteria for SAE
- Date 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 study procedure(s)

- Causality assessment in relation to other medication
- Causality assessment in relation to the investigational product and additional study drugs
- 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 6.4.2. 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 causal relationship between investigational product and/or additional study drug 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 and/or additional study drug?'

For SAEs causal relationship will also be assessed for other medication, study procedures, and additional study drug. 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 the CSP.

Adverse Events based on signs and symptoms

All AEs spontaneously reported by the healthy volunteer or reported in response to the open question from the study personnel: *'Have you/the child 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 protocol mandated laboratory tests and vital signs will be summarized in the Clinical Study Report (CSR). Deterioration as compared to baseline in protocol-mandated laboratory values, and vital signs should therefore only be reported as AEs if they fulfil any of the SAE criteria or are the reason for discontinuation of treatment with the investigational product.

If deterioration in a laboratory value/vital sign is associated with clinical signs and symptoms, the sign or symptom will be reported as an AE and the associated laboratory result/vital sign will be considered as additional information. Wherever possible the reporting 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.

NB. Cases where a healthy volunteer shows an aspartate aminotransferase (AST) **or** alanine aminotransferase (ALT) ≥3x upper limit of normal (ULN) **or** total bilirubin ≥ 2xULN may need to be reported as SAEs, please refer to Appendix D 'Actions required in cases of combined increase of Aminotransferase and Total Bilirubin – Hy's Law', for further instructions.

6.4.4 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 in the course of the study, then Investigators or other site personnel inform appropriate AstraZeneca representatives immediately, or **no later than 24 hours** 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. Investigators or other site personnel inform AstraZeneca representatives of any follow-up information on a previously reported SAE immediately, or **no later than 24 hours** of when he or she becomes aware of it.

6.4.5 Laboratory safety assessment

Blood and urine samples for determination of clinical chemistry, hematology, and urinalysis will be collected at the times indicated in the Study Plan (Table 1). The date and time of collection of all laboratory tests will be recorded in the appropriate eCRF.

The following laboratory variables will be measured:

Clinical chemistry

Serum (S)-Albumin

S-Alanine aminotransferase (ALT)

S-Aspartate aminotransferase (AST)

S-Alkaline phosphatase (ALP)

S-Bilirubin, total

S-Calcium, total

S-Creatinine

S-Fasting glucose

S-Potassium

S-Sodium

Coagulation

International normalised ratio (INR)

Activated partial thrombin time (aPTT)

Thrombin time (TT)

Partial prothrombin time (PT)

Hematology

Blood (B)-Hemoglobin

B-Leukocyte

B-Absolute leukocyte differential count

B-Platelet count

Urinalysis

Urine (U)-Glucose

U-Hemoglobin

U-Protein

Urine Drug Screen

All healthy volunteers will be tested for HIV, hepatitis B surface antigen and antibodies to hepatitis C at screening. In addition, a stool sample will be collected at screening for the detection of any blood present in the stool (fecal occult blood test).

Healthy volunteers enrolled in the relevant clopidogrel cohort should also have been tested regarding their CYP2C19 genotype status.

Urine samples will be screened for alcohol, cotinine, and drugs of abuse at screening (Day -30 to Day -3 and at Day -2). Urine will be tested for the following drugs of abuse: amphetamines, barbiturates, tricyclic anti-depressants, cocaine, methadone, morphine, tetrahydrocannabinol, and opiates. A pregnancy test (as part of the clinical chemistry sample) will be performed at screening and Day -2 for all females. FSH and LH levels of females considered to be of non-childbearing potential will be determined at screening.

Laboratory values outside the reference limit suspected to be of any clinical significance will be repeated. Healthy volunteers, for whom clinical significance is confirmed, will either not be included in the study or if already enrolled, will be monitored until normalisation or for as long as the Investigator considers necessary. Additional laboratory assessments may be performed for safety reasons if judged necessary by the Investigator.

The safety laboratory samples will be analyzed using routine methods at the Physician's Reference Laboratory.

Plateletpheresis will be performed at the Community Blood Center, whilst the platelet transfusion will be performed at St. Luke's South Hospital.

NB. In case a healthy volunteer shows an AST **or** ALT ≥3xULN **or** total bilirubin ≥ 2xULN please refer to Appendix D 'Actions required in cases of combined increase of Aminotransferase and Total Bilirubin – Hy's Law', for further instructions.

For blood volume see Section 7.1.

6.4.6 Physical examination

A complete physical examination will be performed as indicated in the Study Plan (Table 1) and include an assessment of the following: general appearance, respiratory, cardiovascular, skin, head and neck (including ears, eyes, nose, mouth, and throat), lymph nodes, thyroid, abdomen, musculoskeletal (including spine and extremities), and neurological systems.

A brief physical examination (including general appearance, skin, abdomen, cardiovascular and respiratory systems) will be performed on the days of the residential periods and at discharge from the clinic. In the study database only information whether the assessment was performed or not is to be recorded as well as any AEs.

