AstraZeneca

Clinical Pharmacology Study Protocol

Drug substance	AZD6140
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Date	

A Randomized, Double-Blind, Placebo-Controlled Study to Assess the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Single Ascending Oral Doses of AZD6140 Tablets in Healthy Male and Female Japanese and Caucasian Subjects

AstraZeneca Research and Development site representative

Date
(Day Month Year)

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Date
(Day Month Year)

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
1			

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

ASTRAZENECA EMERGENCY CONTACT PROCEDURE

In the case of a medical emergency you may contact the Clinical Study Team Leader. If the Clinical Study Team Leader is not available you may contact the Clinical Study Team Physician.

Role in the study	Name	Address and Telephone number
Clinical Study Team Leader		
Clinical Research Scientists		
Clinical Study Team Physician		

For further clarifications regarding:

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

PROTOCOL SYNOPSIS

A Randomized, Double-Blind, Placebo-Controlled Study to Assess the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Single Ascending Oral Doses of AZD6140 Tablets in Healthy Male and Female Japanese and Caucasian Subjects

Investigator

Study center, type and number of subjects planned

At least 40 healthy male and female subjects to include 20 Japanese and 20 Caucasian subjects from 18 to 45 years of age will be randomized at a single center.

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

Objectives

Primary Objective:

To assess the safety and tolerability of single ascending oral doses of AZD6140 in healthy Japanese and Caucasian subjects by means of incidence and severity of adverse events, 12-lead ECG, vital signs, laboratory parameters and physical examinations.

Secondary Objectives:

To assess and compare the pharmacokinetics following single ascending oral doses of AZD6140 in healthy Japanese and Caucasian subjects by assessment of C_{max} and AUC.

To assess and compare the pharmacodynamics following single ascending oral doses of AZD6140 in healthy Japanese and Caucasian subjects by assessment of platelet aggregation inhibition and bleeding time.

Study design

This is a randomized, double-blind, placebo-controlled study designed to assess the safety, tolerability, pharmacokinetics and pharmacodynamics of single ascending oral doses of AZD6140 in 20 healthy Japanese and 20 healthy Caucasian male and female (non-childbearing potential) subjects.

Investigational product, dosage and mode of administration

The following study drug will be supplied:

- AZD6140 Immediate release tablets 50 mg, 100 mg and 200 mg
- AZD6140 Placebo to match 50 mg, 100 mg and 200 mg

AZD6140/placebo tablets will be administered as a single dose of 50 mg, 100 mg, 200 mg, 300 mg, 400 mg and 600 mg.

Duration of treatment

Subjects in each ethnic group will be divided into 2 cohorts of 10 subjects each. Within each cohort, 8 subjects will receive single ascending doses of AZD6140 on 3 occasions (Cohort A: 50, 200 and 400 mg; Cohort B: 100, 300 and 600 mg). Two subjects in each cohort will receive placebo on 3 occasions. Consecutive single dose administrations will be separated by a washout period of at least 5 days. Progression to the next dose level will not be permitted until all safety data have been reviewed by the Principal Investigator and study team physician of AstraZeneca. Caucasians subjects may be escalated independently of the Japanese subjects.

Outcome variables

Safety and Tolerability

Safety and tolerability will be assessed by means of incidence and severity of adverse events, 12-lead ECG, vital signs, laboratory parameters and physical examinations.

Pharmacokinetics

Blood samples will be analyzed to determine the plasma concentrations of AZD6140 and its active metabolite, AR-C124910XX. The pharmacokinetic parameters Cmax, t_{max}, AUC, AUC_{0-t} and $t_{1/2}$, for AZD6140 and AR-C124910XX, and CL/F and Vz/F for AZD6140 will be estimated.

Pharmacodynamics

The level of platelet aggregation inhibition will be evaluated by optical aggregometry (20 μmol ADP), and bleeding time will be measured.

Pharmacokinetic/Pharmacodynamic Relationship

The relationship between plasma concentrations of AZD6140 and AR-C124910XX and platelet aggregation inhibition measured by optical aggregometry will be determined.

Pharmacogenetics

Genetic analysis of the genes that are involved in the disposition of and response to AZD6140 such as MDR-1 gene will be performed for those subjects providing separate informed consent.

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LIST OF ABBREVIATIONS

The following abbreviations are used in this study protocol.

Abbreviation	Explanation
%PAI	Percent inhibition of ADP-induced platelet aggregation
ADP	Adenosine diphosphate
AE	Adverse event
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
APTT	Activated partial prothrombin time
AST	Aspartate aminotransferase
AUC	Area under the plasma concentration curve from zero to infinity
$AUC_{(0-t)}$	Area under plasma concentration-time curve from zero to last measurable concentration
bid	Twice a day
BMI	Body mass index
CL	Total body clearance
CL/F	Apparent oral clearance
C_{max}	Maximum plasma drug concentration
CRC	Clinical Research Center
CRF	Case report form
DCF	Data clarification form
EC50	Concentration at which 50% of maximum effect is reached
ECG	Electrocardiogram
EDTA	Ethylene diamine tetra-acetic acid
E_{max}	Maximum effect
F	Fraction of dose systemically available
GCP	Good Clinical Practice
GGT	Gamma glutamyltransferase
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IRB	Institutional Review Board
OAE	Other significant adverse event (See Appendix E)

Abbreviation	Explanation
PA_{BL}	Mean response at pre-dose baseline
PA_T	Mean response at time "T"
PD	Pharmacodynamic
PK	Pharmacokinetic
qd	Every day
RBC	Red blood cell
SAE	Serious adverse event
t_{max}	Time to reach peak or maximum concentration or maximum response following drug administration [time]
$t_{½}$	Half-life
Vz/F	Terminal phase volume of distribution
WBC	White blood cell

1. INTRODUCTION

1.1 Background

Adenosine diphosphate (ADP) is an important mediator of platelet activation and aggregation through its binding to at least two distinct subtypes of purinoceptor, designated $P2Y_1$ and $P2Y_{12}$, found on platelets. Two ADP receptor antagonists, clopidogrel and ticlopidine have shown clear benefits for the reduction of clinical thromboembolic events in patients with atherosclerosis due to their ability to block the $P2Y_{12}$-receptor. However, since this blockade is irreversible and usually incomplete, the search continues for agents, which can further improve the clinical outcomes of these patients through improved efficacy or safety.

AZD6140 is a potent, selective $P2Y_{12}$-receptor antagonist (antiplatelet agent) being developed to reduce thromboembolic events in patients with atherosclerosis. It is orally active and does not require metabolic activation. Unlike clopidogrel and ticlopidine, which incompletely block the $P2Y_{12}$-receptor response in humans, pre-clinical studies indicate that AZD6140 can produce long-lasting and complete inhibition of ADP-induced platelet aggregation *ex vivo* following oral dosing.

Five phase I clinical trials conducted primarily in Caucasians have been completed. Two studies (SC-532-5169, SC-532-5171) have examined the tolerability of single oral doses ranging from 0.1 mg to 400 mg in healthy subjects. Complete inhibition of platelet aggregation was achieved with 100 to 400 mg doses. Mean bleeding times increased 3.0, 2.6, 2.5 and 7.4 fold 2 hours following doses of 100, 200, 300 and 400 mg respectively. The primary metabolite of AZD6140, AR-C124910XX, was present in significant (approximately 30% of parent) quantities. *In vitro* studies have shown this metabolite to be equipotent with the parent compound.

Study SC-532-5239 evaluated the safety and tolerability of multiple ascending doses of 50 mg to 600 mg/day AZD6140 in healthy male and female subjects. The safety of AZD6140 was also compared with clopidogrel. In addition, the effect of food on the pharmacokinetics and pharmacodynamics (inhibition of platelet aggregation) of AZD6140 was studied. Once and twice daily dosing regimens of AZD6140 with total daily doses ranging from 50 mg to 600 mg administered for 5 days at each dose level (a total duration of 15 or 20 days) were studied. The pharmacokinetics of AZD6140 following multiple oral dosing was approximately linear over 50 mg to 600 mg. Maximum plasma concentrations (C_{max}) were reached within 2 to 4 hours after dose intake, and the mean terminal half-life ($t_{½}$) of AZD6140 ranged from 6 to 13 hours. The metabolite area under the plasma concentration-time curve (AUC) and C_{max} were about 35% of the corresponding parameters for AZD6140 and were approximately linear over 50 mg to 600 mg dosing of AZD6140. An exploratory food effect determination resulted in <20% increase in AZD6140 C_{max} and <31% increase in AZD6140 AUC. Food did not have an effect on metabolite C_{max} and AUC. Although area under the curve (AUC) values were somewhat higher following administration of AZD6140 with food, there was no obvious

effect of food on pharmacodynamic (PD) response. Greater than 80% inhibition of platelet aggregation was observed at all doses studied. In terms of inhibition of platelet aggregation, twice-daily doses were superior to the equivalent total daily dose given every 24 hours. All total daily doses of AZD6140 above 200 mg were superior to once-daily doses of 75 mg clopidogrel in terms of PD response.

No serious adverse events (SAE) were observed in the 5 trials completed to date, which exposed healthy subjects to daily doses from 0.1 mg to 600 mg for periods of up to 20 days. Approximately 120 healthy subjects were entered into these trials, and 89 healthy subjects received at least 1 dose of AZD6140. Petechiae, tachycardia, and postural hypotension were the most common adverse events reported in some of these trials. However, the causal relationship of these adverse events to AZD6140 is uncertain. Four subjects in Study SC-532-5256 (single 200 mg dose study in healthy subjects) reported rashes that were considered by the investigator to have a reasonable possibility of a causal relationship to study treatment. Two of these subjects were withdrawn because of this adverse event.

In the multiple ascending dose study SC-532-5239, 1 subject had an increase in liver transaminases of more than 3 times the upper limit of normal after 4 daily doses of 300 mg AZD6140, and was discontinued from the study. The transaminases returned to normal over 24 days. Another subject had a milder increase in transaminase after 4 twice-daily doses of 200 mg that improved despite continuation and increase in dose of AZD6140. There were no other laboratory findings, and no ECG, Holter monitor or vital signs findings of concern during any of the studies.

1.2 Rationale

AZD6140 has not been administered to Japanese subjects and the differences in safety/pharmacokinetics/pharmacodynamics between Japanese and Caucasian subjects have not been investigated. The purpose of this study is to investigate the safety of AZD6140 in Japanese subjects and to explore the pharmacokinetic (PK) profile of AZD6140 in groups of healthy subjects of both Japanese and Caucasian ethnicity. Additionally, the level of platelet aggregation inhibition will be evaluated by bleeding time assessment and optical aggregometry. It is anticipated that data from this study will be used to help further clinical development of AZD6140 in Japan, and the use of first, second and/or third generation Japanese data will be included. After single dose administration of AZD6140, the PK parameters of AZD6140 were linear across the dose range of 30 mg to 400 mg in the 2 single ascending dose studies (SC-532-5169 and SC-532-5171) where a good correlation between the plasma concentration of AZD6140 and the degree of inhibition of platelet aggregation was observed. The assessment of both PK and PD endpoints will aid in the analysis should significant differences be observed between the two groups. The cumulative data from the studies in human subjects to date demonstrate a good safety profile for doses up to and including 600 mg. Therefore, if significant differences in pharmacokinetics or pharmacodynamics should be observed between the two groups, we would anticipate an acceptable margin for subject safety.

2. STUDY OBJECTIVES

2.1 Primary objectives

The primary objective of this study is:

- To assess the safety and tolerability of single ascending oral doses of AZD6140 administered to healthy Japanese and Caucasian subjects by means of incidence and severity of adverse events, 12-lead ECG, vital signs, laboratory parameters and physical examinations.

The secondary objectives of this study are:

- To assess and compare the pharmacokinetics in healthy Japanese and Caucasian subjects following single ascending oral doses of AZD6140 by assessment of C_{max} and AUC.

- To assess and compare the pharmacodynamics in healthy Japanese and Caucasian subjects following single ascending oral doses of AZD6140 by assessment of platelet aggregation inhibition and bleeding time.

3. STUDY PLAN AND PROCEDURES

3.1 Overall study design

This study will be conducted at a single center in the United States. It is a randomized, double-blind placebo-controlled study to assess the safety, tolerability, pharmacokinetics and pharmacodynamics of AZD6140 when given as single ascending oral doses to healthy Japanese and Caucasian male and female (non-childbearing potential) subjects. Approximately 40 subjects will be enrolled in order to obtain 20 subjects in each ethnic group. An attempt will be made to randomize an equal number of Japanese and Caucasian female subjects in each cohort.

Subjects in each ethnic group will be divided into 2 cohorts (Cohort A and B) of 10 subjects. Within each cohort, 8 subjects will receive single ascending doses of AZD6140 on 3 occasions. Two subjects in each cohort will receive placebo on 3 occasions. Consecutive single oral doses of either AZD6140 or placebo will be separated by a washout period of at least 5 days.

The study will consist of 5 visits (a screening visit, 3 dosing/PK visits and a follow-up visit). Subjects will receive a single oral dose of AZD6140 or placebo on Day 1 of Visits 2, 3 and 4. For Cohort A dosing is scheduled to progress from 50 mg at Visit 2 to 200 mg at Visit 3 to 400 mg at Visit 4. For Cohort B dosing is scheduled to progress from 100 mg at Visit 2 to 300 mg at Visit 3 to 600 mg at Visit 4 (see Figure 1). **Prior to the subject progressing to the next higher dose level, the safety results from the previous dose level will be evaluated by**

the principal investigator and study team physician of AstraZeneca. Refer to section 3.1.7 for specific stopping criteria.

