AstraZeneca

Clinical Pharmacology Study Protocol

Drug substance	AZD6140
Edition No.	1.0
Study code	D5130C05267
Date	

A Single-blind, Randomized, Placebo-controlled Phase I Study to assess the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Multiple Oral Doses of AZD6140 in Healthy Male Japanese and Caucasian Subjects

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**

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

ASTRAZENECA EMERGENCY CONTACT PROCEDURE

In case of a medical emergency you may contact the Clinical Study Team Leader. If the Clinical Study Team Leader is not available, contact the Clinical Study Team Physician, Drug Safety Physician or Clinical Research Associate/Clinical Research Scientist.

For the Japanese Study Center

Role in the study	Name	Address and Telephone number	After Hours Telephone Number

For US Study Center

Role in the study	Name	Address and Telephone number	After Hours Telephone Number

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 Single-blind, Randomized, Placebo-controlled Phase I Study to assess the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Multiple Oral Doses of AZD6140 in Healthy Male Japanese and Caucasian Subjects

Principal Investigator

Japanese Study Center:

US Study Center:

Study center(s), type and number of subjects planned

Seventy-two healthy male subjects to include 36 Japanese and 36 Caucasian subjects from 20 to 45 years of age will be randomized at a center in Japan for Japanese subjects and a center in the United States (US) for Caucasian subjects.

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

Primary Objectives:

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

To investigate the pharmacokinetics of multiple oral doses of AZD6140 administered to healthy male Japanese and Caucasian subjects by assessment of plasma concentrations of AZD6140 and the active metabolite, AR-C124910XX.

Secondary Objectives:

To investigate the pharmacodynamics of multiple oral doses of AZD6140 administered to healthy male Japanese and Caucasian subjects by assessment of platelet aggregation inhibition and bleeding time.

To investigate the pharmacokinetic/pharmacodynamic relationship between plasma concentrations of AZD6140 and AR-C124910XX and platelet aggregation inhibition following multiple oral doses of AZD6140 administered to healthy male Japanese and Caucasian subjects.

Study design

This will be a single-blind, randomized, placebo-controlled, Phase I study designed to assess the safety, tolerability, pharmacokinetics and pharmacodynamics of multiple oral doses of AZD6140 in Japanese and Caucasian healthy male subjects, performed at 2 study centers – Japanese subjects in Japan and Caucasian subjects in US.

Investigational product, dosage and mode of administration

The following investigational product will be supplied:

- AZD6140 100 mg tablets
- AZD6140 Placebo to match 100 mg tablets

For Cohort A at each study center, the subjects will receive a single 100 mg dose of AZD6140 (n=15 at each center) or matching placebo (n=3 at each center) on Day 1. On Days 4-9, subjects randomized to AZD6140 will receive 100 mg AZD6140 bid and subjects randomized to placebo will receive matching placebo bid. On Day 10, subjects will receive a single 100 mg dose of AZD6140 or matching placebo in the morning only.

For Cohort B at each study center, the subjects will receive a single 300 mg dose of AZD6140 (n=15 at each center) or matching placebo (n=3 at each center) on Day 1. On Days 4-9, subjects randomized to AZD6140 will receive 300 mg AZD6140 bid and subjects randomized to placebo will receive matching placebo bid. On Day 10, subjects will receive a single 300 mg dose of AZD6140 or matching placebo in the morning only.

On Days 1 and 10, investigational products will be administered orally following a 10-hour overnight fast and subjects will remain fasting for 4 hours post dose. On Days 4-9 subjects will receive investigational products at least 1 hour prior to meal.

Duration of treatment

Eight days: A single dose on Days 1 and 10 and twice daily doses for 6 days on Days 4-9 in each subject.

Outcome variables

- **Safety and Tolerability**

  Adverse events, 12-lead ECG, blood pressure/pulse rate, body temperature, laboratory parameters (haematology, clinical chemistry, urinalysis) and physical examinations.

- **Pharmacokinetic**

  Blood samples will be analysed to determine the plasma concentrations of AZD6140 and its active metabolite, AR-C124910XX, on Day 1, Days 6-9 (for pre-dose trough level) and Day 10. The following pharmacokinetic parameters will be calculated;

  Day 1:
  C_{max}, t_{max}, AUC, AUC_{0-t}, AUC_{0-12} and $t_{1/2}$ for AZD6140 and AR-C124910XX, and CL/F and V_z/F for AZD6140.

  Days 6-9:
  Pre-dose trough level for AZD6140 and AR-C124940XX before the morning dose.

  Day 10:
  $C_{ss,min}$ (at pre-dose and 12 h post-dose), $C_{ss,max}$, $C_{ss,av}$, $AUC_{ss,\tau}$, t_{max}, $t_{1/2}$ and accumulation ratio for AZD6140 and AR-C124910XX, and CL_{ss}/F for AZD6140.

- **Pharmacodynamic**

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

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

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

Abbreviation or special term	Explanation
%PAI	Percent inhibition of ADP-induced platelet aggregation
ADP	Adenosine diphosphate
AE	Adverse event
ALT	Alanine aminotransferase
AMOS	AstraZeneca Monitoring System
ANOVA	Analysis of variance
APTT	Activated partial thromboplastine time
AST	Aspartate aminotransferase
AUC	Area under the plasma concentration versus time curve from zero to infinity
AUC_{0-12}	Area under the plasma concentration versus time curve from time zero to 12 hours
AUC_{0-t}	Area under the plasma concentration versus time curve from zero to last measurable concentration
$AUC_{ss,\tau}$	Area under the plasma concentration versus time curve during one dosing-interval at steady state
bid	Twice a day
BMI	Body mass index
BP	Blood pressure
BT	Body temperature
BUN	Blood urea nitrogen
CL	Total body clearance
CL/F	Apparent oral clearance
C_{max}	Maximum plasma drug concentration
CRF	Case report form
$C_{ss,av}$	Observed average plasma concentration at steady state
$C_{ss,max}$	Observed maximum plasma concentration at steady state
$C_{ss,min}$	Observed minimum plasma concentration at steady state
DQF	Data query form
EC_{50}	Concentration at which 50% of maximum effect is reached

Abbreviation or special term	Explanation
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
GI	Gastrointestinal
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human immunodefficiency virus
ICH	International Conference on Harmonization
id	intraduodenal
Investigator(s)	Principal investigator and sub-investigator
IRB	Institutional Review Board
MedDRA	Medical Dictionary for Regulatory Activities
MHW	Ministry of Health and Welfare (present Ministry of Health, Labor and Welfare)
NOAEL	Non Observed Adverse Effects Level
OAE	Other significant adverse event (See Appendix B)
od	once daily
PA_{BL}	Mean response at pre-dose baseline
PAI	Platelet aggregation inhibition
PA_T	Mean response at time “T”
PD	Pharmacodynamic
PK	Pharmacokinetic
po	oral administration
PP population	Per-protocol population
Principal investigator	A person responsible for the conduct of the clinical study at a study site. If a study is conducted by a team of individuals at a study site, the investigator is the responsible leader of the team and may be called the principal investigator.
RBC	Red blood cell
SAE	Serious adverse event
SAP	Statistical analysis plan

Abbreviation or special term	Explanation
Sub-investigator	Any investigator, usually a physician or a dentist, designated and supervised by a Principal Investigator at a Study Site to perform critical study related procedures and/or make important study related decisions
t_{max}	Time to reach peak or maximum concentration or maximum response following drug administration [time]
$t_{½}$	Half-life
V_z/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[1]. Two ADP receptor antagonists, thienopyridine pro-drugs, clopidogrel (under development in Japan) 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[2]. However, this blockade is irreversible and usually incomplete. In addition, there is some safety concerns for the use of ticlopidine. Therefore, the search continues for agents, which can further improve the clinical outcomes of these patients through improved efficacy and/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, different from clopidogrel for which only the metabolite is active. 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.

Preclinical toxicology studies conducted with AZD6140 include single dose studies in mice and rats and repeat dose toxicity studies of 14 days in mice and of up to 3 months' duration in rats and marmosets. In addition, in vitro genotoxicity assays have been conducted with AZD6140 and a major active metabolite, AR-C124910XX, and an *in vivo* genotoxicity study has also been conducted with AZD6140. In repeat dose toxicity studies in rats and marmosets, which were up to one month in duration, the primary dose limiting toxicity was considered to be due to effects on the GI tract. The NOAEL was 20 mg/kg/day in rats due to increased liver weights and slight adrenal vacuolization, and was 200 mg/kg/day in marmosets. In the 3-month oral toxicity studies in rats and marmosets, the dose limiting toxicity was again considered to be due to effects in the GI tract. The NOAEL for both species was less than 20 mg/kg/day (lowest dose tested) based on a minor increase in platelets in rats and the findings of chronic inflammation in the liver of marmosets. The nature of the chronic inflammation of liver, where no liver function abnormalities were detected, was consistent with traumatic injury and is not considered related to treatment with AZD6140. Other no-effect doses are estimated from the 3-month studies for effects on the GI tract (rat: 60 mg/kg/day, marmoset: 100 mg/kg/day), alterations in clinical chemistry parameters for liver function (rat: 60 mg/kg/day), bone marrow fatty atrophy (rat: 180 mg/kg/day, marmoset: 100 mg/kg/day) and increases in foamy alveolar macrophages (rat: 60 mg/kg/day). AZD6140 and its metabolite AR-C124910XX show no genotoxic potential. In addition, safety pharmacology studies on the central, peripheral and autonomic nervous systems and on cardiovascular, respiratory, gastrointestinal and renal systems following acute administration were conducted. AZD6140 had no effect on central nervous system function at doses up to 100 mg/kg po and no statistically significant effects on cardiovascular variables at doses up to 100 mg/kg id. In the oral administration to rats, AZD6140 had no effect on respiratory function at 1 mg/kg, but

dose-relevant, reversible and slight increases in respiration rate occurred at 10 and 100 mg/kg, and a dose of 100 mg/kg decreased intestinal transit. In addition, there was an increase in sodium excretion at doses of 10 and 100 mg/kg and also an increase in chloride excretion and in urine pH at the dose of 100 mg/kg, but no effect on renal function was observed after 4 hours of dosing.

Ten Phase I clinical studies conducted primarily in Caucasian subjects and one Phase I study conducted in Japanese and Caucasian subjects have been completed as of the end of December 2003 (out of 11 studies, the results of 4 studies have not been reported yet). The data from these studies demonstrate a favorable safety profile for AZD6140 over the dose range of 0.1 to 600 mg (for periods of up to 20 days). There is a positive relationship between the plasma concentration of AZD6140 and the degree of inhibition of platelet aggregation, with all volunteers achieving a high level of inhibition of 20 μmol ADP-induced aggregation.

Study D5130C05239 evaluated the safety and tolerability of multiple ascending doses (tablets) of AZD6140 in healthy male and female subjects in Europe. 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, compared with 75 mg clopidogrel. Multiple doses of AZD6140 were well tolerated. The pharmacokinetics (PK) 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. 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 pharmacodynamics (PD) response. In addition, in this study, the effect of food on the PK and PD of AZD6140 was studied in an exploratory way (comparison between the data under fasting condition on Day 15 and under fed condition on Day 16). Plasma concentrations and AUC values of AZD6140 were slightly higher following administration of AZD6140 under fed condition. There did not appear to be any effect on those for the metabolite. Due to the study design, however, the exact magnitude of the effect of food could not be estimated from these data. There was no obvious effect of food on PD response.

No serious adverse events (SAE) were observed in the 11 Phase I studies completed to date. In 9 studies, which have been unblinded and/or reported, 233 healthy subjects were randomised, and 195 healthy subjects received at least 1 dose of AZD6140. Purpura, headache, dizziness, respiratory infection, tachycardia and lymphadenopathy were the most common adverse events reported in some of these trials. However, the causal relationship of these adverse events to AZD6140 is uncertain.

In the multiple ascending dose study D5130C05239, 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 after 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 electrocardiogram (ECG), Holter monitor or vital signs findings of concern during any of the studies.

Study D5130C05266 was a study in healthy Japanese and Caucasian subjects to assess the safety and tolerability, as well as the pharmacokinetics and pharmacodynamics of single oral doses ranging from 50 mg to 600 mg of AZD6140. This study was conducted in the United States (US, Hawaii), with the requirement that Japanese subjects were to have both parents and 4 grandparents who were Japanese. Subjects in both ethnic groups tolerated the range of doses well. The pharmacokinetics of AZD6140 was similar in Japanese and Caucasian individuals following single oral administration of immediate release tablets in the range of 50 mg to 600 mg. After oral dosing, absorption of AZD6140 was rapid in both Japanese and Caucasians as evidenced by the 2 to 3 hour t_{max}. Mean C_{max} of AZD6140 increased linearly over the dose range of 50 mg to 600 mg in Japanese subjects but was linear only up to 400 mg in Caucasians. At the highest dose, 600 mg, C_{max} of AZD6140 seemed to plateau in Caucasians. Mean AUC increased linearly in both Japanese and Caucasian individuals up to 600 mg, with Caucasians exhibiting a slightly lower AUC at 600 mg; unlike C_{max}, mean AUC did not plateau in Caucasians. The mean $t_{1/2}$ of AZD6140 was around 8 to 12 hours in both races. The pharmacokinetics of the active metabolite, AR-C124910XX, was similar between Japanese and Caucasian individuals up to 400 mg. C_{max} and AUC of AR-C124910XX increased linearly over a dose range of 50 mg to 600 mg in Japanese but up to 400 mg in Caucasians. Both C_{max} and AUC values were about 30% lower in Caucasians compared to the Japanese at 600 mg. The platelet aggregation inhibition (PAI) response to AZD6140 was similar in Japanese and Caucasian individuals. Mean PAI greater than 90% was maintained for at least 12 hours at doses 200 mg or greater, with close to 100% inhibition at around t_{max}.

A Phase IIa study where doses of up to 200 mg bid and 400 mg od were given to patients for 28 days has been conducted in Europe and completed recently, however these results are not yet available.

1.2 Rationale

The single ascending doses study (Study D5130C05266) of AZD6140 in Japanese and Caucasian healthy male subjects who live in the United States (Hawaii) demonstrated a favourable safety profile over the dose range of 50 mg to 600 mg and linear PK up to 400 mg. A close correlation between the plasma concentration of AZD6140 and the degree of inhibition of platelet aggregation was also observed. The aim of this study is to investigate the safety, tolerability, PK and PD following multiple doses of AZD6140 in Japanese subjects in Japan and Caucasian subjects in the US. The two doses selected (see section 3.2) have been well tolerated and are considered to adequately cover the dose range to be used in future patient studies. The multiple dosing following the single dosing study design will provide us with necessary additional information to start the next phase in patients in Japan.

In the single dose study (Study D5130C5266), there were no significant differences in the PK and PD of AZD6140 between Caucasian and Japanese subjects who live in the US and it can be expected that there is no intrinsic ethnic factor that may cause the difference in the PK/PD for AZD6140 between Japanese and Caucasians. The other aim of this study is to compare the safety, PK and PD between Japanese subjects in Japan and Caucasian subjects in the US and investigate the differences in extrinsic as well as intrinsic ethnic factors between the two groups at two study centers.

2. STUDY OBJECTIVES

2.1 Primary objectives

The primary objectives of this study are:

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

- To investigate the pharmacokinetics of multiple oral doses of AZD6140 administered to healthy male Japanese and Caucasian subjects by assessment of AZD6140 and the active metabolite, AR-C124910XX, plasma concentration.

2.2 Secondary objectives

The secondary objectives of this study are:

- To investigate the pharmacodynamics of multiple oral doses of AZD6140 administered to healthy male Japanese and Caucasian subjects by assessment of platelet aggregation inhibition and bleeding time.

- To investigate the pharmacokinetic/pharmacodynamic relationship between plasma concentrations of AZD6140 and AR-C124910XX and platelet aggregation inhibition following multiple oral doses of AZD6140 administered to healthy male Japanese and Caucasian subjects.

