AstraZeneca

Amended Clinical Pharmacology Study Protocol	
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A Randomised, Double-Blind, 2-Period Crossover Study to Assess the Effect of Steady-State AZD6140 on the Pharmacokinetics of a Single Oral 500-mg Dose of Tolbutamide, a Substrate of CYP2C9, in Healthy Male and Female Volunteers

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AstraZeneca Research and Development site representative

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

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

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

PROTOCOL SYNOPSIS

A Randomised, Double-Blind, 2-Period Crossover Study to Assess the Effect of Steady-State AZD6140 on the Pharmacokinetics of a Single Oral 500 mg Dose of Tolbutamide, a substrate of CYP2C9, in Healthy Male and Female Volunteers

Investigator

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

A single study centre (PAREXEL International, Baltimore, Maryland) will participate in this study. Up to 24 healthy male and female volunteers will be randomised in order to complete at least 18 healthy volunteers.

Study period	**Phase of development**
Estimated date of first subject enrolled	Clinical Pharmacology-Phase 1
Estimated date of last subject completed	

Objectives

The primary objective of this study is:

1. To assess the effect of steady-state AZD6140 on single-dose pharmacokinetics of tolbutamide by analysis of tolbutamide plasma concentrations.

The secondary objectives of this study are:

1. To assess the effect of steady-state AZD6140 on single-dose pharmacokinetics of 4-hydroxytolbutamide by analysis of 4-hydroxytolbutamide plasma concentrations.

2. To assess the effect of a single dose of tolbutamide on steady-state pharmacokinetics of AZD6140 and AR-C124910XX by analysis of AZD6140 and AR-C124910XX plasma concentrations.

3. To examine the safety and tolerability of AZD6140 and a single dose of tolbutamide by assessment of adverse events, 12-lead ECG, vital signs, laboratory parameters and physical examinations.

Study design

This study will be a randomised, double-blind (with respect to AZD6140), 2-period crossover, single-centre study to compare the safety, tolerability, pharmacokinetic and pharmacodynamic profile of a single dose of tolbutamide alone and in combination with AZD6140 or matching placebo in healthy volunteers aged 18 to 45 years. Approximately 24 healthy men and women will be enrolled to ensure the completion of at least 18 healthy volunteers.

There will be two treatment periods each consisting of an 11-day/10-night inpatient stay. On Day –1 of each treatment period, healthy volunteers will be admitted to the clinical pharmacology unit (CPU) in the evening (at least 12 hours prior to dosing on Day 1). On Day 1 of Period I, healthy volunteers will be randomised to one of two treatments (A or B). Each subject will receive both treatments in a crossover fashion. AZD6140 treatment will be administered double-blind while tolbutamide treatment will be administered open-label. Consecutive treatment periods will be separated by a minimum 14-day washout period.

Investigational product, dosage and mode of administration

Each subject will receive each of the following treatments in cross-over fashion according to the randomisation schedule:

	Treatment A		**Treatment B**	
	Morning	**Evening**	**Morning**	**Evening**
Day 1	180 mg AZD6140 (2 x 90 mg)	180 mg AZD6140 (2 x 90 mg)	2 placebo tablets	2 placebo tablets
Days 2-4[a]	180 mg AZD6140 (2 x 90 mg)	180 mg AZD6140 (2 x 90 mg)	2 placebo tablets	2 placebo tablets
Day 5[a]	180 mg AZD6140 (2 x 90 mg) 500 mg tolbutamide	180 mg AZD6140 (2 x 90 mg)	2 placebo tablets 500 mg tolbutamide	2 placebo tablets
Days 6-9	180 mg AZD6140 (2 x 90 mg)	180 mg AZD6140 (2 x 90 mg)	2 placebo tablets	2 placebo tablets

[a] On Day 4 and 5, study drug will be administered after a minimum 10-hour overnight fast

Over the course of the study, each subject will receive eighteen (18) doses of 180 mg AZD6140 (or 18 doses of matching placebo), and two doses of 500 mg tolbutamide. All study medication will be administered orally with approximately 240 mL room temperature water.

Duration of treatment

The duration of subject participation will be up to approximately 64 days, including the screening and follow-up periods.

Variables

Pharmacokinetic

The following pharmacokinetic parameters will be assessed:

- C_{max}, t_{max}, $t_{1/2}$, AUC_{0-t} and AUC of tolbutamide and 4-hydroxytolbutamide, and CL/F of tolbutamide
- $C_{ss,max}$, $t_{ss,max}$, and $AUC_{ss,\tau}$ of AZD6140 and AR-C124910XX and CL/F of AZD6140, as well as AR-C124910XX to AZD6140 $C_{ss,max}$ and $AUC_{ss,\tau}$ ratios

The primary pharmacokinetics variables will be Cmax and AUC of tolbutamide.

Safety

Safety will be assessed through any of the following variables: incidence and severity of adverse events; incidence of causally related adverse events as assessed by the investigator, incidence of adverse events leading to discontinuation of the medication; vital signs measurements (blood pressure and heart rate); laboratory parameters; physical examination results.

Genetics

The voluntary genetic component of the study serves to generate data for use in future retrospective analyses. Therefore, results of genetic analyses will not form part of the clinical study report for this study. Future analyses will explore genetic factors that may be correlated with the disposition and response of AZD6140, and the effect of AZD6140 on the distribution of tolbutamide. Any results may be pooled to be tested in future studies.

Statistical methods

Pharmacokinetic

Following log-transformation, C_{max} and AUC of tolbutamide and 4-hydroxytolbutamide will be analysed by analysis of variance (ANOVA) fitting terms for sequence, period and treatment. Subject within sequence will be treated as random in the model. Geometric mean ratios of C_{max} and AUC of tolbutamide when administered concurrently with AZD6140 will be compared with these parameters when tolbutamide is administered concurrently with placebo, along with the corresponding 90% confidence intervals. Descriptive statistics will be used to summarize all pharmacokinetic parameters.

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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
ADP	Adenosine diphosphate
AE	Adverse event
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
aPTT	Activated partial thromboplastin time
Assessment	An observation made on a variable involving a subjective judgment
AST	Aspartate aminotransferase
AUC_{0-t}	Area under the plasma concentration-time curve from time zero to last sampling time "t"
AUC	Area under the plasma concentration-time curve from time zero to infinity
$AUC_{ss,\tau}$	Area under the plasma concentration-time curve within a dosing interval at steady state
BMI	Body mass index
CGG	Clinical genotyping group
CL/F	Apparent oral clearance
CL_{ss}/F	Steady state oral clearance
C_{max}	Maximum plasma concentration following a single dose
CPU	Clinical pharmacology unit
CRF	Case report form
CRO	Contract research organization
CSR	Clinical study report
$C_{ss,max}$	Maximum plasma concentration within a dosing interval at steady state
CYP	Cytochrome P450 enzyme
eCRF	Electronic case report form
ECG	Electrocardiogram
FSH	Follicle stimulating hormone
GCP	Good clinical practice
GGT	γ-glutamyltransferase
HBsAg	Hepatitis B surface antigen

Abbreviation or special term	Explanation
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
ICH	International conference on harmonisation
IRB	Institutional review board
LLOQ	Lower limit of quantitation
Measurement	An observation made on a variable using a measurement device
MedDRA	Medical dictionary for regulatory activities
NSAIDs	Non-steroidal anti-inflammatory drugs
OAE	Other significant adverse event (ie, adverse events of particular clinical importance, other than SAEs and those AEs leading to discontinuation of the subject from study treatment; see definition in Section 4.6.1.1).
OTC	Over the counter
PD	Pharmacodynamic
PGx	Pharmacogenetics
pH	Potential of hydrogen; the logarithm of the reciprocal of hydrogen-ion concentration in gram atoms per liter; provides a measure on a scale from 0 to 14 of the acidity or alkalinity of a solution (where 7 is neutral, greater than 7 is acidic, and less than 7 is basic)
PK	Pharmacokinetic
Principal investigator	A person responsible for the conduct of a clinical study at a study site. Every study centre has a principal investigator
PT	Prothrombin time
SAE	Serious adverse event
$t_{½}$	Mean terminal half-life
t_{max}	Time of maximum observed plasma concentration
$t_{ss,max}$	Time to maximum plasma concentration within a dosing interval at steady state
ULN	Upper limit of normal
WBDC	Web based data capture
WHO	World Health Organisation

1. INTRODUCTION

1.1 Background

Adenosine diphosphate (ADP) is an important mediator of platelet activation and aggregation through its binding to at least 2 distinct subtypes of purinoceptor, designated $P2Y_1$ and $P2Y_{12}$, found on platelets. Two ADP receptor antagonists, thienopyridine pro-drugs, PLAVIX™ (clopidogrel) and TICLID™ (ticlopidine) have shown clear benefits for the reduction of clinical thrombotic events in patients with atherosclerosis due to their ability to block the $P2Y_{12}$ receptor. However, this blockade is irreversible and usually incomplete. 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, reversible, 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 metabolites are active. Unlike clopidogrel and ticlopidine, which incompletely block the $P2Y_{12}$-receptor response in humans, pre-clinical studies indicate that AZD6140 can produce reversible and complete inhibition of ADP-induced platelet aggregation *ex vivo* following oral dosing. Additionally, AZD6140 has shown greater and more consistent inhibition of platelet aggregation compared to clopidogrel in both healthy volunteers and patients. It has also demonstrated a faster onset and offset of antiplatelet effect. These properties suggest that AZD6140 may be able to reduce the occurrence of thrombotic events compared to clopidogrel with an acceptable safety profile.

AZD6140 binds to plasma proteins (>99.7%), and is extensively metabolised by CYP3A, with little parent drug excreted unchanged in the urine. AZD6140 has a number of drug–drug interactions of clinical relevance since it is a substrate, inhibitor, and activator of CYP3A4 and a substrate and inhibitor of the P-glycoprotein transporter. Following an oral dose of ^{14}C-labelled AZD6140 in humans, approximately 27% was excreted in the urine and 57.8% in the faeces. The elimination half-life ($t_{½}$) of the parent compound is approximately 11 hours after single dose administration.

One of the primary metabolites, AR-C124910XX, is considered equipotent to the parent drug in the in vitro studies. The time course of the pharmacologically active metabolite approximately parallels AZD6140; the area under the plasma concentration curve (AUC) and maximum plasma drug concentration after single dose administration (C_{max}) of AR-C124910XX is typically 30 to 40% of the corresponding parameters for AZD6140.

Plavix™ (Clopidogrel bisulfate) is a registered trademark of Sanofi-Synthelabo and distributed by Bristol-Myers Squibb/Sanofi Pharmaceuticals Partnership, NY, NY.

TICLID™ (ticlopidine) is a registered trademark of Roche Pharmaceutical, Inc, Nutley, NJ.

Adverse events (AEs) reported with a frequency of at least 2% in Phase 1 studies with at least 3 days of AZD6140 dosing include: headache, somnolence, dizziness, epistaxis, nausea, abdominal pain, back pain, dyspnoea, ecchymosis, lethargy, pharyngolaryngeal pain, blurred vision, postural dizziness, pollakiuria (frequent urination), and increased tendency to bruise. Two serious adverse events (SAE) occurred during the Phase 1 program; an occurrence of chronic mediastinitis not considered by the investigator to be related to study drug, and an episode of sinus pause (high grade atrioventricular block and ventricular escape rhythm associated with syncope) in a volunteer who received a single 1260 mg dose of AZD6140. The volunteer recovered without sequelae, and the event was considered to be medically important and related to study drug.

Refer to the Investigator Brochure for further details on AZD6140 exposure, pharmacokinetic, and safety findings. These data support the further development of AZD6140 as an oral antiplatelet agent, which may be able to prevent more thrombotic events than clopidogrel by sustaining higher levels of $P2Y_{12}$ receptor blockade with an acceptable safety profile.

1.2 Rationale

The ability of AZD6140 to both inhibit and induce cytochrome P450 (CYP) enzymes was assessed in a series of in vitro studies. AZD6140 had no inhibitory effect on human CYP1A2, 2C19 or 2E1 enzymes. Moderate inhibition (approximately 50%) of CYP2C9 and CYP2D6 was observed at high levels of AZD6140 (50 μM) using tolbutamide 4-hydroxylation and bufuralol 1'-hydroxylation as markers of these enzyme activities. However, the greatest extent of human cytochrome P450 enzyme (CYP) inhibition was observed for CYP2C9-mediated diclofenac hydroxylation (IC50 = 10.5 μM).

Tolbutamide is an oral sulfonylurea that is used in the treatment of non-insulin-dependent diabetes mellitus. It is a substrate for CYP2C9, and has long been used as the probe drug in vitro and in vivo to evaluate the ability of a second compound to inhibit CYP2C9. This inhibition is measured by a change in the plasma pharmacokinetics of tolbutamide and its metabolite, 4-hydroxytolbutamide, in the presence of the second compound.

This study, therefore, is being conducted to examine the effect of in vivo AZD6140 on the pharmacokinetics of tolbutamide and 4-hydroxytolbutamide, the former of which is a substrate of CYP2C9.