At Visits 2, 3 and 4, subjects will remain at the Clinical Research Center (CRC) for 48 hours following dosing. There will be a washout period of at least 5 days between Visits 2 and 3 and between Visits 3 and 4. A follow-up visit (Visit 5) will be scheduled 7 to 14 days following the last dose of study drug.

In order to ensure that the **lowest** dose of study drug is the first dose administered, the subjects assigned to Cohort A will be randomized and dosed prior to the subjects assigned to Cohort B. Subjects assigned to Cohort B will be randomized and dosed **after** the results of the safety evaluations from Cohort A have been assessed and a decision to progress to the next dose level has been made.

The schedule of procedures and assessments are shown in Table 1.

3.1.1 Visit 1 Screening Period

In order to establish eligibility to participate in this study, subjects will undergo all screening procedures and assessments within 28 days prior to Visit 2 (first dosing visit). Subjects will have the study design fully explained to them. Each subject will provide written informed consent **prior** to any study related procedures or assessments. Subjects will be given a cohort assignment (A or B) at this time.

Please refer to Table 1 and Section 4 for the timing and detailed descriptions of the screening assessments.

3.1.2 Visit 2 (Days –2, –1, 1, 2, 3 and 4)

Note: Throughout the study the site will supply standardized meals while the subjects remain at the CRC. Menus will be approved by the sponsor.

Subjects will arrive at the Clinical Research Center (CRC) on the evening of Day –2 as directed. At this time the subjects will be reassessed with regard to the study inclusion/exclusion criteria. In addition, on Day –2, a blood sample for genetic testing will be collected from those subjects who have given written consent.

On Day –2 the subjects will begin fasting at least 10 hours prior to the start of the baseline serial ECGs scheduled for Day –1 (refer to Table 1). On the morning of Day –1 baseline serial ECGs will begin.

On Day –1, subjects will begin fasting at least 10 hours prior to receiving study drug on Day 1 and remain fasting until 4 hours following dosing. On the morning of Day 1, the subjects will be randomized to receive either AZD6140 or placebo to AZD6140. The first dose of AZD6140 (50 mg for Cohort A or 100 mg for Cohort B or matching placebo) will be administered by site personnel. Subjects will remain at the CRC until the completion of all the assessments and procedures scheduled for Day 3. At this time the subjects will be

permitted to return to their home environment. They will be instructed to return to the CRC the next morning as directed for the assessments scheduled for Day 4. Please refer to Table 1 and Sections 3.4.2 and 4 for the timing and detailed descriptions of the assessments and procedures to be performed at Visit 2.

3.1.3 Visit 3 (Days –1, 1, 2, 3 and 4)

Subjects will arrive at the CRC on the evening of Day –1. At this time the subjects will be reassessed with regard to the study inclusion/exclusion criteria.

On Day –1, subjects will begin fasting at least 10 hours prior to receiving study drug on Day 1 and remain fasting until 4 hours following dosing. On the morning of Day 1, a single oral dose of AZD6140 (200 mg for Cohort A or 300 mg for Cohort B or matching placebo) will be administered by site personnel. Subjects will remain at the CRC until the completion of all the assessments and procedures scheduled for Day 3.

Upon the completion of all the assessments and procedures scheduled for Day 3 the subjects will be permitted to leave the CRC. The subjects will be instructed to return to the CRC the next morning (Day 4) for the assessments and procedures scheduled for Day 4.

Please refer to Table 1 and Sections 3.4.2 and 4 for the timing and detailed descriptions of the assessments and procedures to be performed at Visit 3.

3.1.4 Visit 4 (Days –1, 1, 2, 3 and 4)

Subjects will arrive at the CRC on the evening of Day –1. At this time the subjects will be reassessed with regard to the study inclusion/exclusion criteria.

On Day –1, subjects will begin fasting at least 10 hours prior to receiving study drug on Day 1 and remain fasting until 4 hours following dosing. On the morning of Day 1, a single oral dose of AZD6140 (400 mg for Cohort A or 600 mg for Cohort B or matching placebo) will be administered by site personnel. Subjects will remain at the CRC until the completion of all the assessments and procedures scheduled for Day 3.

Upon the completion of all the assessments and procedures scheduled for Day 3 the subjects will be permitted to leave the CRC. The subjects will be instructed to return to the CRC the next morning (Day 4) for the assessments and procedures scheduled for Day 4.

At the completion of the assessments and procedures for Day 4 of **Visit 4**, the subjects will be discharged from the CRC for a **7 to 14-**day period following study drug dosing after which subjects will be instructed to return to the CRC for the follow-up visit (Visit 5).

Please refer to Table 1 and Sections 3.4.2 and 4 for the timing and detailed descriptions of the assessments and procedures to be performed at Visit 4.

3.1.5 Washout Periods

At the completion of the assessments and procedures for Day 4 of **Visits 2 and 3**, the subjects will be discharged from the CRC and enter a washout period of at least 5 days from study drug dosing. Subjects will return to the CRC on the evening prior to receiving the next dose of study drug (Day –1).

3.1.6 Visit 5 Follow-up Visit

Seven to 14 days following the last dose of study drug, on Day 1 of Visit 4, subjects will return to the CRC for a follow-up visit.

Please refer to Table 1 and Section 4 for the timing and detailed descriptions of the assessments and procedures to be performed at this visit.

At the completion of the assessments and procedures scheduled for Visit 5 subjects will be discharged from the study.

3.1.7 Stopping criteria for dose escalation

Prior to the subject progressing to the next dose level, the safety results from the previous dose level will be evaluated by the investigator and study team physician of AstraZeneca. Written approval from the sponsor will be obtained before dose escalation. Adverse events, with particular attention to events that may be caused by increases in bleeding times, clinical safety labs, and clinically significant changes in ECGs will be reviewed for each dose group. If the clinical safety laboratory measurements or adverse event rate with regard to possible effect on bleeding time is significantly disproportionate between the Caucasian group and the Japanese group to suggest that there may be an exaggerated difference in pharmacodynamic response to AZD6140 and if it is anticipated that subjects may experience significant risk from further dose escalations, then that dose group may be stopped from escalating after discussions with the investigator and the sponsor have taken place. If at least 50 % of any dose group have significant adverse events or laboratory abnormalities related to prolongation of bleeding effects (as judged by the investigator and AZ) escalation for that dose group may be stopped.

Figure 1 Study flow chart

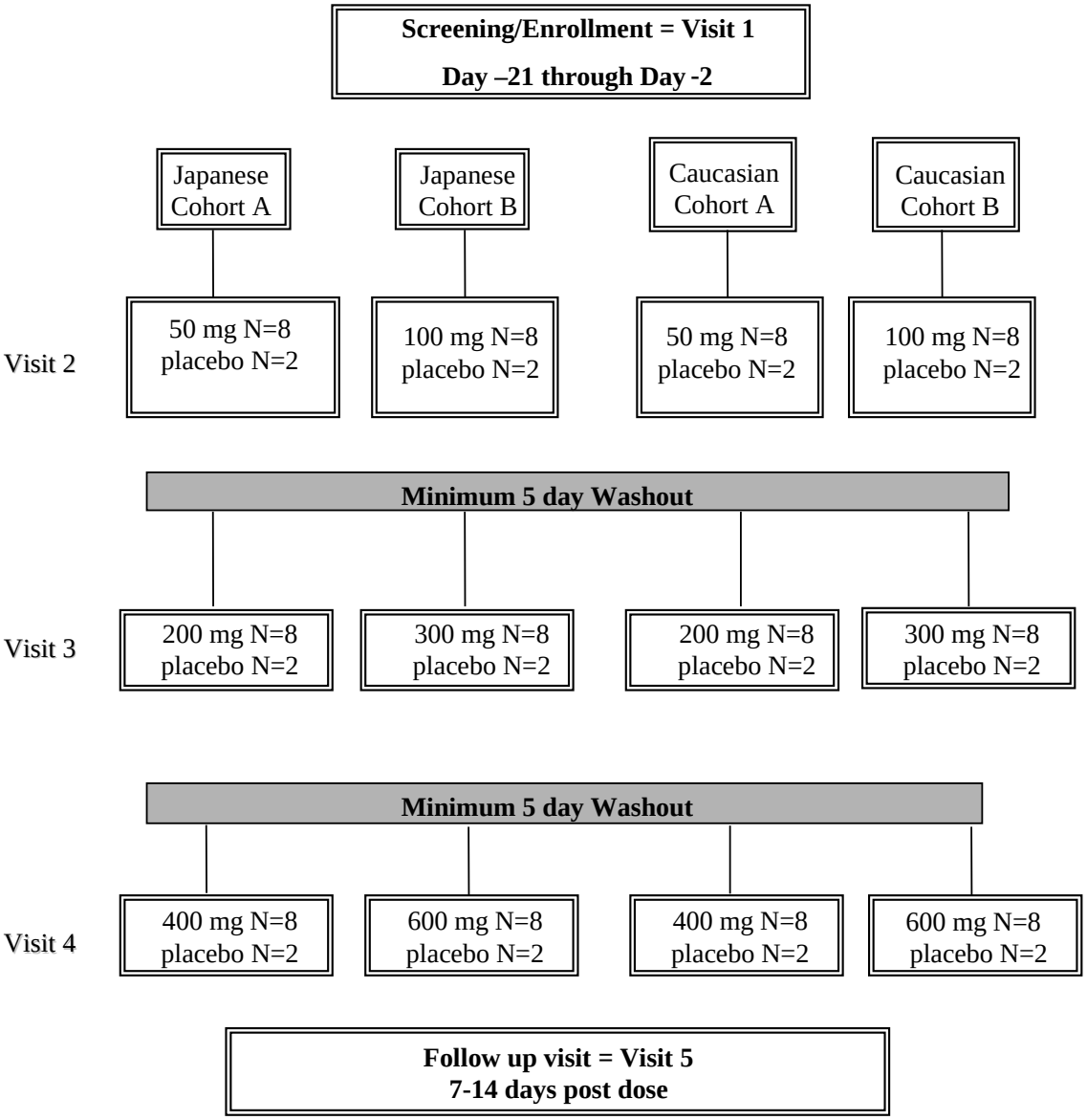

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Table 1 Study plan

Assessment	**Screening Visit 1**	**Visits 2, 3 and 4**						**Follow-up Visit 5**
	Max. 28 days prior to Day 1 of Visit 2	**Day -2 (Visit 2 only)**	**Day -1**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	7 - 14 days after last dose
Informed Consent	X							
Demographics	X							
Inc/Excl. Criteria	X	X	X [j]					
Concomitant Medication	X	X	X	X	X	X	X	X
Medical/Surgical History	X							
Physical Exam	X	X						X
Brief Physical Exam			X [j]					
HIV, HBsAg, Hep. C Screen	X							
Drugs of abuse	X	X	X[j]					
12-lead ECG [a]	X		X	X	X	X	X	X
Vital Signs [b]	X	X	X	X	X	X	X	X
Pregnancy test (female subjects only) [k]	X	X	X [j]					X
Clinical laboratory assessments [c]	X	X	X [j]		X			X
Limited chemistry panel [d]				X				
Randomization [e]				X				
PK Sampling [f]				X	X	X	X	
ADP induced platelet aggregation [g]				X	X			
Bleeding Times [h]	X			X	X			
AZD6140/Placebo Administration				X				
Adverse event questioning		X	X	X	X	X	X	X
Genetic sampling [i]		X						

a ECGs will be performed after 10 minutes in the supine position.
A single ECG will be performed at the following time points:
Visit 1 (Screening)
Visit 2: 48 and 72 hours post dose
Visits 3 and 4: Day –1 and at 48 and 72 hours post dose
Visit 5 (Follow-up)
Two ECGs approximately 1 minute apart will be performed at each of the following time points:
Visit 2: Day –1 at 1, 2, 3, 4, 8, 12 and 24 hours post theoretical dose.
Visits 2, 3 and 4: Day 1 at 1, 2, 3, 4, 8, 12 and 24 hours post dose.

b **Sitting blood pressure and pulse (after sitting for 5 minutes) will be taken at the following times:**
Visit 1 (Screening)
Visits 2: Day –2, Visits 2, 3 and 4: Days –1, 1, 2, 3 and 4
Visit 5 (Follow-up)
Orthostatic blood pressure assessments (after 10 minutes supine and after 2 minutes standing) will be performed at the following times:
Visits 2, 3 and 4: 4 hours post dose

c Blood samples for clinical chemistry, hematology and urinalysis will be obtained at the following times:
Visit 1 (Screening)
Visit 2: Day –2 and at 24 hours post dose
Visits 3 and 4: Day –1 and at 24 hours post dose
Visit 5 (Follow-up)

d Blood samples for ALT, AST, GGT, alkaline phosphatase and total bilirubin will be obtained at the following times:
Visits 2, 3 and 4: predose

e Randomization will occur at Visit 2 only.

f Blood samples for pharmacokinetics will be collected at the following time points:
Visits 2, 3 and 4: Pre-dose, 0.5, 1, 2, 3, 4, 6, 8, 12, 18, 24, 36, 48, and 72 hours post dose.

g Blood samples for platelet aggregometry will be collected at the following time points:
Visits 2, 3 and 4: pre-dose, 2, 4, 12, and 24 hours post dose.

h Bleeding time will be assessed at the following time points:
Visit 1 (Screening)
Visits 2, 3 and 4: pre-dose, 4.5 and 24 hours post dose.

i A single blood sample for genetic analysis will be obtained prior to dosing at Visit 2 only.

j Visits 3 and 4 only

k Serum pregnancy tests will be performed at Visit 1 (Screening) and Visit 5 (Follow-up).
Urine pregnancy tests will be performed at Visit 2, Day –2 and at Visits 3 and 4, Day –1.