3. STUDY PLAN AND PROCEDURES

3.1 Overall study design

This will be a single-blind, randomized, placebo-controlled, Phase I study in healthy male Japanese and Caucasian subjects, performed at 2 study centers – Japanese in Japan and Caucasians in the US. The study will consist of two sequential study cohorts at each study center with 3 visits (a screening visit, a dosing/PK visit and a follow-up visit) for each cohort. (See Figure 1) A total of 72 subjects (36 Japanese and 36 Caucasian) will be randomized to AZD6140 or placebo. There will be 18 subjects within each cohort at each study center.

In each ethnic group, 36 healthy male subjects will be divided into two sequential cohorts of 18 subjects. Within each cohort, 15 subjects will receive AZD6140 and 3 subjects will receive placebo.

On Days 1 and 10, investigational products will be administered orally following a 10-hour overnight fast and subjects will remain fasting 4 hours post dose. On Days 4-9, subjects will receive AZD6140 or matching placebo at least 1 hour prior to having a meal.

The subjects for Cohort A in each center will be randomized to receive either AZD6140 or placebo before the morning dose on Day 1 of Visit 2. Subjects will receive a single 100 mg dose of AZD6140 (n=15 at each study center) or matching placebo (n=3 at each study center) on Day 1. Prior to dosing in the morning of Day 4, the principal investigator will evaluate the safety of each subject, based on all results of the safety assessment as well as the presence and type/intensity of adverse event (AE). After the safety for the subjects is confirmed, the principal investigator will allow the subjects to enter the multiple dose period (See Section 3.1.1 and Section 3.3.5.1). **At the Japanese study center** the principal investigator will record the results of this safety confirmation. From Days 4-9, subjects randomized to AZD6140 will receive 100 mg AZD6140 bid and subjects randomized to placebo will receive matching placebo bid. On Day 10, subjects will receive a single 100 mg dose of AZD6140 or matching placebo in the morning only.

Prior to dosing subjects assigned to Cohort B, the safety results of Visit 2 in Cohort A will be evaluated by the principal investigator at each study center and the appropriate AstraZeneca study team physician of AstraZeneca. Refer to section 3.1.2 for specific stopping criteria.

The subjects for Cohort B will be randomized to receive either AZD6140 or placebo before the morning dose on Day 1 of Visit 2. Subjects will receive a single 300 mg dose of AZD6140 (n=15 at each study center) or matching placebo (n=3 at each study center) on Day 1. Prior to dosing in the morning of Day 4, the principal investigator will evaluate the safety results for each subject, based on all results of the safety assessment as well as the presence and type/intensity of AE. After the safety results for the subjects are confirmed, the principal investigator will allow the subjects to enter the multiple dose period. (See Section 3.1.1 and Section 3.3.5.1) **At the Japanese study center** the principal investigator will record the

results of this safety confirmation. From Days 4-9, subjects randomized to AZD6140 will receive 300 mg AZD6140 bid and subjects randomized to placebo will receive matching placebo bid. On Day 10, subjects will receive a single 300 mg dose of AZD6140 or matching placebo in the morning only.

At Visit 2, subjects will remain at the study center for 72 hours following the last dose of investigational product. A follow-up visit (Visit 3) will be scheduled within 5 to 10 days following the last dose of AZD6140 or placebo.

The schedules of procedures and assessments are shown in Table 1-Table 4.

Visit 1 Screening Period

In order to establish eligibility to participate in this study, subjects will undergo all screening procedures and assessments within 3 to 28 days prior to the first dosing of Visit 2. 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.

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

Visit 2 (Days –2 to 13)

Subjects will arrive at the study center on Day –2 as directed. At this time the subjects will be reassessed with regard to the study inclusion/exclusion criteria. On the morning of Day –1 baseline serial ECGs will begin.

On the morning of Day 1, subjects will be randomized to receive either AZD6140 or matching placebo. The single dose on Days 1 and 10 and twice daily dose for 6 days from Days 4-9 of AZD6140 (100 mg or matching placebo for Cohort A or 300 mg or matching placebo for Cohort B) will be administered by study center personnel. Subjects will remain at the study center until the completion of all the assessments and procedures scheduled for Day 13. At the completion of the assessments and procedures for Day 13 of **Visit 2**, the subjects will be discharged from the study center for a **5 to 10**-day period following the last investigational product dosing after which subjects will be instructed to return to the study center for the follow-up visit (Visit 3).

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

Note: Throughout the study the study center will supply standardized meals while the subjects remain at the study center. Although the menus may differ between study centers, they should be similar in calories and fat content. The same meals will be served on study Day 1 and 10. Menus will be approved by the sponsor in advance.

Visit 3 Follow-up Visit

Within 5 to 10 days following the last dose of investigational product on Day 10 of Visit 2, subjects will return to the study center 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 3, subjects will be discharged from the study.

Figure 1 Study flow chart

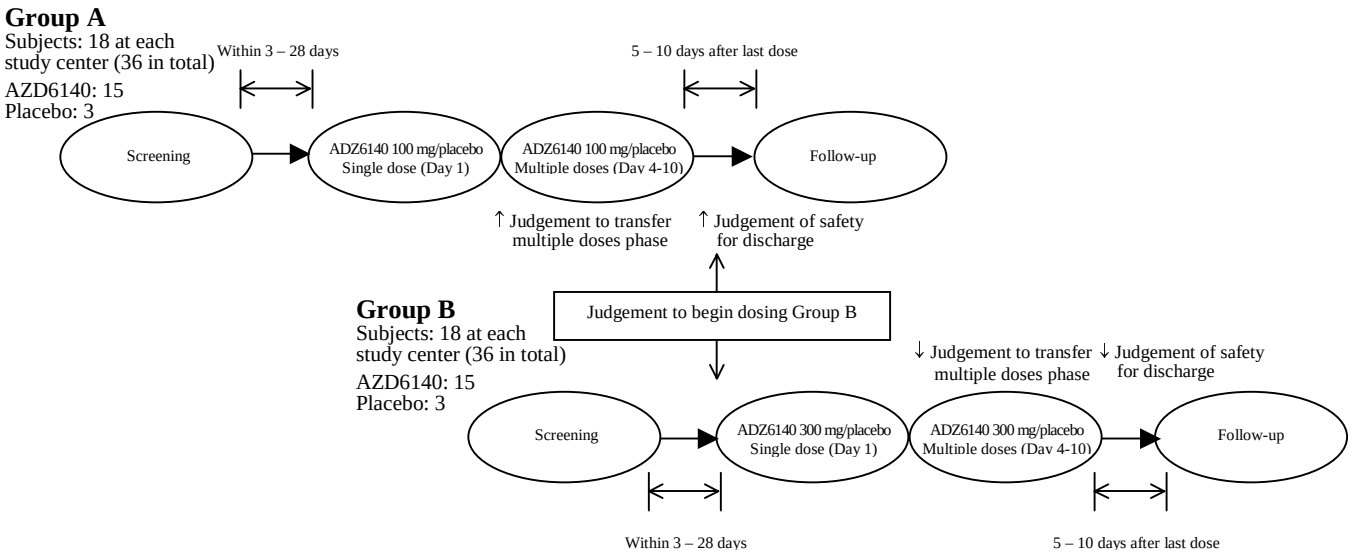

Table 1 Study plan

Assessment	Screening Visit 1	Visit 2 (Study Day)															Follow-up Visit 3
	≤ -28~ -3 days prior to Day 1	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	5-10 days after last dose
				Single dose phase			Multiple dose phase										
Informed consent	✓																
E-Code	✓																
Inc/Excl. criteria	✓	✓		✓[1)]													
Concomitant medication check	✓	<-	--	--	--	--	--	--	--	--	--	--	--	--	--	->	✓
Medical/surgical history	✓																
Demography	✓																
Body weight/height	✓																
Complete Physical exam[2)]	✓	✓		✓			✓									✓	✓
Brief physical exam[2,3)]				✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		
HIV, HBsAg, hepatitis C and syphilis antibody screen	✓																
Drugs of abuse	✓	✓[4)]															
Alcohol breath test		✓[4)]															
12-lead ECG	✓		✓[5)]	✓	✓[3)]		✓	✓[3)]	✓[3)]	✓[3)]	✓[3)]	✓[3)]	✓[5)]	✓		✓	✓
BP, pulse, body temperature	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Haematology/clinical chemistry, urinalysis	✓		✓	✓	✓	(✓)[6)]	✓			✓			✓	✓	(✓)[6)]	✓	✓
Haemoccult test[3,7)]		✓		<-	--	->							<-	--	->		
PK blood samples				✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	
Optical aggregometry samples	✓	✓		✓	✓								✓	✓	✓	✓	
Bleeding time	✓			✓	✓								✓	✓	✓[8)]		
Randomization				✓													
AZD6140/Placebo administration				✓			✓	✓	✓	✓	✓	✓	✓				
Adverse event recording		<-	--	--	--	--	--	--	--	--	--	--	--	--	--	--	->
Genotyping blood sample				✓													

1) To be randomized before dosing only on Day 1 at Visit 2. Before the randomization, eligibility of the subjects with regard to inclusion and exclusion criteria will be confirmed.
2) "Open, standardized AE questioning" will also be performed at the same time.
3) Japanese subjects only
4) Caucasian subjects only
5) Serial ECGs will be recorded.
6) The laboratory tests at the 48-hours post dose on Day 3 or 12 will be performed for the subjects who have significant abnormalities at the 24-hours post dose on Day 2 or 11, accordingly.
7) Faecal samples collected on Day –2 and during the period between Days 1-3 and Days 10-13 will be tested.
8) The assessment at 48-hours post dose measurement on Day 12 will be performed for the subjects whom bleeding time at the 24-hours post dose on Day 11 shows longer then 12 minutes.

Table 2 Schedule of assessments on Days –2, 1 and 2 of Visit 2

Study day	**Day -2**	**Day -1**						**Day 1**											**Day 2**		
Protocoled time		**-1h**	**0h**	**2h**	**4h**	**8h**	**12h**	**Pre-dose[1)]**	**0h**	**0.5h**	**1h**	**2h**	**3h**	**4h**	**6h**	**8h**	**12h**	**18h**	**24h**	**28h**	**36h**
Inc/Excl. criteria	✓								✓												
Concomitant medication	<-	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Complete physical exam[2)]	✓							✓													
Brief physical exam[2,3)]												✓		✓		✓			✓		
Drugs of abuse	✓[4)]																				
12-lead ECG		✓[5)]		✓[5)]	✓[5)]	✓[5)]	✓[5)]	✓				✓		✓		✓[3)]	✓[3)]		✓[3)]		
Blood pressure, pulse	✓							✓				✓		✓		✓			✓		
Body temperature	✓							✓											✓		
Haematology/clinical chemistry, urinalysis		✓						✓											✓		
Haemoccult test[3,6)]	✓																<-	--	--	--	--
PK blood samples								✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓
Optical aggregometry samples	✓							✓				✓		✓			✓		✓		
Bleeding times								✓						✓					✓		
Randomization									✓												
AZD6140/Placebo administration									✓												
Adverse event recording	<-	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Genotyping blood sample[7)]								✓													
Meal[8)]	D				L		D							L			D		B	L	D

1) Within 2 hours before AZD6140/Placebo administration
2) "Open, standardized AE questioning" will also be performed at the same time.
3) Japanese subjects only
4) Caucasian subjects only
5) Serial ECGs (two ECGs approximately 1 minute apart will be measured at each of these timepoints) will be recorded.
6) Faecal samples collected on Day –2 and during the period between 12h and 60h after dosing on Day 1 will be tested.
7) A single blood sample for genetic analysis will be obtained prior to dosing on Day 1 (See Appendix E).
8) Standardized meals, breakfast (B), lunch (L) and dinner (D), will be served after performing all assessments at each time point.

Table 3 Schedule of assessments Days 3, 4, 5, 6, 7, 8 and 9 of Visit 2

Study day	**Day 3**			**Day 4**									**Day 5**			**Days 6 ~ 9**								
Protocoled time	**48h**	**52h**	**60h**	**Pre-dose**	**0h**	**1h**	**2h**	**4h**	**5h**	**8h**	**12h**	**13h**	**Pre-dose**	**0h**	**1h**	**5h**	**12h**	**13h**	**Pre-dose**	**0h**	**1h**	**5h**	**12h**	**13h**
Concomitant medication	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Complete physical exam[1)]				✓[3)]																				
Brief physical exam[1,2)]	✓						✓	✓		✓			✓[3)]						✓[3)]					
12-lead ECG				✓[3)]									✓[2,3)]						✓[2,3)]					
Blood pressure, pulse	✓			✓[3)]									✓[3)]						✓[3)]					
Body temperature	✓			✓[3)]									✓[3)]						✓[3)]					
Haematology/clinical chemistry, urinalysis	(✓)[6)]			✓[4)]															✓[4,5)]					
Haemoccult test[2, 7)]	--	--	->																					
PK blood samples	✓			✓[4)]															✓[4)]					
AZD6140/Placebo administration					✓						✓			✓			✓			✓			✓	
Adverse event recording	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Meal[8)]	B	L	D			B			L			D			B	L		D			B	L		D

1) "Open, standardized AE questioning" will also be performed at the same time.
2) Japanese subjects only
3) Within 2 hours before AZD6140/Placebo administration
4) Just before dosing
5) Day 7 only
6) The laboratory tests at the 48-hours post dose on Day 3 will be performed for the subjects who have significant abnormalities at the 24-hours post dose on Day 2.
7) Faecal samples collected during the period between 12h and 60h after the first dosing on Day 1 will be tested.
8) Standardized meals, breakfast (B), lunch (L) and dinner (D), will be served after performing all assessments at each time point.

Table 4 Schedule of assessments on Days 10, 11, 12 and 13 of Visit 2

Study day	**Day 10**											**Day 11**			**Day 12**			**Day 13**
Protocoled time	**Pre-dose**	**0h**	**0.5h**	**1h**	**2h**	**3h**	**4h**	**6h**	**8h**	**12h**	**18h**	**24h**	**28h**	**36h**	**48h**	**52h**	**60h**	**72h**
Concomitant medication	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	->
Complete physical exam[1)]																		✓
Brief physical exam[1,2)]	✓[4)]				✓		✓		✓			✓			✓			
12-lead ECG	✓[3,4)]				✓[3)]		✓[3)]		✓[3)]	✓[3)]		✓						✓
Blood pressure, pulse	✓[4)]				✓		✓		✓			✓			✓			✓
Body temperature	✓[4)]											✓			✓			✓
Haematology/clinical chemistry, urinalysis	✓[5)]											✓			(✓)[6)]			✓
Haemoccult test[2,7)]										<-	--	--	--	--	--	--	->	
PK blood samples	✓[5)]		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓			✓
Optical aggregometry samples	✓[4)]				✓		✓			✓		✓			✓			✓
Bleeding times	✓[4)]						✓					✓			(✓)[8)]			
AZD6140/Placebo administration		✓																
Adverse event recording	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	(until Visit 3)
Meal[9)]							L			D		B	L	D	B	L	D	

1) "Open, standardized AE questioning" will also be performed at the same time.
2) Japanese subjects only
3) Serial ECGs (two ECGs approximately 1 minute apart will be measured at each of these timepoints) will be recorded.
4) Within 2 hours before AZD6140/Placebo administration
5) Just before dosing
6) The laboratory tests at the 48-hours post dose on Day 11 will be performed for the subjects who have significant abnormalities at the 24-hours post dose on Day 12.
7) Faecal samples collected during the period between 12h and 60h after the last dosing on Day 10 will be tested.
8) The assessment at 48-hours post dose measurement on Day 12 will be performed for the subjects whom bleeding time at the 24-hours post dose on Day 11 shows longer than 12 minutes.
9) Standardized meals, breakfast (B), lunch (L) and dinner (D), will be served after performing all assessments at each time point.

