AstraZeneca

Revised Clinical Study Protocol	
Drug Substance	AZD6244
Study Code	D1532C00066
Edition Number	1
Date	

A Phase I, Single-center, Randomized, Open-label, Crossover Study to Compare the White (Current Phase II) and Blue (Planned Phase III) Capsule Formulations of AZD6244 Hyd-Sulfate in Healthy Male Subjects

Sponsor:

AstraZeneca Research and Development
site representative

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

Amendment No.	Date of Amendment	Local Amendment No.	Date of local Amendment
1	17 July 2012		

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

AstraZeneca

PROTOCOL SYNOPSIS

A Phase I, Single-center, Randomized, Open-label, Crossover Study to Compare the White (Current Phase II) and Blue (Planned Phase III) Capsule Formulations of AZD6244 Hyd-Sulfate in Healthy Male Subjects

Principal Investigator

Study center and number of subjects planned

This study will be conducted at a single study center in the United States. Up to 30 (5 per sequence) healthy volunteers will be enrolled in order to ensure 24 complete this study.

Study period		Phase of development
Estimated date of first subject enrolled	Q2 2012	I
Estimated date of last subject completed	Q3 2012	

Objectives

Primary objective

The primary objective of this study is to compare the pharmacokinetics of AZD6244 after oral administration of single doses of white (current Phase II) and blue (planned Phase III) capsule formulations.

Secondary objectives

The secondary objectives of this study are to:

- Investigate the safety and tolerability of single doses of AZD6244 in healthy male volunteers
- Estimate within-volunteer variability in the pharmacokinetics of AZD6244

- Establish the relative bioavailability of AZD6244 after oral administration of single doses of blue capsules (planned Phase III) with respect to an oral solution formulation

- Characterize the pharmacokinetics of *N*-desmethyl AZD6244 after oral administration of single doses of AZD6244 white (current Phase II) and blue (planned Phase III) capsules

Exploratory objectives

The exploratory objectives of this study are to:

- Characterize the pharmacokinetics of AZD6244 amide metabolite after an oral administration of single doses of AZD6244 white and blue capsules

- Collect and store deoxyribonucleic acid for future exploratory research into genes/genetic variation that may influence response (ie, distribution, safety, tolerability, and efficacy) of AZD6244 (optional part of the study and, if performed, will be reported separately from the Clinical Study Report)

Study design

This is an open-label, randomized, 3-treatment, 4-period, 6-sequence crossover study conducted at a single study center to compare the pharmacokinetics of AZD6244 after oral administration of single doses of white (current Phase II) and blue (planned Phase III) capsule formulation. Volunteers will receive 1 single oral dose each of AZD6244 of Treatment A and Treatment C and 2 doses of Treatment B each separated by washout periods of a minimum of 7 days between doses.

- Treatment A will consist of three 25-mg AZD6244 white (current Phase II) capsules.

- Treatment B will consist of three 25-mg AZD6244 blue (planned Phase III) capsules.

- Treatment C will consist of 35 mg AZD6244 solution.

Serial blood samples for the determination of AZD6244 pharmacokinetics will be collected up to 36 hours following the AZD6244 dose. Volunteers will be admitted to the study center on Day -1 and will remain resident until completion of Day 2 procedures for each treatment period. A washout period of at least 7 days will occur between each AZD6244 administration. A follow-up visit will be conducted at least 7 days following study center discharge of the volunteer's fourth and final treatment.

Target subject population

Healthy male volunteers between the ages of 18 and 55 years, inclusive, with a minimum weight of 50 kg and a body mass index between 18 and 30 kg/m^2, inclusive, are eligible for

study participation. Up to 30 healthy volunteers will be enrolled in order to ensure 24 volunteers complete this study.

Investigational product, dosage, and mode of administration

This study will consist of 4 single oral doses of AZD6244. Volunteers will receive Treatment A and Treatment C on 1 occasion and Treatment B on 2 occasions. Treatment A will consist of three 25-mg AZD6244 white (current Phase II) capsules. Treatment B will consist of three 25-mg AZD6244 blue (planned Phase III) capsules. Treatment C will consist of 35 mg AZD6244 solution.

Comparator, dosage, and mode of administration

Not applicable.

Duration of treatment

The duration of the study for each volunteer will be approximately 67 days, including a screening period (Visit 1) of 28 days or less, 4 residential periods (Visits 2, 3, 4, and 5) of 3 days (from check-in on Day -1 until discharge on Day 2), and at least a 7-day washout period will occur between each treatment, and a follow-up visit (Visit 6) at least 7 days after the last dose.

Outcome variables:

- Pharmacokinetics

  The following pharmacokinetic parameters will be calculated (if estimable) from AZD6244 plasma concentration during each period: area under the plasma concentration-time curve from time zero extrapolated to infinity (AUC), maximum observed plasma concentration (C_{max}), time to C_{max} (t_{max}), area under the plasma concentration-time curve from time zero to the time of the last quantifiable concentration [$AUC_{(0-t)}$], area under the plasma concentration-time curve from time zero to 12 hours postdose [$AUC_{(0-12)}$], apparent terminal rate constant (λ_z), apparent terminal half-life ($t_{1/2}$), apparent oral clearance from plasma (CL/F), apparent volume of distribution (V_z/F), and apparent volume at distribution equilibrium (V_{ss}/F).

  The following pharmacokinetic parameters will be calculated (if estimable) *N*-desmethyl AZD6244 and AZD6244 amide metabolites plasma concentration during each period: AUC, C_{max}, t_{max}, $AUC_{(0-t)}$, $AUC_{(0-12)}$, λ_z, and $t_{½}$.

- Safety

  Safety will be assessed based on adverse events, clinical laboratory assessments (clinical chemistry, hematology, and urinalysis), vital signs (blood pressure and pulse rate), physical examinations, digital and paper electrocardiograms, left ventricular ejection fraction, and ophthalmology assessments.

Statistical methods

The primary pharmacokinetic parameters are AUC and C_{max} of AZD6244. If AUC is not reportable in more than 60% of the 30 enrolled volunteers (ie, 18 reportable AUCs) then $AUC_{(0-t)}$ will be used in the inferential analysis.

Utilizing data from the first 3 treatment periods only, the relative bioavailability between treatments will be assessed between test (Treatment B) and reference (Treatments A) treatments. Analyses will be performed using a linear mixed-effect analysis of variance model using the logarithm of AUC and C_{max}. Transformed back from the logarithmic scale, geometric least-squares means together with confidence intervals (2-sided 95%) for AUC and C_{max} will be estimated and presented. Also, ratios of geometric least-squares means together with confidence intervals (2-sided 90%) will be estimated and presented. Using a similar model as above, comparisons will be made for dose-normalized AUC and C_{max} between test (Treatment B) and reference (Treatments C) treatment. AUC and C_{max} will be dose-normalized prior to the ln-transformation.

Using Treatment B data only, estimates of between- and within-volunteer variability will be obtained by employing a linear mixed-effect analysis of variance model using the logarithm of AUC and C_{max}. Estimates for the between- and within-volunteer variability will be present together with the associated 90% confidence intervals.

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

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

Abbreviation or special term	Explanation
AE	Adverse event (see definition in Section 6.3.1)
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
%AUCex	Percentage of AUC obtained by extrapolation
AUC	Area under the plasma concentration-time curve from time zero to infinity
$AUC_{(0-t)}$	Area under the plasma concentration-time curve from time zero to the time of the last quantifiable concentration
$AUC_{(0-12)}$	Area under the plasma concentration-time curve from time zero to the time of 12 hours
BRAF	v-raf murine sarcoma viral oncogene homolog B1
BLQ	Below the Lower Limit of Quantitation
BMI	Body mass index
BNP	Brain natriuretic peptide
CI	Confidence interval
CK-MB	Creatine kinase myocardial band
CL/F	Apparent oral plasma clearance
CPA	Clinical Pharmacology Alliance
CPK	Total creatine phosphokinase
C_{max}	Maximum plasma concentration
CRCL	Creatinine clearance
CSA	Clinical Study Agreement
CSP	Clinical Study Protocol
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Event
CV	Coefficient of variation
DAE	Discontinuations due to AE
dECG	Digital electrocardiogram
DNA	Deoxyribonucleic acid

Abbreviation or special term	Explanation
ECG	Electrocardiogram
EClysis©	User-interactive, modular computer-based system for dECG data processing, analysis, and measurement of ECG intervals and wave amplitudes, exports, and reports, used by the AstraZeneca ECG Centre
eCRF	Electronic Case Report Form
GCP	Good Clinical Practice
GLS	Geometric least-square
HIV	Human immunodeficiency virus
HR	Heart rate
ICH	International Conference on Harmonization
IP	Investigational product
KRAS	v-Ki-ras2 Kirsten rat sarcoma vital oncongene homolog
LLOQ	Lower Limit of Quantitation
LVEF	Left ventricular ejection fraction
MedDRA	Medical Dictionary for Regulatory Activities
N	Number of data points
n	Number of observations
NA	Not applicable
NC	Not calculable
NSCLC	Nonsmall cell lung cancer
OAE	Other Significant Adverse Event (see definition in Section 11.1.2)
pECG	Paper electrocardiogram
PK	Pharmacokinetic(s)
PR(PQ)	Electrocardiogram interval measured from the onset of the P wave to the onset of the QRS complex
QRS	Electrocardiogram interval measured from the onset of the QRS complex to the J point
QT	Electrocardiogram interval measured from the onset of the QRS complex to the end of T wave
QTcF	QT interval corrected for heart rate using Fridericia's formula
RR	The time between corresponding points on 2 consecutive R waves on ECG
Rsq	Coefficient of determination
SAE	Serious adverse event (see definition in Section 6.3.2).

Abbreviation or special term	Explanation
SD	Standard deviation
SOP	Standard Operating Procedure
$t_{1/2}$	Terminal half-life
TBL	Total bilirubin
t_{max}	Time to C_{max}
TPGS	D-α-tocopherol polyethylene glycol 1000 succinate
ULN	Upper limit of normal
V_{ss}/F	Apparent volume at distribution equilibrium
V_z/F	Apparent volume of distribution

1. INTRODUCTION

Selumetinib is a potent, selective, uncompetitive inhibitor of mitogen-activated protein kinase, licensed for development by AstraZeneca Pharmaceuticals from Array BioPharma. Array BioPharma was responsible for the first-administration-into-man study. The remainder of the clinical development program for oncology indications is the responsibility of AstraZeneca. Phase II monotherapy studies commenced in 2006.

Note, Selumetinib and AZD6244 Hyd-sulfate are one in the same and will be referred to as AZD6244 throughout this protocol.

1.1 Background

1.1.1 Nonclinical experience with AZD6244

Nonclinical experience with AZD6244 is described in the current version of the AZD6244 Investigator's Brochure. Nonclinical experience with AZD6244 in lung cancer models and in combination with docetaxel is summarized below.

AZD6244 had strong antitumor activity in multiple nonclinical models, including human lung cancer tumors (A549). AZD6244 has demonstrated potent inhibition of v-raf murine sarcoma viral oncogene homolog B1 (BRAF) or v-Ki-ras2 Kirsten rat sarcoma vital oncongene homolog (KRAS) positive cell line viability and inhibition of xenograft growth both as monotherapy and in combination with a number of cytotoxic and targeted agents, including docetaxel. In 3 xenograft models of a *KRAS* positive tumor (SW620 colorectal cancer, HCT-116 colorectal cancer, and A549a nonsmall cell lung cancer [NSCLC]), a beneficial effect of the combination of AZD6244 with cytotoxic drugs (eg, docetaxel and temozolomide) or targeted agents (eg, gefitinib) was observed when compared with either agent as monotherapy.

1.1.2 Clinical experience with AZD6244

Clinical experience with AZD6244 as monotherapy and in combination with other anticancer agents is described in the current version of the AZD6244 Investigator's Brochure. Clinical experience with AZD6244 in combination with docetaxel is summarized below.