3.2 Rationale for study design, doses and control groups

This study will be the first study to explore the PK and PD profile of AZD6140 in Japanese subjects. Healthy subjects are chosen to minimize the effect of concurrent illness or medication on the pharmacokinetic or pharmacodynamic effect. Placebo control subjects are included in order to evaluate safety and tolerability. In the Phase I clinical studies conducted with AZD6140 in Europe to date, single or multiple oral doses of up to 600 mg (qd, for 5 days) administered to healthy male Caucasian subjects were well tolerated, and without significant safety concerns.

The dose levels of AZD6140 selected for this study are based on data from animal toxicology studies, and the results of 5 previous human clinical studies. These doses are expected to give sufficiently high plasma concentrations to allow accurate estimation of PK parameters. Furthermore, these dose levels are not expected to cause any safety concerns based on experience in previous studies, and will include doses that would be anticipated to be therapeutically relevant.

The starting dose in this study will be 50 mg (50 mg/person = 0.8 mg/kg, assuming the average body weight is 60 kg) which is 1/24 of the No Effect Dose Level (20 mg/kg/day) estimated in 1-month, repeated-dose toxicity studies with rats and marmosets. Although this ratio is higher than the ordinary theoretical ratio (1/60) for the selection of initial dose in Japanese studies, doses of AZD6140 up to 600 mg were well tolerated when administered to healthy male subjects in the previous human clinical studies. Therefore, the lowest initial dose in this study is anticipated to be a safe starting dose also for healthy Japanese subjects who will receive AZD6140 for the first time.

The highest dose of 600 mg was chosen based on safety and tolerability of multiple oral doses of 600 mg in Caucasians. The results in the repeated dosing study with healthy male Caucasian subjects (50 mg od, 200 mg od, 50 mg bid) showed that C_{max} of AZD6140 at steady state were approximately 1.0, 1.3 and 1.5 times higher than those after the single administration of the same dose and C_{max} of AR-C124910XX 1.2, 1.4 and 1.5 times higher, respectively. The future daily dose is now assumed to be around 400 mg. However, to fully explore the tolerability, safety, pharmacokinetics and pharmacodynamics of the Japanese subjects 600 mg was chosen as the upper exposure limit to ensure comparable safety and tolerability between Japanese and Caucasian subjects.

A genetic analysis will also be carried out to explore genetic factors important in the disposition of AZD6140 and response to AZD6140. Since this study is not designed or powered for genetic analysis, the resultant genetic data are considered exploratory and will not form part of the clinical study database or the clinical study report. The results of this study may be pooled with genetic results from other studies on AZD6140 to generate hypotheses to be tested in future studies.

3.3 Selection of study population

3.3.1 Study screening record log

The investigator must keep a record of all subjects who were considered for enrollment (signed an informed consent form). Subjects who do not successfully complete screening must not be re-screened without prior approval from AstraZeneca.

3.3.2 Inclusion criteria

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

1. Provide signed written informed consent.

2. Be healthy male or post-menopausal (cessation of menses for at least 12 months) or surgically sterilized female between the ages of 20 and 45 inclusive.

3. Be Caucasian or Japanese. Japanese is defined as having both parents and 4 grandparents who are Japanese. This includes second and third generation Japanese whose parents or grandparents are living in a country other than Japan.

4. Have a Body Mass Index (BMI) between 18 and 30 kg/m2 and weigh at least 50 kg unless approved by the sponsor and investigator.

5. Have normal physical examination, laboratory values, 12-lead ECG and vital signs unless the investigator considers an abnormality to be clinically irrelevant.

6. Be willing to communicate with the investigator and comply with all study procedures.

3.3.3 Exclusion criteria

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

1. History of neurological, hematological, psychiatric, gastrointestinal, hepatic or renal disease or other condition known to interfere with the absorption, distribution, metabolism or excretion of drugs as determined by the investigator.

2. Current and/or past history of intolerance or hypersensitivity to drugs with a similar chemical structure or mechanism of action to AZD6140 or any ingredient in its formulation (refer to section 3.4.1). These drugs include, but are not limited to clopidogrel (Plavix) and ticlopidine (Ticlid).

3. Dietary practices that may be considered extreme in the judgment of the investigator.

4. Symptoms of any clinically significant illness within 2 weeks of screening.

5. Clinically significant out-of-range values for prothrombin time (PT) or activated partial prothrombin time (APTT) as judged by the investigator.

6. Have a bleeding time of ≥ 9 minutes (inclusive) by Simplate II method.

7. A personal or family history of bleeding diatheses or a reasonable suspicion of vascular abnormalities including aneurysms.

8. A personal history of severe hemorrhage, hematemesis, melena, hemoptysis, severe epistaxis or intracranial hemorrhage.

9. History of significant rectal bleeding, as determined by the investigator, within the past 3 months.

10. Blood donation and/or sampling in excess of 200 mL of whole blood within the preceding 4 weeks, 400 mL of whole blood within the preceding 12 weeks and/or 1200 mL of whole blood within the preceding 12 months prior to Screening for **Japanese** subjects.

11. Blood donation and/or sampling in excess of 500 mL within the previous 12 weeks or in excess of 1200 mL within the preceding 12 months prior to Screening for **Caucasian** subjects.

12. Use of any prescribed medication or over the counter preparations including herbal preparations and vitamins in the 2 weeks prior to randomization and throughout the study unless otherwise approved by the sponsor and investigator. Use of hormone replacement therapy for female subjects is permitted as long as there has been no change in the dosing regimen for at least 3 months prior to screening.

13. Use of tobacco or history of use of tobacco or nicotine-containing products in the 3 months prior to randomization.

14. Consumption of aspirin, ibuprofen or any other drug known to increase the propensity for bleeding within 2 weeks prior to randomization and throughout the study.

15. History of surgery or significant trauma within 3 months prior to randomization.

16. Current and/or past history of alcohol abuse.

17. An average consumption of >3 units of alcohol per day. (A unit of alcohol is defined as 1 beer, 1 wine [5 oz] or 0.75 oz alcohol.)

18. Current and/or past history of drug abuse or a positive test for drugs of abuse.

19. Positive results of HIV, HBsAg or Hepatitis C antibody testing.

20. Positive results of pregnancy test.

21. Use of any other investigational compound or participation in another clinical trial within 60 days prior to randomization.

22. Employees of the CRC conducting this study.

23. Subjects, who, in the opinion of the investigator, should not participate in this study,

3.3.4 Restrictions

Subjects will be required to:

1. Maintain a consistent level of physical activity throughout the study.

2. Abstain from consumption of caffeine-containing foods or beverages (eg, coffee, tea, chocolate, cocoa and cola) within 48 hours of dosing and while attending the CRC.

3. Abstain from consumption of alcoholic beverages for 48 hours prior to dosing and while attending the CRC.

4. Abstain from the intake of grapefruit juice and Seville oranges for 1 week prior to randomization and throughout the study.

5. Refrain from taking aspirin, ibuprofen or any other drug known to increase the propensity for bleeding for 2 weeks before randomization and throughout the study.

6. Refrain from taking prescribed or over-the-counter medications including herbal remedies and vitamin preparations for 2 weeks prior to randomization and throughout the study unless approved by the sponsor and the investigator.

7. Fast for a period of ten hours prior to each dose of study drug (Visits 2, 3 and 4) and remain fasting for 4 hours after dosing. This fasting schedule will also be observed beginning on the evening of Day –2 (Visit 2) and continuing until the morning of Day –1 (Visit 2). Water will be allowed during the fast except from 2 hours before study drug administration until 2 hours after study drug administration.

3.3.5 Discontinuation of subjects from treatment or assessment

3.3.5.1 Criteria for discontinuation

Subjects may be discontinued from study treatment and assessments at any time, at the discretion of the investigator(s). Specific reasons for discontinuing a subject from this study are:

1. Voluntary discontinuation by the subject, who is, at any time, free to discontinue his/her participation in the study.

2. Safety reasons as judged by the investigator or AstraZeneca (refer to section 3.1.7).

3. Protocol noncompliance, which, in the judgment of the sponsor and investigator, has the potential to significantly affect the integrity of the data.

3.3.5.2 Procedures for discontinuation

Subjects who discontinue from the study should always be asked about the reason(s) for their discontinuation and about the presence of any adverse events. If possible, they should be seen and assessed by the investigator in addition to completing the procedures and evaluations scheduled for the Follow-up Visit (Refer to Table 1 and Section 4). Adverse events should be followed until resolution or until the investigator decides that no further follow-up is necessary.

The case report forms (CRFs) should be completed to reflect the reason for the subject's discontinuation from the study.

If a subject is withdrawn or drops out, he/she may be replaced at the discretion of the sponsor. (See Section 3.4.3)

3.4 Treatment(s)

The investigator or institution has the responsibility to establish a system for handling study drug so as to ensure that:

- Deliveries of study drug from AstraZeneca are correctly received by the investigator or designee.
- Such deliveries are recorded on a drug log.
- Study drug is handled and stored properly.
- Study drug is only dispensed to study subjects in accordance with the protocol.
- Unused study drug is accounted for and returned to the designated facility.

3.4.1 Investigational product(s)

3.4.1.1 Identity of investigational product

AstraZeneca will provide AZD6140 tablets and placebo in subject specific bottles.

(a) 50 mg AZD6140 tablets (formulation number FDN 240)

Compound number: AZD6140

Dosage form: Immediate Release Tablets, each single tablet containing 50 mg AZD6140

Manufacturer: AstraZeneca R&D Charnwood.

Ingredients: See table below:

Ingredient	**Quantity per unit dose Unit dose (mg)**	**Quantity (%w/w or w/v)**
AZD6140	50.00	14.286
Lactose monohydrate	189.74	54.210
Microcrystalline cellulose	79.10	22.600
Polyvinylpyrrolidone K30	15.40	4.400
Croscarmellose sodium	14.00	4.000
Magnesium stearate	1.75	0.500
	Quantity per unit area (mg/cm^2)	**Quantity %w/w based upon a 10mm round tablet**
Opadry Pink (ref 33G24513)	4.63-6.17	3-4

(b) 100 mg AZD6140 tablets (formulation number FDN 239)

Compound number: AZD6140

Dosage form: Immediate Release Tablets, each single tablet containing 100 mg AZD6140

Manufacturer: AstraZeneca R&D Charnwood.

Ingredients: See table below:

Ingredient	**Quantity per unit dose Unit dose (mg)**	**Quantity (%w/w or w/v)**
AZD6140	100.00	28.57
Lactose monohydrate	139.76	39.93
Microcrystalline cellulose	79.10	22.60
Polyvinylpyrrolidone K30	15.40	4.40
Croscarmellose sodium	14.00	4.00
Magnesium stearate	1.75	0.50
	Quantity per unit area (mg/cm^2)	**Quantity %w/w based upon a 10mm round tablet**

Opadry Pink (ref 33G24513)	4.63-6.17	3-4

(c) 200 mg AZD6140 tablets (formulation number FDN 266)

Compound number: AZD6140

Dosage form: Immediate Release Tablets, each single tablet containing 200 mg AZD6140

Manufacturer: AstraZeneca R&D Charnwood.

Ingredients: See table below:

Ingredient	**Quantity per unit dose Unit dose (mg)**	**Quantity (%w/w or w/v)**
AZD6140	200.00	57.143
Lactose monohydrate	77.20	22.057
Microcrystalline cellulose	43.75	12.500
Polyvinylpyrrolidone K30	13.30	3.800
Croscarmellose sodium	14.00	4.000
Magnesium stearate	1.75	0.500
	Quantity per unit area (mg/cm^2)	**Quantity %w/w based upon a 10mm round tablet**
Opadry Pink (ref 33G24513)	4.63-6.17	3-4

(d) AZD6140 placebo tablets (formulation number FDN 234)

Dosage form: Tablet, containing zero active therapy (identical in appearance to active tablets)

Manufacturer: AstraZeneca R&D Charnwood.

Ingredients: See table below:

Ingredient	Quantity (%w/w or w/v)
Lactose spray dried	69.6%
Avicel PH102	30.0%
Magnesium stearate	0.40%
Opadry Pink (ref 33G24513)	2-4% (of core weight)

AZD6140 50 mg, 100 mg, 200 mg and placebo tablets are all size matched. All of the tablets are pink film coated so they will be identical in appearance.

3.4.1.2 Labeling

AZD6140 and matching placebo tablets will be manufactured by AstraZeneca R&D Charnwood and will be supplied in 50 mL HDPE (high density polyethylene) bottles. Each subject will be assigned (according to the randomization schedule) a carton containing 3 bottles. Each bottle will contain 1, 2 or 3 tablets which corresponds to 1 dose of study drug or placebo. One bottle is to be dispensed at Visits 2, 3 and 4. Labeling will be in accordance with GMP. Bottle labels will have a tear off portion, which will be inserted into the CRF at the time of administering supplies to subjects.