Height will be measured in centimetres and weight in kilograms. All weight measurements should be taken without shoes on a calibrated scale. The BMI will be calculated from the height and weight.

6.4.7 ECG

Electrocardiograms will be performed at the time points indicated in Table 1.

6.4.7.1 Resting 12-lead ECG

A 12-lead paper ECG for safety review by the Investigator will be performed after 10 minutes supine rest 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.

6.4.8 Vital signs

Supine blood pressure will be measured using a semiautomatic blood pressure recording device with an appropriate cuff size. The healthy volunteers will be required to rest in a supine position for at least 10 minutes prior to blood pressure, and pulse rate measurements. For timings of assessments refer to the Study Plan (Table 1).

6.4.8.1 Pulse and blood pressure

Blood pressure (DBP and SBP) and pulse rate will be measured using standard equipment with an appropriate cuff size after 10 minutes supine rest on a bed. For timing of the measurements see the Study Plan (Table 1).

6.5 Patient reported outcomes (PRO) (Not applicable)

6.6 Pharmacodynamics

6.6.1 Collection of pharmacodynamic markers

Blood samples will be collected to determine IPA (by LTA and VerifyNow™) as outlined in Table 2 to Table 5. The measurement of platelet aggregation will be done at the Phase I Unit, Quintiles

Samples will be collected, labelled, stored and processed as detailed in the Laboratory Manual.

For blood volume for LTA and VerifyNow see Section 7.1.

6.7 Pharmacogenetics

6.7.1 Collection of pharmacogenetic samples

All healthy volunteers screened for the study will have to donate a blood sample for the mandatory pharmacogenetic analysis, if they are to be screened for the relevant clopidogrel cohort. Healthy volunteers needed for the relevant clopidogrel cohort will be assessed for their CYP2C19 genotype status to ensure that they carry 2 functional (*1) alleles (2C19 *1/*1). Blood samples for this analysis will only be collected after the healthy volunteer has provided a separate genetic informed consent for the genetics sample collection.

Blood samples will be collected during the pre-screening period (Days -35 to -30) to ensure that the results will be available in time to determine if the healthy volunteer could be enrolled in the study.

Healthy volunteers will be excluded from participating in the study if they refuse to consent to the genetic analysis.

The analysis and results from the pharmacogenetic testing will be used to determine which healthy volunteers will be eligible for the relevant clopidogrel cohort (cohort 3b, cohort 4a, or cohort 4b).

Only 1 sample should be collected per healthy volunteer (relevant clopidogrel cohort only) for genetic research during the study.

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

For blood volume see Section 7.1.

6.8 Health economics (Not applicable)

7. BIOLOGICAL SAMPLING PROCEDURES

7.1 Volume of blood

The total volume of blood to be drawn from each healthy volunteer included in this study is as follows:

Table 8 Volume of blood to be drawn from each healthy volunteer

Assessment		Sample volume (mL)	No. of samples	Total volume (mL)
Safety	Clinical chemistry	8.5	8	68
	Hematology	4	8	32
	Endocrinology	3.5	1	3.5
	Coagulation	2.7	8	21.6
Other	Virology	8.5	1	8.5
	Complete blood count	4	1	4
	Genotyping**	10	1	10
	Platelet counts	2	3	6
Pharmacodynamic				
ADP - induced platelet aggregation (LTA)		4.5	29	130.5
VerifyNow™ $P2Y_{12}$ assay*		2	58	116
	Discard tube	2	28	56
Total				**456.1**

* VerifyNow™ samples will be collected in duplicate. The duplicate sample will only be used if the first sample is compromised

** Only for healthy volunteers screened for the relevant clopidogrel cohort

Table 9 Volume of blood to be drawn from each healthy volunteer for platelet apheresis

Assessment	Sample volume (mL)	No. of samples	Total volume (mL)
Platelet apheresis	250	1	250
Blood Center testing	45	1	45
Total			**295 mL**

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 here.

The results from future analysis will not be reported in the CSR.

7.2.1 Pharmacodynamic samples

Pharmacodynamic samples will be disposed of after the clinical study report has been finalised, unless retained for future analyses, see below.

7.2.2 Pharmacogenetic samples

The processes adopted for the coding and storage of samples for genetic analysis are important to maintain healthy volunteer confidentiality. Samples will be stored for a maximum of 15 years, from the date of the last healthy volunteer's last visit, after which they will be destroyed. Deoxyribonucleic acid (DNA) is a finite resource that may be used up during analyses. Samples will be stored and used until no further analyses are possible or the maximum storage time has been reached.

For all samples irrespective of the type of coding used the DNA will be extracted from the blood sample. The DNA sample will be assigned a unique number replacing the information on the sample tube. Thereafter, the DNA sample will be identifiable by the unique DNA number only. The DNA number is used to identify the sample and corresponding data at the AstraZeneca genetics laboratories, or at the designated contract laboratory. No personal details identifying the individual will be available to any person (AstraZeneca employee or contract laboratory staff working with the DNA).