The combination of AZD6244 and docetaxel (75 mg/m^2 every 21 days) has been investigated in a Phase I study (D1532C00004) in 35 non-Asian patients with advanced solid tumors (Kim et al 2011, EORTC-AACR abstract). Combination therapy did not appear to affect the pharmacokinetics (PK) of AZD6244 or docetaxel. The tolerability profile of the combination treatment was largely consistent with AZD6244 or docetaxel administered as monotherapy: the most common adverse events (AEs) were peripheral edema (71%), diarrhea (69%), fatigue (63%), nausea (49%), vomiting (46%), neutropenia (43%), and dermatitis acneiform (40%). The most common Grade 3 or greater AEs were hematological events (51%), infections (26%), fatigue/asthenia (23%), peripheral edema (10%), and gastrointestinal events (10%). Dose-limiting toxicities of neutropenia and febrile neutropenia were reported, and AZD6244

75-mg twice daily with docetaxel 75 mg/m^2 (without granulocyte colony-stimulating factor primary prophylaxis) was the recommended Phase II dose. Clinical responses were observed in 5/28 patients (18%) receiving AZD6244 75-mg twice daily with docetaxel.

AZD6244 75-mg twice daily with docetaxel (75 mg/m^2 every 21 days) has been investigated as second-line treatment for non-Asian patients with *KRAS* mutation-positive locally advanced or metastatic NSCLC in a randomized double-blind Phase II study (D1532C00016). A numerically greater increase in overall survival (not statistically significant) was reported in patients receiving AZD6244 plus docetaxel compared with those receiving placebo plus docetaxel, and statistically significant improvements in favor of AZD6244 were observed for the secondary endpoints of progression-free survival, objective response rate and patients alive, and progression free at 6 months.

The most common AEs in patients receiving AZD6244 plus docetaxel were consistent with the monotherapy profiles of each agent: diarrhea (72.7%), infections (50%), nausea (43.2%), vomiting (43.2%), peripheral edema (40.9%), rash (mainly dermatitis acneiform, 38.6%), and stomatitis (oral mucositis, 36.4%). Neutrophil counts below the lower limit of normal were reported in similar proportions of patients in each treatment group (86.1% versus 78.6%). Higher incidences of Common Terminology Criteria for AE (CTCAE) Grade 4 low neutrophil counts (44.2% versus 23.8%), febrile neutropenia (15.9% versus 0%), and CTCAE Grade 3 infections (20.5% versus 2.4%) were reported in patients receiving AZD6244 plus docetaxel compared with those receiving placebo plus docetaxel. The incidence of deaths due to AEs was low and similar between treatment groups (9.1% versus 7.1%). Common Terminology Criteria AE Grade 5 events were respiratory failure and pneumonia in the AZD6244 plus docetaxel group (none considered related to AZD6244 by the Investigator), and respiratory failure plus pneumonitis and cardiac arrest in the placebo plus docetaxel group.

Details of the safety profile in monotherapy are presented in Section 1.3.2.

This section lists those events that are to be regarded as expected for regulatory reporting purposes as documented in Section 5.4 of the Investigator's Brochure.

- Gastrointestinal disorders: diarrhea, nausea, vomiting, and stomatitis (oral mucositis)
- Skin and subcutaneous tissue disorders: rashes (including dermatitis acneiform and exfoliative rash) and dry skin
- General disorders: facial and/or peripheral edema, fatigue/asthenia, and pyrexia
- Respiratory disorders: dyspnea
- Vascular disorders: increased blood pressure
- Eye disorders: blurred vision.

- Investigations: increases in serum aspartate aminotransferase (AST) or alanine aminotransferase (ALT)

- Metabolism disorders: hyperphosphataemia, which may be associated with an increase in calcium x phosphate product requiring therapeutic intervention

Refer to the Investigator's Brochure for more information.

1.2 Rationale for conducting this study

AZD6244 is presented as the anhydrous, crystalline hydrogen sulfate salt. For Phase II studies the material was formulated as a dispersion of drug substance in D-α-tocopherol polyethylene glycol 1000 succinate (TPGS) encapsulated in size 4 hypromellose capsules, with strength of 25 mg. The excipients employed in this formulation are pharmacopoeial grade and generally recognized as safe listed. At the current dose of 75 mg, AZD6244 is considered to be a Biopharmaceuticals Classification System Class IV compound.

Prior to initiation of Phase III studies, a number of minor changes to the formulated product are being introduced, with an additional change of site and scale of drug product manufacture. The risk that these changes will have a major impact on the bioavailability of AZD6244 is considered to be low. However, it is considered prudent to confirm this using a clinical study.

As well as the primary comparison of the white (current Phase II) and blue (Study D1532C00029; planned Phase III study) capsule formulations, the opportunity will also be taken to determine within-volunteer variability in the availability of AZD6244 from the blue capsule and to examine the formulation effects on PK by using a solution formulation.

Safety and tolerability will be investigated to extend the safety database for AZD6244 for the situation where AZD6244 is given to healthy volunteers in support of future clinical pharmacology studies that may be required later in development.

1.3 Benefit/risk and ethical assessment

1.3.1 Safety considerations

Volunteers will be instructed to use sunscreen (greater than 30 sun protection factor) for up to 14 days after last dose of AZD6244 due to the photoxicity risk. Skin rash algorithm, retinal abnormality algorithm, and dyspnea investigation algorithm are provided to address the investigation and management of safety concerns. A left ventricular ejection fraction (LVEF) management algorithm is currently being developed.

1.3.2 Adverse event profile

Adverse events potentially relevant to healthy volunteers include:

- Increases in ALT and AST

- Diarrhea, nausea, and vomiting
- Dermatitis acneiform (rash)
- Blurred vision
- Increases in blood pressure
- Photosensitivity
- Increase in blood phosphate levels
- Decrease in blood calcium levels
- Effects on embryos in pregnant women
- Fatigue
- Dyspnea/pneumonitis
- Decreases in LVEF
- Peripheral edema

For patient data refer to the Investigator's Brochure Section 5.2.3.

Adverse events within 24 hours of AZD6244 treatment

A summary of AEs reported within 24 hours of a single dose of 75-mg AZD6244 monotherapy treatment is included (Table 1). Adverse event data are available from studies D1532C00005 and D1532C00020 (65 advanced cancer patients in total). The most frequent AEs were decrease blood potassium 3/65 (4.5%) patients, diarrhea, headache, and nausea (each reported from 2/65 [3%] patients). The event of decrease blood potassium occurred in 3 patients who each had blood potassium values below the lower limit of normal at the screening visit and therefore these events are not considered to be a clinically significant finding. All other AEs were reported in only 1 patient each. These AEs are commonly reported as background comorbidities in advanced cancer patient studies.

Number and percentage of subjects with AEs within 24 hours of AZD6244 treatment

MedDRA preferred term	Total (n=65)		D1532C00005[a] (n=7)		D1532C00005[b] (n=28)		D1532C00020[c] (n=30)	
	n	%	n	%	n	%	n	%
Blood potassium decreased	3	4.5	0	0	0	0	3	9.9
Diarrhea	2	3.0	1	14.3	0	0	1	3.3
Headache	2	3.0	0	0	0	0	2	6.6
Nausea	2	3.0	1	14.3	0	0	1	3.3
Abdominal pain	1	1.5	1	14.3	0	0	0	0
Anemia	1	1.5	0	0	0	0	1	3.3
Constipation	1	1.5	1	14.3	0	0	0	0
Decreased appetite	1	1.5	0	0	0	0	1	3.3
Dehydration	1	1.5	1	14.3	0	0	0	0
Dry skin	1	1.5	1	14.3	0	0	0	0
Dyspnea exertional	1	1.5	1	14.3	0	0	0	0
Dysuria	1	1.5	1	14.3	0	0	0	0
Fatigue	1	1.5	0	0	0	0	1	3.3
Frequent bowel movements	1	1.5	0	0	0	0	1	3.3
Pain in extremity	1	1.5	0	0	0	0	1	3.3
Somnolence	1	1.5	0	0	0	0	1	3.3
Syncope vasovagal	1	1.5	0	0	0	0	1	3.3
Tachycardia	1	1.5	1	14.3	0	0	0	0
Vision blurred	1	1.5	0	0	1	3.6	0	0
Vomiting	1	1.5	0	0	0	0	1	3.3
Wheezing	1	1.5	0	0	0	0	1	3.3

[a] Part A, dose escalation phase of study.
[b] Part B, expansion phase of study.
[c] Fed/fasted study with AZD6244.
MedDRA Medical Dictionary for Regulatory Activities.

AstraZeneca will immediately notify the Investigator if any additional safety information becomes available during the study.

Further information on the investigational product (IP) can be found in the Investigator's Brochure.

Adverse effects related to fetal development and survival in mice

Preliminary reproductive toxicology data indicate that AZD6244 can have adverse effects on embryo fetal development and survival at dose levels that do not induce maternal toxicity in mice

1.3.3 Safety monitoring

Hematology, clinical chemistry (including troponin, brain natriuretic peptide [BNP], creatine kinase myocardial band [CK-MB], and total creatine phosphokinase [CPK]), and urinalysis will be taken. Telemetry and paper printout ECGs (pECGs) for immediate safety assessment as well as digital ECGs (dECGs) for pharmacodynamic assessments will be performed. Baseline and as needed eye examination will be performed. Blood pressure and pulse will be recorded. Echocardiograms will be performed (BNP, troponin, CPK, and CK-MB blood draw will be performed on the same day as the echocardiograms). Collection of AE reports throughout the study will also be monitored.

2. STUDY OBJECTIVES

2.1 Primary objective

The primary objective of this study is to compare the PK of AZD6244 after oral administration of single doses of white (current Phase II) and blue (planned Phase III) capsule formulations.

2.2 Secondary objectives

The secondary objectives of this study are to:

- Investigate the safety and tolerability of single doses of AZD6244 in healthy male volunteers
- Estimate within-volunteer variability in the PK of AZD6244
- Establish the relative bioavailability of AZD6244 after oral administration of single doses of blue capsules (planned Phase III) with respect to an oral solution formulation
- Characterize the PK of *N*-desmethyl AZD6244 after oral administration of single doses of AZD6244 white (current Phase II) and blue (planned Phase III) capsules

2.3 Exploratory objectives

The exploratory objectives of this study are to:

- Characterize the PK of AZD6244 amide metabolite after an oral administration of single doses of AZD6244 white and blue capsules

- Collect and store deoxyribonucleic acid (DNA) for future exploratory research into genes/genetic variation that may influence response (ie, distribution, safety, tolerability, and efficacy) to AZD6244 (optional part of the study and, if performed, will be reported separately from the Clinical Study Report [CSR])

3. STUDY PLAN AND PROCEDURES

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

3.1 Overall study design and flow chart

This is an open-label, randomized, 3-treatment, 4-period, 6-sequence crossover study conducted at a single study center to compare the PK of AZD6244 after oral administration of single doses of white (current Phase II) and blue (planned Phase III) capsule formulations. Volunteers will receive 1 single oral dose of AZD6244 each of Treatment A and Treatment C and 2 doses of Treatment B each separated by washout periods of a minimum of 7 days between doses. Treatment A will consist of three 25-mg AZD6244 white (current Phase II) capsules. Treatment B will consist of three 25-mg AZD6244 blue (planned Phase III) capsules. Treatment C will consist of 35 mg AZD6244 solution.

For Dosing Period 1, 2 volunteers will receive AZD6244 doses at least 2 days before the remainder of the volunteers.

Serial blood samples for the determination of AZD6244 PK will be collected up to 36 hours following the AZD6244 dose. Volunteers will be admitted to the study center on Day -1 and will remain resident until completion of Day 2 procedures for each treatment period. A follow-up visit will be conducted at least 7 days following study center discharge of the volunteer's final treatment.