A retrospective analysis of the polymorphisms of genes may be correlated with the disposition of and response to AZD6140. Certain polymorphisms of CYP2C9 genes are associated with reduced the metabolism of tolbutamide. CYP2C9 polymorphisms may contribute to the variability of the effect of AZD6140 on the pharmacokinetics of tolbutamide. Therefore, it is important that all healthy volunteers randomised in the study donate blood samples for genetic analysis.

2. STUDY OBJECTIVES

2.1 Primary objective

The primary objective of this study is to assess the effect of steady-state AZD6140 on single-dose pharmacokinetics of tolbutamide by analysis of tolbutamide plasma concentrations.

2.2 Secondary objective(s)

The secondary objectives of this study are:

1. To assess the effect of steady-state AZD6140 on single-dose pharmacokinetics of 4-hydroxytolbutamide by analysis of 4-hydroxytolbutamide plasma concentrations.

2. To assess the effect of a single dose of tolbutamide on steady-state pharmacokinetics of AZD6140 and AR-C124910XX by analysis of AZD6140 and AR-C1249XX plasma concentrations.

3. To examine the safety and tolerability of AZD6140 and a single dose of tolbutamide by assessment of adverse events, 12-lead ECG, vital signs, laboratory parameters and physical examinations.

3. STUDY PLAN AND PROCEDURES

3.1 Overall study design

This study will be a randomised, double-blind (with respect to AZD6140), 2-period crossover, single-centre study to compare the safety, tolerability, pharmacokinetic and pharmacodynamic profile of a single dose of tolbutamide alone and in combination with AZD6140 or matching placebo in healthy volunteers aged 18 to 45 years. Approximately 24 healthy men and women will be enrolled to ensure that at least 18 healthy volunteers complete the study.

Tolbutamide will be dosed open-label. AZD6140 will be administered in a double-blind manner. The study will consist of two 11-day periods (Periods I and II) separated by a washout period of at least 14 days. During Period I, healthy volunteers will be randomised to receive Treatment A or B; the healthy volunteers will receive the alternate treatment during Period II. Treatments A and B are outlined below.

Treatment A:

Day 1-4: 180 mg AZD6140 will be administered in the morning. Twelve hours later, 180 mg AZD6140 will be administered (total daily dose: 360 mg).

Day 5: After a 10-hour overnight fast, 180 mg AZD6140 will be administered in the morning concurrently with a single 500 mg dose of tolbutamide. Twelve hours later, 180 mg AZD6140 will be administered (total daily dose of AZD6140: 360 mg).

Days 6–9: 180 mg AZD6140 will be administered every 12 hours (total daily dose: 360 mg).

Treatment B:

Day 1-4: 2 placebo tablets (to match the AZD6140 tablets) will be administered in the morning. Twelve hours later, 2 placebo tablets will be administered (total daily dose: 4 tablets).

Day 5: After a 10-hour overnight fast, 2 placebo tablets will be administered in the morning concurrently with a single 500 mg dose of tolbutamide. Twelve hours later, 2 placebo tablets will be administered (total daily dose: 4 tablets).

Days 6–9: 2 placebo tablets will be administered every 12 hours (total daily dose: 4 tablets).

The morning dose of AZD6140/placebo on Day 4 and tolbutamide/AZD6140/placebo on Day 5 of Periods I and II will be administered following a 10-hour overnight fast. Healthy volunteers will be administered the study medications with approximately 240 mL of room temperature water. Healthy volunteers will remain fasting until four (4) hours post-dose and water will be restricted, except water for dosing, from two (2) hours pre-dose until two (2) hours post-dose.

Blood glucose will be measured pre-dose and at selected time points after dosing of tolbutamide on Day 5 (hourly, up to eight (8) hours, then as needed as determined by the investigator). Glucose tablets (eg, BD™ Glucose Tablets) will be administered when blood glucose concentrations have fallen below 70 mg/dL whether or not symptoms of hypoglycaemia are present. Healthy volunteers who exhibit clinical signs of hypoglycaemia will receive glucose tablets or parenteral glucose at the judgment of the investigator regardless of blood glucose concentrations.

A washout interval of at least 14 days will occur between Periods I and II.

Figure 1 Study flow chart

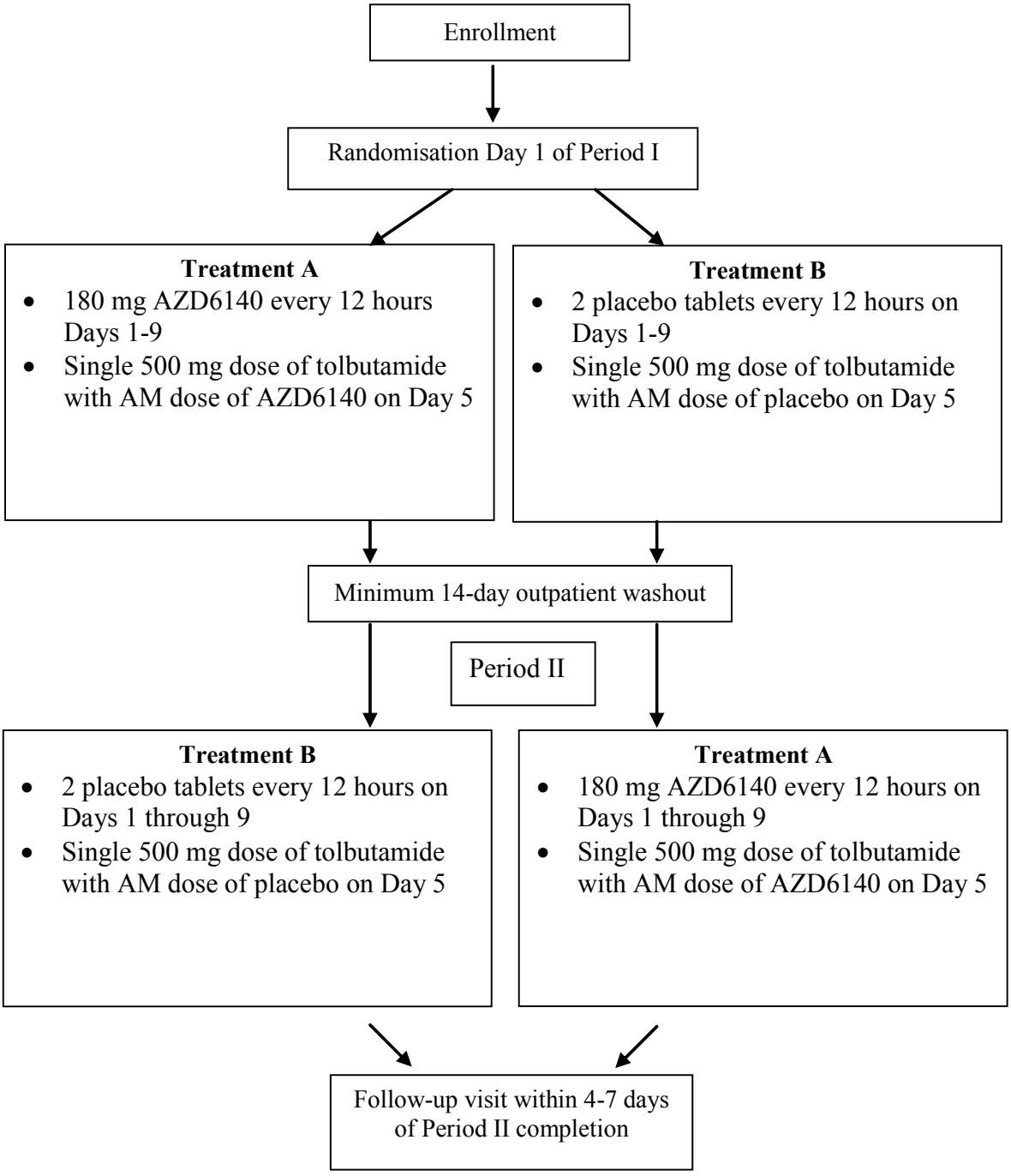

Table 1 Study plan

Assessment	Screening Visit 1	Visits 2 and 3 (Periods I and II) (Study Day)											Follow-up Visit 4
	≤ 21 days prior to Day −1	-1	1	2	3	4	5	6	7	8	9	10	4-7 days after Period II, Day 10
Admission/inpatient stay		✓											
Inclusion/exclusion criteria	✓	✓											
Informed consent	✓												
Demographics	✓												
Height and weight	✓												
Vital signs (heart rate, blood pressure, and oral temperature)	✓	✓	✓[a]	✓[a]	✓[a]	✓[a]	✓[a]	✓[a]	✓[a]	✓[a]	✓[a]	✓	✓
Medical and surgical history	✓												
Clinical lab assessments	✓	✓					✓					✓	✓
aPTT and PT assessments	✓												
Physical examination (complete)	✓												✓
Physical examination (brief)		✓										✓	
Serum alcohol screen	✓	✓											
Urine drug screen	✓	✓											
Screening for HIV and hepatitis	✓												
Serum pregnancy test (women only)	✓	✓											✓
12-lead ECG	✓	✓											✓
Randomisation			✓										

Assessment	Screening Visit 1	Visits 2 and 3 (Periods I and II) (Study Day)											Follow-up Visit 4
	≤ 21 days prior to Day –1	-1	1	2	3	4	5	6	7	8	9	10	4-7 days after Period II, Day 10
Admission/inpatient stay		✓											
Genetic blood sample[b]			✓										
Concomitant medications	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Adverse events[c]	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
AZD6140 or placebo administration[d]			✓	✓	✓	✓	✓	✓	✓	✓	✓		
AZD6140/AR-C124910XX PK sample[d]				✓	✓	✓	✓						
Tolbutamide 500 mg dosing[d]							✓						
Blood glucose measurements (see Table 2)							✓						
Tolbutamide/4-hydroxytolbutamide PK sample[d]							✓	✓	✓	✓	✓	✓	

[a] To be measured within 30 minutes prior to AZD6140/placebo dosing

[b] Sample to be drawn prior to morning dose administration on Day 1 of Period I only if the volunteer signs the separate genetic informed consent form. If for some reason the sample cannot be drawn on Day 1, it may be collected at anytime after randomisation through the end of the subject's participation in the study.

c Serious adverse events will be collected from signature on the informed consent through completion of the follow-up visit. Adverse events will be collected from the first dose of study drug until the completion of the follow-up visit

d Refer to Table 2 for exact timing of dose administrations and sample collections

Table 2 Schedule of assessments for Days –1 through 10, Periods I and II

	Day -1	Day 1			Days 2, 3			Days 4, 5			Day 6			Days 7, 8, 9		Day 10
Assessment		**Predose**	**0h**	**12h**	**Predose**	**0h**	**12h**	**Predose**	**0h**	**12h**	**18h**	**24h**	**36h**	**0h**	**12h**	**0h**
Inclusion/exclusion criteria	✓	✓														
Admission/inpatient stay	✓	←	—	→												✓
Clinical lab assessments	✓									✓ [b]						
12-lead ECG	✓															
Vital signs[a]	✓	✓			✓			✓		✓ [b]		✓		✓		✓
Serum alcohol screen	✓															
Urine drug screen	✓															
Serum pregnancy test (women only)	✓															
Randomisation[c]		✓														
Genetic blood sample[d]		✓														
Physical examination (brief)	✓															
Concomitant medications	✓	←	—	→												✓
Adverse event recording	✓	←	—	→												✓
AZD6140 180 mg or placebo dose			✓	✓		✓	✓		✓	✓		✓	✓	✓	✓	
AZD6140/AR-C124910XX PK sample[e]					✓			✓		✓		✓		✓		✓
Blood glucose measurements								✓[f]	✓[f]	✓[f]						
Tolbutamide 500 mg dosing									✓ [b]							

	Day -1	Day 1			Days 2, 3			Days 4, 5			Day 6			Days 7, 8, 9		Day 10
Assessment		**Predose**	**0h**	**12h**	**Predose**	**0h**	**12h**	**Predose**	**0h**	**12h**	**18h**	**24h**	**36h**	**0h**	**12h**	**0h**
Tolbutamide/4-hydroxytolbutamide PK sample[g]								✓		✓	✓	✓	✓	✓		✓

[a] On dosing days, blood pressure, heart rate, and oral temperature will be taken within 15 minutes prior to AZD6140/placebo dose.
[b] Day 5 only.
[c] Immediately before dosing on Day 1 of Period I only.
[d] Sample to be drawn prior to morning dose administration on Day 1 of Period I only. If for some reason the sample cannot be drawn on Day 1, it may be collected at anytime after randomisation through the end of the subject's participation in the study.
[e] In addition to samples noted in table, on Days 4 and 5, samples obtained at pre-dose (within 15 minutes of dose), 0.5, 1, 2, 3, 4, 6, 8, 10, and 12 hours post morning dose.
[f] On Day 5 only, samples obtained at pre-dose (within 15 minutes of dose), 1, 2, 3, 4, 5, 6, 7, 8 and 12 hours post tolbutamide dose.
[g] In addition to samples noted in table, on Day 5, samples obtained at pre-dose (within 10 minutes of dose), 0.5, 1, 2, 3, 4, 6, 8, 10, and 12 hours post morning dose. No samples will be obtained on Day 4.