The samples and data for genetic analysis in this study will be single coded. The link between the healthy volunteer's enrolment/randomization code and the DNA number will be maintained and stored in a secure environment, with restricted access WITHIN the Clinical Genotyping Group Laboratory Information Management System (LIMS) at AstraZeneca. The link will be used to identify the relevant DNA samples for analysis, facilitate correlation of genotypic results with clinical data, allow regulatory audit and to trace samples for destruction in the case of withdrawal of consent when the healthy volunteer has requested disposal/destruction of collected samples not yet analyzed.

7.3 Labelling and shipment of biohazard samples

The Principal Investigator ensures that samples are labelled 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 of this CSP 'International Airline Transportation Association (IATA) 6.2 Guidance Document'.

Any samples identified as Infectious Category A materials are not shipped and no further samples will be collected from the healthy volunteer unless agreed with AstraZeneca and appropriate labelling, 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 lifecycle.

The Principal Investigator keeps full traceability of collected biological samples from the healthy volunteers 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 study centers 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 healthy volunteer withdraws consent to the use of donated biological samples, the samples will be disposed of/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 biological samples is an integral part of the study, then the healthy volunteer is withdrawn from further study participation.

The Principal Investigator:

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

AstraZeneca ensures the central laboratory holding the samples is informed about the withdrawn consent immediately and that samples are disposed of/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 the ethical principles that have their origin in the Declaration of Helsinki and are consistent with International Conference on Harmonisation (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.

AstraZeneca will not provide individual genotype results to healthy volunteers, any insurance company, any employer, their family members, general physician or any other third party, unless required to do so by law**.**

Extra precautions are taken to preserve confidentiality and prevent genetic data being linked to the identity of the healthy volunteer. In exceptional circumstances, however, certain individuals might see both the genetic data and the personal identifiers of a healthy volunteer. For example, in the case of a medical emergency, an AstraZeneca Physician or an Investigator might know a healthy volunteer's identity and also have access to his or her genetic data. Also Regulatory authorities may require access to the relevant files, though the healthy volunteer's medical information and the genetic files would remain physically separate.

Care should be taken since the CYP2C19 genotype status of the healthy volunteers included in the relevant clopidogrel cohort will be known.

8.3 Ethics and regulatory review

An Institutional Review Board (IRB) should approve the final CSP, including the final version of the Informed Consent Forms (main and genetic) and any other written information and/or materials to be provided to the healthy volunteers. 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 enrolment of any healthy volunteer into the study.

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

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

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

Before enrolment of any healthy volunteer into the study, the final CSP, including the final version of the Informed Consent Forms, are 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, IRBs and the Principal Investigator with safety updates/reports according to local requirements, including suspected unexpected serious adverse reactions (SUSARs), where relevant.

Each Principal 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 Principal Investigator so that he/she can meet these reporting requirements.

8.4 Informed consent

The Principal Investigator will:

- Ensure each healthy volunteer is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study
- Ensure each healthy volunteer is notified that they are free to discontinue from the study at any time
- Ensure that each healthy volunteer is given the opportunity to ask questions and allowed time to consider the information provided
- Ensure each healthy volunteer provides signed and dated informed consent before conducting any procedure specifically for the study
- Ensure the original, signed Informed Consent Forms are stored in the Investigator's Study File
- Ensure a copy of the signed Informed Consent Forms are given to the healthy volunteer
- Ensure that any incentives for healthy volunteers who participate in the study as well as any provisions for healthy volunteers harmed as a consequence of study participation are described in the informed consent form that is approved by an IRB

8.5 Changes to the protocol and informed consent form

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

If there are any substantial changes to the CSP, then these changes will be documented in a protocol amendment and where required in a new version of the CSP (Revised Clinical Study Protocol).

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

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

If a protocol amendment requires a change to the Informed Consent Forms, AstraZeneca and the study center's IRB are to approve the revised Informed Consent Forms before the revised form is used.

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

8.6 Audits and inspections

Authorized representatives of AstraZeneca, a regulatory authority, or an 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 protocol, GCP, guidelines of the ICH, 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

Quintiles will manage the study on behalf of AstraZeneca.

9.1 Pre-study activities

Before the first healthy volunteer is entered into the study, it is necessary for a representative of AstraZeneca to visit the investigational study center to:

- Determine the adequacy of the facilities
- Determine availability of appropriate healthy volunteers for the study

- Discuss with the Investigator (and other personnel involved with the study) their responsibilities with regard to CSP adherence, and the responsibilities of AstraZeneca or its representatives. This will be documented in a CSA between AstraZeneca and the Investigator.

9.2 Training of study center personnel

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

The Principal 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 Principal 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, an AstraZeneca representative will have regular contacts with the study center, including visits to:

- Provide information and support to the Investigator(s)
- Confirm that facilities remain acceptable
- Confirm that the investigational team is 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 study drug accountability checks are being performed
- Perform source data verification (a comparison of the data in the eCRFs with the healthy volunteer's medical records at the hospital or practice, and other records relevant to the study) including verification of informed consent of participating healthy volunteers. This will require direct access to all original records for each healthy volunteer (eg, clinic charts)
- Ensure withdrawal of informed consent to the use of the healthy volunteer's biological samples is reported and biological samples are identified and disposed of/destroyed accordingly, and the action is documented, and reported to the healthy volunteer.