Safety assessments will include AEs, clinical laboratory assessments (clinical chemistry, hematology, and urinalysis), vital signs (blood pressure and pulse rate), physical examinations, telemetry, pECGs, dECGs, LVEF, and ophthalmology assessments.

Genetic analysis of the genes related to AZD6244 disposition and safety may be performed. Participation in the exploratory genetic sampling is optional for the volunteer.

The study flow chart is presented in Figure 1 and the study plan is presented in Table 2. Specific timing for PK, dECG, blood pressure, and laboratory assessments are in Table 3 and a detailed time schedule for dECG assessments during the residential period is presented in Table 4.

Figure 1 Study flow chart

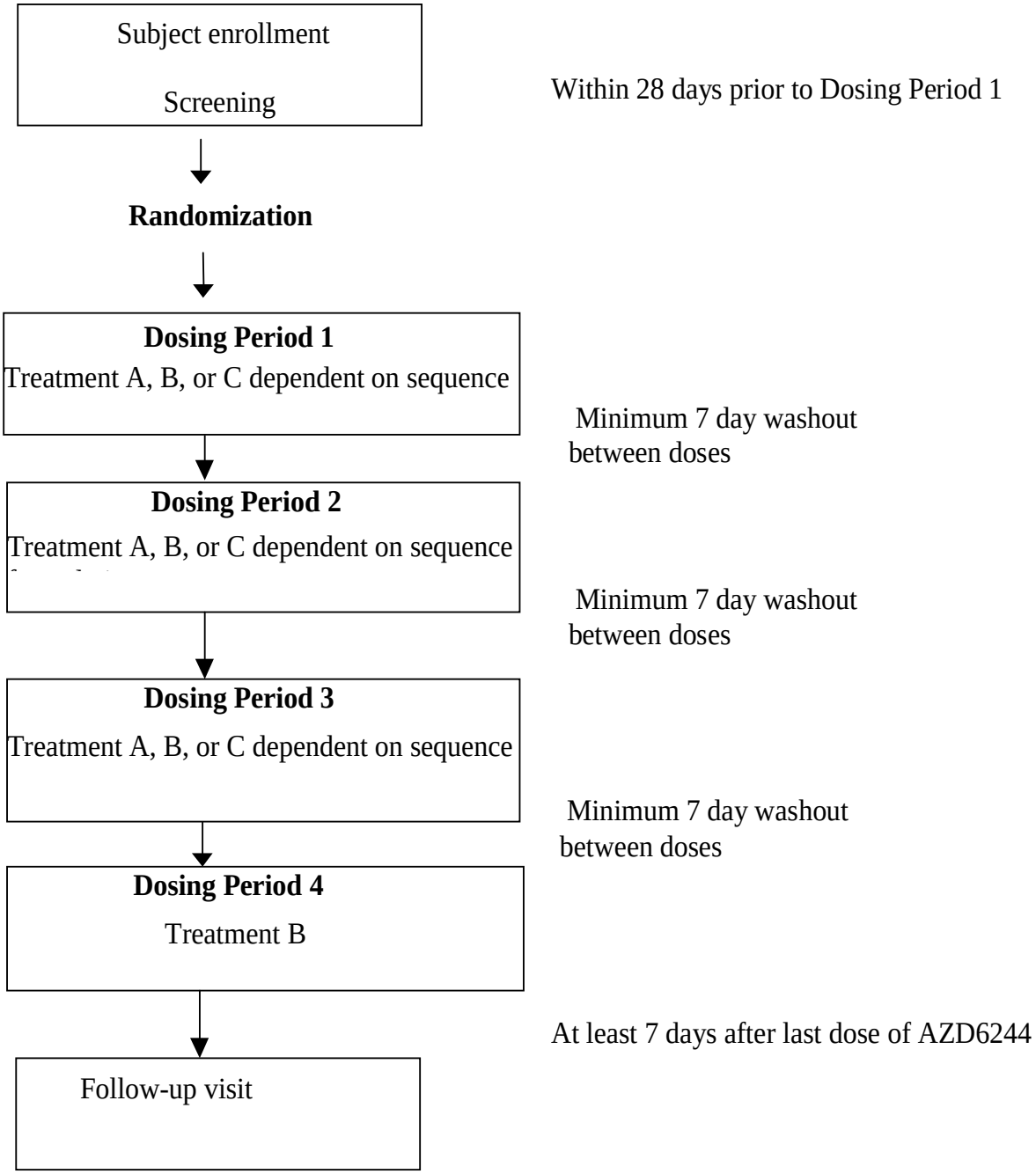

Table 2 Study plan

Assessment	**Screening**	**Dosing Periods 1, 2, 3, and 4[a]**			**Follow-up**
Visit	**1**	**2, 3, 4, and 5**			**6**
Days Assessment	**Day -28 to -2**	**-1**	**1**	**2**	**At least 7 days after the last dose**
Informed consent[b]	X				
Optional pharmacogenetic informed consent[c]	X				
Inclusion/exclusion criteria	X	X			
Demography	X				
Height and calculation of BMI	X				
Weight	X				X
Medical/surgical/smoking history	X				
Hepatitis B, C, and HIV screen	X				
Admission to the study center		X			
Prior/concomitant medications	X	X	X	X	X
Physical examination[d]	X	X		X	X
Eye examination[e]	X				
Laboratory assessments (clinical chemistry, hematology, and urinalysis[f])	X[g]	X			X
Cardiac biomarkers[h,n]	X	X	X		X
Drug screening	X	X			

Table 2 Study plan

Assessment	**Screening**	**Dosing Periods 1, 2, 3, and 4[a]**			**Follow-up**
Visit	**1**	**2, 3, 4, and 5**			**6**
Days	**Day -28 to -2**	**-1**	**1**	**2**	**At least 7 days after the last dose**
Assessment					
Vital signs (including blood pressure and pulse rate)[k]	X	X	X[j]	X	X
12-lead dECG[k]			X[l]	X	
12-lead pECG	X	X	X[l]	X	X
Telemetry[m]		X[r]	X		
Echocardiogram[n]	X	X			X
Randomization			X[o]		
Dose administration			X[p]		
PK blood sampling[q]			X	X	
Optional pharmacogenetic sampling			X[r]		
SAE recording	X	X	X	X	X
AE recording		X	X	X	X
Discharge				X	

[a] At least 7-day washout period will occur between each AZD6244 administration.
[b] Informed consent will be collected prior to any study-specific procedures being performed.
[c] Pharmacogenetic informed consent is required if the subject volunteers for the pharmacogenetic blood sampling. Period 1, Day 1 only.
[d] Complete physical examinations will be performed at screening and at follow-up. A brief physical examination will occur on Day -1 and discharge of each treatment.
[e] Ophthalmological examination will be performed any time prior to the first dose of IP and on any occurrence of a visual AE.
[f] Microscopy will be performed if abnormal urinalysis results.
[g] Subjects will fast for 4 hours prior to clinical laboratory evaluations at screening. Creatinine clearance will be estimated using Cockroft-Gault formula at screening.

[h] Includes laboratory assessments for troponin, BNP, CPK, and CK-MB which are be collected on the same day as the echocardiogram. On Day -1 and at follow-up troponin, BNP, CPK, and CK-MB may be determined as part of the clinical chemistry panel. On Day 1, only troponin, BNP, CPK, and CK-MB will be collected at the sampling times specified in Table 3.

[i] Supine blood pressure and pulse rate will be evaluated after the subject has rested in the supine position for at least 10 minutes prior to the evaluation. If possible, the same arm and equipment should be used for each evaluation.

[j] Vital signs will be recorded at predose and 1.5, 6, and 24 hours postdose.

[k] A 12-lead dECG and vital signs will be performed after the subject has rested in a supine position for at least 10 minutes prior to the evaluation.

[l] Digital and paper ECGs will be performed predose and at 1.5, 6, and 24 hours postdose.

[m] Telemetry to be performed for 4 hours on Day -1 during Period 1 only. For each period, telemetry will occur approximately 30 minutes predose until 24 hours postdose.

[n] Additional cardiac blood biomarkers may be collected along with an ad hoc echocardiogram on occurrence of cardiac AE.

[o] Randomization will occur in Dosing Period 1 on Day 1, predose only.

[p] Subjects will receive a single dose on Day 1 of Dosing Periods 1, 2, 3, and 4 according to their randomized treatment sequence.

[q] Plasma samples will be collected predose and 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24, and 36 hours postdose on Dosing Periods 1, 2, 3, and 4.

[r] Period 1 only.

BMI body mass index; HIV human immunodeficiency virus; SAE serious AE.

Table 3 Detailed study plan for Day 1 of Dosing Periods 1, 2, 3, and 4

t (hours)	PK blood collection	dECG	Supine blood pressure	Troponin, BNP, CPK, and CK-MB
0	X	X	X	X
0.5	X			
1	X			
1.5	X	X	X	
2	X			
4	X			
6	X	X	X	X
8	X			
12	X			
24	X	X	X	
36	X			

Table 4 Time schedule for digital ECG (dECG) assessments during residential period

Study Days	ECG Number	Time: Start hour: minute[a]	Dose	Time: Stop hour: minute	dECG continuous[bcd]	Other
1		-01:30		-01:00		Apply the electrodes[c]
1		-00:40		-00:30		Rest in bed
1	1	-00:30	Predose	-00:20	10 minutes	
1		-00:20		-00:05		Toilet use recommended
1		00:00	Admin of AZD6244/ placebo			
1	2	01:25		01:30	5 minutes[d]	
1	3	05:55		06:00	5 minutes[d]	
2	4	23:55		24:00	5 minutes[d]	

[a] Time points for dECG may be adjusted according to emerging PK data.
[b] The subject must be in the same supine body position (maximum 30 degrees flexion in the hip) at each time point and at all visits. Subject's feet should not contact the footboard of the bed.
[c] Skin must be cleaned, and electrode positions marked with an indelible pen. Electrodes should be applied at least 30 minutes before first recording.
[d] Subject must rest in bed for at least 10 minutes prior to each ECG time point.
Admin administration; dECG digital electrocardiogram; ECG electrocardiogram; PK pharmacokinetic.

3.2 Individual stopping criteria

The individual stopping criteria are:

- Any volunteer who experiences a 10% drop in LVEF from baseline taking the value to below 55% must be withdrawn from the study and referred to a cardiologist for management and monitoring.

- Any volunteer with an AE of dyspnea will be withdrawn from this study. The event must be fully investigated according to the AZD6244 dyspnea algorithm.

- Any volunteer experiencing a visual AE should have a full ophthalmological examination. If a retinal abnormality is identified, volunteers should be withdrawn and an OCT scan performed. All abnormalities should be followed up to resolution. The assessments will be repeated 28 days later to document resolution.

- In the event of a cardiac AE or a clinically significant increase in troponin and CK-MB at the 6 hour postdose time point, the volunteer should have an echocardiogram performed, a follow-up blood sample for troponin, BNP, CPK, and CK-MB taken.

The volunteer must be withdrawn from the study and referred to a cardiologist for management and monitoring.

3.3 Rationale for study design, doses, and control groups

This study will be conducted in healthy, male volunteers to avoid interference from disease processes or other drugs. The selection criteria are defined such that volunteers selected for participation in this study are known to be free from any significant illness.

The study will investigate the differences of 25-mg white capsule formulation in relation to the 25-mg blue capsule formulation of AZD6244. The oral dose of 75-mg AZD6244 (administered as 3 capsules) was selected because 75 mg is the dose level used in the Phase II and the dose identified for the Phase III study. The predicted mean exposure from a single 75-mg (three 25-mg capsules) dose of AZD6244 has been calculated from data from the approximately 1250 patients so far exposed to AZD6244 in clinical studies. Review of the safety and tolerability profile from these advanced cancer patients have indicated that the healthy volunteers are considered unlikely to experience acute significant AEs. Table 1 indicates that acute AEs within the first 24 hours tend to be either low grade and manageable (such as headache) or related to the underlying disease (and hence will not be expected in healthy volunteers). The intensive safety monitoring included in this study will aim to identify any potential AEs early and include specific management algorithms for specific events that have been associated with significant toxicity in cancer patients receiving long-term AZD6244.