3.1.1 Discontinuation criteria before the multiple dose period at each cohort

Prior to the multiple dose phase at each dose level, the safety results up to 72 hours after the single dose on Day 1 will be evaluated by the principal investigator. All safety related results will be reviewed for each subject. The review will include the following:

- All complete and brief physical examinations
- Laboratory tests
  (The laboratory tests at the 48-hours post dose on Day 3 will be performed for the subjects who have significant abnormalities at the 24-hours post dose on Day 2.)
- Pulse, blood pressure (BP), body temperature (BT) and ECG
- The presence and type/intensity of AE
- Bleeding times

Subjects will be discontinued if any of the following symptoms are observed.

1. Aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) increase ≥ 3 times upper limit of reference range at the study center for which the causality of AZD6140 can not be excluded
2. Significant prolongation of the bleeding time (e.g. >30 minutes increase compared to baseline), as judged by the investigator, together with moderate to severe bleeding events and/or significant abnormalities of laboratory findings/vital signs, for which the causality of AZD6140 can not be excluded
3. Severe and serious adverse events for which the causality of AZD6140 can not be excluded

After the safety of the subjects is confirmed, the principal investigator will allow the subjects to enter the repeated dosing period (Refer to Section 3.3.5.1).

3.1.2 Stopping criteria for dose escalation

Prior to dosing in Cohort B, the safety results up to 72 hours after the final dose of Visit 2 in Cohort A will be evaluated by the principal investigator and study team physician of AstraZeneca in each site. **In Japan**, written approval from the sponsor will be obtained before dose escalation. Adverse events and all the other safety related data, 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 Cohort A. If it is anticipated that subjects may experience risk from further dose escalations, then the escalation to the next dose group may be stopped after discussions between the principal investigator and the sponsor's physician have taken place.

The dose escalation for Cohort B will be discontinued if any of the following tolerability or safety symptoms occur in at least three individuals who received AZD6140.

- AST and/or ALT increase ≥ 3 times upper limit of reference range at the study center for which the causality of AZD6140 can not be excluded

- Significant prolongation of the bleeding time (e.g. >30 minutes increase compared to baseline), as judged by the investigator, together with moderate to severe bleeding events and/or significant abnormalities of laboratory findings/vital signs, for which the causality of AZD6140 can not be excluded

The discontinuation of dose escalation will be considered if the following tolerability or safety symptoms occur in at least one subject.

- Severe and serious adverse events for which the causality of AZD6140 cannot be excluded

3.1.3 Criteria for discharge

Prior to discharging subjects on Day 13 of Visit 2, the safety results up to 72 hours after the final dose will be evaluated by the principal investigator. The following will be evaluated:

- All complete and brief physical examination

- Laboratory tests
  (If the results of the 24-hour post-final dose laboratory test (Day 11) shows a significant abnormality, as determined by the principal investigator, the test will be repeated at 48-hours post-final dose (Day 12). If these results show clinically significant abnormalities, the principal investigator will contact the sponsor's physician to discuss the appropriate course of action.)

- Pulse, BP, BT and ECG

- The presence and type/intensity of AE

- Bleeding times
  (The principal investigator must assess bleeding time for every subject before discharge. In principal, the principal investigator must not discharge any subjects whose bleeding time is longer than 12 minutes. Bleeding time will be performed at 24-hour (Day 11) after final dosing of investigational product. If the bleeding time at 24-hour after final dosing is longer than 12 minutes, bleeding time will be repeated at 48-hours (Day 12) after final dosing. If the bleeding time at 48-hour after final dosing is shows longer than 12 minutes, the principal investigator will contact the sponsor's physician to discuss the appropriate course of action.)

3.2 Rationale for study design, doses and control groups

The aim of this study is to investigate the safety, tolerability, PK and PD following multiple doses of AZD6140 in Japanese subjects living in Japan and Caucasian subjects living in the US. A randomized, placebo-controlled design has been chosen in order to minimise subject bias when evaluating drug safety and tolerability. This is a single-blind study because the study personnel performing the platelet aggregation and bleeding time assessments may become unblinded due to the nature of these measurements.

The two doses of AZD6140 chosen for use in this study are felt to adequately cover the dose range expected to be used in future studies and for which good tolerability has been confirmed. The data from the previous clinical studies mainly in healthy Caucasian subjects indicate that AZD6140 is well tolerated when given as a single daily dose of 0.1 to 600 mg and when administered as multiple doses up to a total daily dose of 600 mg (total duration of 15 or 20 days). In a single-dose study, where AZD6140 was administered to Japanese and Caucasian healthy male subjects living in the US (Hawaii, D5130C5266) in doses ranging from 50 to 600 mg, AZD6140 was well tolerated in both Japanese and Caucasian subjects and there was little difference in PK/PD between the two races.

The initial single dose of 100 mg (1.7 mg/kg/day, assuming the average body weight is 60 kg) on Day 1 of Cohort A in this study is $1/12^{th}$ of the NOAEL (20 mg/kg/day) estimated in the 1-month oral toxicity study in rats and $1/120^{th}$ of that (200 mg/kg/day) estimated in the 1-month study in marmosets. The second multiple dose of 300 mg bid (Cohort B), daily dose of 600 mg, is $1/2^{nd}$ and $1/20^{th}$ of the NOAELs, respectively. Although the chronic liver inflammation seen in 3-month studies in marmosets is considered the result of dosing trauma and not treatment-related, the treatment relationship of the other findings related to potential effects in the liver observed in preclinical and clinical studies conducted to date (increased ALP, ALT in 3-month studies in rats, and liver transaminase increases in the clinical multiple ascending dose study conducted in Europe, D5130C05239) is not well established. However, frequent monitoring (Days 1, 2, 4, 7, 10, 11, 13) of liver function laboratory tests is scheduled in this study, and the stopping criteria for ALT and AST is specified, such that if these observations are related to effects of ADZ6140 in the liver in the clinic, the effects will be detected at the earliest opportunity and stopping criteria is set adequately. Therefore, the safety of subjects in this study will be ensured.

In a multiple dose study in Caucasians (D5130C05239), the results in the 50 mg twice daily dosing group showed that the mean AUC_t for AZD6140 and AR-C124910XX at steady state were about 1.8 and 1.9 times higher than those after single administration of the same dose, respectively. The results from the single dose study in Japanese and Caucasians (D5130C5266) showed approximate linearity of pharmacokinetic parameters of plasma concentrations of AZD6140 and AR-C124910XX with increasing dose in the range of 50 to 600 mg of AZD6140 in Japanese subjects. These results indicate that in Japanese subjects, AUC_t and C_{max} at steady state following 300 mg bid should not exceed those for a single 600 mg dose for which good tolerability has been shown. In Caucasians, the safety of repeatedly administered AZD6140 of up to 600 mg has also been demonstrated.

Since this is the first multiple dosing study in Japanese subjects and AZD6140 will be given to Japanese subjects living in Japan for the first time, careful consideration has been given to safety. Physical examinations and ECGs will be performed extensively for Japanese subjects during the study period. A haemoccult test will be performed only for Japanese subjects in order to detect any possible effects of AZD6140 in the gastrointestinal tract at the earliest opportunity. In order for each subject to proceed to the multiple dose phase of the study a safety evaluation will be conducted according to the criteria stated in Section 3.1.1. In addition, dose escalation will be decided following the safety assessment according to the criteria shown in Section 3.1.2.

Considering these, multiple oral dosing of 100 and 300 mg bid for 7 days is thought not to jeopardize the health of Japanese and Caucasian male subjects in this study.

The interval between the single dose and the first dosing of multiple dose is 72 hours in this study since the plasma concentrations at 72 hours after single dosing are expected to be almost eliminated because the $t_{1/2}$ of AZD6140 and AR-C12490XX was about 8-12 hours in the single dose study (D5130C5266). The duration of multiple dose administration is 7 days in this study since the steady state of plasma AZD6140 and AR-C124910XX concentrations is expected to be achieved between 3 to 5 days of twice daily dosing estimated from the $t_{1/2}$ of AZD6140 and AR-C12490XX in the single dose study.

3.3 Selection of study population

3.3.1 Study selection record

The principal 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. Provision of written informed consent.

2. Be male Japanese at Japanese center and male Caucasian at US center

3. Be aged between 20 and 45 years of age.

4. Have a Body Mass Index (BMI): ≥18 <28 kg/m^2.

5. Have no clinically significant abnormal findings upon physical examination, laboratory testing*, 12-lead ECGs, or vital sign assessments.

   * Basically within reference range. When a value deviates from the reference range, the investigator(s) must judge whether or not the value is clinically significant.

6. Have a minimum body weight of 50 kg.

Rationale for inclusion criteria

For the criterion of No 4, the criterion is to reduce potential variability caused by the difference in BMI and avoids possible risks of complications associated with obesity. This range was determined taking mean BMIs of both Japanese and Caucasians into account.

3.3.3 Exclusion criteria

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

1. History or presence of neurological, haematological, psychiatric, gastrointestinal, hepatic or renal disease or other condition known to interfere with the absorption, distribution, metabolism or excretion of drugs, which may interfere with the study objectives, as judged by the principal investigator.

2. History or presence of intolerance or hypersensitivity to drugs with a similar chemical structure (e.g., adenine nucleoside antivirals and immunosuppressant drugs) or their excipients.

3. Symptoms of any clinically significant illness within 2 weeks prior to Visit 1 until randomization.

4. Clinically significant out-of-range values for prothrombin time or activated partial thromboplastin time as judged by the investigator(s). Have a bleeding time of > 9 minutes by Simplate® R method.

5. A personal or family history of bleeding diatheses or a reasonable suspicion of vascular abnormalities including aneurysms. A personal history of severe haemorrhage, haematemesis, melaena, haemoptysis, severe epistaxis, or intracranial haemorrhage. Rectal bleeding within the 3 months prior to Visit 1 until randomization.

6. Have platelet aggregation of < 70% by optical aggregometry (final extent), using 20 μmol ADP as the agonist.

7. Use of any prescribed medication or over the counter preparations or any herbal preparations and vitamins in the 2 weeks prior to Visit 1 until randomization.

8. Use of tobacco or nicotine-containing products in the 3 months prior to Visit 1 until randomization.

9. Surgery or significant trauma within 3 months prior to Visit 1 until randomization.

10. **For Japanese subjects**, 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 Visit 1 until randomization.

For Caucasian subjects, donation of blood or plasma in total >500 mL within the previous 3 months or >1200 mL in the year prior to Visit 1 until randomization.

11. History or presence of alcohol abuse.

12. History or presence of drug abuse or a positive drug test.

13. Evidence of having serum hepatitis or positive test results for hepatitis B surface antigen or hepatitis C antibodies, human immunodefficiency virus (HIV) antibodies or syphilis.

14. **For Japanese subjects**, participation in any clinical study and administration of the other investigational product within 4 months prior to Visit 1 until randomization.

    For Caucasian subjects, participation in any clinical study and administration of the other investigational product within 2 months prior to Visit 1 until randomization.

15. Subjects who, in the opinion of the principal investigator should not, for reasons of safety, participate in the study.

Rationale for exclusion criteria

For criteria 4 and 5, the criteria are to secure the safety of subjects since this compound inhibits platelet activation and aggregation.

For criterion 6, the criterion is to exclude non- or inadequate responders to platelet aggregation with 20 μmol ADP as the agonist.

3.3.4 Restrictions

Subjects will be required to:

1. Maintain a consistent level of physical activity throughout the study. Avoid strenuous physical activity (eg, conduct sports) throughout the study.

2. Have standardized meals served by the study center. Taking any other meals and snacks is not permitted.

3. Abstain from consumption of caffeine-containing foods or beverages (e.g., coffee, tea, chocolate, cocoa and cola) for 48 hours prior to dosing and while attending the study center unless approved by sponsor and the investigator(s).

4. Abstain from consumption of alcoholic beverages for 48 hours prior to dosing and while attending the study center.

5. Abstain from the intake of grapefruit and grapefruit juice and Seville oranges, and health food containing St. John's wort from 1 week prior to randomization to the follow-up visit.

6. Abstain from smoking from 3 months prior to Visit 1 to the follow-up visit.

7. Refrain from taking prescribed or over-the-counter medications including herbal remedies and vitamin preparations from 2 weeks prior to Visit 1 to the follow-up visit unless approved by the sponsor and the investigator(s).

3.3.5 Discontinuation of subjects from treatment or assessment

3.3.5.1 Criteria for discontinuation

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

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

2. Safety reasons for example the occurrence of severe or serious AE, which may compromise the subject's health judged by the investigator(s) or AstraZeneca (Refer to Section 3.1.2).

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

4. Intolerance to study procedures

5. Incorrectly enrolled or randomized subject

6. AST and/or ALT increase $\geq$ 3 times upper limit of normal for which the causality of AZD6140 can not be excluded

7. Significant prolongation of the bleeding time (e.g. >30 minutes increase compared to baseline), as judged by the principal investigator, together with moderate to severe bleeding events and/or significant abnormalities of laboratory findings/vital signs, for which the causality of AZD6140 can not be excluded

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(s) 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(s) decides that no further follow-up is necessary. Should protocoled dosing be stopped during the study, the

investigator(s) will perform the best possible observation(s), test(s) and evaluation(s) as well as give appropriate medication and all possible measures for the safety of the subject. In addition, they will record on the case report form (CRF) the date of withdrawal, the reasons, manifestation and treatment at the time of withdrawal and also course, treatment and outcome after withdrawal. They will also immediately inform AstraZeneca of the withdrawal. Any serious adverse event should be communicated to AstraZeneca according to the procedures defined in Section 4.7.2.

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

3.4 Treatment(s)

3.4.1 Investigational product(s)

3.4.1.1 Identity of investigational product

AZD6140 and matching placebo will be provided as tablets for oral administration.

The identity of investigational products is summarized in Table 5. Each bottle of AZD6140 and placebo contains 60 tablets.

Table 5 Identity of investigational product

Investigational product	Dosage form and strength	Raw Materials	Pack	Manufacturer
AZD6140 tablet	100 mg tablet	AZD6140, Lactose monohydrate, Microcrystalline cellulose, Polyvinylpyrrolidone K30, Croscarmellose sodium, Magnesium stearate, Hydroxypropylmethylcellulose 2910, Triacetin, Titanium dioxide, Red iron oxide, Yellow iron oxide, Black iron oxide, Polyethylene glycol 4000	50 mL white HDPE bottles with tamper evident, child resistant closure	AstraZeneca R&D Charnwood, UK
Placebo tablet to AZD6140	Matching appearance to active tablets	Lactose monohydrate, Microcrystalline cellulose, Magnesium stearate, Hydroxypropylmethylcellulose 2910, Triacetin, Titanium dioxide, Red iron oxide, Yellow iron oxide, Black iron oxide, Polyethylene glycol 4000	50 mL white HDPE bottles with tamper evident, child resistant closure	AstraZeneca R&D Charnwood, UK

3.4.1.2 Labelling

The packaging and labelling will be carried out by Investigational Products (IPS), AstraZeneca R&D Charnwood, UK. All labels will be applied in line with Good Manufacturing Practice (GMP) and local regulatory requirements.