The AstraZeneca representative will be available between visits if the Investigator(s) or other staff at the study center needs information and advice about the study conduct.

9.3.1 Source data

Refer to the CSA for the location of the source data.

9.4 Study agreements

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

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

9.4.1 Archiving of study documents

The Investigator follows the principles outlined in the CSA.

9.5 Study timetable and end of study

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

The study is expected to start in Q4 2012 and to end by Q2 2013.

The study may be terminated 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

Data management will be performed by Quintiles Phase I Unit,

The Data Management Plan will describe the methods used to collect, check and process the clinical data in detail. It will also clarify the roles and responsibilities for the different functions and personnel involved in the data management process. Furthermore the Data Management Plan will describe the data flow and timelines within the study.

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

Adverse events and medical/surgical history will be classified according to the terminology of the latest version of the Medical Dictionary for Regulatory Activities (MedDRA). Medications will be classified according to the AstraZeneca Drug Dictionary. All coding will be performed by Quintiles.

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, signed, and locked, clean file will be declared. Any treatment revealing data may thereafter be added and the final database will be locked.

Any genotype data generated in this study will be stored in the AstraZeneca genotyping LIMS database, or other appropriate secure system within AstraZeneca and/or third party contracted to work with AstraZeneca to analyse samples. The results from this genetic research may be reported in the CSR for the main study as the data will be applicable to the eligibility criteria for healthy volunteers to be included in the study.

Genotype data will be transferred to the clinical database, and merged with the clinical data from the main study, prior to the statistical analysis and reporting of the study.

11. EVALUATION AND CALCULATION OF VARIABLES

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 -2 for the given period
- Vital signs: Day 1, predose for the given period

If a healthy volunteer 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 (OAE)

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 DAEs. Based on the expert's judgement, significant adverse events of particular clinical importance may, after consultation with the Global Safety Physician, be considered OAEs and reported as such in the Clinical Study Report. A similar review of laboratory/vital signs/ECG 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 patient reported outcome variables (not applicable)

11.3 Calculation or derivation of pharmacokinetic variable (not applicable)

11.4 Calculation or derivation of pharmacodynamic variable(s)

Pharmacodynamic analyses will be the responsibility of Biostatistician at Quintiles, Early Clinical Development,

The following measures collected from LTA and Verify Now tests will be used for further statistical analysis:

- Percent of inhibition of ADP induced platelet aggregation (LTA) at protocol scheduled time points
  - Percent inhibition of platelet aggregation will be calculated using data obtained at baseline (Day 1 predose) and all other subsequent time points using the following formula.

$$IPA\ (\%) = 100 \times \frac{PA_b - PA_t}{PA_b}$$

  - Where PA_b is the response at baseline and PA_t is platelet aggregation at any post treatment time point. Percent inhibition will be restricted to the closed interval [0,100]; any data falling outside this range will be truncated to the appropriate limit (i.e., any value less than 0 will be set to 0).
- Platelet reactivity as measured by P2Y12 Reaction Units (PRU) using VerifyNow™ at the protocol scheduled time points

11.5 Calculation or derivation of pharmacogenetic variables (Not applicable)

11.6 Calculation or derivation of health economic variables (not applicable)

12. STATISTICAL METHODS AND SAMPLE SIZE DETERMINATION

12.1 Description of analysis sets

12.1.1 Safety analysis set

All healthy volunteers who receive at least 1 administration of the investigational product and for whom any postdose data are available will be included in the safety analysis set

12.1.2 Pharmacodynamic analysis set

The PD analysis set will be used for all analyses of PD data. The PD analysis set is defined as all healthy volunteers in the safety analysis set who have no major protocol deviations (including those that affect the integrity of the PD results; eg, non-compliance with study drug).

12.2 Methods of statistical analyses

12.2.1 General principles

The PD and safety summaries and the statistical analysis will be the responsibility of the study biostatistician at Quintiles Global Phase 1 (using SAS® Version 9.2 or higher and, where appropriate, additional validated software). Graphics may be prepared with SAS® Version 9.2, or higher; SigmaPlot® 9.0, or higher (Systat Software, Inc., San Jose, California, United States); or WinNonlin Professional 5.2, or higher. Quantitative continuous variables will be summarized by treatment using descriptive statistics, including n, mean, standard deviation (SD), median, minimum, and maximum values.

In general, descriptive statistics will follow the rounding convention in Quintiles Global Phase 1 SOPs.

12.2.2 Safety Analyses

All safety data (scheduled and unscheduled) will be presented in the data listings. Continuous variables will be summarized by cohort, treatment, and time point using descriptive statistics (n, mean, SD, minimum, median, maximum). Categorical variables will be summarized by cohort, treatment, and time point in frequency tables (frequency and proportion). Safety variables (eg, clinical laboratory values and vital signs) will be reported to the same precision as the source data. Derived variables will be reported using similar precision to those from which they were derived (eg, QTc derived from QT interval).