3.2 Rationale and risk/benefit assessment

3.2.1 Rationale for study design, doses and control groups

1. The objective of this study is to examine the effect of AZD6140 on the pharmacokinetics of tolbutamide, a substrate of CYP2C9. In vitro metabolism data indicate that AZD6140 inhibits CYP2C9 in vitro at high concentrations.

2. A randomised crossover design is chosen to examine the pharmacokinetic parameters of tolbutamide alone and in combination with AZD6140 and to minimize bias.

3. The pharmacokinetics of AZD6140 and its metabolite AR-C124910XX, and of tolbutamide and its metabolite 4-hydroxytolbutamide, will be examined to assess the magnitude of the interaction.

4. A dose of 180 mg every 12 hours of AZD6140 was chosen because this is expected to be a clinically relevant dosing regimen. The tolbutamide dose of 500 mg on Day 5 was chosen because it is expected to be safely tolerated in healthy volunteers and should yield measurable tolbutamide and 4-hydroxytolbutamide plasma concentrations. The standard therapeutic dose of tolbutamide is 1-2 g daily.

5. The duration of AZD6140 dosing is to ensure that steady state levels of AZD6140 have been achieved, since AZD6140 will be given as a chronic therapy to patients.

6. The rationale for choosing healthy volunteers is to investigate the effects of AZD6140 on CYP2C9 activity without adding additional confounding factors, such as disease state and concomitant medications.

7. A genetic analysis may be carried out to explore genetic factors important in the disposition of AZD6140, response to AZD6140 and the potential interaction between AZD6140 and tolbutamide. This may include, but not be limited to, an analysis of P-glycoprotein and CYP2C9 polymorphisms. The result of the genetic study will not form part of the clinical study database or the clinical study report. The results of this study may be pooled with genetic results from other studies on AZD6140 to generate hypotheses to be tested in future studies.

8. The pre-specified (20%) limits (0.8-1.25) are chosen based on the FDA guidance on drug interaction trials (In Vivo Drug Metabolism/Drug Interaction Studies - Study Design, Data Analysis, and Recommendations for Dosing and Labeling). Selection of study population

3.2.2 Risk/benefit and ethical assessment

This study will not provide any direct medical benefits to the volunteer who participates. The benefit derived from this study will allow further understanding of the pharmacokinetic properties of AZD6140, which may be of benefit to further development of the drug and patients with ACS who may potentially receive AZD6140 in the future. Volunteers will be monitored under supervision in a clinical pharmacology unit (CPU), where management of any adverse events can take place.

Investigator's Brochure for AZD6140 contains the information supporting the overall risk/benefit assessment of the test product and is available as a reference. It contains a summary of all relevant pharmaceutical, non-clinical and clinical findings with AZD6140.

Risks observed after administration of AZD6140 to healthy volunteers in Phase I studies are listed below.

Phase I experience: The most common adverse events with an incidence of at least 2% reported to date in Phase I studies with at least 3 days of AZD6140 dosing include: headache, somnolence, dizziness, epistaxis, nausea, abdominal pain, back pain, dyspnea, ecchymosis, lethargy, pharyngolaryngeal pain, blurred vision, postural dizziness, pollakiuria (frequent urination), and increased tendency to bruise. Other adverse events reported less frequently include: elevations in liver enzymes, tachycardia, orthostatic hypotension, rash, and gingival bleeding as possible adverse events associated with AZD6140 administration.

3.3 Selection of study population

3.3.1 Study selection record

Investigator(s) must keep a record of healthy volunteers who were considered for enrolment but never enrolled, eg, subject screening log- according to local procedures. This information is necessary to establish that the subject population was selected without bias.

3.3.2 Inclusion criteria

For inclusion in the study healthy volunteers must meet all of the following criteria:

1. Give written informed consent and agree to comply with all requirements of the study.

2. Men or women aged between 18 and 45 years inclusive. Female subjects must meet one of the following conditions:

   - have been surgically sterilized at least 12 months prior to screening
   - are postmenopausal having had no regular menstrual bleeding for at least one (1) year prior to inclusion. Menopause will be confirmed by a plasma FSH level of > 40 IU/L at screening.

3. Weigh at least 50 kg and have a body mass index (BMI) between 18 and 30 kg/m^2 inclusive. BMI will be calculated as weight in kg/height squared (m^2) and will not be reported on the case report form (CRF).

4. Must have normal physical examination, laboratory values and vital signs, unless the investigator considers an abnormality to not be clinically significant

5. Must be able to communicate with the investigator, and to understand and comply with all study requirements

3.3.3 Exclusion criteria

Each of the following is a criterion for a subject's exclusion from the study:

1. History of clinically significant hypoglycaemia, as determined by the investigator

2. History of significant head trauma within 30 days of study entry

3. History of haemophilia, von Willebrand's disease, lupus anticoagulant or other diseases/syndromes that can either alter or increase the propensity for bleeding

4. Known poor metaboliser of CYP2C9 substrates

5. History of a known sulfonylurea hypersensitivity

6. Use of prescription medication for a chronic medical condition within 3 months of Day 1 of Period I

7. Use of prescription medication for an acute medical condition within 4 weeks of Day 1 of Period I

8. Use of over-the counter preparations (except three (3) doses of acetaminophen of up to 1 g within 7 days of Day 1, Period I) including all herbal remedies such as Cordyceps sinensis, dan shen, feverfew, Ganoderma lucidum, ephedra, echinacea, St. John's Wort, and garlic [aged extract taken on an ongoing basis], ginseng, ginkgo, and vitamin preparations within 7 days of Day 1, Period I

9. A 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 as determined by the investigator.

10. A significant history of alcohol or substance abuse within the past year as determined by the investigator

11. Surgery or significant trauma within 3 months prior to Day 1 of Period I as determined by the investigator

12. Current tobacco use or history of tobacco or nicotine containing products use in the past 6 months

13. Positive test results for the human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg) or hepatitis C antibody (anti-HCV)

14. Positive urine drug screen for drugs of abuse

15. Receipt of an investigational drug within 60 days prior to Day 1 of Period I

16. Previous participation in an AstraZeneca AZD6140 study

17. Consumption of Seville oranges (eg, orange marmalade) or grapefruit-containing products, alcohol, caffeine, medicines or nutritional supplements within 7 days of Day 1, Period I

18. Blood donation within 90 days before Day 1 of Period I

19. History of hypersensitivity or adverse reaction to dicalcium phosphate and lactose excipients

20. A personal history of vascular abnormalities including aneurysms; a personal history of severe haemorrhage, haematemesis, melena, haemoptysis, severe epistaxis, or intracranial haemorrhage; rectal bleeding within 3 months prior to screening

21. History suggestive of peptic ulcer disease

22. History of fainting episodes or syncope

23. Platelet count < 150,000/mm^3 at screening

24. Clinical judgment by the investigator that the subject should not participate in the study

25. A suspected/manifested infection according to World Health Organization (WHO) risk categories 2, 3 and 4 (see Appendix D)

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

3.3.4 Restrictions

Healthy volunteers are to adhere to the following restrictions:

1. No alcohol consumption from 72 hours prior to and during each treatment period

2. No consumption of caffeine-containing products from 7 days prior to and during each treatment period

3. No consumption of tobacco-containing products during the entire time of study participation

4. No consumption of Seville oranges and grapefruit-containing products which will be restricted from 1 week prior to and during each treatment period.

5. No strenuous physical exercise within 72 hours prior to and during each treatment period

6. No over-the-counter medications or preparations, including herbal remedies such as Cordyceps sinensis, dan shen, feverfew, Ganoderma lucidum, ephedra, echinacea, St. John's Wort, and garlic (aged extract taken on an ongoing basis), ginseng, ginkgo, and vitamin preparations within 7 days prior to Treatment Period I, and through the completion of the follow-up visit

7. No taking aspirin/NSAIDS or any other drug known to increase the propensity for bleeding for 1 week before Treatment Period I and through the completion of the follow-up visit (exception: up to three (3) doses of acetaminophen are allowed during Periods I and II)

8. No scheduling surgery, including dental surgery, at any time following the screening visit, and through the completion of the follow-up visit

3.3.5 Discontinuation of subjects from treatment or assessment

3.3.5.1 Criteria for discontinuation

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

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

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

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

- Incorrect enrolment (ie, the subject does not meet the required inclusion/exclusion criteria) or randomisation (ie, the subject is not allocated study drug as described in the protocol) of the subject.

- Subject lost to follow-up

- Healthy volunteers may be discontinued if they have a suspected or manifested infection according to WHO risk categories 2, 3, and 4 (see Appendix D)

- Subject lost to follow-up

Specific reasons for discontinuing a subject from the genetic research when genetics is a secondary objective of the study are:

- Withdrawal of consent for genetic research. A subject may withdraw from this genetic research at any time, independent of any decision concerning participation in other aspects of the main study described in this protocol. Voluntary discontinuation by the subject will not prejudice further treatment.

3.3.5.2 Procedures for discontinuation

Healthy volunteers who discontinue should always be asked about the reason(s) for their discontinuation and the presence of any adverse events (AEs). If possible, they should be seen and assessed by an investigator using the assessments detailed in the final study visit. Adverse events should be followed up and the subject should return any diary cards, questionnaires, and investigational products.

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

3.3.5.3 Procedures for handling incorrect enrolled subjects

Volunteers not meeting the inclusion/exclusion criteria for this study must, under no circumstances, be enrolled into this study; there can be no exceptions to this rule. Where volunteers not meeting the study criteria are enrolled in error, incorrectly randomized, or where volunteers subsequently fail to meet the criteria for the study, the volunteer should return for procedures and assessments scheduled for the follow-up visit if study drug has been administered.

3.3.5.4 Procedures for discontinuation from genetic aspects of the study

Healthy volunteers who discontinue from the study should always be asked specifically whether they are withdrawing or continuing their consent for the linked genetic research. It must be established whether the subject:

- Agrees to the genetic sample and any DNA extracted from the sample being kept for genetic analyses in the future.

- Withdraws consent for the sample to be kept for genetic analysis in the future and wishes the sample to be destroyed. Destruction of the sample (or the DNA extracted from the sample) will only be possible so long as the particular sample is traceable. In the event that DNA analysis has already been performed, AstraZeneca

will retain the results and associated data for regulatory reasons but these will not be used in any subsequent analyses.

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

The investigator will contact the study site monitor in the first instance, and then, depending upon the agreed set-up of the study, the monitor will forward this notification at the earliest possible opportunity to the head of the Clinical Genotyping Group (CGG). The CGG will provide a written confirmation of the action taken.

The address of the CGG is as follows:

Clinical Genotyping Group (CGG)
Block 17, Mereside
Alderley Park, Macclesfield, UK, SK10 4TG
Tel: +44 (0) 1625 230959
Fax: +44 (0) 1625 230958

3.4 Treatment(s)

3.4.1 Investigational product(s)

3.4.1.1 Identity of investigational product

The investigational product, AZD6140, and the matching placebo are described in Table 3 below. Data for tolbutamide are summarized in Table 4.

Table 3 Identity of investigational product

Investigational product	Dosage form and strength	Manufacturer	Formulation number	Batch number
AZD6140	90 mg immediate release, round, pale yellow, film-coated tablet	AstraZeneca R & D Charnwood, UK	334	*
Placebo	Tablet, inactive (identical in appearance to active tablets)	AstraZeneca R&D Charnwood	322	*

* The batch number will be recorded in the study master file and identified in the Clinical Study Report.

Table 4 Identity of test drug, Tolbutamide

Dosage form and strength	Manufacturer	NDC #	Ingredients
500 mg tablets	Mylan Pharmaceuticals, USA	0378-0215-01	See product information (Appendix C)

3.4.1.2 Labelling

AZD6140 will be supplied in bulk containers. The supplies will be labelled with the following information in accordance with Good Manufacturing Practice and local regulatory requirements:

- Name of sponsor and address
- Product name, dosage form, and quantity of dosage units
- Study code
- Keep out of reach of children
- Directions for use
- Storage conditions
- Lot number
- The following statement: "Caution: New Drug – Limited by Federal (or USA) Law to Investigational Use"

3.4.1.3 Storage

AZD6140 tablets should not be stored above 30°C (86°F). All investigational products must be kept in a secure place. The storage location will be locked and only accessible to authorized study personnel.

3.4.1.4 Accountability

The investigator (or delegate) is responsible for maintaining drug accountability records for study drugs. Drug accountability for this study will be carried out in accordance with standard procedures at the study centre.

The medication provided for this study is for use only as directed in the protocol. Investigational site personnel will return all unused drugs and empty containers to a vendor delegated by the sponsor. The investigational site personnel will account for all drugs dispensed and returned. Certificates of delivery and return must be signed.

The investigator will provide the tolbutamide tablets. Generic tolbutamide is marketed by Mylan Pharmaceuticals, Inc., Pittsburgh, PA. The tablets should have the same lot number and be stored in accordance with the manufacturer's instruction. Drug accountability for this study will be carried out in accordance with standard procedures at the study centre.