The labels of bottles and cartons for transportation will include the following information in Japanese for the Japanese center and English for the US center:

For the Japanese Study Center:

Bottle label

- Name and address of sponsor (AstraZeneca KK)
- AZD6140 100 mg/placebo, dosage form and quantity of dosage units
- Contents: 60 tablets
- Study code
- Storage conditions and expiry date
- Lot Number
- The following standard statements
  - "For clinical study use only"

Carton label

- Name and address of sponsor (AstraZeneca KK)
- Study code
- Storage conditions and expiry date
- Carton contents: 17 bottles/6 bottles containing AZD6140 100 mg tablets/placebo
- The following standard statements
  - "For clinical study use only"

For US Study Center:

Bottle label

- Name and address of sponsor (AstraZeneca)
- AZD6140 100 mg/placebo, dosage form and quantity of dosage units
- Contents: 60 tablets
- Study code
- Directions for use
- Storage conditions
- Lot Number
- The following standard statements
  - Caution: New Drug - Limited by Federal (United States) Law to Investigational Use.
  - "Keep out of reach of children"

Carton label

- Name and address of sponsor (AstraZeneca)
- Study code
- Contents: 17 bottles/6 bottles containing AZD6140 100 mg/placebo
- Directions for use
- Storage conditions
- Lot Number
- 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 must be stored between 15°C and 30°C (59°F to 86°F), protected from light and high humidity.

The principal investigator or study center has the responsibility to establish a system for handling investigational product so as to ensure that:

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

3.4.1.4 Accountability

The medication provided for this study is for use only as directed in the protocol. The study center personnel will account for all drugs dispensed. Certificates of delivery must be signed.

In Japan, the investigational products will be distributed to the study center following agreement of the contract for the study. The drug storage manager at the Japanese study center will be responsible for the management of the investigational products and maintaining drug accountability records for AZD6140 and placebo tablets according to the "Procedures for accountability of investigational product (including procedures of storage conditions for investigational product)" prepared by AstraZeneca KK. Following reconciliation of all drugs by the sponsor, the drug storage manager will return all unused drugs as well as empty cartons and packaging to AstraZeneca KK.

In the US, the investigator(s) (or delegate) is responsible for maintaining drug accountability records for AZD6140 and placebo tablets. The study monitor will check such documents routinely. On study completion, all original dispensing records will be stored in the Investigator's Study File. Following reconciliation of all drugs by the sponsor, US study center personnel will return all unused study drug to the sponsor or a vendor designated by the sponsor.

3.4.2 Doses and treatment regimens

A total of 72 subjects (36 Japanese and 36 Caucasian) will be randomized in this study. Eighteen subjects in each ethnic group will be assigned by the study center to each dosage group (Cohort A or Cohort B) before the first dosing on Day 1 of Visit 2. Cohort A will be

the first group to dose. Cohort B will begin following the approval to escalate based on the safety results from Cohort A. Within each cohort the subjects will be randomized to receive either active investigational product or placebo as follows:

(a) Cohort A – AZD6140 100 mg bid or placebo

A total of 36 subjects (18 Japanese and 18 Caucasian) will be assigned to Cohort A and will receive a single 100 mg oral dose of AZD6140 (n=15 at each study center) or matching placebo (n=3 at each study center) on Day 1 of Visit 2. From Days 4-9 of Visit 2, subjects randomized to AZD6140 will receive 100 mg AZD6140 bid and subjects randomized to placebo will receive matching placebo bid. On Day 10, subjects will receive a single 100 mg oral dose of AZD6140 or matching placebo in the morning only.

(b) Cohort B – AZD6140 300 mg bid or placebo

A total of 36 subjects (18 Japanese and 18 Caucasian) will be assigned to Cohort B and will receive a single 300 mg oral dose of AZD6140 (n=15 at each study center) or matching placebo (n=3 at each study center) on Day 1 of Visit 2. From Days 4-9 of Visit 2, subjects randomized to AZD6140 will receive 300 mg AZD6140 bid and subjects randomized to placebo will receive matching placebo bid. On Day 10, subjects will receive a single 300 mg oral dose of AZD6140 or matching placebo in the morning only.

All doses will be administered by study center personnel as oral tablets with 240 mL of room temperature water. On Days 1 and 10, investigational products will be administered orally following a 10-hour overnight fast and subjects will remain fasting 4 hours post dose. On Days 4-9 subjects will receive investigational products at least 1 hour prior to meal. Subjects 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 except when undergoing planned examinations. Water intake will be restricted, other than what is required for dosing, from 2 hours before until 2 hours after dosing except for a drink supplied with a meal.

3.4.3 Method of assigning subjects to treatment groups

Written informed consent will be obtained before the time of enrollment. The subjects will be identified with an enrollment number starting with **E0001001 for Japanese subjects** and **E0002001 for Caucasian subjects**, 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.**

The sponsor will provide the principal investigator with a copy of the treatment randomization list which will be generated using the AZ Global Randomization System (GRand) by AstraZeneca LP. Subjects will be randomized to receive either active drug or placebo (within cohort and ethnic group) according to the treatment randomization list, 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(s) must inform AstraZeneca personnel. The subject will be replaced if the anticipated number of subjects who received AZD6140 from each ethnic group completing the study falls below 12. The replacing subject will be given a randomization number from the randomization list (the lowest randomization number available for active treatment of the cohort at the ethnic group). In this case the subject/randomization number assigned to a replacement subject will not necessarily be assigned sequentially.

3.4.4 Blinding and procedures for unblinding the study

3.4.4.1 Methods for ensuring blinding

This is a single-blind study because the study personnel performing the platelet aggregation and bleeding time assessments may become unblinded due to the nature of these measurements. It is important that the investigator(s) and other study personnel make every effort to maintain the blind to subjects so as not to compromise the study results.

The matching placebo tablets will be indistinguishable in appearance from AZD6140 100 mg tablets. The placebo tablets will be supplied to maintain the single-blind.

3.4.4.2 Methods for unblinding the study

Not applicable since the study is single blind, i.e. blind for the subjects (who will not know if he receives active drug or placebo) and open for the investigator(s).

3.4.5 Concomitant medication

Subjects should refrain from taking prescribed or over-the-counter medications including herbal remedies and vitamin preparations throughout the study unless approved by the sponsor and the investigator(s).

Any medication, which is considered necessary for the subject’s safety and well-being, may be given at the discretion of the investigator(s). The administration of all medication (including investigational products) must be recorded in the appropriate sections of the CRF from 2 weeks prior to Visit 1.

3.4.6 Treatment compliance

Compliance will be ensured by supervised administration of the investigational product by the principal 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-Table 4). 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, PD and safety laboratory samplings are scheduled for the same timepoint, the PD sample will be collected first and the PK will be collected second, and then safety laboratory samplings at the protocol time. 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 2 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.)

5) Physical examination and body temperature

4.1 Screening and demographic measurements

Each subject will undergo the screening procedures and assessments within 28 days prior to randomization. Demographic information (date of birth, sex, 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, eligibility for the study 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).

4.2 Pharmacokinetic measurements

Venous blood samples (2 mL) for PK analysis will be collected at the times listed in Table 6. Blood samples will be collected, labeled, processed and shipped as detailed in Sections 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 of biological samples

4.2.1.1 Plasma samples for determination of AZD6140 and AR-C12491010XX concentrations

Blood samples (2 mL each) will be collected into ***plastic lithium heparin Vacuette® tubes*** at the defined time points (see Table 6) until 72 hours after the last dose at Visit 2.

After applying a tourniquet, venous blood will be taken. If the study center 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. A normal saline solution will be used to keep catheters patent (heparin must not be used). 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 1500 g. The resulting plasma will be transferred into a polypropylene tube. 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).

The plasma concentration of AZD6140 and AR-C124910XX will be determined using liquid chromatography and MS-detection after protein precipitation. The limit of quantification (LOQ) is set to 1 and 2.5 ng/mL, respectively. Plasma samples for measurement of AZD6140 and its metabolite AR-C124910XX concentration will be analyzed using validated bioanalytical methods by York Bioanalytical Solutions. Details of the method used will be provided in the clinical study report.

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

4.2.2 Labeling of plasma samples

The labels will include the following information:

- Study Number: D5130C05267
- Randomization/Subject Number
- Cohort (A or B)
- Scheduled/Protocol Date and Time
- Type of sample: PK

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. 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.

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

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 Mondays – Wednesdays. 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:

Notification to:

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

Table 6 Schedule of PK blood sampling and a maximum acceptable deviation from the scheduled time

Scheduled Time Relative to Morning Dose		
Day 1	**Days 6-9**	**Day 10**
Pre-dose (within 120 min. before dosing)	Pre-dose (0 hour: within 5 min. before dosing)	Pre-dose (0 hour: within 5 min. before dosing)
0.5 hour post dose (± 5 min.)		0.5 hour post dose (± 5 min.)
1 hour post dose (± 5 min.)		1 hour post dose (± 5 min.)
2 hours post dose (± 5 min.)		2 hours post dose (± 5 min.)
3 hours post dose (± 5 min.)		3 hours post dose (± 5 min.)
4 hours post dose (± 10 min.)		4 hours post dose (± 10 min.)
6 hours post dose (± 10 min.)		6 hours post dose (± 10 min.)
8 hours post dose (± 15 min.)		8 hours post dose (± 15 min.)
12 hours post dose (± 15 min.)		12 hours post dose (± 15 min.)
18 hours post dose (± 15 min.)		18 hours post dose (± 15 min.)
24 hours post dose (± 15 min.)		24 hours post dose (± 15 min.)
36 hours post dose (± 15 min.)		36 hours post dose (± 15 min.)
48 hours post dose (± 15 min.)		48 hours post dose (± 15 min.)
72 hours post dose (± 15 min.)		72 hours post dose (± 15 min.)

4.3 Pharmacodynamic measurements

4.3.1 ADP-induced platelet aggregation

Two 4.5 mL blood samples will be collected into ***siliconized glass Vacutainer***® tubes which contain 0.5 mL of 0.105M (equivalent to 3.2%) buffered citrate solution. These samples will be used to measure inhibition of platelet aggregation by optical aggregometry, using 20 μmol ADP as the agonist and run in duplicate, at the defined time points (See Table 7) at Visit 2.

Final and maximum extent of aggregation will be recorded. Final extent of aggregation will be measured 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 the principal investigator prior to the start of the study.

%PAI from pre-dose on Day 1 will be calculated at each time using the following formula for:

$$\%PAI = 100 \; x \; \frac{(PA_{BL} - PA_T)}{PA_{BL}}$$

- where PA_T is the mean response at time 'T', and PA_{BL} is the mean response at pre-dose on Day 1. Percentage inhibition will be restricted to limits 0 and 100. Any data falling outside this range will be truncated to the appropriate limit.

Table 7 Schedule of ADP-induced platelet aggregation sampling and a maximum acceptable deviation from the scheduled time

Scheduled Time Relative to Morning Dose			
Screening	**Day -2**	**Day 1**	**Day 10**
Within 3 to 28 days prior to randomization	After arriving at the study center	Pre-dose (within 120 min. before dosing)	Pre-dose (0 hour: within 5 min. before dosing)
		2 hours post dose (± 5 min.)	2 hours post dose (± 5 min.)
		4 hours post dose (± 10 min.)	4 hours post dose (± 10 min.)
		12 hours post dose (± 15 min.)	12 hours post dose (± 15 min.)
		24 hours post dose (± 15 min.)	24 hours post dose (± 15 min.)
			48 hours post dose (± 15 min.)
			72hours post dose (± 15 min.)

4.3.2 Bleeding time assessment

Bleeding time measurements using Simplate® R method will be determined at the times indicated in Table 8. If any bleeding time measurement is >60 minutes, the procedure will be stopped and the bleeding time will be recorded as being greater than 60 minutes (>60 mins) on the appropriate CRF. If the pre-dose bleeding time on Day 1 is >9 minutes the subject should not be dosed and will be discontinued from the study.

The assessment at 48-hours post dose measurement on Day 12 will be performed for the subjects whose bleeding time at the 24-hours post dose on Day 11 is longer than 12 minutes.

The methodology is detailed in Appendix D.

Table 8 Schedule of starting time for bleeding time measurements and a maximum acceptable deviation from the scheduled time

Visit, Day	Scheduled Time
1 (Screening)	Within 3 to 28 days prior to randomization
2, Day 1	Pre-dose (within 120 min. before dosing)
	4 hours post dose (± 15 min.)
	24 hours post dose (± 15 min.)
2, Day 10	Pre-dose (within 120 minutes of dosing)
	4 hours post dose (± 15 min.)
2, Day 11	24 hours post dose (± 15 min.)
(2, Day 12)	48 hours post dose (± 15 min.)

4.4 Screening and safety assessments and procedures

For timing of individual assessments and procedures refer to study plan/schedule (Table 1-Table 4).

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 in order to establish and confirm his eligibility to participate or continue in the study. Before the randomization on Day 1 at Visit 2, eligibility of the subjects with regard to inclusion and exclusion criteria will be reconfirmed.

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/schedule (Table 1-Table 4) 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:

Samples will be collected in the following tubes and at the volumes specified

	Japan	**US**
Clinical chemistry	6 mL of blood on no anti-coagulant	10 mL of blood on no anti-coagulant
Clinical chemistry (P-Glucose)	1 mL of blood on NaF	Not applicable
Haematology	2 mL of blood on EDTA-2K	5 mL of blood on EDTA-2K
Haematology (PT/APTT)	1.8 mL of blood on 3.13 % sodium citrate	4.5 mL of blood on 3.2% sodium citrate
HIV, HBsAg, hepatitis C and syphilis	6 mL of blood on no anti-coagulant	10 mL of blood on no anti-coagulant

4.4.3.1 Chemistry

Clinical chemistry assessments will include the followings:

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

4.4.3.2 Hematology

Hematology assessments will include the following:

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

For the Japanese study center, the samples for prothrombin time and activated partial thromboplastin time will be sent to and analysed at SRL Nishinihon, Inc.

4.4.3.3 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.

4.4.3.4 Haemoccult test

A faecal sample will be obtained for the presence of blood using the chemical method (Guaiac test) at **only the Japanese study center**.

4.4.4 HIV, HBsAg, hepatitis C and syphilis

Blood samples for HIV, HBsAg, hepatitis C and syphilis antibody will be obtained at screening. If a test result is positive the subject will not be permitted to continue his

participation in the study. For the Japanese study center, the samples for HIV, HBsAg, hepatitis C and syphilis antibody will be sent to and analysed at SRL Nishinihon, Inc.

4.4.5 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 participation in the study. The following drugs will be screened:

Amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, methadone (**for Caucasian subjects only**), opiates, phencyclidine and propoxyphene (for Caucasian subjects only).

4.4.6 Medical/surgical history

A detailed medical and surgical history including medication history will be recorded for each subject at screening. Relevant 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 prescribed medications and over-the-counter products (including vitamins and herbal remedies) taken within 2 weeks prior to Visit 1 are to be recorded on the appropriate page of the CRF.

4.4.7 Physical examination

4.4.7.1 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 at screening only.

4.4.7.2 Brief physical examination

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

4.4.8 Resting 12-lead ECG

For timing of individual assessments refer to study plan/schedule (Table 1-Table 4).

4.4.8.1 Methods of assessment

All ECGs will be evaluated by the investigator(s) for safety as normal or abnormal at each study center. If indicated, as necessary for the safety of the subject, additional ECG assessments can be made at the discretion of the investigator(s).

Baseline serial ECGs (two ECG approximately 1 minute apart at each timepoint) will be recorded on Day –1 of Visit 2 (Refer to Table 2). Then, serial ECGs will be recorded on Day 10 of Visit 2. The serial ECGs collected on Days –1 and 10 will be evaluated by the investigator(s) for safety and electronically sent to a cardiologist at e Research Technologies, 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.

4.4.8.2 ECG machine

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

4.4.8.3 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 study 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.

4.4.8.4 Body positioning during ECG monitoring

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

4.4.8.5 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. All other ECGs will be recorded once at each time point, and no electronic recording is necessary

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, 1 photocopy of each tracing will be made. One copy will be available for the sponsor. Each ECG will be labeled with the study code, subject initials (for the US study center only), enrollment/randomization number, scheduled day, scheduled time, and actual time recorded.