Extra measurements (such as unscheduled or repeat assessments) will not be included in the descriptive statistics by scheduled time point, but will be included in data listings. All AEs, ECG outliers, and clinical laboratory outliers that occur following the first administration of the investigational product will be included in the tabulations of AEs and outlier events, including episodes that occur at unscheduled evaluations.

All available data from volunteers in the safety analysis set will be included in the safety analyses. No adjustment or imputation will be utilized for missing values or for volunteers who withdraw prior to completing the study, neither will analyses be restricted to volunteers with complete data.

All AEs will be collected for each volunteer from the time when informed consent is obtained until the follow-up visit. Adverse events that occur before dosing will be reported separately.

Adverse events will be summarized by Preferred Term and System Organ Class using Medical Dictionary for Regulatory Activities (MedDRA) vocabulary (Version 12.0 or higher) by cohort and treatment, and across cohorts and all treatments, as appropriate. Furthermore, listings of SAEs and AEs that lead to withdrawal will be presented and the number of volunteers who have any AEs, SAEs, AEs that lead to withdrawal, and AEs with severe intensity will be summarized.

Tabulations and listings of data for vital signs, clinical laboratory tests, ECGs, and physical examination findings will be presented. All continuous safety data will be summarized by cohort and treatment at each scheduled assessment and for the corresponding change from baseline. For clinical laboratory tests, listings of values for each volunteer will be presented with abnormal or out-of-range values flagged. Clinical laboratory data will be reported in Système International units in the CSR.

12.2.3 Pharmacodynamic Analyses

Listings of PD blood sampling times will be generated. LTA and VerifyNow™ results as well as IPA determined by LTA and VerifyNow™ will be listed by cohort, treatment and time.

Where data are available, IPA (LTA) and IPA (VerifyNow™) will be summarized as follows.

IPA curves vs. time (t=0, predose until t=96h) will be plotted on healthy volunteer level (one healthy volunteer per plot), with treatment A (transfusion) and treatment B (no transfusion) indicated in the same plot (cohort 1, 2 and potential clopidogrel cohort 3b, 4a or 4b. Treatment A only for potential cohort 3a).

Arithmetic treatment mean (± SD) of IPA will be plotted vs time by cohort, with treatment A and treatment B indicated in the same plot (Treatment A only for potential cohort 3a). Corresponding descriptive statistics (n, mean, SD, minimum, median, maximum) will be presented by IPA method, cohort, treatment and time.

Descriptive statistics (n, mean, SD, minimum, median, maximum) of within healthy volunteer differences between treatment A and treatment B for cohort 1, 2 and potential clopidogrel cohort (3b, 4a or 4b) will be presented by IPA method, cohort and time.

Primary Analyses

To assess the effect of platelet transfusion on IPA (LTA), 12-hour posttransfusion values will be compared by cohort. Comparisons between the platelet transfusion treatment (test) and the without platelet transfusion treatment (reference) will be performed by cohort for cohorts 1, 2 and potential cohort 3a. For cohort 1 and 2, IPA will be analyzed using a mixed effects model with treatment, period and sequence as fixed effects and healthy volunteer nested within sequence as a random effect. For cohort 3a 12-hour posttransfusion values (platelet transfusion treatment [treatment A]) will be compared to the 24-hour posttreatment values (without platelet transfusion treatment [treatment B]) from both cohorts 1 and 2 using a fixed effects model with treatment as fixed effect. Least squares means and associated 95% confidence intervals (CI's) will be calculated and presented. Also, the difference between platelet transfusion and without transfusion treatments and associated 95% CI's will be calculated and presented. In case of deviation from normality, transformation of IPA data or nonparametric methods will be considered.

Secondary Analyses

For IPA (LTA), 12-hour posttransfusion values for the potential clopidogrel cohort (3b, 4a or 4b) and IPA (VerifyNow™) for all cohorts will be compared by cohort using the same approach as described for the primary analyses.

To evaluate a potential rebound in anti-platelet effect, change in IPA from pretransfusion time point (48, 24 or 12 hours postdose as indicated in Table 2, Table 3, and Table 4) will be calculated and presented descriptively (n, mean, SD, minimum, median, maximum) by cohort, posttransfusion sampling time and treatment.

12.2.4 Interim analyses

Cohorts 1 (24-hour transfusion) and 2 (48-hour transfusion) will be analysed as described in Section 12.2.3. Based on the results for cohort 1 and 2, inclusion of an additional 12-hour transfusion cohort (3a) and/or clopidogrel cohort (3b, 4a or 4b) will be decided as described in Section 3.1. If potential cohort 3a is run, analyses as described in Section 12.2.3 will be performed for cohort 3a before running cohort 4a or 4b.

12.3 Determination of sample size

The sample size is based on the desire to obtain adequate safety, tolerability and PD data to achieve the objectives of the study whilst exposing as few healthy volunteers as possible to the study treatment and procedures.