The bottle labels will include the following information:

- Name of sponsor (AstraZeneca)
- AZD6140/placebo, dosage form, route of administration and quantity of dosage units
- Study code
- Subject/Randomization number
- Visit number
- Administer as directed
- Storage conditions
- Space for date dispensed
- Lot Number
- The following standard statements

- Caution: New Drug - Limited by Federal (United States) Law to Investigational Use.
- "Keep out of reach of children"

The carton labels will contain at least the following information:

- Name of sponsor (AstraZeneca)
- Study code
- Subject/randomization number
- Carton contents: 3 bottles containing AZD6140 tablet (s) or placebo
- The following standard statements
  - Caution: New Drug - Limited by Federal (United States) Law to Investigational Use.
  - "Keep out of reach of children"

3.4.1.3 Storage

All investigational products must be kept in a secure place under appropriate storage conditions. AZD6140 tablets and placebo must be stored between 15°C and 30°C (59°F to 86°F), protected from light and high humidity. The storage location will be locked and accessible only to authorized study site personnel.

3.4.1.4 Accountability

The study drugs provided for this study are for use only as directed in the protocol. The investigational site personnel are responsible for maintaining drug dispensing records and will account for all drugs received and returned. All packaging will be retained throughout the course of the study until drug accountability and reconciliation has been performed by the AstraZeneca monitor following completion of the study. The AstraZeneca monitor will return all unused drugs to a vendor designated by the sponsor. Certificates of delivery and return must be signed.

3.4.2 Doses and dosing regimens

Twenty subjects in each ethnic group will be assigned by the site at screening to one of 2 dose level groups (Cohort A or Cohort B). Cohort A, which includes the lowest dose of study drug, will be the first group to dose. Cohort B will begin following the approval to escalate based on the safety results from the first dose group in Cohort A. Within each cohort the subjects will be randomized to receive either active study drug or placebo as follows:

(a) Cohort A – AZD6140 50 mg, 200 mg and 400 mg or placebo

Eight Japanese or Caucasian subjects assigned to Cohort A will receive AZD6140 50 mg at Visit 2, 200 mg at Visit 3 and 400 mg at Visit 4. Two Japanese or Caucasian subjects will receive placebo at Visits 2, 3 and 4.

(b) Cohort B – AZD6140 100 mg, 300 mg and 600 mg or placebo

Eight Japanese or Caucasian subjects assigned to Cohort B will receive AZD6140 100 mg at Visit 2, 300 mg at Visit 3 and 600 mg at Visit 4. Two Japanese or Caucasian subjects will receive placebo at Visits 2,3 and 4.

All doses will be administered by site personnel as oral tablets with 240 mL of room temperature water after an overnight fast of at least 10 hours. Subject will be instructed not to crush or chew the tablets. The tablets will be administered while subjects are sitting upright or in a semi-recumbent position. Subjects must remain either sitting or semi-recumbent for at least 2 hours and will continue to fast for at least 4 hours following dosing. Water intake will be restricted, other than what is required for dosing, from 2 hours before until 2 hours after dosing.

3.4.3 Method of assigning subjects to treatment groups

Written informed consent will be obtained at the time of enrollment. The subjects will be identified with an enrollment number starting with E0001001 and continuing in a consecutive sequence. **Japanese subjects** assigned to Cohort A and fulfilling the eligibility criteria at Visit 2 will be assigned a randomization/subject number starting with number **101**. **Caucasian subjects** assigned to Cohort A and fulfilling the eligibility criteria at Visit 2 will be assigned a randomization/subject number starting with number **201.**

Japanese subjects assigned to Cohort B and fulfilling the eligibility criteria at Visit 2 will be assigned a randomization/subject number starting with number **301**. **Caucasian subjects** assigned to Cohort B and fulfilling the eligibility criteria at Visit 2 will be assigned a randomization/subject number starting with number **401.**

Subjects will be randomized to receive either active drug or placebo (within cohort and ethnic group), strictly sequentially as subjects are eligible for randomization. If a subject discontinues from the study neither the enrollment number nor the subject number will be re-used and the subject will not be allowed to re-enter the study.

In the event of dropouts or withdrawals, the investigator must call AstraZeneca personnel. The subject will be replaced if the anticipated number of subjects from each ethnic group completing the study falls below 8. An unblinded individual at Investigational Products Department at AstraZeneca will determine the appropriate subject number for the replacement subject (in this case the subject/randomization number assigned to a replacement subject will not necessarily be assigned sequentially) so that each replacement subject will be assigned to the appropriate dosage level and will receive the same study drug or placebo as the subject being replaced.

3.4.4 Blinding and procedures for unblinding the study

3.4.4.1 Methods for ensuring blinding

This is a double-blind study. The active study drug or placebo assignment will be blinded both to the subjects and to the investigator. The study drug will be blinded by providing AZD6140 and matching placebo tablets, which will be indistinguishable in appearance. Packaging, labeling, and preparation of investigational products will be performed in a way that will ensure the blinding throughout the study. Neither the sponsor's representative responsible for monitoring the study, the study personnel, nor the investigator will know whether study drug or placebo has been allocated for each subject.

3.4.4.2 Methods for unblinding the study

Individual treatment codes (scratch-off cards with the concealed randomization information), indicating the treatment randomization for each randomized subject will be supplied, by AstraZeneca to the investigator or pharmacist at the research center and to AstraZeneca Drug Safety Department.

The individual treatment codes must not be broken except in medical emergencies when the appropriate management of the subject necessitates knowledge of the treatment randomization. The investigator must document and report to AstraZeneca any breaking of the treatment code. AstraZeneca retains the right to break the code in order to report serious adverse events to regulatory authorities.

Treatment codes will not be broken for the planned analyses of data until all decisions on the evaluability of the data from each individual subject have been made and documented.

3.4.5 Concomitant medication

Subjects should refrain from taking prescribed or over-the-counter medications including herbal remedies and vitamin preparations from 2 weeks prior to randomization and throughout the study unless approved by the sponsor and the investigator. Use of hormone replacement therapy for female subjects is permitted as long as the therapy has been stable for at least 3 months prior to screening.

Aspirin, ibuprofen or any other drug known to increase the propensity for bleeding is prohibited from 2 weeks before the first dose of study drug until completion of the study.

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

3.4.6 Treatment compliance

Compliance will be ensured by supervised administration of the study drug by the investigator or his/her designee.

4. MEASUREMENT OF STUDY VARIABLES

The following study measurements will be obtained. The timing of these measurements is detailed in the study plan (Table 1). The following sequence of events will be in effect when more than one assessment is required at a particular timepoint:

1) ECG

2) Vitals (blood pressure followed by pulse)

3) Pharmacokinetic sampling (Note: PK sampling must be performed at the precise scheduled time. When the PK and PD samplings are scheduled for the same timepoint, the PD sample will be collected first. The exact time for the PK and PD samples will be recorded in the CRF.

4) Bleeding time assessments (Note: Pre-dose bleeding time evaluations may take place up to 1 hour prior to dosing. Post-dose bleeding time evaluations should be taken as soon as possible **after** the corresponding PD/PK blood samples are obtained.

4.1 Screening and demographic measurements

Each subject will undergo the screening procedures and assessments within 21 days prior to randomization. Demographic information (date of birth, sex, ethnicity, race and date of informed consent) will be collected on the appropriate CRF for **all** subjects who are enrolled. Information collected for screen failures will be limited to demographics and the reason for failure of screening including all entrance criteria that were not met and the reason for discontinuation. Please refer to the Study Plan (Table 1) for the list of procedures and assessments to be performed at screening. Refer to section 4.4 for a detailed description of the screening and safety assessments.

4.2 Pharmacokinetic measurements

Venous blood samples (2 mL) for PK analysis will be collected at the times listed in Table 2. Blood samples will be collected, labeled, processed and shipped as detailed in Section 4.2.1, 4.2.2 and 4.2.3. The date and actual time of collection will be recorded on the appropriate page of the CRF.

4.2.1 Collection and processing of plasma samples

Venous blood samples will be collected for the determination of AZD6140 and metabolite AR-C124910XX concentrations. Blood samples (2 mL) will be collected at the defined time points (see Table 2) for 72 hours post dose at Visits 2, 3 and 4.

After applying a tourniquet, venous blood will be taken. Individual draws for each timepoint may be performed or an indwelling catheter may be used. Blood samples (2 mL) will be collected. If the site chooses to collect blood samples from an indwelling catheter, the first 1

mL of blood drawn will be discarded and a total of 3 mL will be collected. AstraZeneca will provide the collection tubes and labels to be used. The sample will be placed on ice until centrifugation, which will begin within 30 minutes after the sample is obtained. The samples will be centrifuged for 10 minutes at 4°C at a relative centrifugal force of 1500g. The resulting plasma will be divided between 2 polypropylene tubes (screw cap). This process will provide 2 complete sets of plasma samples for analysis (set A and B). The samples will be immediately frozen upright at -20°C or below in a non frost-free freezer and kept frozen at this temperature before, during and after transport to the designated laboratory (refer to section 4.2.3).

Plasma samples for measurement of AZD6140 and its metabolite AR-C124910XX concentration and PK parameters will be analyzed using validated bioanalytical methods by York Bioanalytical Solutions. Details of the method used will be provided in the clinical study report.

4.2.2 Labeling of plasma samples

The labels will include the following information:

Study Number: D5130C05266

Randomization/Subject Number

Visit Number: (Visit 2, 3 or 4)

Tube and Set Number (A or B)

Scheduled/Protocol Time

The label must only be used for the intended sample and the pre-printed information must not be changed.

4.2.3 Shipping of plasma samples

All PK plasma samples will be shipped via World Courier. Plasma samples will be shipped to the address below. **The 2 sets (set A and B) of plasma samples will not be shipped together in the same shipment.** Samples must be packed and shipped in accordance with the Department of Transportation (DOT) Regulation UN3373 for diagnostic specimens in compliance with International Air Transport Association (IATA) Packing Instruction 650. All applicable shipping regulations must be followed.

Samples must be placed in protective bubble wrap and then enclosed in a sealed watertight plastic bag. The sealed bag must include absorbent material to contain any leaks. The sealed bag must then be placed into a watertight TYVEC container.

The samples must be packed securely to avoid breakage during transit and packed with a sufficient quantity of dry ice to ensure they remain frozen for at least 72 hours. All applicable shipping regulations must be followed.

Documentation sufficient to identify each sample must be included in the shipment. The primary contact at AstraZeneca and the designated laboratory identified below must be notified **before samples are shipped.**

Ship samples on Saturday, Monday and Tuesday only. Do not ship on or the day before a legal holiday.

All batches of plasma samples, accompanied by the corresponding documentation, shall be addressed to:

and the recipient must be notified by phone or fax at the time the samples are shipped.

Table 2 Schedule of PK blood sampling

Scheduled Time Relative to Dose at Visits 2, 3 and 4	**Tube and Set Number**
Pre-dose (within 30 minutes of dosing)	**1 A/B**
0.5 hour post dose	**2 A/B**
1 hour post dose	**3 A/B**
2 hours post dose	**4 A/B**
3 hours post dose	**5 A/B**
4 hours post dose	**6 A/B**
6 hours post dose	**7 A/B**
8 hours post dose	**8 A/B**
12 hours post dose	**9 A/B**
18 hours post dose	**10 A/B**
24 hours post dose	**11 A/B**
36 hours post dose	**12 A/B**
48 hours post dose	**13 A/B**
72 hours post dose	**14 A/B**

4.3 Pharmacodynamic measurements

4.3.1 ADP induced platelet aggregation

Two 4.5 mL blood samples will be collected into a blue-top Vacutainer® tube which contains 3.2% 0.105M buffered citrate. These samples will be used to measure inhibition of platelet

aggregation by optical aggregometry, using 20μmol ADP as the agonist and run in duplicate, at the defined time points (see Table 3) at Visits 2, 3 and 4.

Final and maximum extent of aggregation will be recorded. Final extent of aggregation will be measured after 6 minutes or at a point where the curve has been constant for at least 1 minute. The date and time of collection will be recorded on the appropriate CRF. Details of the methods used will be agreed upon in writing by the sponsor and investigator prior to the start of the study.

Table 3 Schedule of ADP induced platelet aggregation sampling

Scheduled Time Relative to Dose at Visits 2, 3 and 4
Pre-Dose (within 30 minutes of dosing)
2 hours post dose
4 hours post dose
12 hours post dose
24 hours post dose

4.3.2 Bleeding time assessment

Bleeding time measurements using Simplate II® method will be determined at the times indicated in Table 4. Times will be recorded for each of the 2 incisions made by the device. If the pre-dose bleeding time is >9 minutes the subject should not be dosed and will be discontinued from the study.

If at the 24-hours post dose measurement the bleeding time is >60 minutes, a repeat assessment will be performed 48 hours post-dose. Additional repeat bleeding time assessments may be performed at the discretion of the investigator.

The methodology is detailed in Appendix C.

Table 4 Schedule of bleeding time sampling

Visit	Scheduled Time
1 (Screening)	Within 21 days prior to randomization
2	Pre-dose (within 60 minutes of dosing)
	4.5 hours post dose
	24 hours post dose
3	Pre-dose (within 60 minutes of dosing)
	4.5 hours post dose
	24 hours post dose
4	Pre-dose (within 60 minutes of dosing)
	4.5 hours post dose
	24 hours post dose

4.4 Screening and safety assessments and procedures

For timing of individual assessments and procedures refer to study plan (Table 1).

4.4.1 Informed consent

The subject's signed and dated written informed consent must be obtained before conducting any study-specific procedure.