The time of recording of the ECG and the safety evaluation (normal/abnormal) will be recorded on the CRF. On Days 1 and 2, the following data will be collected (for timing of collections on Days 1 and 2 at each study center refer to study plan of Table 2):

- PR interval (ms)
- RR interval (ms)
- QRS interval (ms)

- QT interval (ms)
- Heart rate (beats/min)
- Safety evaluation: normal/abnormal
- Sinus rhythm, extra systoles, conduction and ST-T changes including specification interpreted by the investigator(s) at the study center

If the ECG is abnormal, a comment will be entered onto the CRF.

4.4.8.6 Determination of RR and QT intervals

A central cardiologist at eResearch Technology, inc., who is blinded to the study will provide an interpretation of the serial ECGs collected on Days –1 and 10 of Visit 2. The interpretation will include the RR, PR, QT, and QRS intervals from the serial ECGs. Manual over-read of ECGs will be performed on digitized ECGs with electronic annotations. Non-serial ECGs, i.e., those performed on all other days will be assessed for safety, and will be interpreted by the investigator(s) at each study center. Additional data will be collected for the ECGs performed on Days 1 and 2 as outlined in Section 4.4.8.5 above.

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.

4.4.8.7 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.

4.4.8.8 QT interval correction factors

The primary QTc correction will be through Bazett's formula. The Fridericia correction for QT will be applied as a secondary correction factor. Bazett and Fridericia correction for all ECGs analysed 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)

4.4.9 Vital signs

4.4.9.1 Blood pressure and pulse rate

Supine blood pressure and pulse will be recorded using an electronic automated sphygmomanometer with an appropriate cuff size at the times indicated in Table 1-Table 4. For each subject, throughout the study, supine blood pressure and pulse will be measured after the subject has been supine for 5 minutes.

4.4.10 Body temperature

Body temperature (oral temperature) will be measured at the timepoints in accordance with the study plan/schedule (Table 1-Table 4).

4.5 Genetic sampling and storage

Genetic analysis will be performed for those subjects who give separate informed consent. Details of this sub-study are described in Appendix E and F, and the data obtained will not form part of the clinical study database.

If consent is obtained, a single 9 mL venous blood sample will be collected for genetic analysis.

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 9 Volume of blood to be drawn from each subject

Japanese Study Center

Assessment	Sample volume (mL)	No. of samples	Total volume (mL)
PK samples	2	32	64
ADP-induced platelet aggregation	9	14	126
HIV, HBsAg, hepatitis C and syphilis	6	1	6
Clinical chemistry	7	10	70
Hematology	3.8	10	38
Genetic sample	9	1	9
Sub-total			313
Flushing volume[1)]	1	18	18
Total			**331**

1) The first 1 mL from an indwelling catheter will be discarded at sampling time for PK samples taken between 0.5~12h after dosing on Days 1 and 10.

US Study Center

Assessment	Sample volume (mL)	No. of samples	Total volume (mL)
PK samples	2	32	64
ADP-induced platelet aggregation	9	14	126
HIV, HBsAg, hepatitis C and syphilis	10	1	10
Clinical chemistry	10	10	100
Hematology	9.5	10	95
Genetic sample	9	1	9
Total			**404**

4.7 Adverse Events

The definitions of adverse events (AEs), serious adverse events (SAEs) and other significant adverse events (OAEs) are given in Appendix B. It is of the utmost importance that all study staff 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(s) or the study staff. Open, standardized AE questioning such as "Have you had any health problems since the previous medical examination?" will be done by the investigator(s) or the study staff 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 adverse events will be collected from Day –2 of Visit 2 until the completion of the follow-up visit (Visit 3). 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 the study staff will be recorded in the CRF. Any AE including clinical findings not resolved at Visit 3 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(s) considers it to be of such clinical importance as to merit recording it as an AE. Abnormalities in these variables will be comprehensively evaluated at AstraZeneca US as part of the preparation of the clinical study report. 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 investigational product 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(s) will evaluate and report the

onset (date and time), resolution (date and time), maximum intensity, causality (yes or no), action taken, outcome (if applicable), whether it constitutes an SAE or not, and whether or not it caused the subject to discontinue the study.

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 AstraZeneca Monitoring System (AMOS).

4.7.2 Reporting of serious adverse events

When the investigator(s) 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 (ie, immediately but no later than the end of the next business 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 principal investigator is responsible for informing the Institutional Review Board (IRB) **in the US** and the IRB through the head of the study center **in Japan**, and/or the Regulatory Authority of the SAE as per local requirements.

The AstraZeneca representative will work with the investigator(s) 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.

In Japan, the AstraZeneca representative will inform the AstraZeneca Drug Safety Department (Japan) of it by day one, and will work with the investigator(s) to compile all the necessary information and ensure that the AstraZeneca Drug Safety Department (Japan) receives a report within 4 calendar days.

In the US, the AstraZeneca representative will inform the AstraZeneca Drug Safety Department (US) of it within five calendar days.

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 Harmonisation (ICH) document "Good Clinical Practice: Consolidated Guideline".

In Japan, the monitoring of this study will also be performed in accordance with Japanese GCP (Ministry of Health and Welfare Ordinance No. 28, 27 March 1997).

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.

There is no data for which the CRFs will act as the source data for the purpose of this study.

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 assure that the principal investigator has submitted the CRFs to AstraZeneca. For any change or amendment in the collected CRFs, the monitor will assure that the principal investigator has reported to the sponsor the change (or amendment), date, and the reason in a written form.

In Japan, the head of the study center and the investigator(s) should cooperate for monitoring and audit by AstraZeneca, and accept inspection by the IRB or regulatory authorities. All study documents such as raw data should be open for direct access to source data at the request of the monitor and the auditor of AstraZeneca, the IRB, or regulatory authorities.

5.2 Archiving of study documentation

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

In **Japan**, the head of the study center will retain all documentation pertaining to this study for the longer period of either at least 15 years after study completion or the designated period in GCP. The records should be managed by a responsible person appointed by the head of the study centre. However this is not always applied to those that are not preservable such as blood samples.

In the **US**, The principal investigator will retain all documentation pertaining to this study for the longer period of either at least 15 years after study completion or the designated period in GCP.

5.3 Audits and inspections

Authorised representatives of AstraZeneca, a regulatory authority, an 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, analysed, and accurately reported according to the protocol, GCP guidelines of the ICH and any applicable regulatory requirements. The investigator(s) 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 study staff, and that any new information of relevance to the performance of this study is forwarded to the study 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 of the study protocol must be notified to or approved by each IRB, and, if applicable, also the local regulatory authority, before implementation. Local requirements must be followed. It is also possible to create a new version of the study protocol (Amended protocol) if needed in that case.

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

AstraZeneca will distribute amendments (and new versions of the protocol, Amended Protocol, if needed) to each principal investigator who in turn is responsible for the distribution of these documents to the IRB, and to the study 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 at each center must comply with all the terms, conditions, and obligations of the clinical study agreement for this study. In the event of any inconsistency between this protocol and the study agreement, this protocol shall prevail.

5.7 Study timetable and termination

The study is expected to start on and to be completed by

Discontinuation or suspension of the whole study program

AstraZeneca will examine appropriateness of continuation of the study when the following situation occurs:

- When AstraZeneca obtains the information related to the quality, efficacy and safety of the investigational product and other important information for adequate conduct of the study.
- When the protocol requires changes and the study center cannot comply with the changed protocol.
- When AstraZeneca cannot approve the request of an amendment in the protocol from the head of the study center according to the opinions of the IRB.
- When the IRB determines that the study should not be continued and subsequently the head of the study center instructs the discontinuation of the study.
- When the study center violates significantly or continuously the GCP, the protocol or study contract.
- When any of the stopping criteria for dose escalation (see Section 3.1.1) are met and the dose escalation is decided to be stopped.

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

The investigator(s) 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 investigator(s) will report in writing the completion of the study as well as a summary of the results to the head of the study center in accordance with the study center's rules. The head of the study center 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 printed on four-part 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, initialled and dated. Correction fluid or covering labels must not be used. The top original (white) and the first (yellow) and second (green) copies of each completed CRF will be collected and returned to the AstraZeneca designee; the investigator(s) will retain the third copy (manila).

The AstraZeneca Monitor will check data at the monitoring visits to the study center. The principal investigator, together with the AstraZeneca Monitor, will ensure that the data in the CRFs are accurate, complete and legible.

AstraZeneca Data Management will enter the CRF data on an ongoing basis into their standard commercial database. The data will be verified and reviewed 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(s) via the site monitor using Data Query Forms. 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.

6. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

6.1 Statistical evaluation

A comprehensive Statistical Analysis Plan (SAP) will be prepared and finalized before database lock.

6.2 Description of outcome variables in relation to hypotheses

6.2.1 Safety and Tolerability

Incidence and severity of adverse events, 12-lead ECG, blood pressure/pulse rate, body temperature, laboratory parameters (haematology, clinical chemistry, urinalysis, haemoccult for Japanese subjects) and physical examination.

6.2.2 Pharmacokinetics

Plasma concentrations of AZD6140 and its metabolite AR-C124910XX will be listed and depicted graphically as a function of time relative to the first dose (Day 1) after single dose and relative to Day 10 morning dose for all subjects receiving AZD6140. Following single dose administration on Day 1 pharmacokinetic parameters C_{max}, t_{max}, $t_{1/2}$, AUC_{0-12}, AUC_{0-t} and AUC for AZD6140 and AR-C124910XX and AZD6140 CL/F (apparent oral clearance) and V_z/F (terminal phase volume of distribution) will be estimated by non-compartmental analysis. Following multiple dosing, on Day 10 pharmacokinetic parameters $C_{ss,max}$, $C_{ss,min}$, $C_{ss,av}$, t_{max}, $t_{1/2}$ and $AUC_{ss,\tau}$ for AZD6140 and AR-C124910XX and, AZD6140 steady state CL_{ss}/F will be estimated by non-compartmental analysis.

AZD6140 and AR-C124910XX C_{max} and $C_{ss,max}$ will be estimated as the highest measured concentration after Day 1 single oral dose and in a 12-hour dosing interval on Day 10 at steady-state, respectively. Correspondingly, t_{max} will be the time to maximum concentration following a single oral dose or within a dosing interval at steady state. $C_{ss,min}$ will be the concentration at the end of a dosing interval at steady state. AZD6140 and AR-C124910XX $C_{ss,av}$, the average concentration in a dosing interval at steady state will be estimated as the ratio of $AUC_{ss,\tau}$ and dosing interval. 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 following single oral dose 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. $AUC_{ss,\tau}$ will be calculated using the linear trapezoidal method over the 12 hour dosing interval at steady state. AZD6140 CL/F and CL_{ss}/F will be estimated as the ratio of AZD6140 dose and AUC following the single oral dose and as the ratio of AZD6140 dose and Day 10 $AUC_{ss,\tau}$ at steady state, respectively. The terminal phase volume of distribution, V_z/F, of AZD6140 will be estimated as the ratio of AZD6140 CL/F to λ_z. Accumulation ratio of AZD6140 and AR-C124910XX will be estimated as a ratio of Day 10 $AUC_{ss,\tau}$ to Day 1 AUC_{0-12}.

6.2.3 Pharmacodynamics

6.2.3.1 ADP-induced inhibition of platelet aggregation

Percentage inhibition, %PAI, from pre-dose baseline will be calculated at each time post-dose using the following formula for ADP-induced aggregation:

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

where PA_T is the response at time 'T', and PA_{BL} is the response at pre-dose baseline on Day 1. Percentage inhibition will be restricted to the closed interval [0,100]; any data falling outside this range will be truncated to the appropriate limit.

6.2.3.2 Bleeding times

The bleeding time, as measured by the Simplate® R technique, will be read directly from the CRF. Bleeding times which are recorded as being greater than 60 minutes in the CRFs 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.

6.3 Description of analysis sets

There are two populations in this study. All subjects randomized to treatment who receive at least one dose of study drug will be included in the safety population. This population will allocate subjects to the treatment actually received and not treatments randomized (although the two should concur). The per-protocol (PP) population is defined as a subset of the safety population, excluding those subjects with a protocol deviation felt to significantly influence either the pharmacokinetics or the pharmacodynamics of the drug. Definitions of all such protocol deviations will be made in the SAP. Examples include, but are not limited to, major changes in the administration of study drug or co-administration of medications expected to affect the pharmacokinetics or pharmacodynamics (however these are not all deviations excluded from PP population).

All safety data will be summarized using the safety population. Pharmacokinetic data will be summarized using the PP population excluding subjects who received placebo. Aggregometry and bleeding time data will be summarized using the PP population. In the case of the PP and safety populations being identical, only the safety population will be used throughout; this decision will be taken at the time of clean file and documented in the SAP.

6.4 Methods of statistical analyses

Statistical analysis will be carried out by, or under the guidance of, the biostatistical group at AstraZeneca using the SAS system, version 8.2. Pharmacokinetic analysis will be carried out by, or under the guidance of, the Pharmacokinetic Section, Experimental Medicine, at AstraZeneca.

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

Where treatment group is referenced, this will refer to the dosage regime being given at that time. Therefore, the following treatment groups will be referenced:

AZD6140 100 mg bid (Cohort A)

AZD6140 300 mg bid (Cohort B)

AZD6140 placebo (Cohorts A and B)

For reporting purposes, the AZD6140 placebo groups in Cohorts A and B will be combined.

(a) Demographic and baseline data

All demographic and baseline data, including medications, will be listed and summarized using standard descriptive statistics by group and ethnic group. No hypothesis test comparing groups will be made.

(b) Phamacokinetics

Pharmacokinetic data (including plasma concentrations and summary parameters for both parent and metabolite) will be summarized and listed by treatment group and ethnic group. The plasma concentrations and pharmacokinetic parameters, except for $t_{1/2}$, t_{max}, and V_z/F, will be summarized using geometric mean, coefficient of variation (CV) and the standard descriptive statistics. $t_{1/2}$ and V_z/F will be summarized by standard descriptive statistics. t_{max} will be summarized using standard descriptive statistics except for (arithmetic) mean and standard deviation.

The primary pharmacokinetic parameters ($AUC_{ss,\tau}$, $C_{ss,max}$) with / without weight adjustment will be compared in a factorial design between the Japanese and Caucasian subjects adjusting for dose level if appropriate (no significant interaction between dose and ethnic group). Similarity in exposure between ethnic groups will be concluded if the 90% confidence interval for the ratio (Japanese/Caucasian) of geometric mean AZD6140 $AUC_{ss,\tau}$ and $C_{ss,max}$ are completely contained within the interval (0.7-1.43) for all comparisons. The comparison of these parameters with/without BMI adjustment might be considered.

(c) Bleeding time

Bleeding time data will be summarized and listed using standard descriptive statistics by treatment group and ethnic group. Graphical representations of the data will also be produced.

(d) Optical aggregometry data

Optical aggregotmetry data will be summarized by treatment group and ethnic group and listed, both as absolute values and as percentage inhibition, calculated from the formula given in section 4.3.1 of this protocol. Graphical representations of the data will also be produced.

(e) Adverse events

Adverse events will be summarized by System Organ Class and Preferred Term, using MedDRA by treatment group and ethnic group. All adverse event data will be listed for all subjects. Separate listings of all serious adverse events, deaths or other significant adverse events will be presented.

(f) Laboratory data

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

Continuous laboratory data will be summarized using standard descriptive statistics. Both absolute values and change from pre-dose baseline will be summarized. In addition, shift plots will be produced for each variable. All summaries will be by treatment group and ethnic group.

Discrete laboratory data will be summarized using standard descriptive statistics. In addition, shift tables showing change from pre-dose baseline will be produced. All summaries will be by treatment group and ethnic group.

(g) 12-lead ECG data

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

(h) Vital signs

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

(i) Physical examination

Physical examination abnormalities will be listed.

6.5 Determination of sample size

Each ethnic group-cohort (dose) arm of the study will include 18 subjects (15 active and 3 placebo). Seventy-two subjects will be randomized in total.