3.4.2 Doses and treatment regimens

All doses of AZD6140/placebo will be administered fasting at least 1 hour before a meal with the exception of the morning doses on Days 4 and 5 as described below:

Periods I and II

Day 4: The morning dose of AZD6140/placebo will be administered with approximately 240 mL of room temperature water after an overnight fast of at least 10 hours. Healthy volunteers are to remain fasting for 4 hours after the AZD6140 dose. Consumption of water will be restricted, other than water for dosing, from 2 hours prior to the AM dose to 2 hours post AM dose on Day 4.

Day 5: The morning dose of AZD6140/placebo will be administered concurrently with the dose of tolbutamide with approximately 240 mL of room temperature water. Study medication will be administered after an overnight fast of at least 10 hours. Healthy volunteers are to remain fasting for 4 hours after the AZD6140 dose. Consumption of water will be restricted, other than water for dosing, from 2 hours prior to the AM dose to 2 hours post AM dose on Day 5.

3.4.3 Method of assigning subjects to treatment groups

Written informed consent will be obtained before enrolment and the healthy volunteers will be identified with an enrolment number starting with E0001001. Healthy volunteers fulfilling the eligibility criteria will be assigned randomisation codes, starting with number 101.

QDS will generate the treatment randomisation code using its internal SAS v. 8.2-based randomisation application. QDS will complete the necessary AstraZeneca Global Randomisation (GRand) request form and transfer the form and treatment randomisation to AstraZeneca for importation into the GRand system. QDS will retain the original treatment randomisation code. QDS will generate additional randomisation treatment assignments for replacement subjects if necessary and follow the process as previously described.

The study will be dosed as double-blind with respect to AZD6140 and open label with respect to tolbutamide. Before study drug administration on Day 1 of Period I, healthy volunteers who are eligible to continue the study will be randomised to one of two treatment sequences (AB, BA), in a balanced fashion, strictly sequentially. If the healthy volunteer should be incorrectly randomised, randomisation should continue with no attempt to correct the error. If a healthy volunteer discontinues from the study, the randomisation number will not be re-used, and the healthy volunteer will not be allowed to re-enter the study. Volunteers who discontinue prematurely will not be replaced unless it appears that sufficient volunteers will

not complete the study. The randomisation of replacement volunteers will be at the discretion of the sponsor or delegate.

3.4.4 Blinding and procedures for unblinding the study

3.4.4.1 Methods for ensuring blinding

A copy of the random scheme will be made available to the pharmacist/appropriate personnel to allow the dispensing of tolbutamide and AZD6140 to take place, and to the PK analyst to enable the analysis of samples from healthy volunteers who have received active treatment to be prioritised. This documentation will be kept in a secure location until the end of the study.

3.4.4.2 Methods for unblinding the study

Individual treatment codes, indicating the treatment randomisation for each randomised subject, will be available to the investigator(s) or pharmacists at the study centre.

The individual treatment codes must not be broken except in medical emergencies when the appropriate management of the subject necessitates knowledge of the treatment randomisation. The investigator(s) must document and report to AstraZeneca any breaking of the treatment code. AstraZeneca retains the right to break the code for SAEs suspected to be causally related to an investigational product and that potentially require expedited reporting to regulatory authorities.

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

3.4.5 Concomitant medication

No concomitant medication or therapy will be allowed (exception: up to three (3) doses of acetaminophen will be allowed during Periods I and II). The healthy volunteers must be instructed that no additional medication will be allowed without the prior consent of the investigator.

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.

3.4.6 Treatment compliance

Compliance will be assured by supervised administration of the investigational product by the investigator and/or his or 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 2). The following 'priority order' will be in effect when more than one assessment is required at a particular time point:

- Pharmacokinetic blood sampling will take priority over all study procedures except for study drug administration. Pre-dose samples should be obtained shortly (within 15 minutes) prior to dose administration.

- Safety assessments (vital signs, 12-lead ECGs, safety laboratory tests and adverse event questioning) may be performed within 15 minutes prior to the protocol time-point.

4.1 Medical examination and demographic measurements

4.1.1 Enrolment medical examination and demographic measurements

Each subject will undergo an enrolment medical examination in the 21 days prior to the first treatment period (Period I, Day -1). This will consist of:

- Recording of demographic data - date of birth, gender, height, weight, race

- A standard medical/surgical history and a complete physical examination

- Blood samples will be collected for standard clinical chemistry and haematology assessments and a mid-stream urine sample will be obtained for urinalysis. In addition, blood samples will be collected for HIV and hepatitis screening and urine will be tested for drugs of abuse although these results will not be recorded in the CRF. Women will also undergo serum pregnancy testing. Serum alcohol testing will be performed on all healthy volunteers

- A resting blood pressure and heart rate measurement.

- Oral temperature measurement

- 12-lead ECG

4.1.2 Periods I and II

Healthy volunteers will be housed in the CPU on Day -1 to 10 for each of the treatment periods. Healthy volunteers will be admitted on Day -1, at least 12 hours prior to dosing on Day 1 for each period, and will remain in the clinical unit under supervision until the 216-hour post-dose pharmacokinetic samples are obtained on Day 10 of each treatment period. There will be a minimum 14-day washout period between treatment periods. On Day 1 of each treatment period, healthy volunteers will begin AZD6140 or placebo dosing with 180 mg (2 x 90 mg) AZD6140 or 2 placebo tablets in the morning. This will be followed by 180 mg AZD6140 or 2 placebo tablets every 12 hours through the evening dose on Day 9. The morning AZD6140 or placebo dose on Days 4 and 5 will be administered under fasting conditions as explained in Section 3.4.2. In the morning on Day 5, in addition to the AZD6140 or placebo dose, healthy volunteers will receive a single 500 mg dose of tolbutamide. Safety, PK, and PD assessments are to be performed as detailed in the Study Plan (Table 1) and the Schedule of Assessment for Periods I – II (Table 2).

4.1.3 Post-study medical examination

A post-study follow-up visit will be conducted 4-7 days after the last inpatient day (Period II, Day 10). A complete physical examination, vital sign measurements, 12-lead ECG and laboratory assessments (including a pregnancy test for female subjects) will be completed as indicated in Table 1.

At the completion of this follow-up visit, healthy volunteers may be discharged from the study at the discretion of the investigator.

4.2 Pharmacokinetic measurements

For timing of individual samples refer to Table 2.

4.2.1 AZD6140 and AR-C124910XX

4.2.1.1 Determination of drug concentration in biological samples

Samples for measurement of drug concentration of AZD6140 and its metabolite, AR-C124910XX, in plasma will be analysed by York Bioanalytical Solutions, Upper Poppleton, York, UK, on behalf of Development DMPK & Bioanalysis, Mölndal, AstraZeneca R&D Mölndal, using fully validated bioanalytical methods. The lower limit of quantification (LLOQ) will be 5 and 2.5 ng/mL, respectively. Details of the methods used will be provided in the clinical study report (CSR). Samples will be disposed of after the CSR is finalized.

4.2.1.2 Determination of AZD6140 and AR-C124910XX concentrations in plasma samples

Venous blood samples (4 mL) for determination of AZD6140 and AR-C124910XX concentrations in plasma will be taken at the times presented in the study plan (Table 1). After blood collection, centrifugation and preparation of plasma, as detailed below, the sample will be split into two aliquots. One of the aliquots will be labeled and shipped as detailed below. The other aliquot will be labeled and retained at the site as backup and shipped for analysis if needed. If not needed for analysis, this sample will be destroyed when the analysis is considered final or maximum of 4 weeks after first shipment.

Blood will be collected according to site procedure. Disposable needles and disposable lithium heparinised tubes (22-040-069 Greiner VACUETTE North America No. 454029) shall be used. Individual venipunctures for each time point may be performed or an indwelling catheter may be used. If an indwelling catheter is used, the catheter should be kept patent with isotonic saline; the saline will be withdrawn (1 mL) and discarded prior to the blood sample being taken. Blood samples (4 mL) will be collected into a lithium-heparinised tube. The heparin and blood will be carefully mixed. The sample will be placed on ice until centrifugation, which will begin within 30 minutes after the sample is obtained. The sample will be centrifuged for 10 minutes at 4°C at a relative centrifugal force of 1500g. The resulting plasma will be split into two aliquots and transferred to two 1.8 mL polypropylene tubes (Nunc Cryovial, Fisher Scientific No 12-565-163N, NNI No. 375418) with screw cap

and 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.

Samples should be stored at -20°C or below before, during and after the transport, and analysed within the timeframe after collection for which the stability in the samples has been validated and found acceptable. Results from analyses stored longer than the period stated will not be reported.

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

Table 5 Periods I and II: Schedule of AZD6140/AR-C124910XX pharmacokinetic blood sampling and tube numbers

Study Day	Analyte	Scheduled Time Relative to AZD6140 Dose (Hours)	Tube Number
Day 2	AZD6140/AR-C124910XX	Pre-dose	1
Day 3	AZD6140/AR-C124910XX	Pre-dose	2
Day 4	AZD6140/AR-C124910XX	Pre-dose	3
	AZD6140/AR-C124910XX	0.5	4
	AZD6140/AR-C124910XX	1	5
	AZD6140/AR-C124910XX	2	6
	AZD6140/AR-C124910XX	3	7
	AZD6140/AR-C124910XX	4	8
	AZD6140/AR-C124910XX	6	9
	AZD6140/AR-C124910XX	8	10
	AZD6140/AR-C124910XX	10	11
	AZD6140/AR-C124910XX	12	12
Day 5	AZD6140/AR-C124910XX	Pre-dose	13
	AZD6140/AR-C124910XX	0.5	14
	AZD6140/AR-C124910XX	1	15
	AZD6140/AR-C124910XX	2	16
	AZD6140/AR-C124910XX	3	17
	AZD6140/AR-C124910XX	4	18
	AZD6140/AR-C124910XX	6	19
	AZD6140/AR-C124910XX	8	20
	AZD6140/AR-C124910XX	10	21
	AZD6140/AR-C124910XX	12	22
Day 6	AZD6140/AR-C124910XX	24	23
Day 7	AZD6140/AR-C124910XX	48	24

Study Day	Analyte	Scheduled Time Relative to AZD6140 Dose (Hours)	Tube Number
Day 8	AZD6140/AR-C124910XX	72	25
Day 9	AZD6140/AR-C124910XX	96	26
Day 10	AZD6140/AR-C124910XX	120	27

4.2.2 Tolbutamide

4.2.2.1 Determination of tolbutamide and 4-hydroxytolbutamide concentrations in plasma samples

Samples for measurement of drug concentration of tolbutamide and 4-hydroxytolbutamide in plasma will be analysed by York Bioanalytical Solutions, Upper Poppleton, York, UK, on behalf of Development DMPK & Bioanalysis, Mölndal, AstraZeneca R&D Mölndal, using fully validated bioanalytical methods. The lower limit of quantification (LLOQ) will be 10 ng/mL. Details of the method used will be provided in the CSR. Samples will be disposed of after the CSR is finalized.

4.2.2.2 Sample collection and processing for determination of tolbutamide and 4-hydroxytolbutamide in plasma

Venous blood samples (4 mL) for determination of tolbutamide and 4-hydroxytolbutamide concentrations in plasma will be taken at the times presented in the study plan (Table 1). After blood collection, centrifugation and preparation of plasma, as detailed below, the sample will be split into two aliquots. One of the aliquots will be labeled and shipped as detailed below. The other aliquot will be labeled and retained at the site as backup and shipped for analysis if needed. If not needed for analysis, this sample will be destroyed when the analysis is considered final or maximum of 4 weeks after first shipment.

Blood will be collected according to site procedure. Disposable needles and disposable lithium heparinised tubes (22-040-069 Greiner VACUETTE North America No. 454029) shall be used. Individual venipunctures for each time point may be performed or an indwelling catheter may be used. If an indwelling catheter is used, the catheter should be kept patent with isotonic saline; the saline will be withdrawn (1 mL) and discarded prior to the blood sample being taken. Blood samples (4 mL) will be collected into a lithium-heparinised tube. The heparin and blood will be carefully mixed. The sample will be placed on ice until centrifugation, which will begin within 30 minutes after the sample is obtained. The sample will be centrifuged for 10 minutes at 4°C at a relative centrifugal force of 1500g. The resulting plasma will be split into two aliquots and transferred to two 1.8 mL polypropylene tubes (Nunc Cryovial, Fisher Scientific No 12-565-163N, NNI No. 375418) with screw cap and 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.

Samples should be stored at or below -20°C, and analysed within the timeframe after collection for which the stability in the samples has been validated and found acceptable. Results from analyses stored longer than the period stated will not be reported.