The oral solution proposed for use in this study has not previously been administered to humans in a clinical study. The dose for use in study D1532C00066 was selected based upon preclinical experience of the solution performance in both dog and monkey and the bioavailability observed. The relative bioavailability of the capsule formulation compared to the Phase I AZD6244 free base in Captisol® suspension ("mix and drink") was comparable in preclinical studies in dog and in a clinical study in patients (D1532C00005). Therefore the relative bioavailability of the oral solution to the capsule in dog was considered to be predictive of the expected performance in human and this was used to derive a suitable dose. This preclinical relative bioavailability predicted a potential for up to a 2-fold difference in exposure (both C_{max} and AUC), and this also took into account that the oral solution is not dissolution rate limited and a more rapid and complete absorption is possible, resulting in a higher C_{max}. Therefore, in order to minimize the risk of exceeding the mean AUC and C_{max} observed with a 75-mg dose of the AZD6244 capsule formulation, a solution dose of 35 mg (approximately half that of the capsule) was recommended.

For Dosing Period 1, 2 volunteers will receive AZD6244 doses at least 2 days before the remainder of the volunteers.

4. SUBJECT SELECTION CRITERIA

The Investigator should keep a record, the volunteer screening log, of volunteers who entered prestudy screening.

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

4.1 Inclusion criteria

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

2. Healthy male volunteers aged 18 to 55 years with suitable veins for cannulation or repeated venipunctures. (Healthy as determined by medical history, physical examination, laboratory parameters, pECG, and eye examination performed before first dose administration.)

3. Have a BMI between 18 and 30 kg/m^2 inclusive and weigh at least 50 kg and no more than 100 kg, inclusive

4. Calculated creatinine clearance (CRCL) greater than 50 mL/min using Cockcroft-Gault formula

For inclusion in the genetic component of the study, volunteers must fulfill the following additional criteria:

5. Provision of signed, written, and dated informed consent for optional genetic research. If a volunteer declines to participate in the genetic component of the study, there will be no penalty or loss of benefit to the volunteer. The volunteer will not be excluded from other aspects of the study described in this CSP.

4.2 Exclusion criteria

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

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

3. Any clinically important illness, medical/surgical procedure, or trauma within 4 weeks of the first administration of IP

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

5. Any positive result on screening for serum hepatitis B surface antigen, hepatitis C antibody, and HIV

6. Abnormal vital signs, after 10 minutes supine rest, defined as any of the following (note, vital signs may be repeated at the discretion of the Investigator):

   - Systolic blood pressure less than 90 mmHg or 140 mmHg or more
   - Diastolic blood pressure less than 50 mmHg or 90 mmHg or more
   - Pulse rate less than 40 or greater than 85 beats per minute

7. Any clinically important abnormalities in rhythm, conduction, or morphology of the resting ECG and any clinically important abnormalities in the 12-lead ECG as considered by the Investigator that may interfere with the interpretation of QTc interval changes, including abnormal T-wave morphology, particularly in the CSP-defined primary lead; marked early repolarization with ST segment elevation and prominent, tall, or peaked T-waves; prominent U-waves that make the offset of the T-wave difficult to measure or left ventricular hypertrophy

8. Prolonged QTcF greater than 450 ms or shortened QTcF less than 340 ms or family history of long QT syndrome

9. PR(PQ) interval shortening less than 120 ms (volunteers with PR[PQ] greater than 110 ms but less than 120 ms are acceptable if there is no evidence of ventricular preexcitation and no other ECG findings that would exclude the volunteer)

10. PR(PQ) interval prolongation (greater than 240 ms) intermittent second or third degree AV block

11. Incomplete bundle branch block complete or intermittent complete or intraventricular conduction delay with QRS greater than 110 ms (volunteers with QRS greater than 110 ms but less than 115 ms are acceptable if there is no evidence of ventricular hypertrophy and no other ECG findings that would exclude the volunteer)

12. Digital ECG findings suggesting a metabolic or other noncardiac condition that may confound interpretation of serial changes (such as hypokalemia)

13. Current or past history of central serous retinopathy or retinal vein thrombosis, intraocular pressure greater than 21 mmHg or uncontrolled glaucoma

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

15. Current smokers or those who have smoked or used nicotine-containing products within the previous 3 months

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

17. Positive screen for drugs of abuse or cotinine (nicotine) or positive screen for alcohol at screening or on admission to the study center on Day -1 of each treatment period

18. History of severe allergy/hypersensitivity or ongoing clinically important allergy/hypersensitivity, as judged by the Investigator, or history of hypersensitivity to drugs with a similar chemical structure or class to AZD6244

19. Excessive intake of caffeine-containing drinks or food eg, coffee, tea, chocolate, Red Bull, or cola (more than 6 units of caffeine per day). One caffeine unit is contained in the following items: 1 (6 oz) cup of coffee, 2 (12 oz) cans of cola, 1 (12 oz) cup of tea, ½ (4 oz) cup of energy drink (eg, Red Bull), or 3 oz of chocolate

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

21. Use of any prescribed or nonprescribed medication including antacids, analgesics other than paracetamol/acetaminophen 1 g 4 times a day for occasional use, herbal remedies, vitamins, and minerals during the 2 weeks or five half-lives of the compound, whichever is longer, prior to the first administration of IP

22. Any intake of grapefruit, grapefruit juice, Seville oranges, Seville orange marmalade, or other products containing grapefruit or Seville oranges within 7 days of the first admission on Day -1 of Period 1

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

24. Has received another new chemical entity (defined as a compound which has not been approved for marketing) or has participated in any other clinical study that included drug treatment within at least 3 months of the first administration of IP in this study. The period of exclusion begins 3 months after the final dose or 1 month after the last visit whichever is the longest. Note: volunteers consented and screened, but not randomized in this study or a previous Phase I study, are not excluded.

25. Left ventricular ejection fraction less than 55%

26. Previous randomization to treatment in the present study

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

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

29. Volunteers who have previously received AZD6244

30. Volunteers who are vegans or have medical dietary restrictions

31. Volunteers who cannot communicate reliably with the Investigator

In addition, any of the following is regarded as criteria for exclusion from the genetic research

32. Previous bone marrow transplant.

33. Non-leukocyte depleted whole blood transfusion within 120 days of the date of the genetic sample collection

For procedures for handling incorrectly randomized volunteers see Section 5.3.

5. STUDY CONDUCT

5.1 Restrictions during the study

The following restrictions apply during specified times during the study periods:

1. On Day 1 of each treatment period, volunteers will be fasted from midnight before the day of dosing through 4 hours after dosing. No fluids will be allowed from 1 hour prior to dosing until 1 hour after dosing, except the water needed to consume the IP (see Section 5.5.2).

2. The volunteer should be semi-supine (minimum of 30 degrees) for 1 hour following each dose unless otherwise required for study procedures

3. Volunteers should not engage in any strenuous activity from 72 hours prior to dose until final follow-up

4. Volunteers will be asked to abstain from alcohol, grapefruit, grapefruit juice, Seville oranges, and quinine (eg, tonic water) within 7 days prior to check-in on Day -1 until follow-up.

5. During admission periods volunteers will receive a standard diet (and beverages) which excludes all alcohol and grapefruit-containing products. No additional food or beverages must be consumed while in the study center.

6. During the volunteers' outpatient periods, volunteers should abstain from consuming alcohol, high-energy drinks (eg, red bull), food containing poppy seeds, and any over-the-counter medication or herbal preparations until after their final follow-up visit is completed. Volunteers should also limit their caffeine intake to equivalent of 3 cups of coffee per day (1 cup = 12-oz soda, 6-oz coffee, or 8-oz tea).

7. Volunteers will be required to abstain from blood or plasma donation until 3 months after the final medical examination at the study follow-up.

8. If a volunteer does not comply with these restrictions or tests positive to any laboratory tests (eg, drug or alcohol) they will be excluded or withdrawn from the study.

9. Male volunteers with sexual partners who are pregnant or who could become pregnant (ie, women of child-bearing potential) should use barrier methods of contraception for 14 days after completing the study to avoid pregnancy and/or potential adverse effects on the developing embryo. Volunteers should avoid sperm donation during and for 14 days after study completion. Reliable methods of contraception should be used consistently and correctly. Acceptable methods for volunteers or their partners, include:

   - Implants, injectables, combined oral contraceptives (which must all be combined with barrier methods of contraception), some intrauterine devices, vasectomized partner (which must all be combined with barrier methods of contraception), and sexual abstinence.

   - Volunteers will be required to use reliable methods of contraception for the duration of the study and until 14 days after the last dose of AZD6244 treatment

10. Throughout the study, volunteers should avoid the addition of any concomitant medications, in particular any that are likely to affect the metabolism of AZD6244 (eg, *CYP1A2* or *3A4* inhibitors or inducers), unless considered clinically essential for management of concurrent conditions

11. Volunteers should avoid excessive sun exposure and use adequate sunscreen protection, if sun exposure is anticipated. Volunteers should use sunscreen protection for up to 14 days after the last dose of AZD6244.

12. Volunteers will be required to avoid excessive exercise during the study

5.2 Subject enrollment, randomization, and initiation of investigational product

The Investigator (or designee) will:

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

2. Assign potential volunteers a unique enrollment number, beginning with 'E0001001'

3. Determine volunteer eligibility. See Sections 4.1 and 4.2.

4. Assign eligible volunteers a unique randomization code (volunteer number), beginning with '1001'

If a volunteer withdraws from participation in the study, then his randomization code cannot be reused. If the volunteer is replaced, the replacement volunteer will be assigned the next available volunteer number from the randomization.

Procedures for randomization

Up to 30 healthy male volunteers (aged 18 to 55 years) will be enrolled. For Dosing Period 1 only, the first two volunteers enrolled will be dosed at least 2 days before the remainder of the volunteers. A randomization scheme will be produced
Global Phase I using the global randomization system. Volunteers will be randomized to 1 of the 6 sequences as shown in Table 5.

Table 5 Randomization sequences

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

Treatment A will consist of three 25-mg AZD6244 (current Phase II) capsules.
Treatment B will consist of three 25-mg AZD6244 blue (planned Phase III) capsules.
Treatment C will consist of 35 mg AZD6244 solution.

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

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

Where volunteers who do not meet the selection criteria are incorrectly started on treatment, or where volunteers subsequently fail to meet the study criteria post-initiation, a discussion should occur between the AstraZeneca Clinical Pharmacology Alliance (CPA) Physician and the Investigator regarding whether to continue or discontinue the volunteer's treatment. The AstraZeneca CPA Physician is to ensure all such decisions are appropriately documented. In situations where an agreement cannot be reached, further administration of the IP should be stopped.

5.4 Blinding and procedures for unblinding the study

5.4.1 Methods for ensuring blinding

The randomization list will be kept in a secure location.

5.4.2 Methods for unblinding the study

This is an open label study.

5.5 Treatments

5.5.1 Identity of investigational product(s)

Investigational product	Dosage form and strength[a]	Manufacturer
White capsule (Phase II formulation)	25-mg oral capsule	
Blue capsule (planned Phase III formulation)	25-mg oral capsule	
AZD6244 Hyd-Sulfate in 25% w/v aqueous Captisol® Oral Solution (0.7 mg/g)	35-mg (50 g) oral solution	Extemporaneous preparation at

[a] Expressed as AZD6244 free base.