With 12 active evaluable subjects per arm in a factorial design $C_{ss,\,max}$ and $AUC_{ss,\,\tau}$ of AZD6140, the study has been planned to have at least 90% power to show that the 90% confidence limits for the ratio (Japanese/Caucasian) of geometric mean will fall within the interval 70% to 143%, if the hypothesis of equality is true assuming that there is no significant

interaction between dose and ethnic group. Based on a previous multiple ascending dose study in Caucasian subjects (Study No D5130C05239: In this study the estimates of CV ranged from 12% to 69% for C_{max} and 26% to 54% for AUC_{τ} in 13 treatment groups. The mean CVs weighted by number of subjects in each treatment groups for C_{max} and AUC_{τ} were 38.1% and 37.8%, respectively), the estimate for the between subject CV is assumed to be 38.1% for both parameters. The sample size calculation is based on log-transformed data and two, one-sided t-tests with 5% significance levels. 72 subjects will be randomized (12 to placebo [3/arm]). 60 of those subjects will be randomized to receive active AZD6140 in order to ensure at least 48 subjects receiving AZD6140 complete the study (this assumes a 20% withdrawal rate).

In the event that a significant interaction is detected and pooling across doses is not possible, there will be at least 99% power to show that the 90% confidence limits for the ratio (Japanese/Caucasian) of geometric mean will fall within the interval 50% to 200% (ruling out more than a dose doubling effect of ethnic group), if the hypothesis of equality is true.

6.6 Interim analyses

There will be no interim analysis for this study.

6.7 Data Presentation

Presentation of the data will be detailed in the Statistical Analysis Plan.

6.8 Data or safety monitoring committee

Not Applicable.

7. ETHICS

7.1 Ethics review

The final study protocol and the final version of the Written Informed Consent Form (**in Japan**, and sample CRFs) must be approved or given a favourable opinion in writing by the IRB as appropriate. **In the US**, the investigator(s) must submit written approval to AstraZeneca before he or she can enroll any subject into the study. **In Japan**, the head of the study center must submit the written approval provided by the IRB to AstraZeneca before the investigator(s) can enroll any subject into the study.

In Japan, the study center and AstraZeneca must have a signed or sealed, valid contract between the study center and AstraZeneca before the investigator(s) can enroll any subject into the study.

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

In the US, either the principal investigator or AstraZeneca must submit progress reports to the IRB according to local regulations and guidelines. Furthermore the principal investigator must also provide the IRB with any reports of serious adverse events from the study center, and is also responsible for providing the IRB with reports of any serious adverse drug reactions from any other study conducted with the investigational product. AstraZeneca will provide this information to the principal investigator.

In Japan, the principal investigator must notify any serious adverse events from the study center in writing to the head of the study center, and verbally or in writing to AstraZeneca immediately. The head of the study center is also responsible for providing the IRB with reports of any serious adverse events from the study center, and any serious and unexpected adverse drug reactions, and any expected fatal or life threatening adverse drug reactions from any other study conducted with the investigational products. AstraZeneca is responsible for providing the principal investigator, the head of the study center and the regulatory agency with reports of any serious and unexpected adverse drug reactions, and any expected fatal or life threatening adverse drug reactions from other study conducted with the investigational product.

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

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 ICH/Good Clinical Practice, applicable regulatory requirements (**In Japan**, 3rd clause of Article 14 and Article 80-2 of the

Pharmaceutical Affairs Law and Good Clinical Practice [Ministry of Health and Welfare (MHW) Ordinance No. 28, 27 March 1997]) and the AstraZeneca policy on bioethics.

In addition, AstraZeneca ensures that special precautions are taken for studies including genetic analysis, with regard to the processes for ensuring confidentiality of data.

7.3 Subject information and consent

The investigator(s) 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 signed 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

For study sites within the US or in studies where non-US subjects' protected health information (subject data) will come into the US through a covered entity (eg, Central Lab/Reader), the Master Informed Consent Form will incorporate, or be accompanied by, a separate document incorporating HIPAA-compliant wording by which subjects authorise the use and disclosure of their Protected Health Information by the principal investigator and by those persons who need that information for the purposes of the study.

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 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 case of a medical emergency you may contact the Clinical Study Team Leader. If the Clinical Study Team Leader is not available, contact the Clinical Study Team Physician, Drug Safety Physician or Clinical Research Associate/Clinical Research Scientist.

For the Japanese Study Center

Role in the study	Name	Address and Telephone number	After Hours Telephone Number

For US Study Center

Role in the study	Name	Address and Telephone number	After Hours Telephone Number

For Serious Adverse event reporting

For the Japanese Study Center

-

For the US Study Center

-

8.2 Procedures in case of medical emergency

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.

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 (Not applicable)

Not applicable. For this study as only male subjects will be included.

9. REFERENCES

1. Hollopeter G, Jantzen HM, Vincent D, Li G, England L, Ramakrishnan V, Yang RB, Nurden P, Nurden A, Julius D, Conley PB. Identification of the platelet ADP receptor targeted by antithrombotic drugs. Nature. 2001;409:202-207.

2. Sharis PJ, Cannon CP, Loscalzo J. The antiplatelet effects of ticlopidine and clopidogrel. Ann Intern Med. 1998;129:394-405.

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Clinical Pharmacology Study Protocol: Appendix A	
Drug substance:	AZD6140
Study Code:	D5130C05267
Appendix Edition No:	1.0
Appendix Date:	

Appendix A
Signatures

ASTRAZENECA SIGNATURE(S)

A Single-blind, Randomised, Placebo-controlled Phase I Study to assess the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Multiple Oral Doses of AZD6140 tablets in Healthy Male Japanese and Caucasian Subjects

I agree to the terms of this study protocol

AstraZeneca Clinical Development Team representative

ASTRAZENECA SIGNATURE(S)

A Single-blind, Randomised, Placebo-controlled Phase I Study to assess the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Multiple Oral Doses of AZD6140 tablets in Healthy Male Japanese and Caucasian Subjects

I agree to the terms of this study protocol

AstraZeneca Research and Development site representative

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A Single-blind, Randomised, Placebo-controlled Phase I Study to assess the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Multiple Oral Doses of AZD6140 tablets in Healthy Male Japanese and Caucasian Subjects

I agree to the terms of this study protocol

AstraZeneca Research and Development site representative

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I agree to the terms of this study protocol

AstraZeneca Research and Developmen
site representative

ASTRAZENECA SIGNATURE(S)

A Single-blind, Randomised, Placebo-controlled Phase I Study to assess the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Multiple Oral Doses of AZD6140 tablets in Healthy Male Japanese and Caucasian Subjects

I agree to the terms of this study protocol

AstraZeneca Research and Development site representative

SIGNATURE OF PRINCIPAL INVESTIGATOR

A Single-blind, Randomised, Placebo-controlled Phase I Study to assess the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Multiple Oral Doses of AZD6140 tablets in Healthy Male Japanese and Caucasian Subjects

I agree to the terms of this study protocol. I will conduct the study according to the procedures specified herein, and according to the principles of Good Clinical Practice (GCP) and local regulations.

Centre No.: 001

Signature:

_____ _____

Date
(Day Month Year)

SIGNATURE OF PRINCIPAL INVESTIGATOR

A Single-blind, Randomised, Placebo-controlled Phase I Study to assess the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Multiple Oral Doses of AZD6140 tablets in Healthy Male Japanese and Caucasian Subjects

I agree to the terms of this study protocol. I will conduct the study according to the procedures specified herein, and according to the principles of Good Clinical Practice (GCP) and local regulations.

Centre No.: 002

Signature:

_____ _____

Date
(Day Month Year)

AstraZeneca

Clinical Pharmacology Study Protocol: Appendix B	
Drug substance:	AZD6140
Study Code:	D5130C05267
Appendix Edition No:	1.0
Appendix Date:	

Appendix B
Additional Safety Information

1. DEFINITIONS OF ADVERSE EVENTS, SERIOUS ADVERSE EVENT AND OTHER SIGNIFICANT ADVERSE EVENTS

Adverse event (AE)

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.

In Japan, for cases where it could be suspected that a tissue-derived medicine has been contaminated by pathogen, information about any of the above conditions (including infection) should be collected.

Serious adverse event (SAE)

A serious adverse event is an AE occurring during any study phase (ie, 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-patient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability or incapacity
- Is a congenital abnormality or birth defect
- Is an important medical event that may jeopardise the subject or may require medical intervention to prevent one of the outcomes listed above.

The causality of SAEs (ie, their relationship to study treatment) will be assessed by the investigator(s), who in completing the relevant case report form must answer “yes” or “no” to the question “Do you consider that there is a reasonable possibility that the event may have been caused by the drug?” For further guidance on the definition of a SAE and a guide to the interpretation of the causality question is provided in section 2 and 3 in this Appendix.

Other Significant Adverse Events (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 haematological 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.

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

Life threatening

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

Hospitalisation

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

Important medical event or medical intervention

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

Examples of such events are:

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

3. A GUIDE TO INTERPRETING THE CAUSALITY QUESTION

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

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

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

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

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

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

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

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

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

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

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

- Is there a known mechanism

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

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Clinical Pharmacology Study Protocol: Appendix C	
Drug substance:	AZD6140
Study Code:	D1530C05267
Appendix Edition No:	1.0
Appendix Date:	

Appendix C
Investigators and Study Administrative Structure

STAFF AT THE STUDY SITE (JAPAN)

Centre No.	Centre address	Name (First name, Last name)	Qualifications	Present position	Role in this study
1					

STAFF AT THE STUDY SITE (US)

Centre No.	Centre address	Name (First name, Last name)	Qualifications	Present position	Role in this study
2					

ASTRAZENECA STUDY PERSONNEL (JAPAN)

Address	Name (First name, Last name)	Qualifications	Present position	Role in this study

ASTRAZENECA STUDY PERSONNEL (US)

Address	Name (First name, Last name)	Qualifications	Present position	Role in this study

OTHER PARTICIPANTS

Organisation and address	Role in this study

AstraZeneca

Clinical Pharmacology Study Protocol: Appendix D	
Drug substance:	AZD6140
Study Code:	D5130C05267
Appendix Edition No:	1.0
Appendix Date:	

Appendix D
Capillary bleeding time - method description

BLEEDING TIME MEASUREMENT USING SIMPLATE R DEVICE

Description below consists of sections taken from the Simplate® R product insert.

Introduction

The bleeding time measurement is one of few biological blood coagulation tests available and is a simple and basic haemostatic function test.

It is defined as the time period measured between an initial small skin incision and the moment the subsequent bleeding stops[1, 2)].

Factors involved in bleeding time are platelet count, platelet function (adhesion, aggregation and release), capillary function and coagulation-fibrinolytic system. Bleeding time is known to reflect platelet count and function in the primary haemostasis most strongly[3)].

Known bleeding time tests are Duke's[4)], Ivy[5)] and template methods[6)]. In Japan Duke's method in which the bleeding time is measured by puncturing the earlobe with a lancet is widely used, but its reliability, reproducibility and sensitivity are slightly problematic.

Simplate products are sterile, disposable medical devices designed to always make uniform incisions permitting highly reproducible bleeding time measurement. Ethylene oxide is used for sterilisation.

Product overview

Simplate® R can be handled safely because a spring-loaded blade is contained in a plastic shell and automatically retract to the shell after use.

When triggered on the forearm, Simplate® R is designed to provide one incision 5 mm long and 1 mm deep. Simultaneously, a timer is started and the blood from the incision is blotted at 30-second intervals. The time required for the bleeding to cease is estimated to the nearest half-minute and recorded.

Additional materials required

1. Sphygmomanometer
2. Timer (stopwatch)
3. Whatman No 1 filter paper disc or equivalent
4. Alcohol swab
5. Butterfly bandage

Assessment of results

Because of the complexity of the primary haemostatic 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.

Reference expected range: 2.3-9.5 minutes.

Procedural notes and precautions

1. If thrombocytopenia is present, the bleeding time may be prolonged (less than 75,000/mm^3 platelets)[7].

2. Since the bleeding time of many people is increased after the ingestion of aspirin, 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.

3. Before testing, every patient should be informed that the possibility of faint scarring exists with any bleeding time procedure. Keloid formation, though rare, may occur with certain idiosyncratic patients.

4. Use once and discard.

5. Although the automatically retractable blade in Simplate R eliminates a potential hazard, after use all Simplate devices should be handled as if capable of transmitting infectious agents.

Test procedure

1. Seat the subject with arm supine on a steady support with the volar surface exposed. Select a site on the muscular area of the forearm distal to the antecubital fossa , taking care to avoid surface veins, scars, and bruises. Cleanse with an alcohol swab and allow to air dry for at least 30 seconds. 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 and twist off the white, tear-away stopper on the side of the device.

   Note: 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.

4. Depress the trigger and simultaneously start the timer. Remove Simplate approximately one second after triggering.

5. At 30 seconds, blot the flow of blood with filter paper. 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. Stop timer.

6. Remove cuff, cleanse around the incision with an alcohol swab, and apply a butterfly bandage across the incision. 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 used device to the opened blister pack, and discard.

References

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6. Mielke CH, Kanschire MM, Maher IA. et a.: The standardized normal Ivy bleeding time and its prolongation by aspirin. Blood., 34: 204, 1969.

AstraZeneca

Clinical Pharmacology Study Protocol: Appendix E	
Drug substance:	AZD6140
Study Code:	D5130C05267
Appendix Edition No:	1.0
Appendix Date:	

Appendix E
Genetic Research Component to the Main Study Protocol

TABLE OF CONTENTS PAGE

LIST OF IN-TEXT TABLES PAGE

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS FOR GENETIC STUDIES

The following abbreviations and special terms are used in this appendix to the clinical study protocol.

Abbreviation or special term	Explanation
DNA	Deoxyribonucleic acid
LIMS	Laboratory information management system
AstraZeneca	AstraZeneca LP, US for US center and AstraZeneca KK, Japan for Japanese center

The other abbreviations and terms listed in the main clinical study protocol are also applied.

1. INTRODUCTION

1.1 Background

Genetic variation within a population can be an important contributory factor in determining inter-individual differences in drug safety, efficacy and response, as well as acting as a marker for disease susceptibility and prognosis. Characterisation of such variation can help clarify the biology of drug action and elucidate processes that may influence safety and tolerability of a drug or class of drugs[1]. In the clinical use of thienopyridine ADP receptor antagonist, such as clopidogrel and ticlopidine, and aspirin, while non-responders to the drugs are sometimes observed, some significant adverse drug reactions have been reported and have been serious issues for some of those drugs such as ticlopidine. Therefore, if we could know before the administration of drugs whether or not the drugs would be effective to the patient and whether or not the exposure of the drug would be possibly higher in the patient, the adequate treatment could be given to each patient and the contribution to the medical treatment would be extremely high.

There is evidence that AZD6140 is a substrate for the p-glycoprotein (PgP) efflux transporter coded for by the *MDR1* gene (alternative names/symbols; *PGP, ABCB1, PGY1, MDR1 GP170*). A polymorphism within the *MDR1* gene has been described (C3435T, exon 26) which affects expression levels and *in vitro* activity of the resultant PgP protein. The frequency of this polymorphism varies between different ethnic groups, with frequencies of the high expressing C/C genotype being 0.26 in Caucasians, 0.83 in West Africans and 0.34 in Japanese[2]. Due to overlapping substrate specificities for the drug transporter proteins it is feasible that other drug transporters may also have a role in AZD6140 transport.