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

Table 6 Periods I and II: Schedule of tolbutamide and 4-hydroxytolbutamide blood sampling and tube numbers

Study Day	Analyte	Scheduled Time Relative to AZD6140 Dose (Hours)	Tube Number
Day 5	tolbutamide/4-hydroxytolbutamide	Pre-dose	1
	tolbutamide/4-hydroxytolbutamide	0.5	2
	tolbutamide/4-hydroxytolbutamide	1	3
	tolbutamide/4-hydroxytolbutamide	2	4
	tolbutamide/4-hydroxytolbutamide	3	5
	tolbutamide/4-hydroxytolbutamide	4	6
	tolbutamide/4-hydroxytolbutamide	6	7
	tolbutamide/4-hydroxytolbutamide	8	8
	tolbutamide/4-hydroxytolbutamide	10	9
	tolbutamide/4-hydroxytolbutamide	12	10
	tolbutamide/4-hydroxytolbutamide	18	11
Day 6	tolbutamide/4-hydroxytolbutamide	24	12
	tolbutamide/4-hydroxytolbutamide	36	13
Day 7	tolbutamide/4-hydroxytolbutamide	48	14
Day 8	tolbutamide/4-hydroxytolbutamide	72	15
Day 9	tolbutamide/4-hydroxytolbutamide	96	16
Day 10	tolbutamide/4-hydroxytolbutamide	120	17

4.2.3 Labelling and shipment of all pharmacokinetic plasma samples

The following sections (4.2.3.1 and 4.2.3.2) describe how the AZD6140/AR-C124910XX and tolbutamide/4-hydroxytolbutamide pharmacokinetic plasma samples are to be labelled and shipped.

4.2.3.1 Labelling of all pharmacokinetic plasma samples

Appropriate labels must be applied to all plasma sample tubes. Labels obtained will consist of a printed paper component and a freezer proof shrink film over wrap, which is intended to cover the printed paper label of samples stored at -20ºC. The purpose of the label over wrap

film is to prevent loss of printed text by samples rubbing against each other on shipment. The labels should include the following information:

Study Code: D5130C00051

Subject number:

Tube number:

Period/Study Day:

Scheduled time:

Matrix: PLASMA

4.2.3.2 Shipment of all pharmacokinetic plasma samples

All PK plasma samples accompanied by the specimen shipment logs will be shipped via an agreed-upon overnight courier (World Courier). The frozen samples must be packed securely to avoid breakage during transit; they should be double bagged to contain leaks and packed with a sufficient quantity of dry ice to ensure they remain frozen for at least 72 hours to allow for delays in shipment. The samples from each subject will be placed in separate bags for each analyte (AZD6140/AR-C124910XX or tolbutamide/4-hydroxitolbutamide) labelled as instructed in Section 4.2.3.1. All applicable shipping regulations must be followed. Documentation sufficient to identify each sample must be included in the shipment.
The primary contact, Henrik Sillén, MSc., Development DMPK & Bioanalysis, Mölndal, AstraZeneca R&D, Mölndal, Sweden, and the designated laboratory identified below must be notified by e-mail OR fax at the time samples are shipped.

Samples should only be shipped on Monday through Wednesday. Do not ship on or the day before a legal holiday.

Plasma samples should be shipped to:

Notification to DMPK and Bioanalytical Chemistry, Sweden:

4.3 Safety measurements

Please refer to the schedule of assessment (Table 2) for the precise timing of safety measurements.

4.3.1 Laboratory safety measurements

Blood and urine samples for determination of clinical chemistry, haematology and urinalysis parameters will be taken at the times given in the schedule of assessments (Table 2). The date and time of collection will be recorded on the appropriate CRF.

If any of the tests performed on the samples taken after investigational product administration show clinically abnormal results, as judged by the investigator, new blood samples will be taken and repeated until the results return to baseline or the cause is assessed. The investigator will provide an evaluation of the clinical importance of the deviation. The development of any clinically relevant deterioration in any laboratory test may constitute an AE if it leads to discontinuation of the study drug, or if it fulfils the criteria of seriousness.

If ALT, AST, or bilirubin elevations are > 3 x ULN at any time, AstraZeneca should be informed immediately.

The following laboratory variables will be measured:

Table 7 Laboratory variables to be measured

Clinical chemistry		Haematology	Urinalysis
Glucose	Magnesium	Haemoglobin	PH
Total bilirubin	Potassium	Haematocrit	Occult blood
Alkaline phosphatase	Uric acid	Red blood cell count	Protein
Creatinine		White blood cell count with differential	Microscopic analysis of formed elements [a]
AST		Platelet count	
ALT		Differential leukocytes	
GGT		PT (screening visit only)	

Sodium	aPTT (screening visit only)

[a] If a urine sample is positive for protein or blood, a microscopic examination of the urine sediment will be performed. Further investigations may be undertaken at the discretion of the investigator.

4.3.2 Urine drug screen

Urine will be tested for the following drugs of abuse: benzodiazepines, cocaine and/or metabolites, amphetamines, tetrahydrocannabinol (THC), opiates, methamphetamines (including ecstasy), phencyclidine (PCP), and barbiturates at the times indicated in the study plan (Table 1). The results of these tests will be documented in the subjects' study file; however, they will not be recorded in the CRFs.

If a subject tests positive for drugs of abuse, they will be excluded from entering, or continuing in the study.

4.3.3 HIV and hepatitis screens

Testing for the HIV antibody, HB_sAg, and Hepatitis C antibody will be performed on all healthy volunteers at screening only. If a test result is positive, the subject will not be allowed to enter the study.

Note: Although the results of the HIV and hepatitis screens must be documented in the subject's files, they will not be collected on the CRFs, and will not be recorded in the study database.

4.3.4 Serum alcohol testing

Serum alcohol will be measured at the times specified in the schedule of assessments (Table 2) and, if the result is positive, the subject will not be permitted to proceed in the study. The results of these tests will be documented in the subjects' study file; however, they will not be recorded in the CRFs.

4.3.5 Serum pregnancy test

Female healthy volunteers will have a serum β-HCG test performed on the days specified in the study plan (Table 1). If at any point a pregnancy test result is positive, the subject will not be allowed to proceed in the study. Refer to Section 8.4 for instructions regarding the reporting and follow up of pregnancies. No study medication may be given to a woman who has not had negative results on the initial and subsequent serum pregnancy tests. The results of the pregnancy tests will be recorded on the CRF as "positive" or "negative."

4.3.6 Blood Glucose Measurements

Blood glucose levels will be measured using a glucometer on Day 5 as indicated in Table 2. If glucose levels fall below 70 mg/dL, glucose tablets will be administered whether or not symptoms of hypoglycaemia are present. For healthy volunteers who show signs of

hypoglycaemia, glucose tablets or parenteral glucose will be administered regardless of serum glucose levels. The administration of glucose tablets and/or parenteral glucose will be documented in the healthy volunteers' CRFs. The investigator will supply the glucose tablets.

4.3.7 Electrocardiographic measurements

For timing of individual measurements refer to the schedule of assessments (Table 2). All ECGs will be obtained using the same model of ECG machine with the capacity to record 12 leads simultaneously. In addition, the same, specific ECG machine should be used on a subject throughout the study unless extenuating circumstances (eg, equipment failure) prevent this. A minimum of 5 heartbeat complexes should be recorded.

Leads will be placed in the standard 12-lead configuration. The investigative staff will make a reasonable effort to ensure that the ECG leads are placed in the same location for all ECGs performed on a subject. Marks may be made on the skin with an indelible pen if needed to help ensure the leads are placed in the same location. Electrode and skin preparation will remain consistent throughout the study.

For all ECGs, the original tracing should be retained in the subject's file for source data verification. Each tracing should be labelled with the AstraZeneca study code, enrolment number, subject initials, visit number, date and time of recording. A photocopy of each ECG tracing should be made available to the monitor.

4.3.7.1 Resting 12-lead ECG

Resting 12-lead ECGs will be obtained after the subject has been lying down for at least 10 minutes in each case. Screening and follow-up ECGs will be documented in the CRF by recording date, time, heart rate, QRS duration, PR interval, QT and QTc intervals.

All ECGs will be recorded and evaluated by the investigator. If indicated, additional ECG assessments can be made at the discretion of the investigator. These assessments should be entered as an unscheduled assessment on the appropriate CRF.

The investigator will judge the overall interpretation as normal or abnormal. If abnormal, it will be decided as to whether or not the abnormality is clinically significant or not clinically significant. The reason for the abnormality will be recorded on the CRF.

4.3.8 Vital signs

For timing of procedures, refer to the schedule of assessments (Table 2). Vital signs assessments, in addition to those discussed below can be made at the discretion of the investigator in order to follow the subject's clinical condition. These assessments should be entered as unscheduled assessments in the appropriate sections of the CRF.

4.3.8.1 Blood pressure and heart rate

For timing of individual measurements refer to schedule of assessments (Table 2).

Blood pressure and heart rate will be measured using an automated blood pressure machine with an appropriate cuff size after the subject has been sitting for at least 5 minutes. As much as possible, for each subject throughout the study, blood pressure will be measured using the same arm.

4.3.8.2 Oral temperature

Oral temperature will be measured in degrees Celsius (°C).

4.3.8.3 Height and weight

Height (cm) and weight (kg) will be measured without shoes.

4.3.9 Physical examination

4.3.9.1 Complete physical examination

The complete physical examination will include an assessment of the following: general appearance, skin, head and neck (including eyes, ears and throat), lymph nodes, thyroid, musculoskeletal/extremities (including spine), cardiovascular, lungs, abdomen, and neurological systems.

Complete physical examination data to be recorded on the CRF will include:
1) normal/abnormal, and 2) a description of any abnormalities. Except for the screening examination, if there has been no change from the previous exam, only that information should be recorded.

4.3.9.2 Brief physical examination

The brief physical examination will include an assessment of the following: general appearance, abdomen, lungs, and the cardiovascular system.

Brief physical examination data to be recorded on the CRF will include: 1) normal/abnormal, and 2) a description of any abnormalities. If there has been no change from the previous exam, only that information should be recorded.

4.4 Genetic measurements and co-variables

4.4.1 Collection of samples for genetic research

Healthy volunteers will provide a blood sample as per the inclusion criteria and visit schedule. A single 9 mL venous blood sample will be collected into a polypropylene EDTA tube and gently inverted a minimum of five times to mix thoroughly. Tubes will be labelled with the protocol study number, centre number, enrolment code and/or randomisation number and date of sample collection. No personal identifiers (subject name, initials or date of birth) will be placed on the tube or accompanying documentation. A record of the date of the subject consent to the genetic research and the date of the blood sample collection will be recorded in the appropriate section of the CRF.

Genotype is a stable parameter, therefore if for any reason the blood sample is not drawn on Day 1 of Period I, it may be taken at any visit until the last study visit. The genetic blood sample should ideally be drawn through the same cannula used to draw blood samples required for the main study.

4.4.1.1 Sample processing and shipping

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

Genetic blood samples will be sent to the following address:

Where possible samples should be shipped in batches and shipment should be coordinated with the receiving site to ensure that samples arrive within working hours. A requisition sheet, detailing the protocol study number, centre number, enrolment code and/or randomisation number and date of sample collection, should accompany the shipment.

4.4.1.2 Storage and coding of DNA samples

The processes adopted for the coding and storage of samples for genetic analysis are important to maintain subject confidentiality.

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

The blood samples and data for the voluntary genetic component of this study will be coded. Each blood sample will be labeled with the study number and volunteer number. Only the investigator will be able to link the blood sample to the individual volunteer. The sample and the data will not be labeled with a personal identifier. The link between the volunteer enrollment/randomization code and the DNA number will be maintained. The correlations of genotypic results with clinical data, allow regulatory audit and to trace samples for destruction in the case of withdrawal of consent.

All DNA samples will be stored under secure conditions with restricted access at AstraZeneca. The blood, DNA samples or data derived from the samples may be made available to groups or organisations working with AstraZeneca on this study or as part of the development drug project. However, the samples and any results will remain the property of AstraZeneca at all times. AstraZeneca will not give blood, DNA samples or data derived from the samples to any other parties, except as required by law.

Samples will be stored for a maximum of 20 years, from the date of completion of the study, after which they will be destroyed. DNA is a finite resource that is used up during analyses. Samples will be stored and used until no further analyses are possible. Further samples will not be acquired from healthy volunteers.

4.4.1.3 Summary of genetic assessments and analysis

The purpose of the genetic component of the study is to generate data for use in future retrospective analyses. Future analyses will explore genetic factors that may be correlated with the disposition and response of AZD6140, and the effect of AZD6140 on the disposition of tolbutamide. The results of the genetic analyses will not form part of the clinical study report for this study. The results may be pooled with genetic data from other studies on AZD6140 to generate hypotheses to be tested in future studies.

4.5 Volume of blood sampling

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

Table 8 Volume of blood to be drawn from each subject

Assessment		Sample volume (mL)	No. of samples	Total volume (mL)
AZD6140 PK Samples – Period I*		4	27	108
Tolbutamide PK Samples – Period I		4	17	68
AZD6140 PK Samples – Period II*		4	27	108
Tolbutamide PK Samples – Period II		4	17	68
Blood Samples for Genotyping		9	1	9
Safety	Clinical chemistry	10	8	80
	Haematology	5	8	40
	aPTT and PT	7	1	7
	HIV/ HBsAg, hepatitis C	10	1	10
Total				498

* Indwelling catheters may be used on Days 4 and 5 pre-dose until 12 hours post-dose for each period. Catheters will be kept patent with isotonic saline. Withdrawal of the saline (1mL) prior to the PK sample(s) at each time-point may increase blood volume by up to 1mL per sample (total 40 mL extra for entire study).