4.4.2 Inclusion/exclusion criteria

The inclusion and exclusion criteria must be assessed and reviewed with each subject at screening and on Day –2 of Visit 2 and Day –1 of Visits 3 and 4 in order to establish and confirm his/her eligibility to participate or continue in the study.

4.4.3 Clinical laboratory measurements

Blood and urine samples will be taken for determination of clinical chemistry, hematology and urinalysis parameters. Please refer to study plan (Table 1) for the timing of individual assessments. The date and time of collection will be recorded on the appropriate CRF. The following tests will be performed:

4.4.3.1 Chemistry

Clinical chemistry assessments will include the following:

Sodium, potassium, calcium, chloride, glucose, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, blood urea nitrogen (BUN) total bilirubin, creatinine, gamma glutamyltransferase (GGT) and creatinine kinase.

4.4.3.2 Limited chemistry panel

The limited chemistry panel will consist of the following:

ALT, AST, GGT, alkaline phosphatase and total bilirubin.

4.4.3.3 Hematology

Hematology assessments will include the following:

RBC, platelets, hemoglobin, hematocrit, WBC and differential count (neutrophils, lymphocytes, monocytes, eosinophils and basophils), prothrombin time and activated partial thromboplastin time.

4.4.3.4 Urinalysis

A urine sample will be obtained for the presence of protein, glucose and blood. If positive values are reported for protein or blood, and are considered to be of clinical significance, a microscopic examination of the urine will be performed.

4.4.4 Pregnancy test-Serum and urine

Serum and urine pregnancy tests will be performed on all female subjects. The serum pregnancy test can be collected with the same sample as the blood chemistry. If the result of any pregnancy test is positive, the subject will not be allowed to proceed in the study.

Note: Although the results of the serum and urine pregnancy tests have to be documented in the subject's files, they will not be collected on the CRF's and will therefore not be recorded in the study database.

4.4.5 HIV, HBsAg and hepatitis C

A blood sample for HIV, HbsAg and hepatitis C antibody will be obtained at screening. If a test result is positive the subject will not be permitted to continue his/her participation in the study.

Note: Although the results of the HIV, HbsAg and hepatitis C tests have to be documented in the subject's files, they will not be collected on the CRF's and will therefore not be recorded in the study database.

4.4.6 Drugs of abuse screen

A urine screen for drugs of abuse will be performed on urine samples obtained from each subject. If a test result is positive for any of the substances of abuse, the subject will not be permitted to continue his/her participation in the study. The following drugs will be screened:

Amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, methadone, opiates, phencyclidine and propoxyphene.

Note: Although the results of the urine drug screen have to be documented in the subject's files, they will not be collected on the CRF's and will therefore not be recorded in the study database.

4.4.7 Medical/surgical history

A detailed medical and surgical history including medication history will be recorded for each subject at screening. Significant medical conditions and surgical events are to be recorded on the appropriate CRF.

The medication history must identify any known drug allergies, presence or history of drug or alcohol abuse. All medications and OTC products (including vitamins and herbal remedies) taken within 2 weeks prior to dosing are to be recorded on the appropriate page of the CRF.

4.4.8 Complete physical examination

The complete physical examination will include an assessment of the following: general appearance, skin, head and neck (including eyes, ears, nose and throat), lymph nodes, thyroid, musculoskeletal/extremities (including spine), cardiovascular, lungs, abdomen and neurological (reflexes). Height (cm) and weight (kg) will be measured and BMI will be calculated. Height will be measured and BMI will be calculated at screening only.

4.4.9 Brief physical examination

The brief physical examination will include an assessment of the following: general appearance, abdomen, cardiovascular and lungs. If the subject states changes have occurred related to systems not assessed, then these systems should also be examined.

4.4.10 Resting 12-lead ECG

For timing of individual assessments refer to study plan (Table 1).

4.4.10.1 Methods of assessment

All ECGs will be evaluated by the investigator for safety as normal or abnormal. For timing of individual ECGs please refer to the study plan (Table 1). If indicated, as necessary for the safety of the subject, additional ECG assessments can be made at the discretion of the investigator.

Baseline serial ECGs will be recorded on Day –1 of Visit 2 (refer to Table 1). Serial ECGs will be recorded beginning on Day 1 of Visits 2, 3 and 4. The serial ECGs collected will be evaluated by the investigator for safety as normal or abnormal and sent to a cardiologist for centralized review by manual over-read for evaluation for QT interval measurement. Serial ECGs taken on Day –1 of Visit 2 will serve as a baseline for comparison to subsequent serial ECGs at each timepoint. The types of comparisons to be made will be discussed in the statistical analysis plan.

4.4.10.2 ECG machine

All ECGs are to be obtained using an ECG machine approved by the sponsor (25mm/sec paper speed and a voltage of 10mm/mV) that will record 12 leads simultaneously.

4.4.10.3 Electrode placement

Prior to the first ECG recording of Day –1, the location of the leads on the chest of the subject is to be marked with a water indelible pen. The investigative staff will make a reasonable effort to ensure that the leads are placed in the same location for all subsequent ECGs. Additional marks can be made with an indelible pen if needed to help ensure that the leads are placed in the same location.

4.4.10.4 Body positioning during ECG monitoring

Before starting a recording, the subject is to be in a supine position FOR AT LEAST 10 MINUTES.

4.4.10.5 Recording of ECGs

Serial ECGs are to be recorded electronically. A minimum of 5 to 7 heartbeat complexes should be recorded. Two ECGs approximately 1 minute apart will be collected at each time point for all serial ECGs.

For all ECGs, an original paper printout of the tracing must be obtained. The original tracing will be retained in the subject's file for source data verification. Additionally, 2 photocopies of each tracing will be made. One photocopy will be retained at the site in a separate file and the other copy will be available for the sponsor. Each ECG will be labeled with the study code, subject initials, enrollment/randomization number, scheduled day, scheduled time, and actual time recorded.

All ECGs will be reviewed for safety purposes by the investigator at the CRC. The time of recording of the ECG and the safety evaluation (normal/abnormal) will be recorded on the CRF. If the ECG is abnormal, a comment will be entered onto the CRF.

4.4.10.6 Determination of RR and QT intervals

A central cardiologist blinded to the study will provide an interpretation of the ECG. This will include the RR, PR, QT, and QRS intervals from serial ECGs collected on Visits 2, 3 and 4. Manual over-read of ECGs will be performed on digitized ECGs with electronic annotations.

The preferred lead to be used is Lead II, however, alternative leads (as determined by the central cardiologist) may be used if Lead II is not considered satisfactory.

Three to five complexes will be used for QT interval measurements and the mean value will be used for the estimate of QT interval. The RR interval preceding the QT interval will be measured for up to three to five complexes. The mean RR interval will also be determined.

4.4.10.7 Determination of T & U waves

The central cardiologist will assess the T wave morphology and the presence or absence of U waves will be noted.

4.4.10.8 Calculation of derivation of endpoint

ECG assessment is being conducted for safety purposes in this trial; therefore QT interval analysis is not a primary endpoint. Statistical methods applied to this analysis will be descriptive and are not intended to satisfy a hypothesis.

4.4.10.9 QT interval correction factors

The primary QTc correction will be through Bazett's formula. The Fridericia correction for QT may be applied as a secondary correction factor. Bazett and Fridericia correction for all ECGs analyzed by manual over-read will be reported in the database. The formula is shown below:

$QTc = QT(ms)/[RR(sec)]^{1/2}$ (Bazett)

$QTc = QT(ms)/[RR(sec)]^{1/3}$ (Fridericia)

4.4.11 Vital signs

The subject's sitting and orthostatic blood pressure and pulse will be recorded at the times indicated in Table 1. For each subject, throughout the study, blood pressure and pulse will be measured as follows:

Sitting

Sitting blood pressure and pulse will be measured after the subject has been sitting for 5 minutes.

Orthostatic

Orthostatic blood pressure and pulse will be measured using a blood pressure measuring device with an appropriate cuff size.

- The subject will rest in bed for 10 minutes
- Supine blood pressure and pulse is measured
- Blood pressure and pulse is then recorded again after the subject has been standing up for 2 minutes with arms hanging relaxed down on both sides.
- Any symptoms from the subject should be registered as a comment and if applicable recorded as an adverse event.

4.5 Genetic sampling and storage

A single 9 mL venous blood sample will be collected into a polypropylene EDTA tube at Visit 2 for use in genetic testing. This will be optional and the subject may choose to participate in the study but not to provide a blood sample for genetic analysis. Separate consent will be taken for genetic sampling.

The sample will be frozen as whole blood at -20°C or below in a non frost-free freezer and transported to the assaying laboratory within one month of collection. Processing, labeling and shipping instructions are provided in Appendix D.

4.6 Volume of blood sampling

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

Table 5 Volume of blood to be drawn from each subject

Assessment	Sample volume (mL)	No. of samples	Total volume (mL)
PK samples	2	42	84
Genetic sample	9	1	9
ADP induced platelet aggregation	4.5	30	135
HIV, HBsAg, hepatitis C	10	1	10
Clinical chemistry	10	8	80
Hematology	9.5	8	76
Limited chemistry panel	10	3	30
Serum pregnancy test	1	2	2
Total			**426**

4.7 Adverse events

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

4.7.1 Recording of adverse events

The subjects will be told to report any AE occurring during the study to the investigator or his personnel. Open, standardized AE questioning such as “Have you had any health problems since the previous visit?” will be done by the investigators or their personnel at each contact with the subject. The AE open, standardized questioning should be done discretely in order to prevent the subjects from influencing each other.

Information about AEs will be collected from the first administration of study drug until the completion of the follow-up visit (Visit 5). Serious adverse event information will be collected from the time the subject signs the informed consent until the follow-up visit. Any AEs observed or reported by a subject and/or staff will be recorded in the CRF. Any AE

including clinical findings not resolved on the final dosing day will be followed until resolved or explained.

Laboratory and vital signs abnormalities will not be recorded as an AE unless any criterion for an SAE is fulfilled, the subject discontinues the study due to the result(s), or the investigator considers it to be of such clinical importance as to merit recording it as an AE. If a laboratory value or vital sign is associated with clinical signs or symptoms, the signs or symptoms should be reported as an AE and the associated laboratory value or vital sign should be considered additional information. Any sign or symptom that fulfills SAE definition (Appendix B) or are the reason for discontinuation of study drug should be reported accordingly (refer to Section 4.7.2).

To avoid colloquial expressions, the AE term should be reported in standard medical terminology when possible. For each AE, the investigator will evaluate and report the onset (date and time), resolution (date and time), intensity, causality, action taken, serious outcome (if applicable) and whether or not it caused the subject to discontinue the study.

The following variables will be recorded for each AE:

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

The intensity rating is defined as:

1 = mild (awareness of sign or symptom, but easily tolerated)

2 = moderate (discomfort sufficient to cause interference with normal activities)

3 = severe (incapacitating, with inability to perform normal activities)

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

AEs will be classified using the most current version of the Medical Dictionary for Regulatory Activities (MedDRA). Coding of AEs will be made in the Astra Monitoring System (AMOS).

4.7.2 Reporting of serious adverse events

When the investigator becomes aware of an SAE during the course of the study, the SAE must be reported to the local monitor or other AstraZeneca representative within one (1) day.

All SAEs have to be reported, whether or not considered causally related to the investigational product. All SAEs will be recorded in the case report form. The investigator is responsible

for informing the Ethics Committee and/or the Regulatory Authority of the SAE as per local requirements.

The AstraZeneca representative will work with the investigator to compile all the necessary information to ensure that the appropriate AstraZeneca Drug Safety Department receives a report within one day for all fatal and life-threatening cases and within five days for all other SAEs. Follow-up information on SAEs should also be reported by the investigator in the same time frames. If a non-serious AE becomes serious, this and other relevant follow-up information must also be provided to AstraZeneca within 1 day as described above.

The following procedure must be followed in the case of a serious adverse event. The same contact information is to be used in case of medical emergency.

YOU MUST REPORT ANY SERIOUS ADVERSE EVENT, INCLUDING DEATH DUE TO ANY CAUSE, IMMEDIATELY. COMPLETE THE ASTRAZENECA SERIOUS ADVERSE EVENT REPORT FORM AND CONTACT ONE OF THE PEOPLE LISTED IN THE ASTRAZENECA EMERGENCY CONTACT PROCEDURE ON PAGE 2 OF THIS PROTOCOL.

YOU MUST FOLLOW ALL SUBJECTS WITH A SERIOUS ADVERSE EVENT, INCLUDING DISCONTINUED SUBJECTS, UNTIL RESOLUTION OF THE ADVERSE EVENT.

5. STUDY MANAGEMENT

5.1 Monitoring

5.1.1 Study monitoring

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

5.1.2 Data verification

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

Monitoring will routinely be performed prior to the transfer of data to Data Management.

5.1.3 Direct access to source data

The monitor(s) will verify data from the CRFs against source data before collecting the CRFs to ensure accuracy and completeness of documentation, and to ensure that the principal investigator has submitted the CRFs to AstraZeneca.

5.2 Archiving of study documentation

AstraZeneca will retain all documentation pertaining to this study in the AstraZeneca central files for as long as AZD6140 is available for human consumption.

The investigator will retain all documentation pertaining to this study for at least 15 years.