5.5.2 Doses and treatment regimens

This study will consist of single oral AZD6244 doses administered either as a capsule formulation or as an oral solution as shown below. Treatment A and Treatment C will be administered on 1 occasion and Treatment B will be administered on 2 occasions.

- Treatment A will consist of three 25-mg AZD6244 white (current Phase II) capsules

- Treatment B will consist of three 25-mg AZD6244 blue (planned Phase III) capsules

- Treatment C will consist of 35-mg AZD6244 solution (administered as 35 mg in 50 g of solution)

The treatments will be given according to their randomized sequence. Volunteers will be required to fast from midnight before the day of dosing (Day 1) and should remain fasted until 4 hours after dosing. Water will be restricted up to 1 hour prior to dose through 1 hour postdose.

Oral doses of AZD6244 will be administered with approximately 240 mL water with the volunteer in an upright position. The capsules must be swallowed whole and not crushed, chewed, or divided.

The solution formulation will be administered with water to a total of approximately 240 mL water. The AZD6244 in 25% w/v aqueous Captisol® oral solution will have a weight of 50 g (ie, an approximately 50 mL volume) and volunteers will be provided with at least 190 mL water to rinse the container.

Refer to the Laboratory Handling Instructions for more information.

5.5.3 Labeling

Labels will be prepared in accordance with Good Manufacturing Practice and local regulatory guidelines. Label text will be in English.

5.5.4 Storage

All study drugs should be kept in a secure place under appropriate storage conditions. The IP label on the bottle specifies the appropriate storage.

5.6 Concomitant and poststudy treatment(s)

The use of drugs or substances with enzyme-inducing properties, such as *CYP1A2* or *3A4* inhibitors or inducers are restricted from this study unless considered clinically essential for management of concurrent conditions. Apart from occasional use of acetaminophen/paracetamol, no other concomitant medication or therapy will be allowed from 2 weeks or 5 half-lives of the compound, whichever is longer prior to the first administration of IP until after the final medical examination at the follow-up visit.

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

5.7 Treatment compliance

The date and time of administration of all study drugs (including IP) should be recorded in the appropriate sections of the eCRF. Treatment compliance will be assured by supervised administration of the IP by the Investigator or a delegate. The date and time of administration of the IP will be recorded and checked by the monitor at monitoring visits.

Accountability

The IP provided for this study will be used only as directed in the CSP.

Study center personnel will account for all IP received at the site, unused IP, and appropriate destruction. Certificates of delivery and destruction should be signed.

5.8 Discontinuation of investigational product

Volunteers may be discontinued from IP in the following situations:

- Volunteer decision. The volunteer is at any time free to discontinue treatment, without prejudice to further treatment.
- Adverse event
- Severe noncompliance to the CSP
- Risk to volunteer as judged by the Investigator and AstraZeneca
- Volunteer lost to follow-up
- Any significant and clinically relevant changes in the safety parameters (eg, ECG, vital signs, laboratory assessments, or AEs) making the continuation of the IP unjustified

Procedures for discontinuation of a subject from investigational product

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

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

5.9 Withdrawal from study

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

Withdrawn volunteers will not be replaced.

6. COLLECTION OF STUDY VARIABLES

The study assessments are described in the sections below. The study plan and timing of these assessments are detailed in Table 2.

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

1. 12-lead dECG

2. Vital signs (blood pressure and pulse rate)

3. Pharmacokinetic blood sample (Note: PK sampling must be performed at the precise CSP scheduled time.)

4. Clinical laboratory testing

Predose assessments may be performed 60 minutes prior to administration of the IP.

6.1 Recording of data

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

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

6.2 Data collection at enrollment and follow-up

6.2.1 Enrollment procedures

At enrollment (Visit 1), each potential volunteer will provide written informed consent prior to starting any study-specific procedures.

Demographic data and other characteristics will be recorded and will include date of birth, gender, race, ethnicity, alcohol consumption, and smoking history.

Each volunteer will undergo screening during the 28 days prior to Day -1 to confirm eligibility. This will consist of:

- Review of inclusion and exclusion criteria

- A standard medical, surgical, and smoking history of the volunteer inclusive of prior and concomitant medications

- A complete physical examination
- Eye examination (can be completed any time prior to first dose of IP)
- Height and weight measurements and calculation of BMI
- Vital sign measurements (supine pulse rate and blood pressure)
- Recording of 12-lead pECG
- Echocardiogram
- Collection of blood sample for predose safety laboratory assessments (clinical chemistry [troponin, BNP, CK-MB, CRCL estimated by Cockroft-Gault formula, and CPK] and hematology), and screening for hepatitis B virus surface antigen, antibodies to hepatitis C virus, and HIV. Cardiac biomarkers (troponin, BNP, CK-MB, and CPK will be taken on the day of the echocardiogram.
- Collection of urine sample for routine urinalysis and urine microscopy (if there is an abnormal urinalysis result), drugs of abuse, alcohol, and cotinine
- Serious AE recording

6.2.2 Follow-up procedures

A poststudy follow-up will be performed at least 7 days after discharge from study center. This will include the following:

- Complete physical examination
- Body weight
- Vital sign measurements (supine pulse rate and blood pressure)
- Recording of 12-lead pECG
- Echocardiogram
- Collection of blood samples for safety laboratory assessments (clinical chemistry [troponin, BNP, CK-MB, and CPK] and hematology)
- Collection of urine sample for routine urinalysis and microscopy (if there is an abnormal urinalysis result)
- Serious AE and AE recording
- Use of concomitant medication

6.3 Safety

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

6.3.1 Definition of adverse events

An AE is the development of an undesirable medical condition or the deterioration of a preexisting 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 and chest pain), signs (eg, tachycardia and enlarged liver), or the abnormal results of an investigation (eg, laboratory findings and ECG). 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.

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

6.3.2 Definitions of serious adverse event

An SAE is an AE occurring during any study phase (ie, run-in, treatment, washout, and follow-up), that fulfills 1 or more of the following criteria:

- Results in death
- Is immediately life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions
- Is a congenital abnormality or birth defect
- Is an important medical event that may jeopardize the volunteer or may require medical intervention to prevent 1 of the outcomes listed above

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

6.3.3 Recording of adverse events

Time period for collection of adverse events

All AEs will be collected from Day -1, throughout the treatment periods, and including the follow-up period. Serious AEs will be collected from screening, throughout the treatment periods, and including the follow-up period.

Follow-up of unresolved adverse events

Any AEs that are unresolved at the volunteer's last visit in the study will be followed up by the Investigator for as long as medically indicated, but without further recording in the eCRF.

AstraZeneca retains the right to request additional information for any volunteer with ongoing AE(s)/SAE(s) at the end of the study, if judged necessary.

Variables

The following variables will be collected for each AE:

- Adverse event (verbatim)
- The date and time when the AE starts and stops
- Intensity
- Whether the AE is serious or not
- Investigator causality rating against the IP (yes or no)
- Action taken with regard to IP
- Adverse event caused volunteer's withdrawal from study (yes or no)
- Outcome

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

- Date AE met criteria for SAE
- Date Investigator became aware of SAE
- Adverse event is serious due to
- Date of hospitalization
- Date of discharge
- Probable cause of death
- Date of death
- Autopsy performed
- Causality assessment in relation to study procedure(s)
- Causality assessment in relation to other medication
- Causality assessment in relation to additional IP
- Description of AE

The following intensity ratings will be used:

- 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 6.3.2. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not an SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke but would be an SAE.

Causality collection

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

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

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

Adverse events based on signs and symptoms

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

Adverse events based on examinations and tests

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

If deterioration in a laboratory value/vital sign/ECG is associated with clinical signs and symptoms, the sign or symptom will be reported as an AE and the associated laboratory result/vital sign/ECG will be considered as additional information. Wherever possible the reporting Investigator will use the clinical, rather than the laboratory term (eg, anemia versus

low hemoglobin value). In the absence of clinical signs or symptoms, clinically relevant deteriorations in nonmandated parameters should be reported as AE(s).

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

The aspartate aminotransferase or ALT 3 or more times the upper limit of normal (ULN) **and** total bilirubin (TBL) 2 or more times the ULN at any point during the study irrespective of an increase in alkaline phosphatase (ALP). The elevations do not have to occur at the same time or within a specified time frame. Please refer to Appendix D 'Hy's Law', for further instructions.

6.3.4 Reporting of serious adverse events

All SAEs have to be reported, whether or not considered causally related to the IP, or to the study procedure(s). All SAEs will be recorded in the eCRF.

If any SAE occurs in the course of the study, then Investigators or other site personnel will inform appropriate AstraZeneca representatives immediately, or **no later than 24 hours** of when he or she becomes aware of it.

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

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

6.3.5 Laboratory safety assessment

Blood and urine samples for determination of clinical chemistry, hematology, and urinalysis will be taken at the times indicated in the study plan (see Table 2).

Laboratory variables to be measured are shown in Table 6.

Table 6 Laboratory variables

Hematology	Clinical chemistry	Urinalysis[c]
B-red blood cell count	S-ALT	U-glucose
B-mean corpuscular volume	S-albumin	U-creatinine
B-mean corpuscular hemoglobin	S-ALP	U-leukocyte esterase
B-hematocrit	S-AST	U-occult blood
B-hemoglobin	S-bilirubin, total and unconjugated	U-specific gravity
B-leukocyte count (white blood cell count)	S-blood urea nitrogen	U-total protein
B-leukocyte differential count[a]	S-calcium total	U-cotinine
B-platelet count	S-creatinine[b]	U-methadone
B-reticulocytes	S-gamma glutamyltransferase	U-cannabis
	S-glucose (fasting)	U-cocaine
	S-lactate dehydrogenase	U-benzodiazepines
	S-potassium	U-amphetamine
	S-sodium	U-methamphetamines (including ecstasy)
	S-total protein	U-opiates
	S-uric acid	U-barbiturates
	S-Troponin	U-ethanol
	S-BNP	
	S-CK-MB	
	S-CPK	
	S-phosphate[d]	

[a] The leukocyte differential count will include neutrophils, eosinophils, basophils, lymphocytes, and monocytes.
[b] Estimated CRCL taken only at screening.
[c] Urine microscopy will be performed if there is an abnormal urinalysis result.
[d] Note, calcium phosphate product is obtained by multiplying calcium result with phosphate result (eg, if calcium is 2.0 and phosphate is 1.5) then the product is 2.0 x 1.5 = 3.0.
B blood; S serum; U urine.

Blood will be tested for hepatitis B surface antigen, antibodies to hepatitis C, and antibodies to HIV at the screening visit.

Urine will be tested at the screening visit and on Day -1 of each period for the following drugs of abuse: methadone, cannabis, cocaine, benzodiazepines, amphetamine, methamphetamines

(including ecstasy), opiates, and barbiturates. The urine will be collected at the study center. If a volunteer tests positive for drugs of abuse, a retest may be performed and the volunteer may be excluded from entering the study, as judged by the Investigator. Urine will be screened for cotinine using the qualitative test kit. Volunteers will be tested for alcohol in the urine drug screen.

Laboratory values outside the reference limit suspected to be of any clinical significance will be repeated. Volunteers in whom suspected clinical significance is confirmed will either not be included or if already enrolled will be followed until normalization or for as long as the Investigator considers necessary. Additional laboratory variables may be performed for safety reasons if judged appropriate by the Investigator. Further details are provided in the Laboratory Manual.

The safety laboratory samples will be analyzed using routine methods at the site's accredited clinical laboratory (Physician's Reference Laboratory, Overland Park, Kansas, United States).

The aspartate aminotransferase or ALT 3 or more times the ULN **and** TBL 2 or more times the ULN at any point during the study irrespective of an increase in ALP. The elevations do not have to occur at the same time or within a specified time frame. Please refer to Appendix D 'Hy's Law', for further instructions.

For AEs based on examinations and tests, see Section 6.3.3.