AZD6140 is an active platelet ADP receptor antagonist affecting the $P2Y_{12}$ receptor. AZD6140, whilst active itself is metabolised to a metabolite, AR-C126910XX, which is also active. Genetic variation in the enzymes responsible for this metabolism may be important determinants and predictors of outcome. Many ADME genes are known to be polymorphic and to have the ability to influence PK. Whilst the exact route of metabolism is not yet known for AZD6140, a CYP3A component is thought to be involved due to the overlapping substrate specificities of PgP and the CYP3A enzymes. Within the CYP3A family, two isoforms with overlapping substrate specificities are responsible for drug metabolism; CYP3A4 and CYP3A5. Whilst any molecular basis of inter-individual variability in CYP3A4 is not yet resolved, the genetic basis of CYP3A5 activity is becoming increasingly understood, and a number of polymorphisms have been reported which influence *in vitro* expression and activity of the enzyme. Although the significance of these polymorphisms on *in vivo* activity is still unclear, it is considered that at least *CYP3A5*3* and *CYP3A5*6* are the causal polymorphisms for metabolic activation deficiency[3]. Interethnic differences in allele frequencies exist for *CYP3A5*, which may also prove to be significant. For example, it is reported that, when compared with Caucasians, Japanese have a lower frequency of the *CYP3A5*3* low expression/activity allele (0.74 vs. 0.91)[4].

AZD6140 exerts its action through antagonism of $P2Y_{12}$ receptor. Recently a gain of function haplotype has been reported in the gene coding for the $P2Y_{12}$ receptor, and subjects carrying this haplotype appeared to have a greater maximal aggregation response to ADP[5]. Any effects of this haplotype on response to anti-platelet aggregation drugs such as clopidogrel and AZD6140 remains to be determined. The frequency of the gain of function haplotype was 0.14 in Caucasian populations, and the data on this haplotype in other ethnic groups have not yet been reported .

Variation within these genes could potentially influence the pharmacokinetics of, and pharmacodynamic response to, AZD6140. Genotyping for the major alleles of these genes can provide additional co-variates for PK and PD analysis within the AZD6140 clinical trial programme. In addition it is recognised that meta-analysis across samples collected from a number of trials may be beneficial and therefore the informed consent obtained from subjects will foresee such analyses.

AstraZeneca propose to study such genetic variations and their effect on drug response, safety and efficacy. By utilising this information, the aim is to better understand the impact of genetic variation and how it can be utilised to bring better drugs to the market. To achieve this goal a systematic collection of blood samples for genetic analysis will be applied across a broad range of relevant clinical trials.

1.2 Rationale for genotyping

In study D5130C05267, the main clinical study protocol, the platelet aggregation inhibition, as the pharmacodynamic(PD) variable, as well as pharmacokinetic (PK) variables are planned to be measured. The response on these measurements may show inter-individual differences, which may be partially due to polymorphisms of involved genes. It is considered that analysing genetic polymorphisms of involved genes may be of help in interpreting any possible variation observed in results and help understand the basis of any outlier results identified during the trial.

Genotyping of the following genes will be performed in order to explore how genetic variations may affect clinical parameters associated with AZD6140 use: *MDR1*, *CYP3A*, and *$P2Y_{12}$* (P_{2T}) receptor. The rationales for each genotyping are explained below:

- The MDR1 gene codes for the P-glycoprotein drug transporter protein: AZD6140 is a substrate for the drug-transport pump, p-glycoprotein , which has the potential to impact on the PK, and therefore the PD of AZD6140. Therefore the ability to study PGP polymorphisms as a covariate within the PK data is important.

- Genes coding for the enzymes postulated to be responsible for the metabolism of AZD6140 in the body, CYP3A5: as described above variations in relevant drug metabolising enzymes can have a major impact on inter-individual variability in the PK profile of a drug and can be provide additional covariates to analyse with PK data.

- The gene coding for the drug target, $P2Y_{12}$ (P_{2T}) : as this may influence response to the drugs, and can have a major impact on inter-individual variability in the PD results.

Similar genetic analyses have been included in the clinical pharmacology studies and patients studies with AZD6140 conducted in the Western countries and will be included in future studies as well, throughout the clinical development of this compound. Collecting these data in healthy subjects could provide us with the valuable information for the development of this drug and the treatment of patients in future.

The genetic study outlined here would not be powered to test the hypothesis that this variation is clinically relevant to the target disease in this clinical pharmacology study, but it is planned to carry out some exploratory researches on PK and PD that could be tested in confirmatory way in the future.

Participation of subjects in the genetic study is voluntary. The subject must not be forced to provide blood for genetic study, and blood will be collected only from the subjects who give an additional consent to this genetic research component. Due to the exploratory nature of this study there is unlikely to be any direct benefit to the subject.

2. STUDY OBJECTIVES

The objective of this study is to investigate whether genetic variations are associated with the pharmacokinetic and pharmacodynamic endpoints, and the inter-individual variability in response to AZD6140.

The results will be evaluated in combination with the genetic data from other clinical studies of AZD6140 conducted in Japan and overseas.

It is emphasised that AstraZeneca will only look for markers within genes relevant to mode of action of, and response to AZD6140 under study within the current study protocol. No other testing will ever be performed on the samples.

2.1 Variables

Genotyping of the genes coding for MDR-1(C3435T SNP), CYP3A5 (CYP3A5*3 and CYP3A5*6 alleles) and $P2Y_{12}$ receptor (H2 allele) will be performed in subjects who provided informed consent.

3. STUDY PLAN AND PROCEDURES

3.1 Study plan and procedures

Following written informed consent specific for the genetic research component, a single 9 mL blood sample will be taken from the subjects assigned a randomization/subject number

for the main clinical study at pre-dose (before the first dosing) on Day 1 at Visit 2 for extraction of DNA for planned genetic analysis. This sample is in addition to the blood samples drawn for the main clinical study.

Although genotype is a stable parameter, early sample collection is required to avoid introducing bias through excluding subjects who may withdraw due to an AE, such subjects would be important to include in any genetic analysis. If for any reason the sample is not drawn pre-dose on Day 1 at Visit 2, it may be taken at any visit until Day 13 at Visit 2 and at Visit 3.

Table 1 Study schedule

Assessment	Visit No.	Visit 1	Visit 2		Visit 3
			Day 1	Days 2 ~ 13	
	Hour relative to morning dose		Pre-dose		
Written informed consent - genetic research component		X[a]			
Genetic blood sample			X [b]		

a: Written informed consent can be given at the predose on Day 1, but it must be before the blood sampling for genetic test.

b: If written informed consent for genetic research has been obtained. If, for any reason, the sample is not drawn at this time, it may be taken until Visit 3.

Processing of the genetic samples will be co-ordinated by the Clinical Genotyping Group at the R&D Genetics in AstraZeneca R&D Alderley Park, UK. The samples taken from subjects will be sent for DNA extraction and genetic analysis as a single batch after all subjects have been offered the opportunity to participate in this genetics sub-study.

On receipt of the genetic samples by the Clinical Genotyping Group, R&D Genetics, AstraZeneca R&D Alderley Park, UK, the genetic samples will be de-identified (double-coded). De-identified samples are double-coded and labelled with a unique second number. The link between the clinical subject ID number (enrolment code) and the unique second number is maintained, in a secure database, but is unknown to investigators and subjects. This process is described in more detail in sections 5 and 8.

The de-identification will be done prior to any genotyping analysis. DNA samples will be analysed in the AstraZeneca R&D Genetics Genotyping Facility. Research scientists handling the DNA samples will identify and analyse the samples using only the second code.

The genetic data will not be entered into the database for the main study D5130C05267, which is maintained at AstraZeneca LP, US. However, the genetic data will be entered and retained securely in the genotyping Laboratory Information Management System (LIMS), with access restricted within the R&D Genetics Department at AstraZeneca R&D Alderley Park, UK. The genetic samples and genotype data will be identified by the second subject code, research scientists within the Clinical Genotyping Group will not have access to the link

file and will only be able to identify samples via this second code, and will therefore not be able to link any results to an individual.

3.2 Selection of study population

Subjects' participation in this genetic component is voluntary and any subject's decision not to participate will not exclude them from the main D5130C05267 clinical study. Refusal to participate will involve no penalty or loss of benefits to which the subject would otherwise be entitled.

3.2.1 Study selection record

Please refer to the main clinical study protocol.

3.2.2 Inclusion criteria

For inclusion in the genetic component to the study subjects must fulfil all of the inclusion criteria for the main study, must be assigned a randomization/subject code and

1. Provision of written informed consent for the genetic research component.

3.2.3 Exclusion criteria

To meet all the exclusion criteria as defined in the main protocol. Additional exclusion criteria for the genetic component of the study

1. Previous bone marrow transplant.

3.2.4 Restrictions

Please refer to the main clinical study protocol.

3.2.5 Discontinuation of subjects from the genetic component of the study

Subjects are free to discontinue their participation in the genetic component of the study at any time after they give the consent or after the blood is taken.

Subjects may withdraw from the genetic research component of the study at any time, independent of any decision concerning participation in the main clinical study. If a subject withdraws consent from the genetic research component then:

- Any blood, or DNA extracted from the subject's blood is destroyed. Any results already generated on the sample will have to be retained for audit purposes. If the results have already formed part of any data analyses they will not be removed from the results which the data from the subject constitutes. However, the confidentiality will be kept and this will be explained to the subjects when the informed consent is obtained.

3.2.6 Discontinuation from the main study

For any subject who withdraws their consent to continued participation in the main study, it must be clearly established and documented whether they are withdrawing or continuing their consent to the linked genetics research. Withdrawal of consent will be confirmed using the withdrawal of consent for genetic research form (which will be provided to the investigators by AstraZeneca). If a subject withdraws from the main study, the subject will be given the following options:

- The DNA sample is kept and may be used for genotyping in the future (as per subjects consent).
- The DNA sample is destroyed and no further testing will take place.

If a subject withdraws consent to the genetic research then any blood sample or DNA sample extracted from the subject's blood will be destroyed. Any results already generated on the sample will have to be retained for audit purposes. If the results have already formed part of any data analyses they will not be removed from the results which the data from the subject constitutes. However, the confidentiality will be kept and this will be explained to the subjects when the informed consent is obtained.

3.2.6.1 Procedures for discontinuation

When a subject withdraw his consent after the blood is taken and the sample is sent to UK, the investigator must inform the responsible person of AstraZeneca of it. Requests for sample destruction should be forwarded to Ms. Nancy Robertson at the Clinical Genotyping Group along with copies of the relevant documentation detailing study protocol number, centre number and the clinical subject ID number (enrolment code). The sample will be tracked for destruction using the 'link' between the enrolment code and the re-coded DNA number that was generated during the sample de-identification process. The 'link' makes it possible to destroy samples taken from the subjects who withdraw his consent. Authorisation to access the link must be provided by the clinical study team physician at AstraZeneca, before the Clinical Genotyping Group proceed with sample destruction. The clinical study team and investigator will receive written confirmation from the Clinical Genotyping Group that the genetic sample has been destroyed.

3.3 Treatments

Please refer to the main clinical study protocol. The genetic research programme does not involve any additional drug treatment.

4. MEASUREMENTS OF STUDY VARIABLES AND DEFINITIONS OF OUTCOME VARIABLES

4.1 Screening and demographic data

Please refer to the main clinical study protocol.

4.2 Collection of samples for genetic analysis

4.2.1 Summary of genetics analysis

The genotypes of MDR1, CYP3A and $P2Y_{12}$ receptor polymorphisms will be investigated.

Assays will be performed using standard genotyping technologies.

In this study, the consent for genotyping will be restricted to the above named genes. A GLP-standard LIMS will control access to the samples for genotyping. In practical terms, when the samples are received and entered into the LIMS at the Clinical Genotyping Group, R&D Genetics, AstraZeneca R&D Alderley Park, the consent level will be entered at the same time. The Clinical Study Team Physician of AstraZeneca will be sent a form to check that they agree with the level of consent that has been stored with the samples to allow AstraZeneca to monitor the fact that the Clinical Genotyping Group, R&D Genetics, AstraZeneca R&D Alderley Park has stored the appropriate level of consent for the samples from subjects. The Clinical Genotyping Group will require confirmation, from the Clinical Study Team Physician of AstraZeneca, that the assigned level of consent is correct before any genotyping analyses are performed.

Before AstraZeneca R&D Genetics Genotyping Facility proceed with genotyping, Pharmacogenetics Project Leader, R&D Genetics, AstraZeneca R&D Alderley Park will send AstraZeneca (Clinical Study Team Physician) another form to authorise genotyping for these genes. DNA samples will not be genotyped until authorisation for the analyses is provided by AstraZeneca. When Pharmacogenetics Project Leader, R&D Genetics, AstraZeneca R&D Alderley Park receives authorisation to proceed with genotyping, the LIMS will ensure that the analyses are within the scope of the informed consent.

4.2.2 Sampling for the genetic testing and transport of the samples

Blood samples will be taken , stored, and shipped to UK according to the following procedures.

1. A single venous blood sample (9 mL) will be collected into a polypropylene tube containing ethylenediamine tetra-acetic acid (EDTA) and gently inverted several times to mix thoroughly. The 9/10 mL polypropylene tubes containing the EDTA as an anticoagulant must be used for the samples. Glass tubes and/or heparin must not be used. The appropriate tubes will be supplied by AstraZeneca.

2. The sample tubes will be identified by

   - Study code,
   - Study centre number,
   - Subject ID code (enrolment code),
   - Date of sample taken,

– Serial number and

– Type of sample: ”Blood”

No personal identifiers (such as name, initials, date of birth, social security number, etc) will be placed on the tube.

3. Samples will be frozen as whole blood at –20°C or below and stored at this temperature until shipment. A log recording daily temperature readings of the freezer used to store samples at the centre must be maintained. Samples will be transported, on dry ice after the collection of the last sample.
For the storage, 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.

4. The blood samples must be sent to Clinical Genotyping Group Leader, R&D Genetics, AstraZeneca R&D Alderley Park after the collection of the last sample, as one batch under the freezing condition. The temperature during the transportation of the samples must be lower than –20ºC (with sufficient volume of dry ice). The study site must send a fax to the Clinical Genotyping Group Leader at R&D Genetics, AstraZeneca R&D Alderley Park and the sponsor using defined format just after the sample shipment, so that late arrivals can be investigated promptly. The format includes

– Study code,

– Study center number,

– Number of samples,

– Courier name,

– Airway bill number,

– Date of shipment,

– Shipment condition (dry ice) and

– Contact name and address.

A sample log form detailing a list of sample IDs (enrolment codes), dates of sampling, and confirmation that informed consent has been given for every sample, together with study code and centre number, should also be prepared and faxed.

Please ensure that the shipment is scheduled to arrive during working hours (Monday – Friday, 08:00-18:00). It is advisable to avoid shipping over a weekend

as this can result in delays, and the possibility of sample thawing. Office hours of Customs departments in destination countries should also be taken into account in shipment planning, to allow samples to arrive at a time when they will receive immediate and appropriate attention.

5. On arrival at the Clinical Genotyping Group, R&D Genetics, AstraZeneca R&D Alderley Park, the blood samples will be stored at –20ºC or less until the DNA extraction. Following sample processing, that is DNA extraction and sample de-identification, the DNA samples will be stored in the GLP archive maintained by R&D Genetics, AstraZeneca R&D Alderley Park.

4.2.3 Preparation for transportation

The study site staff should prepare for transportation following the procedures specified below:

- Confirm the samples to be transferred.
- Put sample tubes into the freeze box.
- Wrap the freeze box with double vinyl bag to prevent the samples leaking when the container is damaged.
- Check sufficient dry ice is added to the container to fully bury the freeze boxes and to avoid thawing during transportation.
- Fill necessary items in the Letter to Transmittal (sample: see Appendix 1), sign and keep a copy.
- Pack the Letter to Transmittal (original), the Notice to Receipt (sample: see Appendix 2) and the sample log form (see item 4 in Section 4.2.2) together with the samples in the container. Confirm if the consignee's address for the samples is correct.
- Seal the container.

Samples should be shipped c/o:

4.3 Volume of blood sampling

Blood sample: 9 mL

Please refer to the main clinical study protocol for blood volumes relating to the main study sampling.