4.6 Adverse Events

The methods for collecting adverse events are described below.

4.6.1 Adverse Events

4.6.1.1 Definitions

The definitions of adverse events (AEs), serious adverse events (SAEs) and other significant adverse events (OAEs) are given below. It is of the utmost importance that all staff involved in the study are familiar with the content of this section. The principal investigator is responsible for ensuring this. Serious adverse events will be collected from the time when informed consent is obtained through the follow-up visit. Non-serious adverse events will be collected from the time of the first dose on Day 1 (Period I) through the follow-up visit.

Adverse event

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

Serious adverse event

A serious adverse event is an AE occurring during any study phase (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 any of the following – study medication – other medication?”. For further

guidance on the definition of a SAE and a guide to the interpretation of the causality question, see Appendix B to the Clinical Pharmacology Study Protocol.

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

Other Significant Adverse Events (OAE)

OAEs will be identified by the Study Delivery Team Physician in consultation with the appropriate Global Drug Safety Physician during the evaluation of safety data for the Clinical Study Report. Significant adverse events of particular clinical importance, other than SAEs and those AEs leading to discontinuation of the subject from study treatment, will be classified as OAEs. Examples of these are bleeding events, 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.

4.6.1.2 Recording of adverse events

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

Any AEs observed or reported by a subject and/or staff, will be recorded in the CRF. Any AE including clinical findings not resolved at the follow-up visit, will be followed up at an additional visit or telephone contact within 7 days after the follow-up visit or until resolved or explained.

Laboratory and vital sign abnormalities will not be recorded as AEs, unless any criterion for an SAE is fulfilled, the subject discontinues the study due to the result(s), or the investigator insists that it should be reported as AEs. If a laboratory value or vital sign is associated with clinical signs and symptoms, the signs and symptoms should be reported as an AE and the associated laboratory or vital signs should be considered additional information. Any sign or symptom that fulfils the SAE definition (see Section 4.6.1.1 above) or is the reason for discontinuation of treatment of investigational products should be reported accordingly.

The following variables will be recorded for each AE noted:

- Onset, resolution
- Intensity (mild/ moderate/ severe)
- Action(s) taken
- Outcome of the AE

- Causality of the AE (yes or no)

Whether it constitutes an SAE or not, the intensity rating is defined as:

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

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria in Section 4.6.1.1. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not a SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke but would be a SAE.

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

4.6.1.3 Reporting of serious adverse events

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

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

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

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

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

5. STUDY MANAGEMENT

5.1 Monitoring

5.1.1 Study monitoring

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

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

5.1.2 Data verification

It is a pre-requisite 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.

For this study no original data recorded on the CRF and regarded as source data.

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

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

5.2 Audits and inspections

Authorised representatives of AstraZeneca, a regulatory authority, an Ethics Committee or an Institutional Review Board may visit the centre 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 should contact AstraZeneca or delegate immediately if contacted by a regulatory agency about an inspection at his or her centre.

5.3 Training of staff

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

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

5.4 Changes to the protocol

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

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

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

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

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

5.5 Study agreements

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

Specific reference to genetics should be included in the agreement. The contractual obligations should not include any additional payment for collecting the samples, unless special processing is required.

5.6 Study timetable and end of study

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

5.7 Data management

5.7.1 Case report forms

Data will be entered in the Web Based Data Capture (WBDC) system at the investigational site. The WBDC system will be authorized and approved by AstraZeneca and set up and maintained by QDS. Trained study personnel will be responsible for entering data on the observations, tests and assessments specified in the protocol into the WBDC system and according to the eCRF Instructions within agreed timelines. The eCRF Instructions will also provide the study site with data entry instructions. Data entered in the WBDC system will be immediately saved to a central database and changes tracked to provide an audit trail. When data have been entered, reviewed, edited and Source Data Verification (SDV) has been performed, the Principal Investigator will be notified to sign a hard copy of the eCRF signature page and send it to QDS for imaging. An electronic copy of the eCRF will be provided to the investigational site after the study database has been locked and will be archived at the investigational site.

Data checks will be run and data validation performed continuously by QDS. The investigator should answer any queries raised by AstraZeneca or QDS during the entire duration of the study including the clean-file process.

Adverse events and Medical/Surgical history will be classified according to the terminology of the Medical Dictionary for Regulatory Activities (MedDRA). Medications will be classified according to the AstraZeneca Drug Dictionary. All coding will be performed by the CRO and reviewed and approved at AstraZeneca R&D Wilmington.

It is the responsibility of the Investigator to complete Serious Adverse Event Form when applicable. It is the AstraZeneca monitor's responsibility to ensure that any Serious Adverse Event Form is fully completed.

5.7.2 Electronic data capture at bedside

During the study days, data will be captured electronically at bedside.

The Investigator will ensure that the captured data are correct before transferred to the central database. Any changes made during validation will be documented with a full audit trail within the WBDC application.

Any missing, impossible or inconsistent entries discovered after the data have been transferred to the clinical study database will be referred back to the Investigator using data query forms, and be documented for each individual subject before clean file status is declared.

5.7.3 Genetic data

In the case of genotypic data, only the date the subject gave consent to participation in the genetic research and the date the blood sample was taken from the subject will be recorded in the CRF and database.

The genotypic data generated from the study will be stored in the AstraZeneca LIMS database or other appropriate system. This database is a secure database, which is separate from the database used for the main study. Some or all of the datasets from the main study may be duplicated within the AstraZeneca LIMS database for exploratory genetic analysis.

5.8 Reporting of genotypic results

Results from any genetic research performed will be reported separately from the clinical study 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, unless required to do so by law. The subject's DNA will not be used for any purpose other than those described in the study protocol.

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

6. PHARMACOKINETIC, SAFETY, AND STATISTICAL METHODOLOGY

6.1 Pharmacokinetic evaluation

6.1.1 Calculation or derivation of pharmacokinetic variables

Pharmacokinetic analyses will be performed by Clinical Pharmacokinetics AstraZeneca, Wilmington, DE.

Tolbutamide and 4-hydroxytolbutamide:

The primary objective will be addressed by estimating C_{max} and AUC of tolbutamide. The secondary objective will be addressed by estimating C_{max} and AUC of 4-hydroxytolbutamide. Plasma concentrations of tolbutamide and 4-hydroxytolbutamide will be listed and depicted graphically as a function of time after single-dose administration on Day 5 of both Treatment A and Treatment B. Single-dose pharmacokinetic parameters C_{max}, t_{max}, $t_{½}$, AUC_{0-t} and AUC of tolbutamide and 4-hydroxytolbutamide and tolbutamide CL/F will be estimated by non-compartmental analysis. C_{max} will be estimated as the highest measured concentration, and t_{max} will be the time to maximum concentration following a single dose of tolbutamide. The terminal elimination rate constant (λz) will be calculated by log-linear regression of the terminal portion of the concentration-time profile using at least 3 time points. The terminal elimination half-life ($t_{½}$) will be calculated as $0.693/\lambda z$. AUC will be calculated using the linear trapezoidal method up to the last measurable concentration ($AUC_{0\,t}$) and thereafter by extrapolation of the terminal elimination phase to infinity. Tolbutamide CL/F will be estimated as the ratio of tolbutamide dose and AUC. The ratios of 4-hydroxytolbutamide C_{max} to tolbutamide C_{max} and 4-hydroxytolbutamide AUC to tolbutamide AUC will be calculated for each treatment.

AZD6140 and AR-C124910XX

Plasma concentrations of AZD6140 and its metabolite AR-C124910XX will be listed and presented graphically as a function of time relative to Day 5 dose. Pharmacokinetic parameters listed below will be estimated by non-compartmental analysis.

Day 4 and 5: steady state $C_{ss,max}$, $t_{ss,max}$ and $AUC_{ss,\tau}$ of AZD6140 and AR-C124910XX, AZD6140 steady state CL/F and metabolite to parent $C_{ss,max}$ and $AUC_{ss,\tau}$ ratios.

AZD6140 and AR-C124910XX $C_{ss,max}$ will be estimated as the highest measured concentration and $t_{ss,max}$ will be the time to maximum concentration within a dosing interval on Days 4 and 5. $AUC_{ss,\tau}$ will be calculated using the linear trapezoidal method over the 12 hour dosing interval on Days 4 and 5, respectively. AZD6140 CL/F in the absence and presence of tolbutamide will be estimated as the ratio of AZD6140 dose and $AUC_{ss,\tau}$ on Days 4 and 5, respectively. The ratio of AR-C124910XX $C_{ss,max}$ to AZD6140 $C_{ss,max}$ ratio and, AR-C124910XX $AUC_{ss,\tau}$ to AZD6140 $AUC_{ss,\tau}$ ratio will be calculated for each treatment.

6.2 Safety evaluation

6.2.1 Calculation or derivation of safety variables

Safety and tolerability will be assessed in terms of incidence and severity of AEs, ECGs, vital signs, and safety blood laboratory assessments.

6.3 Genetics as a co-variate (Not applicable)

6.4 Statistical methods and determination of sample size

Statistical analysis will be carried out by, or under the direction of, Biostatistics at AstraZeneca, Wilmington, using SAS (version 8). Summary graphics required for presentation in the text portion of the CSR will be done using SAS and/or SigmaPlot. Other graphics intended for supplemental figures and individual time plots will be done using SAS.

6.4.1 Statistical evaluation

A comprehensive Statistical Analysis Plan will be prepared and finalized prior to database lock.

6.4.2 Description of variables in relation to hypotheses

In order to assess the effects of multiple doses of AZD6140 on the pharmacokinetics of tolbutamide, C_{max} and AUC of tolbutamide and 4-hydroxytolbutamide will be analysed. To aid in this assessment, t_{max}, AUC_{0-t} and $t_{1/2}$ of tolbutamide and 4-hydroxytolbutamide and tolbutamide CL/F will be computed.

In order to assess the effects of single-dose tolbutamide on the steady-state pharmacokinetics of AZD6140, $C_{ss,max}$ and $AUC_{ss,\tau}$ of AZD6140 and AR-C124910XX will be analysed. To aid in this assessment, $t_{ss,max}$ of AZD6140 and AR-C124910XX and AZD6140 CL_{ss}/F will be computed.

Safety and tolerability will be assessed in terms of incidence and severity of AEs, vital signs, ECGs, clinical chemistry, haematology, urinalysis, and physical examination findings.

6.4.3 Description of analysis sets

Pharmacokinetic Analysis Set: All healthy volunteers who complete both periods of study and for whom evaluable C_{max} and AUC_{0-t} tolbutamide data was calculated will be included in the formal statistical analysis and the summaries of the pharmacokinetic data.

All available pharmacokinetic data will be listed.

Safety Analysis Set: All healthy volunteers who receive at least one dose study medication will be evaluated for safety.

6.4.4 Methods of statistical analyses

Pharmacokinetic data:

The primary pharmacokinetics variables will be Cmax and AUC of tolbutamide.

All pharmacokinetic parameters, including tolbutamide and 4-hydroxytolbutamide C_{max}, t_{max}, $AUC_{0-t,}$ AUC and $t_{½}$, metabolite: parent C_{max} and AUC_{0-t}, and AUC ratios and tolbutamide CL/F, as well as, AZD6140 and AR-C124910XX $C_{ss,max}$, $t_{ss,max}$, $AUC_{ss,\tau}$, metabolite: parent $C_{ss,max}$ and $AUC_{ss,\tau}$ ratios and AZD6140 CL/F will be descriptively summarized by treatment. Tolbutamide and 4-hydroxytolbutamide and AZD6140 and AR-C124910XX plasma concentrations will be summarized by treatment and reported as descriptive statistics.

Following log-transformation, C_{max} and AUC_{0-t} of tolbutamide and 4-hydroxytolbutamide will be separately analysed by analysis of variance (ANOVA) fitting terms for treatment, sequence and period. Subject within sequence will be treated as a random effect in the model. Point estimates and 90% confidence intervals for the difference in treatments will be constructed using the appropriate error term. The point estimate and associated 90% confidence intervals will then be exponentially back-transformed to provide point and 90% confidence interval estimates for the ratios of interest (ie, C_{max} and AUC of tolbutamide+AZD6140 to C_{max} and AUC of tolbutamide alone).

$C_{ss,max}$ and $AUC_{ss,\tau}$ of AZD6140 and AR-C124910XX will be analysed in a manner similar to the primary endpoints.

Distributional assumptions underlying the analyses will be assessed by residual plots. Homogeneity of variance and identification of potential outlying observations will be assessed by plotting the studentised residuals against the predicted values from the model, while normality will be examined by normal probability plots. If assumptions are grossly violated, alternative analyses may be performed.