5.3 Audits and inspections

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

5.4 Training of staff

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

5.5 Changes to the protocol

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

If it is necessary for the study protocol to be amended, the amendment and/or a new version of the study protocol (Amended Protocol) must be notified to or approved by each IEC or IRB, and, if applicable, also the local regulatory authority, before implementation. Local requirements must be followed.

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

AstraZeneca will distribute amendments and new versions of the protocol to the principal investigator who in turn is responsible for the distribution of these documents to his or her

IEC or IRB, and to the staff at his or her center. The distribution of these documents to the regulatory authority will be handled according to local practice.

5.6 Study agreements

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

5.7 Study timetable and termination

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

Discontinuation or suspension of the whole study program

If AstraZeneca decides to withdraw or suspend the study, the investigator, the head of the institution and regulatory authorities should be informed of the fact in a written form clarifying the reason.

The principal investigator will immediately notify the decision to the subjects, give appropriate medical treatment, take necessary measures, and record treatment or measures provided on the source documents.

Completion of the study

Upon terminating the study, the principal investigator will report in writing the completion of the study as well as a summary of the results to the head of the institution in accordance with the institution's rules. The head of the institution will then notify, in writing, the IRB and AstraZeneca and will also provide a summary of the results to both parties.

5.8 Data management

5.8.1 Case report forms

CRFs will be provided for the recording of data. The CRFs will be in triplicate with carbonless paper. Data should be recorded directly and legibly from the source documents onto the CRFs, preferably in black ballpoint pen. Corrections to the CRFs should be made legibly, initialed and dated. Correction fluid or covering labels must not be used. The top original (white) and the first copy (yellow) of each completed CRF will be collected and returned to the AstraZeneca designee; the investigator will retain the second copy (manila).

AstraZeneca Data Management will enter the CRF data on an ongoing basis into their standard commercial database. The data will be verified and cleaned with electronic edit checks comprised of validated computer programs and manual data review. Any missing, inconsistent or illegible entries into the CRFs will be referred back to the investigator via the site monitor using Data Query Forms within an agreed number of days upon entering the data. Responses should be received by Data Management and updated within an agreed number of days upon generating the data queries. Clean file will be declared when all of the following have been completed: all data have been accounted for and have been databased; all edit

checks have been run and data discrepancies have been resolved or accepted; all serious adverse events have been reconciled with the clinical database; all coding is complete and has been medically reviewed and approved; and quality control of the database against the CRFs and relevant data sources has been completed.

5.8.2 Pharmacogenetic data

The genotype data will be used to explore genetic factors in the disposition of and response to AZD6140. The results of the genetic study will not form part of the clinical study database or the clinical study report. The results of this study may be pooled with genetic results from other studies on AZD6140 to generate hypotheses to be tested in future studies.

The DNA sample will not be labeled with the subject's name but will only be identified by a code that will link it to other information collected about the study drug during the course of this study. This link will exist to provide a way for regulatory authorities to track and ensure clinical genetic research is being conducted correctly and will be maintained only for the period of time that these authorities require; it will be destroyed after 15 years. If the subject changes his/her mind about this testing after donating a blood sample, this link will be used to track and destroy the sample. The study sponsor can only guarantee the possibility of destroying the sample while the link is maintained.

Test results will be kept confidential in accordance with all applicable laws. None of the test results will be provided to the subject, any insurance company, the subject's employer or family, the investigator or any other physician who is treating the subject or may treat the subject in the future.

The subject's DNA will not be used for any purpose other than genotyping. DNA will not be passed on to any other party and all samples will be retained for a 15-year period.

6. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

6.1 Statistical evaluation

Statistical analysis will be carried out by Biostatistics at AstraZeneca, Wilmington, Delaware using SAS (version 8). Summary graphics required for presentation in the text portion of the Clinical Study Report (CSR) will be done using Sigmaplot 2000. Other graphics intended for use as supplemental figures and individual profile figures will be done using SAS. A comprehensive Statistical Analysis Plan (SAP) will be prepared and finalized before unblinding and analysis of the data.

The goal of the PK analysis is to estimate the effect of ethnicity (Japanese vs. Caucasian) on exposure to AZD6140 and AR-C124910XX over the current dose range of AZD6140. Therefore, no formal hypothesis testing will be performed, though estimation of the ratio of exposure (Japanese/Caucasian) will be supported with two-sided 90% confidence intervals (CI). The analysis of pharmacokinetics will involve subjects included in the primary analysis

set. Estimation of the effect of ethnicity will be performed at each dose level in the study. Graphical methods will be widely used in support of estimation of effects and summary statistics where indicated below. Differences in gender will be addressed descriptively by comparing the PK parameters across gender within an ethnic group.

6.2 Description of outcome variables in relation to hypotheses

The primary objective of this study is to assess the safety and tolerability of single ascending doses of AZD6140.

This study will also compare the pharmacokinetics (C_{max} and AUC) and [$AUC_{(0-t)}$] if there are less than the required number of subjects with AUC estimates]) pharmacodynamics percent ADP induced platelet aggregation inhibition (%PAI) following single ascending oral doses of AZD6140 in healthy Japanese and Caucasian male and female (non-child bearing potential) subjects.

6.2.1 Demographic and baseline data

All demographic variables, including medical and surgical history, physical examination and medications taken before and during the study, as detailed in Section 4.1, will be obtained from the CRF.

6.2.2 Pharmacokinetics

Plasma concentrations of AZD6140 and its metabolite AR-C124910XX will be listed and depicted graphically as a function of time after each single dose administration. PK parameters C_{max}, t_{max}, $t_{½}$, $AUC_{(0-t)}$ and AUC for AZD6140 and AR-C124910XX, and CL/F and Vz/F for AZD6140 will be estimated by non-compartmental analysis. AZD6140 and AR-C124910XX C_{max} will be estimated as the highest measured concentration and t_{max} will be the time to maximum concentration after single dose administration. The terminal elimination rate constant (λ_z) will be calculated by log-linear regression of the terminal portion of the concentration-time profile. The terminal elimination half-life ($t_{½}$) will be calculated as $0.693/\lambda_z$. AUC will be calculated using the linear trapezoidal method up to the last measurable concentration (AUC_{0-t}) and thereafter by extrapolation of the terminal elimination phase to infinity. AZD6140 CL/F will be estimated as the ratio of AZD6140 dose and AUC. The terminal phase volume of distribution, Vz/F, of AZD6140 will be estimated as the ratio of AZD6140 CL/F to λ. The PK parameters for each dose will be summarized by ethnic group - Japanese and Caucasian.

6.2.3 Pharmacodynamics

6.2.3.1 Inhibition of platelet aggregation

%PAI from pre-dose baseline will be calculated at each time using the following formula for:

$$\%PAI = \quad 100 \quad x \quad \frac{(PA_{BL} - PA_{T})}{PA_{BL}}$$

where PA_T is the mean response at time 'T', and PA_{BL} is the mean response at pre-dose baseline. Percentage inhibition will be restricted to limits 0 and 100. Any data falling outside this range will be truncated to the appropriate limit.

6.2.3.2 Bleeding times

The bleeding times as measured by Simplate II® technique will be read directly from the CRF. Bleeding times which are recorded as being greater than 60 minutes will be treated as being exactly 60 minutes in calculating the mean, but all summary statistics associated with that observation will be asterisked and identified with a footnote indicating that the summary statistics are biased.

6.2.4 Safety

The incidence, nature and severity of adverse events will be obtained from the CRF data. All variables relating to laboratory measurements, 12-lead ECG and vital signs, as detailed in Section 4.4, will be included in the analysis. Changes from baseline for each dose level will be relative to the latest pre-dose value on Day –2/–1 at Visits 2, 3 and 4, respectively.

6.3 Description of analysis sets

Subjects will be included in the primary analysis set for the PK or PD variable in question if they complete the trial with no major protocol violations or deviations and they provide adequate data in support of the analyses described below.

Subjects will be included in the analysis of safety, if they have received at least one dose of study medication.

6.4 Methods of statistical analyses

6.4.1 Pharmacokinetics

6.4.1.1 Primary analysis

The primary analysis of AZD6140 AUC will be based on log-transformed data using an ANOVA model with effects for dose, weight and ethnicity and subject within ethnicity as a random effect. The primary model will contain only the terms mentioned above. A model including a dose by ethnicity interaction term will also be explored and a term for gender may also be added depending on gender balance. The effect will be estimated using geometric means and two-sided 90% CIs for the ratio of AUC (Japanese/Caucasians) for each dose will

be estimated based on the primary model. The primary contrast will be between Japanese and Caucasians for each dose level. Because there are 2 dosing sequences (AZD6140 50 mg, 200 mg, 400 mg and AZD6140 100 mg, 300 mg, 600 mg) there will be two models of form similar to that described above one for each set of doses. Geometric mean AZD6140 plasma concentrations versus time by dose and ethnic group will be presented graphically. Individual plasma concentrations versus time by dose will be presented graphically. Adjustment for BMI may also be considered by including them as terms in the model. Individual profiles of AUC versus dose will be presented graphically. Dose normalized AUC versus dose and ethnic group will be presented graphically. Adjustment for BMI or weight may also be considered by including them as terms in the model. In the event that there are less than 6 subjects with estimated AUC, $AUC_{(0\text{-}t)}$ will replace AUC as one of the primary endpoints. $AUC_{(0\text{-}t)}$ and C_{max} will be analyzed in a manner similar to that mentioned above.

AZD6140 plasma concentrations, AUC, $AUC_{(0\text{-}t)}$, C_{max}, t_{max}, $t_{1/2,}$ CL/F and Vz/F will also be summarized by dose using descriptive statistics detailed in the Statistical Analysis Plan.

Assumptions of normality of the log-transformed AUC, $AUC_{(0\text{-}t)}$, and C_{max} data will be explored in the primary analysis and, if necessary an appropriate non-parametric technique will be used to validate the results of the primary analysis.

AR-C124910XX AUC, $AUC_{(0\text{-}t)}$, t_{max}, $t_{1/2}$ and C_{max} will be analyzed in a manner similar to AZD6140.

6.4.1.2 AZD6140 dose proportionality

Dose proportionality will be assessed using C_{max} and AUC [or $AUC_{(0\text{-}t)}$] of AZD6140 as a response variable. The following model will be fit to each ethnic group:

$$AUC = \alpha(dose)^{\beta}\,\varepsilon$$

This model will be fit using log-transformed AUC (and C_{max} separately) and dose data based on an ANOVA model with an effect for log-transformed dose and subject as a random effect. A two-sided 90% CI for β will be constructed.

6.4.2 Pharmacodynamics

Percentage of platelet aggregation inhibition will be summarized using descriptive statistics over time by dose and ethnic group. Mean (or median) percentage of platelet aggregation versus time by dose and ethnic group will be presented graphically. Differences (Japanese – Caucasian) in mean (or median) percentage of platelet aggregation inhibition will be summarized by dose.

6.4.3 Safety

All subjects who receive at least one dose of study medication will be included in the analysis of safety.

6.4.3.1 Adverse events

All adverse events will be listed for each subject including the assigned system organ class (SOC) and preferred term using the Medical Dictionary for Regulatory Activities (MedDRA). All adverse events occurring following the first dose of study drug will be summarized by SOC, preferred term, dose and ethnic group.

6.4.3.2 Bleeding times

Bleeding time and change from baseline in bleeding time will be summarized by time after dose, dose and ethnic group using descriptive statistics. Baseline will be defined as the pre-dose measurement taken at each visit. Differences in change from baseline (Japanese – Caucasians) will also be summarized by time and dose. Mean bleeding time versus time by dose and ethnic group will be displayed graphically. Individual bleeding times versus time will also be displayed graphically by dose and ethnic group.

6.4.3.3 Laboratory and vital sign data

Hematology, clinical chemistry, urinalysis and vital sign data will be listed and summarized by dose, ethnic group and measurement time. Values that are out of the lab reference range will be flagged in the listings. Qualitative urinalysis data will be summarized using the number of subjects with results of negative, trace or positive.

6.4.3.4 ECG

All ECG intervals data will be summarized using descriptive statistics by dose, visit and measurement time. Change from baseline in QTc will be summarized using descriptive statistics by dose and assessment time. There will be a set of baseline measures taken at each visit. Baseline will be the ECG measures taken on the pre-dose day at the same time relative to the expected dosing time for the current visit.

6.4.4 PK/PD relationships

The relationship between AZD6140, AR-C124910XX, total (AZD6140 + AR-C124910XX) concentrations and %PAI will be investigated using an E_{max} model. Data from all doses within an ethnic group will be modeled simultaneously regardless of cohort. Observed and predicted %PAI versus exposure will also be presented graphically. The E_{max} model is a non-linear model of the following form.

$$\%PAI = \frac{E_{\max} C^{\gamma}}{C^{\gamma} + EC_{50}^{\gamma}}$$

Models with sigmoidicity factor γ equal to 1 and where γ is estimated from the data will be explored. A model including a term for ethnicity will be used to explore the effect of ethnicity on model parameters.

6.5 Determination of sample size

Twenty Japanese subjects and 20 Caucasian subjects will be randomized into the trial. Ten subjects from each ethnic group will be assigned to each cohort. Eight subjects will be randomized to receive active AZD6140 (to achieve 6 evaluable) and 2 subjects will be randomized to receive placebo to AZD6140. The ratio of ethnic groups (Japanese/Caucasians) in terms of AUC and C_{max} for the AZD6140 100 mg once daily doses can be estimated using a two-sided 90% confidence interval, assuming the following coefficients of variation (CV) (AUC: 21% C_{max}: 20% [100 mg od based on trial 5130C5238]) with 6 subjects per ethnic group. Assuming these conditions hold, the log-scale precision for each of these intervals will be (0.2186 and 0.2092) and the 90% confidence interval around an observed ratio of 1 would be [AUC (0.80, 1.24) and C_{max} (0.81, 1.23)].