From studies D5130C00006 and D5130C00007, the estimated within subject standard deviation for IPA is not larger than 31%. With 12 subjects a 95% CI for the difference in IPA

will have a half width of 17.5%. Based on the RESPOND study an estimate of the within subject standard deviation for the clopidogrel group is 29% and with 12 subjects a 95 % CI for the IPA difference has a half width of 15.8%.

The number of healthy volunteers that will agree to participate in the genetic research is unknown.

12.4 Data monitoring committee (not applicable)

13. IMPORTANT MEDICAL PROCEDURES TO BE FOLLOWED BY THE INVESTIGATOR

13.1 Medical emergencies and AstraZeneca contacts

The Principal 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 6.4.4.**

In the case of a medical emergency the investigator may contact the CPA Physician. If the CPA Physician is not available, contact the AstraZeneca CPA Programme Director.

Name	Role in the study	Address & telephone number
	CPA Programme Director	Alliance Management Clinical Operations AstraZeneca R&D
	CPA Physician	AstraZeneca R&D
Serious adverse event reporting	24-hour emergency cover at central R&D site	
	Quintiles Principal Investigator	Quintiles Phase I Services
	Quintiles Project Manager	Quintiles Phase I Services

13.2 Overdose

There is no known antidote to ticagrelor. In cases of suspected overdose the healthy volunteer should be treated per standard medical practice based on the judgement of the Investigator. The healthy volunteer should be monitored closely and treated symptomatically.

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 in the course of the study, then Investigators or other study center personnel inform appropriate AstraZeneca representatives **within 1 day**, ie, immediately **but no later than 24 hours** of when he or 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 SAE, standard reporting timelines apply, see Section 6.4.4. For other overdoses, reporting should be done within 30 days.

13.3 Pregnancy

All pregnancies and their subsequent outcome (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth or congenital abnormality) should be reported to AstraZeneca using the appropriate forms.

13.3.1 Maternal exposure

Women of childbearing potential are not allowed to be included in this study. Should a pregnancy still occur, the investigational product should be discontinued immediately and the pregnancy reported to AstraZeneca.

13.3.2 Paternal exposure

Pregnancy of a healthy volunteer's partner is not considered to be an AE. However, any conception occurring from the date of administration of the investigational product until 3 months after the administration of the investigational product should be reported to AstraZeneca and followed up for its outcome.

14. LIST OF REFERENCES

PLAVIX PI 2010
Plavix Prescribing Information, Bristol-Myers Squibb/Sanofi Pharmaceuticals Partnership: 2010 [CV.000-566-087.2.3] (www.plavix.com)

Pruller et al 2011
Pruller F, Drexler C, Archan S, Macher S, Raggam RB, Mahla E. Low platelet reactivity is recovered by transfusion of stored platelets: a healthy volunteer in vivo study. J Thromb Haemost 2011; 9: 1670–3

Vilahur et al 2007
Vilahur G, Choi BG, Zfar MU et al. Normalization of platelet reactivity in clopidogrel-treated subjects. J Thromb Haemostasis 2007;5:82-90

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Clinical Study Protocol Appendix A

Drug Substance	Ticagrelor
Study Code	D5130C00079
Edition Number	1
Date	21 November 2012
Protocol Dated	21 November 2012

Appendix A
Signatures

ASTRAZENECA SIGNATURE(S)

A open label, randomized, cross-over and potential parallel, single dose study of ticagrelor 180 mg and acetylsalicylic acid (ASA) in healthy volunteers followed by autologous *in vivo* platelet transfusion to determine the effects of platelet supplementation on the reversibility of platelet inhibition

This Clinical Study Protocol has been subjected to an internal AstraZeneca peer review.

I agree to the terms of this study protocol/amendment.

AstraZeneca Research and Development site representative

28 - Nov - 2012
Date
(Day Month Year)

ASTRAZENECA SIGNATURE(S)

A open label, randomized, cross-over and potential parallel, single dose study of ticagrelor 180 mg and acetylsalicylic acid (ASA) in healthy volunteers followed by autologous *in vivo* platelet transfusion to determine the effects of platelet supplementation on the reversibility of platelet inhibition

This Clinical Study Protocol has been subjected to an internal AstraZeneca peer review.

I agree to the terms of this study protocol/amendment.

AstraZeneca Research and Development site representative

28 November 2012
Date
(Day Month Year)

Global Product Statistician

SIGNATURE OF PRINCIPAL INVESTIGATOR

A open label, randomized, cross-over and potential parallel, single dose study of ticagrelor 180 mg and acetylsalicylic acid (ASA) in healthy volunteers followed by autologous *in vivo* platelet transfusion to determine the effects of platelet supplementation on the reversibility of platelet inhibition

This Clinical Study Protocol has been subjected to an internal AstraZeneca peer review.

I agree to the terms of this study protocol. I will conduct the study according to the procedures specified herein, and according to the principles of Good Clinical Practice (GCP) and local regulations and I ensure that all relevant site staff follows the instructions given in the latest version of the Laboratory Manual for Investigators.