For blood volume see Section 7.1.

6.3.6 Physical examination

A complete physical examination will be performed at the times specified in the study schedule (see Table 2) and will include an assessment of the following: general appearance, respiratory, cardiovascular, skin, head and neck (including ears, eyes, nose and throat), lymph nodes, thyroid, abdomen, musculoskeletal (including spine and extremities), and neurological systems.

A brief physical examination, which will include assessment of respiratory and cardiovascular systems and general inspection will be performed at the times specified in the study plan (see Table 2).

Height (in centimeters) and weight (in kilograms) will be measured at the times specified in the study plan (see Table 2). Measurements should be taken without shoes. Body mass index will be calculated from the height and weight measurements.

The outcome of the physical examination is to be recorded as normal/abnormal in the eCRF, with any abnormalities specified. Any new or aggravated clinically relevant abnormal medical finding at a physical examination as compared with the baseline assessment will be reported as an AE.

For AEs based on examinations and tests, see Section 6.3.3.

6.3.7 Ophthalmology assessment

A full ophthalmologic examination including a slit-lamp examination, fundoscopy, best corrected near vision, and intraocular pressure measurement must be performed prior to the first dose of IP for all volunteers. If a volunteer experiences visual disturbance the ophthalmological algorithm should be followed for diagnosis and management of these symptoms. Volunteers should undergo an ophthalmological examination and Optical Coherence Tomography scans should be considered. Volunteers who have an ongoing retinal abnormality at the end of the study should have a follow-up eye examination performed 30 days after the last dose of AZD6244 in order to document reversibility.

6.3.8 Electrocardiogram

6.3.8.1 Resting 12-lead pECG

A 12-lead resting pECG will be performed on the days indicated in the study plan (Table 2). Volunteers must rest in a supine position for 10 minutes before each assessment. Overall evaluation (normal/abnormal) will be recorded in the eCRF. If the pECG is abnormal, the abnormality and its clinical significance will be specified in the eCRF. The print-out of the pECG is to be signed, dated, and filed in the Investigator's Study File along with a signed and dated copy (if the print-outs are not on archive-quality paper).

For AEs based on examinations and tests, see Section 6.3.3.

6.3.8.2 Digital electrocardiograms

The AstraZeneca ECG Centre will perform the dECG analysis in this study, using the EClysis© system, version 3.2, or higher.

At CSP-indicated time points (Table 2), 12-lead continuous dECG will be recorded over at least 5 minutes with the Schiller Cardiovit CS-200 recorder (Schiller AG, Baar, Switzerland) and transmitted to the AstraZeneca central dECG repository, according to AstraZeneca ECG Centre´s standard procedures for settings, recording, and transmission of dECGs.

The same recorder will be used for each volunteer at all time points, when possible. Date and time settings must be checked at the start of each study day and aligned with an official timekeeper for all machines used in the study.

Skin preparation must be thorough and electrode positions must be according to standard 12-lead ECG placement. Electrode positions for dECG take precedence over telemetry electrodes. Electrode positions will be marked with an indelible pen at the start of each study day to ensure exact reposition. Permanent electrodes will be applied at least 30 minutes before first study recording and left in place for the duration of each relevant study day.

Volunteers will rest in a supine position for at least 10 minutes before the start of each recording. The volunteer should be in the same supine body position (maximum 30 degrees flexion of the hip and feet not in contact with the footboard) at each recording time points during the study.

The metadata for all dECG files will be checked and approved by the responsible personnel at the study center to ensure that the files transferred to the AstraZeneca central dECG files repository are made accessible to the ECG Scientific Advisors for analysis.

As standard, 10-second ECGs are extracted by the EClysis© system twice per minute from the continuous recording and initially automatically analyzed by the software.

Lead V2 will be analyzed and reported as primary. Lead V5 will be analyzed, for all visits, as backup for the individual where analysis in lead V2 is not deemed possible for predose, for significant parts of whole visits or for whole visits. The analysis is performed blinded to treatment.

The ECG Scientific Advisor will perform all required manual adjustments to the ECG annotations provided automatically by EClysis©.

Finally, an external expert cardiologist will review the totality of data and perform all necessary adjustments before locking the EClysis© data into a read-only state.

The numerical values for ECG intervals and amplitudes will be exported and made accessible on the AstraZeneca central dECG repository to accredited data management specialists for conversion into SAS files.

The following variables will be reported by the AstraZeneca ECG Centre: RR, PR, QRS, and QT intervals from the lead defined as primary in the protocol. Derived parameters (QTcF, HR, and others, as applicable) are calculated by the study statistician or designee.

6.3.8.3 Real time display (telemetry)

Telemetry will be performed for 4 hours on Day -1, while healthy volunteers are awake. A real time ECG will be displayed starting at approximately 0.5 hours before administration of AZD6244 on Day 1 and continuing for at least 24 hours after the administration of AZD6244. Telemetry will be monitored by the Investigator, research nurse, or delegate.

6.3.9 Vital signs

Supine blood pressure and pulse rate will be measured using a semi-automatic blood pressure recording device using the appropriate cuff size after the volunteers have rested for 10 minutes on a bed. For timings of assessments refer to the study plan (Table 2). Additional blood pressure/pulse assessments may be taken for safety at the discretion of the Investigator or delegate.

6.3.10 Echocardiogram

Echocardiogram will be performed to determine LVEF. Echocardiograms will be performed at the times specified in the study plan (Table 2).

A complete high quality standardized 2-D with Doppler echocardiographic examinations should be performed by an experienced sonographer (preferably with the same sonographer

performing all studies for a given volunteer) according to a specified CSP including evaluation of both systolic and diastolic function, and centrally read at a core laboratory for reference. Ejection fraction determinations should be determined quantitatively based on bi-plane measurements of end diastolic and end systolic left ventricular volumes. Echocardiograms should be performed at baseline and repeated during the study, prior to each dosing, and prior to discharge from the study (approximately 1 week later).

6.4 Pharmacokinetics

6.4.1 Collection of samples

Blood samples (approximately 2.0 mL) for determination of AZD6244, *N*-desmethyl AZD6244, and AZD6244 amide in plasma will be taken at the times presented in the study plan (Table 2). Individual venipunctures for each time point may be performed or an in-dwelling catheter may be used. If the study center chooses to use an in-dwelling catheter, the first 1 mL of blood will be discarded and the catheter flushed with saline following the sampling. Heparin may not be used to flush the catheter.

The date and time of collection of each sample will be recorded in the eCRF. Samples will be collected, labeled, stored, and shipped as detailed in Laboratory Manual.

For blood volume see Section 7.1.

6.4.2 Determination of drug concentration

Samples for determination AZD6244, *N*-desmethyl AZD6244, and AZD6244 amide concentrations in plasma will be analyzed b
AstraZeneca R&D, using an appropriate bioanalytical method. Full details of the bioanalytical method used will be described in a separate bioanalytical report.

All samples still within the known stability of the analytes of interest (ie, AZD6244, *N*-desmethyl AZD6244, and AZD6244 amide) at the time of receipt by the bioanalytical laboratory will be analyzed.

6.5 Pharmacodynamics (not applicable)

6.6 Pharmacogenetics

All volunteers enrolled in the study will have the option to provide a blood sample for pharmacogenetic analysis. Volunteers will not be excluded from participating in the study if they decline participation in the genetic analysis. Blood samples for these analyses will be taken only after the volunteer has signed a separate genetic informed consent for sample collection. The analyses and results from these optional studies will be handled and reported separately from the main study.

Collection of pharmacogenetic samples

The blood sample for genetic research will be obtained from the volunteers on Day 1 of Period 1. Although genotype is a stable parameter, early sample collection is preferred to

avoid introducing bias through excluding volunteers who may withdraw due to an AE, such volunteers would be important to include in any genetic analysis. If for any reason the sample is not drawn on Day 1 of Period 1, it may be taken at any visit until the last study visit. Only 1 sample should be collected per volunteer for genetics during the study. Samples will be collected, labeled, stored, and shipped as detailed in the Laboratory Manual.

For blood volume see Section 7.1.

7. BIOLOGICAL SAMPLING PROCEDURES

The approximate total volume of blood that will be drawn from each volunteer in this study is presented in Table 7.

7.1 Volume of blood

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

Table 7 Volume of blood to be drawn from each subject

Assessment		Sample volume (mL)	No. of samples[a]	Total volume (mL)[a]
Safety	Clinical chemistry[b]	8.5	6	51
	Hematology	4	6	24
	Serology	8.5	1	8.5
Pharmacokinetic	AZD6244, *N*-desmethyl AZD6244, and AZD6244 amide	2	44	88
Cardiac biomarkers (troponin, CPK and CK-MB)[c]		5	9	45
Cardiac biomarker (BNP)		4	14	56
Optional pharmacogenetics		10	1	10
Discard[d]		1	44	44
Total				326.5

[a] Blood samples based on all 4 treatment periods, as applicable.
[b] On Day -1 (all 4 periods) and at follow-up, the cardiac biomarkers (troponin, CPK, CK-MB) will be determined as part of the clinical chemistry sample.
[c] On the day of screening echocardiogram and on Day 1 (all 4 periods), the cardiac biomarkers (troponin, CPK, CK-MB) will be collected as a separate sample.
[d] Discard volume calculated for each PK sample, in case an indwelling catheter is used.

The number of samples taken, as well as the volume required for each analysis, maybe changed during the study (ie, if additional samples are drawn for repeated safety assessments).

However, the maximum volume to be drawn from each healthy volunteer will not exceed 450 mL ie, the same volume as would be drawn during a regular blood donation.

7.2 Handling, storage, and destruction of biological samples

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

Biological samples for future research can be retained at the Research and Development site, on behalf of AstraZeneca for a maximum of 25 years following the last volunteer's last visit in the study. The results from future analysis, if performed, will not be reported in the CSR.

7.2.1 Pharmacokinetic samples

Samples will be disposed of after the bioanalytical report finalization or six months after issuance of the draft bioanalytical report (whichever is earlier), unless requested for future analyses. Samples may also be disposed of earlier, pending further notification.

Incurred sample reproducibility analysis, if any, will be performed alongside the bioanalysis of the test samples. The results from the evaluation will not be reported in the Clinical Study Report but separately in a bioanalytical report.

7.2.2 Pharmacogenetic samples

The exploratory genetic research component of the study is optional.

The processes adopted for the coding and storage of samples for genetic analysis are important to maintain volunteer confidentiality. Samples will be stored for a maximum of 25 years, from the date of the last volunteer's last visit, after which they will be destroyed. The DNA is a finite resource that may be used up during analyses. Samples will be stored and used until no further analyses are possible or the maximum storage time has been reached.

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

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

7.3 Labeling and shipment of biohazard samples

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

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

7.4 Chain of custody of biological samples

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

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

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

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

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

7.5 Withdrawal of informed consent for donated biological samples

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

As collection of the pharmacogenetic samples is an optional part of the study, then the volunteer may continue in the study.

The Investigator:

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

- Ensures that the volunteer and AstraZeneca are informed about the sample disposal

AstraZeneca ensures the central and bioanalytical laboratories holding the samples are informed about the withdrawn consent immediately and that samples are disposed of/destroyed and the action documented, and returned to the study center.

8. ETHICAL AND REGULATORY REQUIREMENTS

8.1 Ethical conduct of the study

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

8.2 Subject data protection

The informed consent form(s) will incorporate (or, in some cases, be accompanied by a separate document incorporating) wording that complies with relevant data protection and privacy legislation.

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

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

8.3 Ethics and regulatory review

An ethics committee should approve the final CSP, including the final version of the informed consent forms and any other written information and/or materials to be provided to the volunteers. The Investigator will ensure the distribution of these documents to the applicable ethics committee and to the study center staff.

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

The ethics committee should approve all advertising used to recruit volunteers for the study.