6.6 Interim analyses

No interim analysis is planned.

6.7 Data Presentation

The details of data presentation will be provided in the SAP.

6.8 Data or safety monitoring committee (Not applicable for this protocol)

7. ETHICS

7.1 Ethics review

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

The principal investigator is responsible for informing the IEC or IRB of any amendment to the protocol in accordance with local requirements. In addition, the IEC or IRB must approve all advertising used to recruit subjects for the study. The protocol must be reapproved by the IEC or IRB annually, as local regulations require.

Either the principal investigator(s) or AstraZeneca must submit progress reports to the IEC or IRB according to local regulations and guidelines. The principal investigator must also provide the IEC or IRB with any reports of serious adverse events from the study site.

Under no circumstances will the investigation be extended beyond the limitations defined in this protocol or any subsequent amendments.

The principal investigator is also responsible for providing the IRB with reports of any SAEs from any other study conducted with the investigational product. AstraZeneca will provide this information to the principal investigator(s).

7.2 Ethical conduct of the study

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

7.3 Subject information and consent

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

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

The principal investigator must store the original, signed Written Informed Consent Form. A copy of the Written Informed Consent Form must be given to the subject. If modifications are made according to local requirements, the new version has to be approved by AstraZeneca.

7.4 Subject data protection

The Written Informed Consent Form will explain that study data will be stored in a computer database, maintaining confidentiality in accordance with national data legislation. All data computer processed by AstraZeneca will be identified by subject number/study code. The Written Informed Consent Form will also explain that for data verification purposes, authorized representatives of AstraZeneca, a regulatory authority, an IEC or IRB may require direct access to parts of the hospital or practice records relevant to the study, including subjects' medical history.

8. EMERGENCY PROCEDURES

8.1 Medical emergency contact procedure

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

AstraZeneca Contact	Telephone Number and Fax

For Serious Adverse event reporting

•

The principal investigator is responsible for ensuring that procedures and expertise are available to cope with medical emergencies during the study.

If a medical emergency constitutes a serious adverse event please follow the procedures outlined in Section 4.7.2.

Please refer to Section 3.4.4.2 for unblinding procedures.

8.3 Procedures in case of overdose

There is limited previous human experience regarding the use of AZD6140. In case of overdose, monitoring of cardiac, hepatic and hematological effects is essential. Appropriate standard supportive therapy should be initiated. Since there is no specific antidote to this compound, subjects should be treated symptomatically. For further information see the Clinical Investigator's Brochure.

8.4 Procedures in case of pregnancy

Female subjects must be non-pregnant and of non-child bearing potential (surgically sterilized [eg. tubal ligation, hysterectomy, bilateral oophorectomy] or post menopausal for 12 months prior to screening).

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

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

9. REFERENCES

None

AstraZeneca

Clinical Pharmacology Study Protocol: Appendix B	
Drug Substance	AZD6140
Study Code	D5130C05266
Edition No.	Final Version 1.0
Appendix Date	

Appendix B
Additional Safety Information

DEFINITIONS OF ADVERSE EVENTS

Adverse event

An adverse event 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, electrocardiogram). In clinical studies, an AE can include an undesirable medical condition occurring at any time, including run-in or washout periods, even if no study treatment has been administered.

Serious adverse event

A serious adverse event is an AE occurring during any study phase (eg, run-in, treatment, washout, follow-up), and at any dose of the investigational product, comparator or placebo, that fulfils one or more of the following criteria:

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

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

Other significant adverse event (OAE)

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

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 (e.g. hepatitis that resolved without hepatic failure).

Hospitalization

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

Important medical event or medical intervention

Medical and scientific judgment 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, hospitalization, 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.

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 anemia requiring blood transfusion, etc.) or convulsions that do not result in hospitalization
- 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 etiology 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 recognized 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.

AstraZeneca

Clinical Pharmacology Study Protocol: Appendix C	
Drug Substance	AZD6140
Study Code	D5130C05266
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Appendix C
Instructions for Bleeding Time Evaluations

Test procedure

1. Seat the subject with arm supine on a steady support with the volar surface exposed (Figure 1). Select a site on the muscular area of the forearm distal to the anticubital fossa, taking care to avoid surface veins, scars, and bruises. The lateral aspect is preferred over the medial aspect because the latter has a tendency to be more painful, less reproducible, and has a higher incidence of scarring.[9] Cleanse with an alcohol swab and allow to air dry at least 30 seconds (Figure 2). If the patient has a marked amount of hair, lightly shave the test area. Place a sphygmomanometer cuff on the upper arm.
2. Remove the **Simplate** device from the blister pack (Figure 3), and twist off the white, tear-away tab on the side of the device (Figure 4). *Do not push the trigger or touch the blade slot.* Inflate the sphygmomanometer cuff to 40 mmHg. The time between inflation of cuff and incision should be 30-60 seconds. Monitor frequently to ensure maintenance of pressure during test procedure.
3. Place the device firmly on the forearm. Do not press. Incisions must be made consistently, either parallel or perpendicular to the fold of the elbow. A horizontal incision (parallel to the anticubital fossa) gives a longer bleeding time than a vertical incision (perpendicular to the anticubital fossa).[9]
4. Depress the trigger and simultaneously start the timer (Figure 5). Remove the device approximately one second after triggering (Figure 6).
5. At 30 seconds, blot the flow of blood with filter paper (Figure 7). Bring the filter paper close to the incision without touching the edge of the wound. (Do not disturb the platelet plug.) Blot in a similar manner every 30 seconds until blood no longer stains the filter paper (Figure 8). Stop timer.
6. Remove cuff, clean arm, and apply a butterfly bandage across the incision (Figure 9). An additional covering bandage may be used if desired. Advise patient to keep the butterfly bandage in place for 24 hours. Record the bleeding time to the nearest 30 seconds. Return the device to the opened blister pack; avoid touching the blade area and discard.

LIMITATIONS OF THE PROCEDURE

1. If thrombocytopenia is present, the bleeding time may be prolonged (less than 75,000/mm^3 platelets).[10]
2. Since the bleeding time of many people is increased after the ingestion of aspirin,[5] it is important to determine whether or not the patient has consumed drugs affecting platelet function, such as aspirin or aspirin-containing drugs, within a minimum of one week prior to testing.

EXPECTED RESULTS

Bleeding time studies with **Simplate** devices were conducted in several laboratories to estimate the variation present among normal subjects. These populations consisted of healthy, adult males and females, free from drugs or conditions known to affect the bleeding time. No significant lab-to-lab differences were observed for the various populations; thus, a composite population was formed that consisted of 187 males and females ranging from age 20 to 71 years. After log transformation, the expected range was calculated as a mean, plus or minus two standard deviations.

EXPECTED RANGE: 2.3 to 9.5 minutes

Because of the complexity of the primary hemostatic mechanism, the numerous patient-related factors that may affect the bleeding time (age, skin type, skin condition, vascularity, and temperature) and variation in individual techniques, it is recommended that each laboratory establish its own "expected range."

PERFORMANCE CHARACTERISTICS

The Simplate bleeding time procedure provides results that correlate with the Ivy and Mielke techniques. Correlation coefficients of 0.87 and 0.74 were obtained for Mielke and Ivy comparisons, respectively.

Precision

The precision between duplicate bleeding time determinations was studied in five laboratories. The standard deviation of duplication was not significantly different from one laboratory to the next ($\rho>0.05$) and averaged 0.94 minutes (standard deviation of duplicates).

A study of the sources of variability with the **Simplate** device showed that approximately 40% of the total variability was attributed to day-to-day differences for the same subject; lesser variability was associated with the duplicate and subject-to-subject components. Similar variability was noted in a parallel study with the template procedure.

Appendix D
Instructions for Collection, Storage and Transport of Blood Samples for Genetic Analysis

1. BLOOD SAMPLE COLLECTION

Ideally, blood should be collected into ***9/10 ml polypropylene tubes*** containing the ***anticoagulant EDTA***. Recommended tubes are detailed in the table below. After collection, blood tubes must be gently ***inverted*** several times to ensure thorough mixing of EDTA with the sample to prevent clotting.

Table of recommended blood tubes for genotyping sample collection

Polypropylene Collection Tube	Part #	Comments
	1066 US 1066.001 UK	SARSTEDT Monovette® EDTA KE - 9ml
	368457 USA/UK	Becton-Dickinson Vacutainer™ K2E - 10ml
	455036 USA/UK	Greiner Bio-one Vacuette® K3E EDTA K3 - 9ml

- ***Glass tubes MUST NOT be used*** as they may break during transport and freeze-thaw cycles.
- ***Heparin MUST NOT be used as an anticoagulant*** as it may interfere with downstream genotyping methodology.

The collection tubes must be labeled with the following information:

- Unique sample ID (compliant with protocol)

- Study ID (and Study Center ID, if available)
- Date of sample collection.

2. STORAGE AT THE STUDY CENTER AND TRANSPORT

After collection, blood samples must be stored appropriately at the site of collection and transported to the Central Handling Facility, or Designated DNA Processing Laboratory, ***as soon as possible***. The table below shows guidelines for sample storage and transport:

Table to show the recommended storage conditions for blood samples immediately after collection

Option	***Storage Temperature at Study Center***	***Maximum Duration***	***Transport Temperature***	***Delivery Time***
1	+ 4°C (fridge)	24 hours	0 - 4°C (ice bricks)	24 hours
2	+ 4°C (fridge)	24 hours	Less than -20°C (dry ice)	24-72 hours
3	-20°C (freezer) or -70°C	Up to 1 month	Less than -20°C (dry ice)	24-72 hours

- IF BLOOD SAMPLES ARE TO BE STORED AT -20°C OR LESS, NON-FROST FREE FREEZERS MUST BE USED TO PREVENT REPEATED FREEZE-THAW OF BLOOD WHICH MAY REDUCE YIELD & QUALITY OF THE DNA OBTAINED.
- SAMPLES MUST NOT BE THAWED AND THEN RE-FROZEN AT ANY POINT

The Central Handling Facility, or Designated DNA Processing Laboratory, must be notified of the shipment of any samples prior to dispatch. Ideally, the dispatch note must be sent by either fax or email and must contain the following information:

- Study ID, number of samples and list of sample ID's
- Courier name, airway bill number and date of shipment

- Shipment condition (wet ice or dry ice)
- Contact name and address

Considerations should be made to ensure that the samples are delivered during working hours and within 24-72 hours of dispatch.

3. RECOMMENDED PACKAGING INSTRUCTIONS

For safety reasons all blood samples must be contained. Samples should be individually placed in a clip-lock bag labeled with the sample ID and sealed. Samples may then be batched and again sealed within a second clip-lock bag labeled with the study ID. For ease of further packaging and protection from damage, samples should then be placed within another plastic bag labeled with the study ID and study center ID. A bio-safety label should also be applied.

Sample Shipment.

IATA (International Air Transport Association) approved polystyrene transport boxes must be used.

For samples transported on wet ice:

The box should contain frozen ice blocks and protective packaging (polystyrene flocking), to allow for a minimum of 24 hours transport.

For samples transported on dry ice:

The box should contain dry-ice pellets (if pellets are not available then blocks may be used if protective packaging such as polystyrene flocking is included) to allow for a minimum of 72 hours transport.

Each package must be sealed in a cardboard box labeled with the courier airway bill.

AstraZeneca

Clinical Study Protocol Amendment

Amendment No.	1
Drug Substance	AZD6140
Study Code	D5130C05266
Edition No.	1
Date	

A Randomized, Double-Blind, Placebo-Controlled Study to Assess the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Single Ascending Oral Doses of AZD6140 Tablets in Healthy Male and Female Japanese and Caucasian Subjects

Sponsor:

AstraZeneca Pharmaceuticals LP, Wilmington, Delaware, USA

Centres affected by the Amendment:

001

The protocol for the study is to be amended as follows:

Section of protocol affected:

Page 2: AstraZeneca Emergency Contact Procedure is updated to reflect changes in study personnel at AstraZeneca.

Revised Text:

Role in the study	Name	Address and Telephone number
Clinical Study Team Leader		
Clinical Research Scientist		
Clinical Study Team Physician		

Section of protocol affected:

Protocol Synopsis

The Study Period is updated to reflect changes in the study timeline.

Previous Text:

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

Revised Text:

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

Section of protocol affected:

Section 3.1.1 Visit 1 Screening Period

Previous Text:

In order to establish eligibility to participate in this study, subjects will undergo all screening procedures and assessments within 21 days prior to Visit 2 (first dosing visit).

Revised Text:

In order to establish eligibility to participate in this study, subjects will undergo all screening procedures and assessments within **28 days** prior to Visit 2 (first dosing visit).

Reason for Amendment:

The screening period has been extended from 21 days to 28 days in order to allow more time for recruitment of subjects at the site.

Section of protocol affected:

3.1.2 Visit 2 (Days –2, -1, 1, 2, 3 and 4)

Previous Text:

Subjects will arrive at the Clinical Research Center (CRC) on the evening of Day –2 as directed. At this time the subjects will be reassessed with regard to the study inclusion/exclusion criteria. Subjects will be randomized to receive either AZD6140 or placebo to AZD6140. In addition, on Day –2, a blood sample for genetic testing will be collected from those subjects who have given written consent.