Centre No.:

Signature:

Phil Leese MD
Quintiles Early Clinical Development Services

Fr Nov 20/2

Date
(Day Month Year)

AstraZeneca

Clinical Study Protocol Appendix B

Drug Substance	Ticagrelor
Study Code	D5130C00079
Edition Number	1
Date	21 November 2012

Appendix B
Additional Safety Information

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

Life threatening

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

Hospitalisation

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

Important medical event or medical intervention

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

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

Examples of such events are:

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

A GUIDE TO INTERPRETING THE CAUSALITY QUESTION

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

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

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

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

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

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

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

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

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

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

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

- Is there a known mechanism?

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

Clinical Study Protocol Appendix C

Drug Substance	Ticagrelor
Study Code	D5130C00079
Edition Number	1
Date	21 November 2012

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

LABELLING 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.

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Clinical Study Protocol Appendix D	
Drug Substance	Ticagrelor
Study Code	D5130C00079
Edition Number	1
Date	21 November 2012

Appendix D
Actions Required in Cases of Combined Increase of Aminotransferase and Total Bilirubin - Hy's Law

TABLE OF CONTENTS PAGE

1. INTRODUCTION

During the course of the study the Investigator will remain vigilant for increases in liver biochemistry. The investigator is responsible for determining whether a patient meets potential Hy's Law (PHL) criteria at any point during the study.

The Investigator participates, together with AstraZeneca clinical project representatives, in review and assessment of cases meeting PHL criteria to agree whether Hy's Law (HL) criteria are met. HL criteria are met if there is no alternative explanation for the elevations in liver biochemistry other than Drug Induced Liver Injury (DILI) caused by the Investigational Medicinal Product (IMP).

The Investigator is responsible for recording data pertaining to PHL/HL cases and for reporting Adverse Events (AE) and Serious Adverse Events (SAE) according to the outcome of the review and assessment in line with standard safety reporting processes.

2. DEFINITIONS

Potential Hy's Law (PHL)

Aspartate Aminotransferase (AST) or Alanine Aminotransferase (ALT) ≥3x Upper Limit of Normal (ULN) **and** Total Bilirubin (TBL) ≥2xULN at any point during the study irrespective of an increase in Alkaline Phosphatase (ALP). The elevations do not have to occur at the same time or within a specified time frame.

Hy's Law (HL)

AST or ALT ≥3xULN **and** TBL ≥2xULN, where no other reason, other than the IMP, can be found to explain the combination of increases, eg, elevated ALP indicating cholestasis, viral hepatitis, another drug. The elevations do not have to occur at the same time or within a specified time frame.

3. IDENTIFICATION OF POTENTIAL HY'S LAW CASES

In order to identify cases of PHL it is important to perform a comprehensive review of laboratory data for any patient who meets any of the following identification criteria in isolation or in combination:

- ALT ≥ 3xULN
- AST ≥ 3xULN
- TBL ≥ 2xULN

When a patient meets any of the identification criteria, in isolation or in combination, the central laboratory will immediately send an alert to the Investigator (also sent to AstraZeneca representative).

The Investigator will also remain vigilant for any local laboratory reports where the identification criteria are met, where this is the case the Investigator will:

- Request a repeat of the test (new blood draw) by the central laboratory
- Complete the appropriate unscheduled laboratory CRF module(s) with the original local laboratory test result

When the identification criteria are met from central or local laboratory results the Investigator will without delay:

- Determine whether the patient meets PHL criteria (see Section 2 of this Appendix for definition) by reviewing laboratory reports from all previous visits (including both central and local laboratory results)

The Investigator will without delay review each new laboratory report and if the identification criteria are met will:

- Notify the AstraZeneca representative
- Determine whether the patient meets PHL criteria (see Section 2 of this Appendix for definition) by reviewing laboratory reports from all previous visits
- Promptly enter the laboratory data into the laboratory CRF

4. FOLLOW-UP

4.1 Potential Hy's Law Criteria not met

If the patient does not meet PHL criteria the Investigator will:

- Inform the AstraZeneca representative that the patient has not met PHL criteria.
- Perform follow-up on subsequent laboratory results according to the guidance provided in the Clinical Study Protocol.

4.2 Potential Hy's Law Criteria met

If the patient does meet PHL criteria the Investigator will:

- Determine whether PHL criteria were met at any study visit prior to starting study treatment in the presence of liver metastases (See Section 6)

- Notify the AstraZeneca representative who will then inform the central Study Team

The Study Physician contacts the Investigator, to provide guidance, discuss and agree an approach for the study patients' follow-up and the continuous review of data. Subsequent to this contact the Investigator will:

- Monitor the patient until liver biochemistry parameters and appropriate clinical symptoms and signs return to normal or baseline levels, or as long as medically indicated

- Investigate the etiology of the event and perform diagnostic investigations as discussed with the Study Physician

- Complete the three Liver CRF Modules as information becomes available

- If at any time (in consultation with the Study Physician) the PHL case meets serious criteria, report it as an SAE using standard reporting procedures

5. REVIEW AND ASSESSMENT OF POTENTIAL HY'S LAW CASES

The instructions in this Section should be followed for all cases where PHL criteria were met.