Geometric mean plasma concentration time plots for tolbutamide and 4-hydroxytolbutamide, AZD6140 and AR-C124910XX will be produced by treatment. Further, individual plasma

concentration time plots for tolbutamide and 4-hydroxytolbutamide, and for AZD6140 and AR-C124910XX will be produced.

Safety data:

No formal statistical analysis of the safety data will be performed. Safety and tolerability data, including baseline demographics, medical history, physical examinations, vital signs, laboratory and ECG parameters and subject disposition will be summarized descriptively by treatment and presented in tabular and/or graphical form. All adverse event data will be listed individually and summarized using MedDRA terminology.

6.4.5 Determination of sample size

According to published data 12 healthy male healthy volunteers received a single oral 500 mg dose of tolbutamide (Jorga KM, et al. 2000). The inter-subject coefficients of variation (CV) for C_{max} and AUC for these healthy volunteers were estimated to be 27.1% and 33.2%, respectively. Assuming similar variability and an intra-subject correlation of 0.65 (resulting in within-subject CV estimates for C_{max} and AUC of 16% and 20%, respectively), a sample size of 18 healthy volunteers will provide approximately 90% power that the 90% confidence interval for the ratios of interest (C_{max} or AUC) of tolbutamide administered with AZD6140 to that of tolbutamide alone) will be completely contained within the pre-specified equivalence range of 0.80 to 1.25. These calculations are based on a two one-sided testing procedure at an alpha level of 0.05, assuming a true ratio of 1.0. No adjustments for pre-planned multiple comparisons were made.

6.5 Interim analyses (Not applicable)

6.6 Data monitoring committee (Not applicable)

7. ETHICS

7.1 Ethics review

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

The Principal Investigator 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 re-approved by the IRB annually, as local regulations require.

The Principal Investigator is also responsible for providing the IRB with reports of any serious and unexpected adverse drug reactions from any other study conducted with the

investigational product. AstraZeneca or delegate will provide this information to the Principal Investigator.

Progress reports and notifications of serious and unexpected adverse drug reactions will be provided to the IRB according to local regulations and guidelines.

Where there is a genetic research, approval must be obtained for this genetic research and the associated genetic informed consent from the IRB. It must be clearly stated in the approval that this genetic research is approved. The investigator must submit written approval to AstraZeneca or delegate before any subject participates in this genetic research.

7.2 Ethical conduct of the study

The study will be performed in the spirit of the Declaration of Helsinki and are consistent with Good Clinical Practice, applicable regulatory requirements 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 Informed Consent

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

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

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

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

The genetic research is optional and the subject may participate in the main study without participating in the genetic component. To participate in the genetic component of the study the subject must sign and date both the consent form for the main study (non-genetic components of the study) and the genetic component of the study. Copies of both signed and dated consent forms must be given to the subject and the original filed at the study centre. The principal investigator(s) is responsible for ensuring that consent is given freely and that the subject understands that they may freely discontinue the genetic aspect of the study at any time.

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

7.4 Subject data protection

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

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

The Master Informed Consent Form will also explain that for data verification purposes, authorised representatives of AstraZeneca or delegate, 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.

All data protection and confidentiality principles, described in the main study protocol, are applicable to this genetic research. Reference to participation in this genetic research should not be recorded into the subjects' general medical records. All notes should be kept within the clinical study records.

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

Extra precautions are taken to preserve confidentiality and prevent genetic data being linked to the identity of the subject, however, it must be recognised that there are exceptional circumstances where individuals may see both genetic data and a subject's personal identifier, for example in the case of a medical emergency, when AstraZeneca Physicians and investigators might know the subjects' identity and might have access to the genetic data, or during regulatory audit where designated authorities must be permitted access to the relevant files.

8. PROCEDURES IN CASE OF EMERGENCY, OVERDOSE OR PREGNANCY

8.1 Emergency contact procedure

This information has been placed in a separate document titled, "Supplement 2: Study Delivery Team Contacts in the Event of Emergency".

8.2 Procedures in case of medical emergency

The principal investigator(s) is responsible for ensuring that procedures and expertise are available to cope with medical emergencies during the study. **A medical emergency usually constitutes an SAE and should be reported as such, see Section 4.6.1.3.**

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 haematological effects is essential. Appropriate standard supportive therapy should be initiated. Since there is no specific antidote to this compound, healthy volunteers should be treated symptomatically. For further information see the Investigator's Brochure.

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

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

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

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

8.4 Procedures in case of pregnancy

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

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

9. REFERENCES

Jorga KM, et al. Lack of interaction between tolcapone and tolbutamide in healthy volunteers. J Clin Pharmacol 2000; 40:544-551.

Clinical Pharmacology Study Protocol: Appendix B

Drug Substance	AZD6140
Study Code	D5130C00051
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Appendix B
Additional Safety Information

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

Life threatening

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

Hospitalisation

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

Important medical event or medical intervention

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

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

Examples of such events are:

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

A GUIDE TO INTERPRETING THE CAUSALITY QUESTION

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

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

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

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

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

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

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

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

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

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

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

- Is there a known mechanism?

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

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Clinical Pharmacology Study Protocol: Appendix C	
Drug Substance	AZD6140
Study Code	D5130C00051
Appendix Edition Number	1.0
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Appendix C
Tolbutamide Product Information

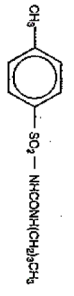
1. PACKAGE INSERT

TOLBUTAMIDE TABLETS, USP

500 mg

℞ only

Description: Tolbutamide is an oral blood-glucose-lowering drug of the sulfonylurea class. Tolbutamide is a pure, white, crystalline compound which is practically insoluble in water. The chemical name is benzenesulfonamide, N-[(butylamino)-carbonyl]-4-methyl-. its structure can be represented as follows:



M.W. 270.35 $C_{12}H_{18}N_2O_3S$

Tolbutamide is supplied as compressed tablets containing 500 mg of tolbutamide, USP.

Each tablet for oral administration contains 500 mg of tolbutamide and the following inactive ingredients: colloidal silicon dioxide, magnesium stearate, microcrystalline cellulose, sodium lauryl sulfate and sodium starch glycolate.

CLINICAL PHARMACOLOGY:

Actions: Tolbutamide appears to lower the blood glucose acutely by stimulating the release of insulin from the pancreas, an effect dependent upon functioning beta cells in the pancreatic islets. The mechanism by which tolbutamide lowers blood glucose during long-term administration has not been clearly established. With chronic administration in Type II diabetic patients, the blood-glucose-lowering effect persists despite a gradual decline in the insulin secretory response to the drug. Extrapancreatic effects may be involved in the mechanism of action of oral sulfonylurea hypoglycemic drugs.

Some patients who are initially responsive to oral hypoglycemic drugs, including tolbutamide, may become unresponsive or poorly responsive over time. Alternatively, tolbutamide may be effective in some patients who have become unresponsive to one or more of the other sulfonylurea drugs.

Pharmacokinetics: When administered orally, tolbutamide is readily absorbed from the gastrointestinal tract. Absorption is not impaired and glucose lowering and insulin releasing effects are not altered if the drug is taken with food. Detectable levels are present in the plasma within twenty minutes after oral ingestion of a 500 mg tolbutamide tablet, with peak levels occurring at three to four hours and only small amounts detectable at 24 hours. The half-life of tolbutamide is 4.5 to 6.5 hours. As tolbutamide has no p-amino group, it cannot be acetylated, which is one of the common modes of metabolic degradation for the antibacterial sulfonamides. However, the presence of the p-methyl group renders tolbutamide susceptible to oxidation, and this appears to be the principal manner of its metabolic degradation in man. The p-methyl group is oxidized to form a carboxyl group, converting tolbutamide into the totally inactive metabolite 1-butyl-3-p-carboxy-phenylsulfonylurea, which can be recovered in the urine within 24 hours in amounts accounting for up to 75% of the administered dose.

The major tolbutamide metabolite has been found to have no hypoglycemic or other action when administered orally and IV to both normal and diabetic subjects. This tolbutamide metabolite is highly soluble over the critical acid range of urinary pH values, and its solubility increases with increase in pH. Because of the masked solubility of the tolbutamide metabolite, crystalluria does not occur. A second metabolite, 1-butyl-3-(p-hydroxymethyl) phenyl sulfonylurea also occurs to a limited extent. It is an inactive metabolite.

The administration of 3 grams of tolbutamide to either nondiabetic or tolbutamide-responsive diabetic subjects will, in both instances, occasion a gradual lowering of blood glucose. Increasing the dose to 6 grams does not usually cause a response which is significantly different from that produced by the 3 gram dose. Following the administration of a 3 gram dose of tolbutamide solution, non-diabetic fasting adults exhibit a 30% or greater reduction in blood glucose within one hour, following which the blood glucose gradually returns to the fasting level over six to twelve hours. Following the administration of a 3 gram dose of tolbutamide solution, tolbutamide responsive diabetic patients show a gradually progressive blood glucose lowering effect, the maximal response being reached between five to eight hours after ingestion of a single 3 gram dose. The blood glucose then rises gradually and by the 24th hour has usually returned to pretest levels. The magnitude of the reduction, when expressed in terms of percent of the pretest blood glucose, tends to be similar to the response seen in the nondiabetic subject.

INDICATIONS AND USAGE: Tolbutamide is indicated as an adjunct to diet to lower the blood glucose in patients with non-insulin-dependent diabetes mellitus (type II) whose hyperglycemia cannot be controlled by diet alone.

In initiating treatment for non-insulin-dependent diabetes, diet should be emphasized as the primary form of treatment. Caloric restriction and weight loss are essential in the obese diabetic patient. Proper dietary management alone may be effective in controlling the blood glucose and symptoms of hyperglycemia. The importance of regular physical activity should also be stressed, and cardiovascular risk factors should be identified and corrective measures taken where possible.

If this treatment program fails to reduce symptoms and/or blood glucose, the use of an oral sulfonylurea or insulin should be considered. Use of tolbutamide must be viewed by both the physician and patient as a treatment in addition to diet, and not as a substitute for diet or as a convenient mechanism for avoiding dietary restraint. Furthermore, loss of blood glucose control on diet alone may be transient, thus requiring only short-term administration of tolbutamide.

During maintenance programs, tolbutamide should be discontinued if satisfactory lowering of blood glucose is no longer achieved. Judgments should be based on regular clinical and laboratory evaluations.

In considering the use of tolbutamide in asymptomatic patients, it should be recognized that controlling the blood glucose in non-insulin dependent diabetes has not been definitely established to be effective in preventing the long-term cardiovascular or neural complications of diabetes.

CONTRAINDICATIONS: Tolbutamide is contraindicated in patients with:

1. Known hypersensitivity or allergy to the drug.
2. Diabetic ketoacidosis, with or without coma. This condition should be treated with insulin.
3. Type I diabetes, as sole therapy.

WARNINGS: SPECIAL WARNING ON INCREASED RISK OF CARDIOVASCULAR MORTALITY-

The administration of oral hypoglycemic drugs has been reported to be associated with increased cardiovascular mortality as compared to treatment with diet alone or diet plus insulin. This warning is based on the study conducted by the University Group Diabetes Program (UGDP), a long-term prospective clinical trial designed to evaluate the effectiveness of glucose-lowering drugs in preventing or delaying vascular complications in patients with non-insulin-dependent diabetes. The study involved 823 patients who were randomly assigned to one of four treatment groups (Diabetes, 19 (supp.2):747-830, 1970).

UGDP reported that patients treated for 5 to 8 years with diet plus a fixed dose of tolbutamide (1.5 grams per day) had a rate of cardiovascular mortality approximately 2 1/2 times that of patients treated with diet alone. A significant increase in total mortality was not observed, but the use of tolbutamide was discontinued based on the increase in cardiovascular mortality, thus limiting the opportunity for the study to show an increase in overall mortality. Despite controversy regarding the interpretation of these results, the findings of the UGDP study provide an adequate basis for this warning. The patient should be informed of the potential risks and advantages of tolbutamide and of alternative modes of therapy. Although only one drug in the sulfonylurea class (tolbutamide) was included in this study, it is prudent from a safety standpoint to consider that this warning may also apply to other oral hypoglycemic drugs in this class, in view of their close similarities in mode of action and chemical structure.

PRECAUTIONS: General: Hypoglycemia: All sulfonylurea drugs are capable of producing severe hypoglycemia. Proper patient selection, dosage, and instructions are important to avoid hypoglycemic episodes. Renal or hepatic insufficiency may cause elevated blood levels of tolbutamide and the latter may also diminish gluconeogenic capacity, both of which increase the risk of serious hypoglycemic reactions. Elderly, debilitated or malnourished patients, and those with adrenal or pituitary insufficiency are particularly susceptible to the hypoglycemic action of glucose-lowering drugs. Hypoglycemia may be difficult to recognize in the elderly, and in people who are taking beta-adrenergic blocking drugs. Hypoglycemia is more likely to occur when caloric intake is deficient, after severe or prolonged exercise, when alcohol is ingested, or when more than one glucose-lowering drug is used.