On Day –2 the subjects will begin fasting at least 10 hours prior to the start of the baseline serial ECGs scheduled for Day –1 (refer to Table 1). On the morning of Day –1 baseline serial ECGs will begin.

On Day –1, subjects will begin fasting at least 10 hours prior to receiving study drug on Day 1 and remain fasting until 4 hours following dosing. On the morning of Day 1, the first dose of

AZD6140 (50 mg for Cohort A or 100 mg for Cohort B or matching placebo) will be administered by site personnel. Subjects will remain at the CRC until the completion of all the assessments and procedures scheduled for Day 3. At this time the subjects will be permitted to return to their home environment. They will be instructed to return to the CRC the next morning as directed for the assessments scheduled for Day 4. Please refer to Table 1 and Sections 3.4.2 and 4 for the timing and detailed descriptions of the assessments and procedures to be performed at Visit 2.

Revised Text:

Subjects will arrive at the Clinical Research Center (CRC) on the evening of Day –2 as directed. At this time the subjects will be reassessed with regard to the study inclusion/exclusion criteria. In addition, on Day –2, a blood sample for genetic testing will be collected from those subjects who have given written consent.

On Day –2 the subjects will begin fasting at least 10 hours prior to the start of the baseline serial ECGs scheduled for Day –1 (refer to Table 1). On the morning of Day –1 baseline serial ECGs will begin.

On Day –1, subjects will begin fasting at least 10 hours prior to receiving study drug on Day 1 and remain fasting until 4 hours following dosing. **On the morning of Day 1, subjects will be randomized to receive either AZD6140 or placebo to AZD6140. The first dose of AZD6140 (50 mg for Cohort A or 100 mg for Cohort B or matching placebo) will be administered by site personnel.** Subjects will remain at the CRC until the completion of all the assessments and procedures scheduled for Day 3. At this time the subjects will be permitted to return to their home environment. They will be instructed to return to the CRC the next morning as directed for the assessments scheduled for Day 4. Please refer to Table 1 and Sections 3.4.2 and 4 for the timing and detailed descriptions of the assessments and procedures to be performed at Visit 2.

Reason for Amendment:

Randomization of all subjects was moved to Visit 2 Day 1(immediately predose) in order to randomize the subjects immediately before dosing.

Section of protocol affected:

3.1.4 Visit 4 (Days –1, 1, 2, 3 and 4)

Previous Text:

The following sentence is moved to the beginning of Section 3.1.2 for clarity:

Note: Throughout the study the site will supply standardized meals while the subjects remain at the CRC. Menus will be approved by the sponsor.

Section of protocol affected:

Table 1 Study Plan

Previous Text:

Table 1 is updated to reflect the following changes:

The screening period is extended from 21 days to 28 days in order to allow more time for recruitment of subjects at the site.

The Brief Physical Exam scheduled for Visit 2, Day –2 is changed to a Physical Exam scheduled for Visit 2, Day –2 in order to establish a baseline evaluation.

The Limited Chemistry Panel scheduled for Visits 2, 3 and 4, Day –1 is changed to Visits 2, 3 and 4, Day 1 predose.

Randomization of study medication scheduled for Visit 2, Day –2 is changed to Visit 2, Day 1.

Section of protocol affected:

Table 1 Study Plan

Previous Text:

Footnote b:

Sitting blood pressure and pulse (after sitting for 5 minutes) will be taken at the following times:

Visit 1 (Screening)

Visits 2: Day –2, Visits 2, 3 and 4: Days –1, 1, 2, 3 and 4

Visit 5 (Follow-up)

Orthostatic blood pressure assessments will be performed at the following times:

Visits 2, 3 and 4: 4 hours post dose

Revised Text:

Footnote b:

Sitting blood pressure and pulse (after sitting for 5 minutes) will be taken at the following times:

Visit 1 (Screening)

Visits 2: Day –2, Visits 2, 3 and 4: Days –1, 1, 2, 3 and 4

Visit 5 (Follow-up)

Orthostatic blood pressure assessments (after 10 minutes supine and after 2 minutes standing) will be performed at the following times:

Visits 2, 3 and 4: 4 hours post dose

Reason for Amendment:

The positions for orthostatic blood pressure assessments were added for clarity.

Section of protocol affected:

Section 3.3.3 Exclusion Criteria

Previous Text:

2. Current and/or past history of intolerance or hypersensitivity to drugs with a similar chemical structure or mechanism of action of AZD6140 or any ingredient in its formulation (refer to section 2.4.1). These drugs include, but are not limited to clopigrel (Plavix) and ticlopidine (Ticlid).

Revised Text:

2. Current and/or past history of intolerance or hypersensitivity to drugs with a similar chemical structure or mechanism of action of AZD6140 or any ingredient in its formulation (refer to **section 3.4.1**). These drugs include, but are not limited to clopigrel (Plavix) and ticlopidine (Ticlid).

Reason for Amendment:

The section that was referrenced in the previous text was incorrect.

Section of protocol affected:

Section 3.3.3 Exclusion Criteria

Previous Text:

12. Use of any prescribed medication or over the counter preparations including herbal preparation and vitamins in the 2 weeks prior to randomization unless otherwise approved by the sponsor and the investigator. Use of hormone replacement therapy for female subjects is permitted as long as there has been no change in the dosing regimen for at least 3 months prior to screening.

Revised Text:

12. Use of any prescribed medication or over the counter preparations including herbal preparation and vitamins in the 2 weeks prior to randomization **and throughout the study** unless otherwise approved by the sponsor and the investigator. Use of hormone replacement therapy for female subjects is permitted as long as there has been no change in the dosing regimen for at least 3 months prior to screening.

Reason for Amendment:

This statement was added for clarity.

Section of protocol affected:

Section 3.3.3 Exclusion Criteria

Previous Text:

14. Consumption of aspirin, ibuprofen or any other drug known to increase the propensity for bleeding within 2 weeks prior to randomization.

Revised Text:

14. Consumption of aspirin, ibuprofen or any other drug known to increase the propensity for bleeding within 2 weeks prior to randomization **and throughout the study**.

Reason for Amendment:

This statement was added for clarity.

Section of protocol affected:

Section 3.3.4 Restrictions

Previous Text:

7. Fast for a period of ten hours prior to each dose of study drug (Visits 2, 3 and 4) and remain fasting for 4 hours after dosing. This fasting schedule will also be observed beginning on the evening of Day –2 (Visist 2) and continuing until the morning of

Day –1 (Visit 2). Sips of water will be allowed except from 2 hours before until 2 hours after study drug administration.

Revised Text:

7. Fast for a period of ten hours prior to each dose of study drug (Visits 2, 3 and 4) and remain fasting for 4 hours after dosing. This fasting schedule will also be observed beginning on the evening of Day –2 (Visit 2) and continuing until the morning of Day –1 (Visit 2). **Water will be allowed during the fast except from 2 hours before study drug administration until 2 hours after study drug administration.**

Reason for Amendment:

The sentence was changed to provide consistency throughout the protocol.

Section of protocol affected:

4.2.2 Labeling of plasma samples

Previous Text:

Set Number (A or B)

Revised Text:

Tube and Set Number (A or B)

Reason for Amendment:

Tube numbers will be added to the plasma sample labels for identification purposes.

Section of protocol affected:

4.2.3 Shipping of plasma samples

Previous Text:

Ship samples on Mondays-Wednesdays. Do not ship on or the day before a legal holiday

Revised Text:

Ship samples on **Saturday**, **Monday and Tuesday only.** Do not ship on or the day before a legal holiday.

Reason for Amendment:

The samples will be going from Hawaii to Europe and require extra shipping time. Saturday shipping was added to expedite PK sample analysis.

Section of protocol affected:

Table 2 Schedule of PK blood sampling

Previous Text:

Table 2 Schedule of PK blood sampling

Scheduled Time Relative to Dose at Visits 2, 3 and 4
Pre-dose (within 30 minutes of dosing)
0.5 hour post dose
1 hours post dose
2 hours post dose
3 hours post dose
4 hours post dose
6 hours post dose
8 hours post dose
12 hours post dose
18 hours post dose
24 hours post dose
36 hours post dose
48 hours post dose
72 hours post dose

Revised Text:

Table 2 Schedule of PK blood sampling

Scheduled Time Relative to Dose at Visits 2, 3 and 4	**Tube and Set Number**
Pre-dose (within 30 minutes of dosing)	**1 A/B**
0.5 hour post dose	**2 A/B**
1 hours post dose	**3 A/B**
2 hours post dose	**4 A/B**
3 hours post dose	**5 A/B**
4 hours post dose	**6 A/B**
6 hours post dose	**7 A/B**
8 hours post dose	**8 A/B**
12 hours post dose	**9 A/B**
18 hours post dose	**10 A/B**
24 hours post dose	**11 A/B**
36 hours post dose	**12 A/B**
48 hours post dose	**13 A/B**
72 hours post dose	**14 A/B**

Reason for Amendment:

Tube and set number have been added to Table 2 for clarity in regard to labeling PK tubes.

Section of protocol affected:

4.4.4 Pregnancy test-Serum and urine

Previous Text:

Serum and urine pregnancy tests will be performed on all female subjects. The serum pregnancy test can be collected with the same sample as the blood chemistry. If the result of any pregnancy test is positive, the subject will not be allowed to proceed in the study.

Revised Text:

Serum and urine pregnancy tests will be performed on all female subjects. The serum pregnancy test can be collected with the same sample as the blood chemistry. If the result of any pregnacy test is positive, the subject will not be allowed to proceed in the study.

Note: Although the results of the serum and urine pregnancy tests have to be documented in the subject's files, they will not be collected on the CRF's and will therefore not be recorded in the study database.

Reason for Amendment:

This notation was added to explain how pregnacy test results will be collected and filed.

Section of protocol affected:

4.4.5 HIV, HbsAG and hepatitis C

Previous Text:

A blood sample for HIV, HbsAG and hepatitis C antibody will be obtained at screening. If a test result is positive the subject will not be permitted to continue his/her participation in the study.

Revised Text:

A blood sample for HIV, HbsAG and hepatitis C antibody will be obtained at screening. If a test result is positive the subject will not be permitted to continue his/her participation in the study.

Note: Although the results of the HIV, HbsAg and hepatitis C tests have to be documented in the subject's files, they will not be collected on the CRF's and will therefore not be recorded in the study database.

Reason for Amendment:

This notation was added to explain how results of the HIV, HbsAG and Hepatits C results will be collected and filed.

Section of protocol affected:

4.4.6 Drugs of abuse screen

Previous Text:

A urine screen for drugs of abuse will be performed on urine samples obtained from each subject. If a test result is positive for any of the substances of abuse, the subject will not be permitted to continue his/her participation in the study. The following drugs will be screened:

Amphetamines, barbiturates,benzodiazepines, cannabinoids, cocaine, methadone,opiates, phencyclidine and propoxyphene.

Revised Text:

A urine screen for drugs of abuse will be performed on urine samples obtained from each subject. If a test result is positive for any of the substances of abuse, the subject will not be permitted to continue his/her participation in the study. The following drugs will be screened:

Amphetamines, barbiturates,benzodiazepines, cannabinoids, cocaine, methadone,opiates, phencyclidine and propoxyphene.

Note: Although the results of the urine drug screen have to be documented in the subject's files, they will not be collected on the CRF's and will therefore not be recorded in the study database.

Reason for Amendment:

This notation was added to explain how results of the drugs of abuse screen results will be collected and filed.

Section of protocol affected:

4.6 Volume of blood sampling

Previous Text:

Table 5 Volume of blood to be drawn from each subject

Assessment	Sample volume (mL)	No. of samples	Total volume (mL)
PK samples	2	42	84
Genetic sample	9	1	9
ADP induced platelet aggregation	4.5	30	135
HIV, HBsAg, hepatitis C	10	1	10
Clinical chemistry	10	6	60
Hematology	9.5	6	57
Limited chemistry panel	10	3	30
Serum pregnancy test	1	2	2
Total			**387**

Revised Text:

Table 5 Volume of blood to be drawn from each subject

Assessment	Sample volume (mL)	No. of samples	Total volume (mL)
PK samples	2	42	84
Genetic sample	9	1	9
ADP induced platelet aggregation	4.5	30	135
HIV, HBsAg, hepatitis C	10	1	10
Clinical chemistry	10	**8**	**80**
Hematology	9.5	**8**	**76**
Limited chemistry panel	10	3	30
Serum pregnancy test	1	2	2
Total			**426**

Reason for Amendment:

Table 5 is updated to reflect the approximate number of blood draws that the site will perform for the Chemistry and Hematology samples.

Section of protocol affected:

5.7 Study timetable and termination

Previous Text:

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

Revised Text:

The study is expected to start , and to be completed by **.**

Reason for Amendment:

The dates were changed to reflect an update in the study timelines.

Signed agreement to the Amendment:

I agree to the terms of this protocol Amendment.

Study Code: D5130C05266

. . . . .	. . . . .
Date (day month, year)	AstraZeneca Clinical Study Team Physician

I agree to the terms of this protocol Amendment.

Study Code: D5130C05266

Centre No.: 001

. . . . .	. . . . .
Date (day month, year)	AstraZeneca Clinical Study Team Leader

I agree to the terms of this protocol Amendment.

Study Code: D5130C05266

Centre No.: 001

. . . .

Date   Principal investigator
(day month, year)