No later than 3 weeks after the biochemistry abnormality was initially detected, the Study Physician contacts the Investigator in order to review available data and agree on whether there is an alternative explanation for meeting PHL criteria other than DILI caused by the IMP. The AstraZeneca Medical Science Director and Global Safety Physician will also be involved in this review together with other subject matter experts as appropriate.

According to the outcome of the review and assessment, the Investigator will follow the instructions below.

If there **is** an agreed alternative explanation for the ALT or AST and TBL elevations, a determination of whether the alternative explanation is an AE will be made and subsequently whether the AE meets the criteria for a SAE:

- If the alternative explanation is **not** an AE, record the alternative explanation on the appropriate CRF

- If the alternative explanation is an AE/SAE, record the AE /SAE in the CRF accordingly and follow the AZ standard processes

If it is agreed that there is **no** explanation that would explain the ALT or AST and TBL elevations other than the IMP:

- Report an SAE (report term 'Hy's Law') according to AstraZeneca standard processes.
  - The 'Medically Important' serious criterion should be used if no other serious criteria apply
  - As there is no alternative explanation for the HL case, a causality assessment of 'related' should be assigned.

If, there is an unavoidable delay, of over 3 weeks, in obtaining the information necessary to assess whether or not the case meets the criteria for HL, then it is assumed that there is no alternative explanation until such time as an informed decision can be made:

- Report an SAE (report term 'Potential Hy's Law') applying serious criteria and causality assessment as per above
- Continue follow-up and review according to agreed plan. Once the necessary supplementary information is obtained, repeat the review and assessment to determine whether HL criteria are met. Update the SAE report according to the outcome of the review

6. ACTIONS REQUIRED WHEN POTENTIAL HY'S LAW CRITERIA ARE MET BEFORE AND AFTER STARTING STUDY TREATMENT

This section is applicable to patients who meet PHL criteria on study treatment having previously met PHL criteria at a study visit prior to starting study treatment.

At the first on study treatment occurrence of PHL criteria being met the Investigator will:

- Determine if there has been a significant change in the patients' condition[#] compared with the last visit where PHL criteria were met[#]
  - If there is no significant change no action is required
  - If there is a significant change notify the AstraZeneca representative, who will inform the central Study Team, then follow the subsequent process described is Section 4.2 of this Appendix

[#] A 'significant' change in the patient's condition refers to a clinically relevant change in any of the individual liver biochemistry parameters (ALT, AST or total bilirubin) in isolation or in combination, or a clinically relevant change in associated symptoms. The determination of whether there has been a significant change will be at the discretion of the Investigator, this may be in consultation with the Study Physician if there is any uncertainty.

At the first on study treatment occurrence of PHL criteria being, even if there has been no significant change the patient's condition[#] compared with pre-study treatment visits, the Investigator will:

- Notify the AstraZeneca representative who will inform the central Study Team.
- Follow the subsequent process described is Section 4.2 of this Appendix.

[#] A 'significant' change in the patient's condition refers to a clinically relevant change in any of the individual liver biochemistry parameters (ALT, AST or total bilirubin) in isolation or in combination, or a clinically relevant change in associated symptoms. The determination of whether there has been a significant change will be at the discretion of the Investigator, this may be in consultation with the Study Physician if there is any uncertainty.

7. ACTIONS REQUIRED FOR REPEAT EPISODES OF POTENTIAL HY'S LAW

This section is applicable when a patient meets PHL criteria on study treatment and has already met PHL criteria at a previous on study treatment visit.

The requirement to conduct follow-up, review and assessment of a repeat occurrence(s) of PHL is based on the nature of the alternative cause identified for the previous occurrence.

The investigator should determine the cause for the previous occurrence of PHL criteria being met and answer the following question:

- Was the alternative cause for the previous occurrence of PHL criteria being met chronic or progressing malignant disease or did the patient meet PHL criteria prior to starting study treatment and at their first on study treatment visit as described in Section 6 ?

If No: follow the process described in Section 4.2 of this Appendix

If Yes:

Determine if there has been a significant change in the patient's condition[#] compared with when PHL criteria were previously met

- If there is no significant change no action is required
- If there is a significant change follow the process described in Section 4.2 of this Appendix

[#] A 'significant' change in the patient's condition refers to a clinically relevant change in any of the individual liver biochemistry parameters (ALT, AST or total bilirubin) in isolation or in combination, or a clinically relevant change in associated symptoms. The determination of

whether there has been a significant change will be at the discretion of the Investigator, this may be in consultation with the Study Physician if there is any uncertainty.

8. REFERENCES

FDA Guidance for Industry (issued July 2009) 'Drug-induced liver injury: Premarketing clinical evaluation':

http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM174090.pdf