AstraZeneca should approve any modifications to the informed consent forms that are needed to meet local requirements.

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

Before enrollment of any volunteer into the study, the final CSP, including the final version of the informed consent forms, is approved by the national regulatory authority or a notification to the national regulatory authority is done, according to local regulations.

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

AstraZeneca will provide regulatory authorities, ethics committees, and the Investigator with safety updates/reports according to local requirements, including Suspected Unexpected Serious Adverse Reactions, where relevant.

Each Investigator is responsible for providing the ethics committees/Institutional Review Board with reports of any serious and unexpected adverse drug reactions from any other study conducted with the IP. AstraZeneca will provide this information to the Investigator so that he/she can meet these reporting requirements.

8.4 Informed consent

The Investigator or designee will:

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

If any new information on the study medication becomes available which may influence the decision of the volunteer to continue the study, the Investigator should inform the volunteer of such information immediately, record this in a written form, and confirm with the volunteer if he wishes to continue the participation in the study. In addition, if the Investigator deems it necessary to revise the informed consent form(s), they should revise it immediately (refer to Section 8.5). The Investigator should re-explain to the volunteers using updated informed consent form(s) even if the volunteers have already been informed of the new information verbally. Written informed consent to continue participation in the study should be provided separately.

8.5 Changes to the protocol and informed consent form

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

If there are any substantial changes to the CSP, these changes will be documented in a CSP amendment and, where required, in a new version of the CSP (revised CSP).

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

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

If a CSP amendment requires a change to the study center's informed consent form(s), AstraZeneca and the study center's ethics committee are to approve the revised informed consent form(s) before the revised form is used.

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

8.6 Audits and inspections

Authorized representatives of AstraZeneca, a regulatory authority, or an ethics committee may perform audits or inspections at the study center, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents, to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the CSP, GCP, guidelines of the ICH, and any applicable regulatory requirements. The Investigator will contact AstraZeneca immediately if contacted by a regulatory agency about an inspection at the study center.

9. STUDY MANAGEMENT

will manage the study on behalf of AstraZeneca.

9.1 Prestudy activities

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

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

9.2 Training of study site personnel

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

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

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

9.3 Monitoring of the study

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

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

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

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

Source data

Refer to the CSA for location of source data.

9.4 Study agreements

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

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

Archiving of study documents

The Investigator follows the principles outlined in the CSA.

9.5 Study timetable and end of study

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

The study is expected to start in Quarter 2, 2012 and to end by Quarter 3, 2012.

The study may be terminated at the study center if the study procedures are not being performed according to GCP or if recruitment is slow. AstraZeneca may also terminate the entire study prematurely if concerns for safety arise within this study or in any other study with AZD6244.

10. DATA MANAGEMENT

Data management will be performed by .
A 21 Code of Federal Regulations part e used for this study. Electronic CRFs will be produced by for each volunteer.

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

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

will be classified according to the AstraZeneca Drug Dictionary. All coding will be

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

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

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

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

11. EVALUATION AND CALCULATION OF VARIABLES

11.1 Calculation or derivation of safety variable(s)

11.1.1 Calculation of change from baseline

Change-from-baseline variables will be calculated for the safety variables listed below, as the posttreatment value minus the value at baseline. The baseline values will be as follows:

- Clinical laboratory tests: Day -1 in corresponding treatment period
- Vital signs: Day 1, predose in corresponding treatment period
- Digital ECG: Day 1, predose in corresponding treatment period

If a volunteer is missing the baseline collection, the previous nonmissing evaluation will become the baseline value. If no baseline or previous-to-baseline evaluations exist, then the baseline value will be treated as missing and no change-from-baseline value will be calculated.

11.1.2 Other significant adverse events (OAE)

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

Examples of these are marked hematological and other laboratory abnormalities, and certain events that lead to intervention (other than those already classified as serious), dose reduction, or significant additional treatment.

Any abnormal deviation in ECG intervals, amplitude, or morphology that is associated with symptoms is to be reported as an AE.

Any abnormal deviation in ECG/telemetry intervals, amplitudes, or morphology that is deemed to require a change in patient care, surveillance, or study participation is to be reported as an AE/DAE.

Any asymtomatic deviation in ECG/telemetry intervals, amplitudes ,or morphology that is deemed not to constitute a safety concern for the individual volunteer, nor to require a change in volunteer care, surveillance, or study participation is to be reported qualitatively as an outlier value or with a narrative in the CSR. This includes also eg, short, asymptomatic, accidentally found paroxysmal supraventricular tachycardia or nonsustained ventricular tachycardia with characteristics that are normally not associated with test drug exposure:

Extra measurements (such as unscheduled or repeat assessments) will not be included in the descriptive statistics, but will be included in data listings. All AEs, ECG outliers for QTcF (ie, greater than 450, greater than 480, greater than 500 ms, or change from baseline greater than 30 and greater than 60 ms) and clinical laboratory outliers that occur following the first dose of IP will be included in the tabulations of AEs and outlier events, including episodes that occur at unscheduled evaluations or during the washout period between treatments.

11.2 Calculation or derivation of pharmacokinetic variables

The PK analyses of the plasma conce
AZD6244 amide will be performed a
Pharmacokinetic analyses will be con
Procedures (SOPs) for PK analyses if not otherwise specified.

Pharmacokinetic parameters in plasma will be derived using noncompartmental methods with

Actual elapsed time from dosing will be used for final plasma PK parameter calculations. Pharmacokinetic parameter units will be consistent with the plasma concentration units specified in the bioanalytical data. No conversion of units will be made.

If data permits and unless otherwise stated, the following PK parameters will be determined for AZD6244, *N*-desmethyl AZD6244, and AZD6244 amide, following single-dose administration:

AUC	Area under the plasma concentration-time curve from zero to infinity (AUC, ng*h/mL), calculated by linear up/log down trapezoidal summation

$AUC_{(0-t)}$	Area under the plasma concentration-time curve from zero to the time of the last quantifiable concentration [$AUC_{(0-t)}$, ng*h/mL], calculated by linear up/log down trapezoidal summation
$AUC_{(0-12)}$	Area under the plasma concentration-time curve from zero to the time of 12 hours [$AUC_{(0-12)}$, ng*h/mL], calculated by linear up/log down trapezoidal summation
C_{max}	Maximum plasma concentration (C_{max}, ng/mL), obtained directly from the observed concentration versus time data
t_{max}	Time to C_{max} (t_{max}, h)
CL/F	Apparent oral plasma clearance (CL/F, L/h, AZD6244 only)
V_{ss}/F	Apparent volume at distribution equilibrium, MRT*CL/F (V_{ss}/F, L, AZD6244 only)
V_z/F	Apparent volume of distribution (V_z/F, L, AZD6244 only)
$t_{1/2}$	Terminal half-life ($t_{1/2}$, h)
λ_z	Terminal rate constant (λ_z, 1/h).

Metabolite to parent drug ratios will be calculated for the primary PK parameters AUC and C_{max}.

The following PK parameters will be calculated for diagnostic purposes and listed, but will not be summarized:

- The time interval (h) of the log-linear regression to determine $t_{1/2}$ ($t_{1/2,}$ interval)
- Number of data points ($t_{½}$, N) included in the log-linear regression analysis used to determine λ_z. A minimum of 3 data points will be used for λ_z determination
- Coefficient of determination for calculation of λ_z (Rsq). The λ_z and related parameters will be reported only if Rsq is 0.800 or more
- Percentage of AUC obtained by extrapolation (%AUCex); if the extrapolated area ($\%AUC_{ex}$) is greater than 20% then AUC for that specific profile will not be reported

Pharmacokinetic parameters shall be computed and reported if the anomalous predose concentration value is not greater than 5% of the maximum observed concentration (C_{max}) in the profile. If the anomalous predose concentration value is greater than 5% of the maximum observed concentration (C_{max}) in the profile, PK parameters for the profile shall not be reported. If an anomalous concentration value is observed at the terminal phase and is judged as physiologically unreasonable, it will be excluded from computing parameter estimates.

Additional PK parameters may be calculated if deemed appropriate.

12. STATISTICAL METHODS AND SAMPLE SIZE DETERMINATION

12.1 Description of analysis sets

12.1.1 General principles

The analysis of data will be based on different subsets according to the purpose of analysis ie, for safety and PK, respectively.

12.1.2 Safety analysis set

All volunteers who receive at least 1 dose of the AZD6244 and for whom any postdose safety data are available will be included in the safety population.

12.1.3 Pharmacokinetic analysis set

12.1.3.1 Interim analysis

An interim PK analysis will be conducted following Treatment Period 3. All volunteers from each dose that receive active drug will be included in the analysis dataset. The same rules of analysis as described in Section 6.4 will be followed.

General conditions for the interim PK analysis:

- Single analyte of AZD6244 in plasma
- Analysis for estimation of PK parameters using nominal times of sample collections so no merging of bioanalytical data with the database is needed
- Outcome variables to be analyzed are limited to $AUC_{(0-t)}$, C_{max}, and t_{max}, no curve stripping on individual concentration versus time plots will be performed
- Prior to the analysis or receipt of the bioanalytical data, a safety team member or study center staff member will need to provide information of volunteers who had AEs reported or other conditions that would affect the delivery of study drug (ie, primarily emesis post drug administration) during the study in order to evaluate the volunteers for exclusion within the PK analysis
- Informal table and mean figures from the analysis software o reduce involvement of programming team members
- Brief summary by descriptive statistical table, no inferential statistical

Internal review of analysis and outputs but no formal quality control or quality audits

12.1.3.2 Final analysis

The PK analysis set will be a subset of the safety analysis set and will include all healthy volunteers who receive at least 1 dose of IP (AZD6244) without important CSP deviations or violations thought to significantly affect the PK (eg, vomiting within 3 to 4 hours after IP administration on any day in the study; volunteer vomited at or before 2 times median t_{max}; wrong dose administered; prohibited concomitant medication; etc). A strategy for dealing with data affected by CSP violations, deviations, and events will be agreed upon by the pharmacokineticist and statistician prior to PD analysis. Volunteers will be analyzed according to the treatment they actually received.

12.2 Methods of statistical analyses

12.2.1 General principles

The statistical analyses will be performed by

Standard Operating Procedures and Work Instructions will be used as the default methodology if not otherwise specified.

Data from each treatment will be presented separately unless stated otherwise.

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

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

All derived variables/parameters will be rounded for reporting purposes in the summary tables and volunteer listings, as per SOPs.

12.2.2 Pharmacokinetic analyses

Pharmacokinetic blood sample collection times as well as derived sampling time deviations will be listed. Plasma concentration-time data (for AZD6244, *N*-desmethyl AZD6244 and AZD6244 amide) will be listed and summarized by treatment.

Data from volunteers excluded from the PK analysis population will be included in the data listings, but not in the summaries.

Pharmacokinetic variables will be summarized by treatment using appropriate descriptive statistics (eg, n, geometric mean, coefficient of variation [CV], minimum, median, and maximum) for all variables except t_{max} which will use n, arithmetic mean, standard deviation (SD), minimum, median, and maximum. The geometric mean is calculated as the exponential of the arithmetic mean calculated from data on a log scale.

The CV is calculated as:

$$100 \cdot \sqrt{\left(\exp\left(s^2\right)-1\right)}$$

where s is the SD of the data on a log scale.

For descriptive statistics, concentrations below lower limit of quantitation (LLOQ) values will be handled as follows:

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

Figures of arithmetic mean (±SD) plasma concentration-time data (for AZD6244, *N*-desmethyl AZD6244, and AZD6244 amide) up to 36 hours after the dose of each treatment period will be presented by treatment on both the linear and semi-logarithmic scales scale. Individual volunteer concentration-time data will be plotted on linear and semi-logarithmic scales.

Geometric mean and individual AUC and C_{max} values will be presented by treatment. Graphical presentations of other PK data may be added at the discretion of the pharmacokineticist.