4.4 Procedure of discarding samples after analysis

All remaining DNA samples will be discarded confidentially at Clinical Genotyping Group, R&D Genetics, AstraZeneca R&D Alderley Park basically after all planned analysis was completed for all samples. The maximum sample storage period will be 15 years after the sample collection, even if the samples are not discarded before it for any reasons. Authorisation to destroy the samples will be provided by Clinical Study Team Physician of AstraZeneca (or project physician of AztraZeneca, in case that the study team was dissolved). After the samples are destroyed, the certification of sample destruction will be issued by the Clinical Genotyping Group and sent to the study sites in Japan and the US through AstraZeneca.

5. DATA MANAGEMENT

5.1 Sample security and maintenance of subject confidentiality

All genetic information collected will be treated as strictly confidential data to protect confidentiality of individual subjects.

To preserve confidentiality of genetic data, the samples and data for genetic analysis in this study will be de-identified. This will require all samples being double coded and labelled with a unique second code. The link between the clinical study subject number (enrolment code) and the unique second code is maintained, but unknown to investigators, subjects, AstraZeneca KK and AstraZeneca LP.

The process of de-identification is as follows.

1. Blood samples will be taken and labelled at the study site with the enrolment codes, as in standard clinical trial procedures.

2. Blood samples will be transferred to the Clinical Genotyping Group, R&D Genetics, AstraZeneca R&D Alderley Park and DNA will be extracted and archived.

3. Samples will be de-identified (double-coded) using standard procedures. New codes will be assigned within the Clinical Genotyping Group, R&D Genetics, AstraZeneca R&D Alderley Park. The second code (or DNA number) will replace

the original clinical subject numbers (enrolment codes). The 'link' between the enrolment code and the second code will be retained securely within the Clinical Genotyping Group, R&D Genetics, AstraZeneca R&D Alderley Park. Access to the 'link' is restricted to senior members of the R&D Genetics, and the members who can have an access to the 'link' can not have an access to the subjects' identity. Access to the link may be required under special circumstances such as the withdrawal of the consent. Access will be authorised by the Clinical Study Team Physician of AstraZeneca.

4. Before the genotyping, the authorization by the Clinical Study Team Physician is necessary. It is the responsibility of the clinical study team to ensure that the genotyping authorised is appropriate with respect to the subject informed consent.

5. Genotyping will be carried out on an aliquot of the DNA sample, identifiable only by the second code, in the AstraZeneca R&D Genetics Genotyping Facility. Remaining DNA samples will be retained in the GLP archive maintained by R&D Genetics, AstraZeneca R&D Alderley Park, until the samples are destroyed, under the second codes. Genetic data will be entered in to and retained in the LIMS, at R&D Genetics, AstraZeneca R&D Alderley Park. Genetic data in the LIMS is identified by the second code.

6. When the relationship between PK/PD data and polymorphisms of genes is investigated, PK/PD data will be routed to Pharmacogenetics Project Leader, via the Clinical Genotyping Group, where all the subject numbers for PK/PD data will be replaced by the second codes used to label the DNA of the same subjects. The genetic data from this study may be evaluated alongside the genetic data collected in other AZD6140 studies.

7. During analysis, PK, PD, and safety data will be matched with genotypes by Pharmacogenetics Research Group under the second codes. However, only conclusions from the analysis will be presented. Results will represent aggregate genetic data from a group analysis.

Considering the nature of the clinical study being conducted as the main part of this study, AstraZeneca, sponsor, have deemed it reasonable for the study site to manage the study by producing and retaining all source records of this genetic part as original records, together with the data from the other parts of the study D5130C05267; no reports are needed from the study site by use of case report forms (CRFs).

5.2 Genotype data and reporting of the results

Results from any genetic research performed will be reported separately from the clinical trial report. AstraZeneca will not provide individual genotype results to subjects, their family members, any insurance company, an employer, clinical study investigator, general physician or any other third party.

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

6. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

6.1 Statistical evaluation – general aspects

Participation of subjects in the genetic research component of the main study is voluntary. It is therefore not possible to establish beforehand whether a statistically relevant number of subjects will consent. Statistical evaluation of data obtained will therefore be performed in an exploratory manner only.

6.2 Description of outcome variables in relation to objectives and hypotheses

Not applicable.

6.3 Description of analysis sets

Not applicable.

6.4 Method of statistical analysis

Not applicable.

6.5 Determination of sample size

Not applicable.

6.6 Interim analyses

Not applicable.

7. STUDY MANAGEMENT

7.1 Monitoring

Please refer to the main clinical study protocol.

It is a prerequisite of this study that the study monitor has direct access to source record at study site for verification. This will be done by reviewing the source record at the study site to ensure the samples were collected and handled in accordance of this protocol.

In addition, for the genetic research component of the study, before first subject into the study, a representative of AstraZeneca will visit the centre to:

- discuss sampling for DNA extraction for genetic testing, the consequences and procedures associated with the process are to be made clear to the investigator, including the importance of explaining to the subject the background information about genetic studies, procedures to be followed and the intended use of the genetic material.

- check that the appropriate consent documentation for the genetic research component of the study has been completed.

7.2 Audits and inspections

Please refer to the main clinical study protocol.

7.3 Training of staff

Please refer to the main clinical study protocol.

In addition, for studies containing a genetic component, study staff must be familiar with the ethical issues surrounding genotyping and the procedures for the conduct of the study.

7.4 Changes to the protocol

Please refer to the main clinical study protocol.

7.5 Study agreements

Please refer to the main clinical study protocol

7.6 Study timetable and termination

Please refer to the main clinical study protocol.

8. ETHICS

8.1 Ethics review

Please refer to the main clinical study protocol.

IRB approval for this genetic research component and Genetic Written Informed Consent Form must be obtained in writing.

8.2 Ethical conduct of the study

Please refer to the main clinical study protocol.

In addition, in Japan, this genetic study will be conducted well taking into account the principles specified in "The guidance of genetic analysis study", issued from 3 ministries in Japan on March 29, 2001. The samples collected for genetic analysis will be processed according to the principle of GLP.

AstraZeneca ensures that special precautions are taken for studies including genetic analysis, with regard to the processes for ensuring confidentiality of data (Section 5).

8.3 Written informed consent

8.3.1 Subject informed consent for genetic research

A separate written informed consent form will be used for the genetic research component of the study. For each subject, specific written informed consent must be obtained prior to any sample being taken for genetic research, in addition to the subject's consent to participate in the main study if the subject participates in the genetic research component. Subjects who do not wish to participate in the genetic research may still participate in the main clinical study.

The principal investigator(s) must store the original, signed Written Informed Consent Form for the genetic research and a copy must be given to the subject.

Please refer to the main clinical study protocol for further details regarding written informed consent. All details in the main protocol apply equally to the consent for the genetic component of the study.

8.4 Subject data protection

Please refer to the main clinical study protocol.

The study site pays their attention to protect subject' privacy (especially subject's private information, such as address, name and phone number will not be used as the data).

In addition, AstraZeneca ensures that special precautions are taken for this study which includes genetic analysis, with regard to ensuring confidentiality of data.

The Written Informed Consent Form(s) will explain that study data will be stored in a computer database, maintaining confidentiality. Samples in this database will be identified by the second code only. Research scientists at AstraZeneca R&D Genetics, Alderley Park, who have access to the genetic data in the genotyping LIMS, will not have access to any information about the identity of the subject. The genetic data samples and data will be de-identified. The genetic data will be labelled with the second code ensuring that the confidentiality of the subject is maintained at all times.

Details of the procedure specific to this study are in Section 5.

9. ADMINISTRATIVE ASPECTS OF THE STUDY

9.1 Study administrative structure

9.1.1 Study site

9.1.2 Responsible persons at sponsors

Japan:

US:

9.1.3 Analytical site and responsible persons

10. EMERGENCY PROCEDURES

Refer to the main clinical study protocol.

11. REFERENCES

1. Marchall A. 1997. Getting the right drug into the right patient. *Nature Biotechnology*, **15:** 1249-1252.

2. Schaeffeler *et al.*, 2001. Frequency of C3435T polymorphism of *MDR1* gene in African people. *Lancet* **358;** 343-430**.**

3. Kuehl, P.,et al.: Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. *Nature Genet*. **27** : 383-391 (2001)

4. Hiratsuka M et al., 2002. Allele and genotype frequencies of CYP2B6 and CYP3A5 in the Japanese population. Eur J Clin Pharmacol 58(6): 417-21

5. Fontana, P *et al.*, 2003. Adenosine diphosphate-induced platelet aggregation is associated with P2Y12 gene sequence variations in healthy volunteers. *Circulation* **108;** 989-995.

Appendix 1

Letter of Transmittal (Sample)

Date: _____

Please find enclosed samples as listed on the **Sample Log Form**. After checking the sample Qty. and status, please send to Mr. XXXXX at AZ (Fax:+XXXXXX) and me the **Notice of Receipt** by Fax as soon as possible.

STUDY CODE/Trial #: D5130C05267
SPONSOR: AstraZeneca
TEST PRODUCT CODE NAME: AZD6140
STUDY CENTER: _____
Quantities and type of samples: :Subject No. xx - yy
☐blood;Qty= _____

Kind regards,

Signature of the personnel

<name of the personnel>

XXXXX <name>
XXXXX<address>
Phone: +XXXXXX, Fax: +XXXXX

Appendix 2

Notice of Receipt (Sample)

To: <name>XXXX **From:**

Fax: +XXXX

cc: Mr. XXXXXX (AstraZeneca KK)
Fax: +XXXX

I received the following samples and checked the status.

STUDY CODE/Trial #: D5130C05267
SPONSOR: AstraZeneca
TEST PRODUCT CODE NAME: AZD6140
STUDY CETER: _____

RECEIVED SAMPLES;

:Subject No. xx - yy
:☐Blood;Qty= _____
:STATUS; ☐Melted☐Broken ☐Disappeared
☐Others (_____)

If you check **STATUS** √ , please describe the status in detail;

_____
_____
_____

Signature of the personnel

<name of the personnel> _____

DATE RECEIVED : _____

AstraZeneca

Clinical Pharmacology Study Protocol: Appendix F	
Drug substance:	AZD6140
Study Code:	D5130C05267
Appendix Edition No:	1.0
Appendix Date:	

Appendix F
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:

- Study code,
- Study centre number,
- Subject ID code (enrolment code),

- Date of sample taken,
- Serial number and
- Type of sample: ”Blood”

2. STORAGE AT THE STUDY CENTER AND TRANSPORT

The blood samples must be sent to Clinical Genotyping Group Leader, R&D Genetics, AstraZeneca R&D Alderley Park after the collection of the last sample, as one batch under the freezing condition. The table below shows guidelines for sample storage and transport:

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

Storage Temperature at Study Center	***Maximum Duration***	***Transport Temperature***	***Delivery Time***
-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 study site must send a fax to the Clinical Genotyping Group Leader at R&D Genetics, AstraZeneca R&D Alderley Park and the sponsor using defined format just after the sample shipment, so that late arrivals can be investigated promptly. The format includes

- Study code,
- Study center number,
- Number of samples,
- Courier name,
- Airway bill number,
- Date of shipment,

- Shipment condition (dry ice) and
- Contact name and address.

A sample log form detailing a list of sample IDs (enrolment codes), dates of sampling, and confirmation that informed consent has been given for every sample, together with study code and centre number, should also be prepared and faxed.

Please ensure that the shipment is scheduled to arrive during working hours (Monday – Friday, 08:00-18:00). It is advisable to avoid shipping over a weekend as this can result in delays, and the possibility of sample thawing. Office hours of Customs departments in destination countries should also be taken into account in shipment planning, to allow samples to arrive at a time when they will receive immediate and appropriate attention

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. Standard procedures for transporting biological samples as defined by the courier and in compliance with local regulations will be followed if different from recommended packaging instructions.

Sample Shipment.

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

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	D5130C05267
Edition No.	1.0
Date	

A Single-blind, Randomized, Placebo-controlled Phase I Study to assess the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Multiple Oral Doses of AZD6140 in Healthy Male Japanese and Caucasian Subjects

Sponsor:

Centres affected by the Amendment:

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

Section of protocol affected:

Page 46, Section 4.4.3 Clinical laboratory measurements, table

Previous text:

	Japan	**US**
Clinical chemistry	6 mL of blood on no anti-coagulant	10 mL of blood on no anti-coagulant
Clinical chemistry (P-Glucose)	1 mL of blood on NaF	Not applicable
Haematology	2 mL of blood on EDTA-2K	5 mL of blood on EDTA-2K
Haematology (PT/APTT)	1.8 mL of blood on 3.13 % sodium citrate	4.5 mL of blood on 3.2% sodium citrate
HIV, HBsAg, hepatitis C and syphilis	6 mL of blood on no anti-coagulant	10 mL of blood on no anti-coagulant

Revised Text:

	Japan	**US**
Clinical chemistry	6 mL of blood on no anti-coagulant	10 mL of blood on no anti-coagulant
Clinical chemistry (P-Glucose)	1 mL of blood on NaF	Not applicable
Haematology	2 mL of blood on EDTA-2K	5 mL of blood on EDTA-2K
Haematology (PT/APTT)	2.7 mL of blood on 3.13 % sodium citrate	4.5 mL of blood on 3.2% sodium citrate
HIV, HBsAg, hepatitis C and syphilis	6 mL of blood on no anti-coagulant	10 mL of blood on no anti-coagulant

Reason for Amendment:

The sample volume for the analysis of prothrombin time and activated partial thromboplastine time at the Japanese Study Center changed. This change was previously described in CSP Administartive Change 1.0, however, because it concerns the safety of the subjecs, the change is being treated as an amendment of the CSP.

Section of protocol affected:

Page 50, Section 4.6 Volume of blood sampling, Table 9 Volume of blood to be drawn from each subject

Previous text:

Japanese Study Center

Assessment	Sample volume (mL)	No. of samples	Total volume (mL)
PK samples	2	32	64
ADP-induced platelet aggregation	9	14	126
HIV, HBsAg, hepatitis C and syphilis	6	1	6
Clinical chemistry	7	10	70
Hematology	3.8	10	38
Genetic sample	9	1	9
Sub-total			313
Flushing volume[1)]	1	18	18
Total			**331**

1) The first 1 mL from an indwelling catheter will be discarded at sampling time for PK samples taken between 0.5~12h after dosing on Days 1 and 10.

Revised Text:

Japanese Study Center

Assessment	Sample volume (mL)	No. of samples	Total volume (mL)
PK samples	2	32	64
ADP-induced platelet aggregation	9	14	126
HIV, HBsAg, hepatitis C and syphilis	6	1	6
Clinical chemistry	7	10	70
Hematology	4.7	10	47
Genetic sample	9	1	9
Sub-total			322
Flushing volume[1)]	1	18	18
Total			**340**

1) The first 1 mL from an indwelling catheter will be discarded at sampling time for PK samples taken between 0.5~12h after dosing on Days 1 and 10.

Reason for Amendment:

The sample volume for the analysis of prothrombin time and activated partial thromboplastine time at the Japanese Study Center changed. The change was previously described in CSP Administartive Change 1.0, however, because it concerns the safety of the subjecs, the change is being treated as an amendment of the CSP.

Persons who initiated

.

Signed agreement to the Amendment:

I agree to the terms of this protocol Amendment.

Study Code: D5130C05267

I agree to the terms of this protocol Amendment.

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Study Code: D5130C05267

agree to the terms of this protocol Amendment.

Study Code: D5130C05267

I agree to the terms of this protocol Amendment.

Study Code: D5130C05267

I agree to the terms of this protocol Amendment.

Study Code: D5130C05267

Centre No.: 001

…… ……

Date

I agree to the terms of this protocol Amendment.

Study Code: D5130C05267

Centre No.: 002

. . . .
Date