Loss of control of blood glucose: When a patient stabilized on any diabetic regimen is exposed to stress such as fever, trauma, infection, or surgery, a loss of control may occur. At such times, it may be necessary to discontinue tolbutamide and administer insulin.

The effectiveness of any oral hypoglycemic drug, including tolbutamide, in lowering blood glucose to a desired level decreases in many patients over a period of time, which may be due to progression of the severity of the diabetes or to diminished responsiveness to the drug. This phenomenon is known as a secondary failure, to distinguish it from primary failure in which the drug is ineffective in an individual patient when first given. Adequate adjustment of dose and adherence to diet should be assessed before classifying a patient as a secondary failure.

Information For Patients: Patients should be informed of the potential risks and advantages of tolbutamide and of alternative modes of therapy. They should also be informed about the importance of adherence to dietary instructions, of a regular exercise program, and of regular testing of urine and/or blood glucose.

The risks of hypoglycemia, its symptoms and treatment, and conditions that predispose to its development should be explained to patients and responsible family members. Primary and secondary failure should also be explained.

Laboratory Tests: Blood and urine glucose should be monitored periodically. Measurement of glycosylated hemoglobin may be useful.

A metabolite of tolbutamide in urine may give a false positive reaction for albumin if measured by the acidification-after-boiling test, which causes the metabolite to precipitate. There is no interference with the sulfosalicylic acid test.

Drug Interactions: The hypoglycemia action of sulfonylurea may be potentiated by certain drugs including nonsteroidal anti-flammatory agents and other drugs that are highly protein bound, salicylates, sulfonamides, chloramphenicol, probenecid, coumarins, monoamine oxidase inhibitors, and beta adrenergic blocking agents. When such drugs are administered to a patient receiving tolbutamide, the patient should be observed closely for hypoglycemia. When such drugs are withdrawn from a patient receiving tolbutamide, the patient should be observed closely for loss of control.

Certain drugs tend to produce hyperglycemia and may lead to

loss of control. These drugs include the thiazides and other diuretics, corticosteroids, phenothiazines, thyroid products, estrogens, oral contraceptives, phenytoin, nicotinic acid, sympathomimetics, calcium channel blocking drugs, and isoniazid. When such drugs are administered to a patient receiving tolbutamide, the patient should be closely observed for loss of control. When such drugs are withdrawn from a patient receiving tolbutamide, the patient should be observed closely for hypoglycemia.

A potential interaction between oral miconazole and oral hypoglycemic agents leading to severe hypoglycemia has been reported. Whether this interaction also occurs with the intravenous, topical or vaginal preparations of miconazole is not known.

Carcinogenicity and Mutagenicity: Bioassay for carcinogenicity was performed in both sexes of rats and mice following ingestion of tolbutamide for 78 weeks. No evidence of carcinogenicity was found.

Tolbutamide has also been demonstrated to be nonmutagenic in the Ames salmonella/mammalian microsome mutagenicity test.

Pregnancy: ***Teratogenic Effects:*** Pregnancy Category C. Tolbutamide has been shown to be teratogenic in rats when given in doses 25 to 100 times the human dose. In some studies, pregnant rats given high doses of tolbutamide have shown ocular and bony abnormalities and increased mortality in offspring. Repeat studies in other species (rabbits) have not demonstrated a teratogenic effect. There are no adequate and well controlled studies in pregnant women. Tolbutamide is not recommended for the treatment of pregnant diabetic patients.

Serious consideration should also be given to the possible hazards of the use of tolbutamide in women of childbearing age and in those who might become pregnant while using the drug.

Because recent information suggests that abnormal blood glucose levels during pregnancy are associated with a higher incidence of congenital abnormalities, many experts recommend that insulin be used during pregnancy to maintain blood glucose levels as close to normal as possible.

Nonteratogenic Effects: Prolonged severe hypoglycemia (4 to 10 days) has been reported in neonates born to mothers who were receiving a sulfonylurea drug at the time of delivery. This has been reported more frequently with the use of agents with prolonged half-lives. If tolbutamide is used during pregnancy, it should be discontinued at least two weeks before the expected delivery date.

Nursing Mothers: Although it is not known whether tolbutamide is excreted in human milk, some sulfonylurea drugs are known to be excreted in human milk. Because the potential for hypoglycemia in nursing infants may exist, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother. If the drug is discontinued and if diet alone is inadequate for controlling blood glucose, insulin therapy should be considered.

Pediatric Use: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS: Hypoglycemia: See PRECAUTIONS and OVERDOSAGE sections.

Gastrointestinal Reactions: Cholestatic jaundice may occur rarely; tolbutamide should be discontinued if this occurs. Gastrointestinal disturbances, e.g., nausea, epigastric fullness, and heartburn, are the most common reactions and occur in 1.4% of patients treated during clinical trial. They tend to be dose-related and may disappear when dosage is reduced.

Dermatologic Reactions: Allergic skin reactions, e.g., pruritus, erythema, urticaria, and morbilliform or maculopapular eruptions, occur in 1.1% of patients treated during clinical trials. These may be transient and may disappear despite continued use of tolbutamide; if skin reactions persist, the drug should be discontinued.

Porphyria cutanea tarda and photosensitivity reactions have been reported with sulfonylureas.

Hematologic Reactions: Leukopenia, agranulocytosis, thrombocytopenia, hemolytic anemia, aplastic anemia, and pancytopenia have been reported with sulfonylureas.

Metabolic Reactions: Hepatic porphyria and disulfiram-like reactions have been reported with sulfonylureas.

Endocrine Reactions: Cases of hyponatremia and the syndrome of inappropriate antidiuretic hormone (SIADH) secretion have been reported with this and other sulfonylureas.

Miscellaneous Reactions: Headache and taste alterations have occasionally been reported with tolbutamide administration.

OVERDOSAGE: Overdosage of sulfonylureas including tolbutamide can produce hypoglycemia. Mild hypoglycemic symptoms without loss of consciousness or neurologic findings should be treated aggressively with oral glucose and adjustments in drug dosage and/or meal patterns. Close monitoring should continue until the physician is assured that the patient is out of danger. Severe hypoglycemic reactions with coma, seizure, or other neurological impairment occur infrequently, but constitute medical emergencies requiring immediate hospitalization. If hypoglycemic coma is diagnosed or suspected, the patient should be given a rapid intravenous injection of concentrated (50%) dextrose injection. This should be followed by a continuous infusion of a more dilute (10%) dextrose injection at a rate that will maintain the blood glucose at a level above 100 mg/dL. Patients should be closely monitored for a minimum of 24 to 48 hours since hypoglycemia may recur after apparent clinical recovery.

DOSAGE AND ADMINISTRATION: There is no fixed dosage regimen for the management of diabetes mellitus with tolbutamide or any other hypoglycemic agent. In addition to the usual monitoring of urinary glucose, the patient's blood glucose must also be monitored periodically to determine the minimum effective dose for the patient; to detect primary failure, i.e., inadequate lowering of blood glucose at the maximum recommended dose of medication; and to detect secondary failure, i.e., loss of an adequate blood glucose lowering response after an initial period of effectiveness. Glycosylated hemoglobin levels may also be of value in monitoring the patient's response to therapy.

Short-term administration of tolbutamide may be sufficient during periods of transient loss of control in patients usually controlled well on diet.

Usual Starting Dose: The usual starting dose is 1 to 2 grams daily. This may be increased or decreased, depending on individual patient response. Failure to follow an appropriate dosage regimen may precipitate hypoglycemia. Patients who do not adhere to their prescribed dietary regimens are more prone to exhibit unsatisfactory response to drug therapy.

Transfer From Other Hypoglycemic Therapy: ***Patients Receiving Other Antidiabetic Therapy*** Transfer of patients from other oral antidiabetes regimens to tolbutamide should be done conservatively. When transferring patients from oral hypoglycemic agents other than chlorpropamide to tolbutamide, no transition period and no initial or priming doses are necessary. When transferring patients from chlorpropamide, however, particular care should be exercised during the first two weeks because of the prolonged retention of chlorpropamide in the body and the possibility that subsequent overlapping drug effects might provoke hypoglycemia.

Patients Receiving Insulin: Patients requiring 20 units or less of insulin daily may be placed directly on tolbutamide and insulin abruptly discontinued. Patients whose insulin requirement is between 20 and 40 units daily may be started on therapy with tolbutamide with a concurrent 30 to 50% reduction in insulin dose, with further daily reduction of the insulin when response to tolbutamide is observed. In patients requiring more than 40 units of insulin daily, therapy with tolbutamide may be initiated in conjunction with a 20% reduction in insulin dose the first day, with further careful reduction of insulin as response is observed. Occasionally, conversion to tolbutamide in the hospital may be advisable in candidates who require more than 40 units of insulin daily. During this conversion period when both insulin and tolbutamide are being used hypoglycemia may rarely occur. During insulin withdrawal, patients should test their urine for glucose and acetone at least three times daily and report results to their physician. The appearance of persistent acetonuria with glycosuria indicates that the patient is type I diabetic who requires insulin therapy.

Maximum Dose: Daily doses of greater than 3 grams are not recommended.

Usual Maintenance Dose: The maintenance dose is in the range of 0.25 - 3 grams daily. Maintenance doses above 2 grams are seldom required.

Dosage Interval: The total daily dose may be taken either in the morning or in divided doses through the day. While either schedule is usually effective, the divided dose system is preferred by some clinicians from the standpoint of digestive tolerance.

In elderly patients, debilitated or malnourished patients, and patients with impaired renal or hepatic function, the initial and maintenance dosing should be conservative to avoid hypoglycemic reactions (see PRECAUTIONS section).

HOW SUPPLIED: Tolbutamide tablets, USP are available containing 500 mg of tolbutamide. The tablets are white to off-white, scored, round tablets marked with **M13**. They are available as follows:

NDC 0378-0215-01
bottles of 100 tablets

NDC 0378-0215-05
bottles of 500 tablets

Store at 20° to 25°C (68° to 77°F). [See USP for Controlled Room Temperature.]

Protect from light.

Dispense in a tight, light-resistant container as defined in the USP using a child-resistant closure.

MYLAN®

Mylan Pharmaceuticals Inc.
Morgantown, WV 26505

REVISED JANUARY 2006
TOLB:R13

Clinical Pharmacology Study Protocol: Appendix D

Drug Substance	AZD6140
Study Code	D5130D00051
Appendix Edition Number	1.0
Appendix Date	

Appendix D
WHO Risk Categories

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

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

AstraZeneca

Clinical Pharmacology Study Protocol: Appendix E	
Drug Substance	AZD6140
Study Code	D5130C00051
Appendix Edition Number	2.0
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Appendix E
Instructions for Blood Collection, Storage and Transport in Clinical Genetics Studies

1. Blood Sample Collection

Ideally, blood should be collected into ***9/10ml polypropylene tubes*** containing the ***anticoagulant EDTA***. Recommended tubes are detailed in the table below. **Part numbers may vary between countries. Please check tubes are suitable before ordering them**. After collection, blood tubes must be gently ***inverted*** several times to ensure thorough mixing of EDTA with the sample to prevent clotting.

Polypropylene Collection Tube	Part #	Comments
	1066 US 1066.001 UK	SARSTEDT Monovette® EDTA KE - 9ml
	367525 USA/UK	Becton-Dickinson Vacutainer™ K2E - 10ml
	455036 USA/UK	Greiner Bio-one Vacuette® K3E EDTA K3 - 9ml

Table of recommended blood tubes for genotyping sample collection

- ***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 labelled with the following information:

- Unique sample ID (compliant with protocol)
- Study ID (& Study Centre ID, if available)
- Date of sample collection

DNA Processing Laboratories have encountered scanning incompatibility due to inclusion of hidden digits in barcode labels. If barcode labels are to be used, a sample will be requested at a later date. All labels must be freezer-proof.

2. STORAGE AT THE STUDY CENTRE AND TRANSPORT

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

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

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

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

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

- Study ID, number of samples & list of sample ID's
- Courier name, airway bill number & date of shipment
- Shipment condition (wet ice or dry ice)
- Contact name & address

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

3. RECOMMENDED PACKAGING INSTRUCTIONS

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

Sample Shipment.

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

For samples transported on wet ice:

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

For samples transported on dry ice:

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

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

4. STORAGE AT THE CENTRAL HANDLING FACILITY OR DESIGNATED DNA PROCESSING LABORATORY

i. Central Handling Facility (short term storage)

Blood/EDTA samples can be stored temporarily at the Central Handling Facility and subsequently transported to the Designated Processing Laboratory following the guidelines below:

Storage Temperature at Central Handling Facility	Maximum Duration	Transport Temperature	Delivery Time
-20°C (freezer) or -70°C	6 months	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***

ii. Designated DNA Processing Laboratory (final destination)

ON ARRIVAL AT THE DESIGNATED DNA PROCESSING LABORATORY, BLOOD/EDTA SAMPLES SHOULD BE STORED AT -20°C OR -70°C (FREEZER) UNTIL THEY ARE PROCESSED.
IDEALLY, SAMPLES SHOULD BE PROCESSED WITHIN 6 MONTHS OF COLLECTION.