Primary PK parameters are AUC and C_{max} of AZD6244. If AUC is not reportable in more than 60% of the 30 enrolled volunteers (ie, 18 reportable AUCs) then $AUC_{(0\text{-}t)}$ will be used in the inferential analysis.

Utilizing data from the first three treatment periods only, the relative bioavailability between treatments will be assessed between test (Treatment B) and reference (Treatments A) treatments. Analyses will be performed using a linear mixed-effect analysis of variance model using the logarithm of AUC and C_{max} as the response variables, sequence, period, and treatment as fixed effects, and volunteer nested within sequence as a random effect. Transformed back from the logarithmic scale, geometric least-squares means together with confidence intervals (CIs) (2-sided 95%) for AUC and C_{max} will be estimated and presented.

Also, ratios of geometric least squares means together with CI (2-sided 90%) will be estimated and presented.

Using a similar model as above, comparisons will be made for dose-normalized AUC and C_{max} between test (Treatment B) and reference (Treatments C) treatment. AUC and C_{max} will be dose-normalized prior to the ln-transformation.

Comparisons include:

- Treatment B versus A
- Treatment B versus C

Using treatment B data only, estimates of between and within-volunteer variability's will be obtained by employing a linear mixed-effect analysis of variance model using the logarithm of AUC and C_{max} as the response variables and treatment as fixed effect. Volunteer nested within sequence will be a random effect. Treatment will be re-coded as B1 and B2, where B1 will correspond to Treatment B given in the first 3 periods and B2 will correspond to Treatment B given in Period 4. Estimates for the between and within-volunteer variability's will be present together with the associated 90% CIs.

12.2.3 Subject characteristics

Continuous variables (eg, age and height) will be summarized using descriptive statistics (n, mean, SD, minimum, median, and maximum). Categorical variables (eg, sex and race) will be summarized in frequency tables (frequency and proportion) for all volunteers overall.

12.2.4 Safety and tolerability

All safety data (scheduled and unscheduled) will be presented in the data listings. Continuous variables (hematology, clinical chemistry, dECGs, and vital signs) will be summarized using descriptive statistics (n, mean, SD, minimum, median, and maximum) by treatment or scheduled time point, as appropriate. Categorical variables (eg, urinalysis, etc) will be summarized in frequency tables (frequency and proportion) by treatment group or scheduled time point, as appropriate. Where applicable, data will be summarized for the absolute value at each scheduled assessment, and for the corresponding change from baseline. Adverse events will be summarized in frequency tables (frequency and proportion) for each treatment. Graphical presentations may be used, as appropriate.

All AEs will be collected for each volunteer from Day -1 until the follow-up visit. Adverse events that occur before the first dose of IP will be listed only. On-treatment AEs (ie, those beginning after the first dose of IP) will be summarized by preferred term and system organ class using MedDRA vocabulary with volunteer counts and percentages and also by severity and casualty. Furthermore, listings of deaths (if any), SAEs, and AEs that lead to study discontinuation will be made. The number of volunteers who have any AEs, SAEs, AEs that lead to withdrawal, AEs of different intensity, and AEs judged causally related to IP by the Investigator will be summarized.

For clinical laboratory tests, listings of values for each volunteer will be presented with abnormal or out-of-range values flagged. Clinical laboratory data will be reported in Système International units in the CSR. Shifts in clinical laboratory data will be tabulated.

Results from the physical examinations, pECGs, echocardiograms, and eye examinations will be presented in listings only.

The dECG parameters will be summarized for the absolute value at each scheduled assessment, together with the corresponding changes from the predose value. For safety purposes the QT correction factor will be based on the Frederica's formula (QTcF). Categorical summaries of absolute QTcF values (greater than 450 ms, greater than 480 ms, and greater than 500 ms) and change-from-predose values in QTcF values (greater than 30 ms and greater than 60 ms) will be produced. Further, safety data will be judged regarding low/high values or significant changes from predose using the AstraZeneca extended reference limits. Safety variables should be presented in the units indicated by the AstraZeneca extended reference limits. Abnormalities found on physical examination will be listed.

A shift plot showing predose QTcF values on the horizontal axis and last postdose QTcF value on the vertical axis. All 4 treatments will be plotted separately. The diagonal will be indicated on the plot (ie, 45 degree line). A shift plot showing predose QTcF values on the horizontal axis and the QTcF value indicating the maximum increase from baseline on the vertical axis. All 4 treatments will be plotted separately. The diagonal will be indicated on the plot (ie, 45 degree line). Digital ECG parameter graphs of group mean effect per time point by treatment and mean change from baseline per time point by treatment will be produced for all ECG parameters.

Extra measurements (such as unscheduled or repeat assessments) will not be included in the descriptive statistics, but will be included in data listings. All AEs and clinical laboratory and vital sign outliers that occur following the first dose of IP will be included in the tabulations of AEs and outlier events, including episodes that occur at unscheduled evaluations or during the washout period between treatments.

12.3 Determination of sample size

$AUC_{(0\text{-}12)}$ and C_{max} data from Study D1532C00005 (Part A, Day 1 single dose and Day 8 steady state) have been used to provide estimates of within-volunteer variability. Based on the estimate of within-volunteer variability of 0.04, 24 evaluable volunteers (4 per sequence) will provide 95% power to show that the 90% CI for the ratio of the population geometric means, based on log-transformed data, is contained in the limits of 80.00% to 125.00% for AUC. Based on the estimate of within-volunteer variability of 0.13 and a sample size of 24 volunteers, the upper limit of the 80% CI of an observed increase in C_{max} of 25% would be 1.43, which is considered clinically acceptable. In order to ensure that 24 evaluable volunteers complete the study, 30 volunteers (5/sequence) will be recruited.

The number of volunteers who will agree to participate in the genetic research is unknown. It is therefore not possible to establish whether sufficient data will be collected to allow a formal statistical evaluation or whether only descriptive statistics will be generated.

13. IMPORTANT MEDICAL PROCEDURES TO BE FOLLOWED BY THE INVESTIGATOR

13.1 Medical emergencies and AstraZeneca contacts

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

In the case of a medical emergency the Investigator may contact the CPA Physician. If the CPA Physician is not available, the Investigator should contact the CPA Program Director.

13.2 Overdose

For the purposes of this study, exceeding the dosage requirements specified in this CSP represents an overdose. There is no known antidote for AZD6244. In case of suspected

overdose, the volunteer should be treated according to standard medical practice based on the Investigator's judgment. Cases of overdose will be recorded as follows:

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

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

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

For overdoses associated with an SAE, standard reporting timelines apply, see Section 6.3.2. For other overdoses, reporting should be done within 30 days.

13.3 Pregnancy

All outcomes of volunteer's partner pregnancy should be reported to AstraZeneca.

13.3.1 Maternal exposure (Not applicable)

13.3.2 Paternal exposure

Male volunteers should refrain from fathering a child or donating sperm from the first administration of IP until 14 days after last administration of IP. Volunteers must ensure their partners of childbearing potential use a reliable method of contraception, as well as using a barrier method themselves.

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

14. LIST OF REFERENCES

Kim et al 2011, EORTC-AACR abstract
Kim K, Infante J, Cohen R, Burris H, Emeribe U, Curt G et al. A Phase I dose-escalation study of selumetinib in combination with docetaxel in patients with advanced solid tumors. AACR; Mol Cancer Ther 2011;10(11 Suppl).

Clinical Study Protocol Appendix B

Drug Substance	AZD6244
Study Code	D1532C00066
Edition Number	1

Appendix B
Additional Safety Information

FURTHER GUIDANCE ON THE DEFINITION OF A SERIOUS ADVERSE EVENT

Life-threatening

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

Hospitalization

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

Important medical event or medical intervention

Medical and scientific judgment should be exercised in deciding whether a case is serious in situations where important medical events may not be immediately life-threatening or result in death, hospitalization, disability, or incapacity but may jeopardize the volunteer or may require medical intervention to prevent 1 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 judgment must be used.

Examples of such events are:

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

A GUIDE TO INTERPRETING THE CAUSALITY QUESTION

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

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

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

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

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

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

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

A "reasonable possibility" could be considered to exist for an AE where 1 or more of these factors exist.

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

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

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

- Is there a known mechanism?

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

Clinical Study Protocol Appendix C

Drug Substance	AZD6244
Study Code	D1532C00066
Edition Number	1

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

LABELLING AND SHIPMENT OF BIOHAZARD SAMPLES

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

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

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

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

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

Exempt - all other materials with minimal risk of containing pathogens

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

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

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

TABLE OF CONTENTS PAGE

1. INTRODUCTION

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

The Investigator participates, together with AstraZeneca clinical project representatives, in review and assessment of cases meeting PHL criteria to agree whether Hy's Law (HL) criteria are met. Hy's Law criteria are met if there is no alternative explanation for the elevations in liver biochemistry other than drug-induced liver injury (DILI) caused by the investigational product (IP).

The Investigator is responsible for recording data pertaining to PHL/HL cases and for reporting adverse events (AE) and serious AEs (SAEs) according to the outcome of the review and assessment in line with standard safety reporting processes.

2. DEFINITIONS

Potential Hy's Law

Potential Hy's Law is aspartate aminotransferase (AST) or alanine aminotransferase (ALT) 3 or more times the upper limit of normal (ULN) **and** total bilirubin (TBL) 2 or more times the ULN at any point during the study irrespective of an increase in alkaline phosphatase (ALP). The elevations do not have to occur at the same time or within a specified time frame.

Hy's Law

Hy's Law is aspartate aminotransferase or ALT 3 or more times the ULN **and** TBL 2 or more times the ULN, where no other reason, other than the IP, can be found to explain the combination of increases eg, elevated ALP indicating cholestasis, viral hepatitis, or another drug. The elevations do not have to occur at the same time or within a specified time frame.

3. IDENTIFICATION OF POTENTIAL HY'S LAW CASES

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

- ALT 3 or more times the ULN
- AST 3 or more times the ULN
- TBL 2 or more times the ULN

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

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

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

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

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

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

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

4. FOLLOW-UP

4.1 Potential Hy's Law Criteria not met

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

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

4.2 Potential Hy's Law Criteria met

If the volunteer does meet PHL criteria the Investigator will:

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

- Notify the AstraZeneca representative who will then inform the central study team

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

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

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

- Complete the 3 liver eCRF Modules as information becomes available

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

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

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

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

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

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

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

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

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

- Report an SAE (report term 'HL') according to AstraZeneca standard processes

  – The 'Medically Important' serious criterion should be used if no other serious criteria apply

  – As there is no alternative explanation for the HL case, a causality assessment of 'related' should be assigned.

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

- Report an SAE (report term 'PHL') applying serious criteria and causality assessment as above

- Continue follow-up and review according to agreed plan. Once the necessary supplementary information is obtained, repeat the review and assessment to determine whether HL criteria are met. Update the SAE report according to the outcome of the review.

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

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

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

- Determine if there has been a significant change in the volunteer's condition compared with the last visit where PHL criteria were met

  – If there is no significant change no action is required

  – If there is a significant change notify the AstraZeneca representative, who will inform the central study team, then follow the subsequent process described is Section 4.2 of this Appendix

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

At the first on-study treatment occurrence of PHL criteria being met, even if there has been no significant change the volunteer's condition compared with prestudy treatment visit(s), the Investigator will:

- Notify the AstraZeneca representative who will inform the central study team
- Follow the subsequent process described is Section 4.2 of this Appendix

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

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

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

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

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

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

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

If yes: determine if there has been a significant change in the volunteer's condition compared with when PHL criteria were previously met

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

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

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

8. REFERENCES

FDA Guidance for Industry, 2009
FDA Guidance for Industry (issued July 2009) 'Drug-induced liver injury: Premarketing clinical evaluation':
http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM174090.pdf.