Clinical Study Protocol

Drug Substance	AZD9291
Study Code	D5160C00011
Edition Number	1
Date	

A Phase I, Single-centre, Non-Randomised, Open-label, Pharmacokinetic and Mass Balance Study of Orally Administered [^{14}C]-AZD9291 in Healthy Male Volunteers

Sponsor: AstraZeneca AB, 151 85 Södertälje, Sweden

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

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

Administrative Change No.	Date of Administrative Change	Local Administrative Change No.	Date of Local Administrative Change

AstraZeneca

PROTOCOL SYNOPSIS

A Phase I, Single-centre, Non-Randomised, Open-label, Pharmacokinetic and Mass Balance Study of Orally Administered [^{14}C]-AZD9291 in Healthy Male Volunteers

Principal Investigator

Study centre(s) and number of subjects planned

The study will be performed at a single study centre, . It is expected that 8 healthy volunteers will be sufficient to provide a reliable estimate of rates and routes of absorption and excretion and to ensure sufficient data.

Study period	Phase of development
Estimated date of first subject enrolled	1
Estimated date of last subject completed	1

Objectives

Primary objective

To determine the rates and routes of elimination of [^{14}C]-AZD9291 in healthy volunteers by assessment of concentrations of total [^{14}C] radioactivity of AZD9291 and its metabolites in whole blood, plasma, urine, and faeces as well as percentage recovery of the radioactive dose in urine and faeces after oral dosing.

Secondary objectives

1. To calculate the pharmacokinetic parameters of AZD9291, as well as AZ5104 and AZ7550 metabolites, in plasma and urine, and the pharmacokinetic parameters of total whole blood and plasma radioactivity.

2. To compare disposition of drug-related total radioactivity in plasma to whole blood.

3. To provide additional information on the safety and tolerability of a single dose of AZD9291 in healthy male volunteers.

Exploratory objectives

1. To provide samples for subsequent studies to allow the characterisation of the metabolism of [^{14}C]-AZD9291 through the assessment of metabolic profiles of selected plasma and excreta samples.

2. To collect and store an optional pharmacogenetic blood sample from consenting healthy volunteers for future exploratory research into genes/genetic variation that may influence response (ie, distribution, safety, tolerability, and efficacy) to AZD9291.

These results will be reported separately from the clinical study report.

Study design

This will be a Phase I, open-label, single centre study to determine the rates and routes of absorption and elimination of a single dose of [^{14}C]-AZD9291 in healthy male volunteers, by assessment of concentrations of total [^{14}C] radioactivity in whole blood and plasma, AZD9291 (and metabolites AZ5104 and AZ7550) in plasma and urine, and percent recovery of radioactive dose in urine and faeces.

Healthy male volunteers, aged 30 to 65 years (inclusive), will be recruited at one study centre. It is expected that 8 healthy volunteers will be sufficient to provide a reliable estimate of rates and routes of absorption and excretion and to ensure sufficient data are obtained. Volunteers will receive a single oral dose of [^{14}C]-AZD9291 and remain at the study centre for 21 days for blood, urine and faecal collections as well as safety assessments.

Volunteers will return to the study centre for a 24-hour residency on a weekly basis for a further 3 weeks for collection of additional blood, urine and faecal samples. A final 24-hour study visit (including the end of study evaluations) will be conducted on Day 84 to Day 85 and will include collection of additional blood, urine and faecal samples as well as all final poststudy medical assessments. If at any time during return visits the subject is unable to produce a faecal sample, home collections (outpatient basis) of faeces may be requested at the discretion of the investigator and scientific lead for individual subjects. The home collections of faeces should be brought to the study centre. Volunteers will be discharged from the study on Day 85.

Target subject population

Healthy male volunteers aged 30 to 65 years (inclusive), with regular daily bowel movements.

Investigational product, dosage and mode of administration

Each volunteer will receive 20 mg [^{14}C]-AZD9291 oral solution (free base equivalent) containing a nominal dose of 1 μCi (0.037 MBq) activity as a single administration. Volunteers will be fasted for 10 hours prior to investigational product administration and for 4 hours after investigational product administration. Volunteers will not be allowed to consume water up to 1 hour prior to investigational product administration and for 1 hour after investigational product administration.

Comparator, dosage and mode of administration

None

Duration of treatment

Each volunteer will receive 20 mg [^{14}C]-AZD9291 oral solution (free base equivalent) containing a nominal 1 μCi activity as a single administration.

A maximum of 28 days prior to dose administration will be allowed for the screening period (Visit 1). Each volunteer will be admitted to the study centre on Day -1 (Visit 2) and receive a single oral dose of [^{14}C]-AZD9291 on Day 1 after a 10-hour fast. Volunteers will remain as in-patients at the study centre for 21 days after [^{14}C]-AZD9291 administration for collection of urine and faeces, pharmacokinetic and safety assessments. Volunteers will return to the study centre for a 24-hour residency over the next 3 weeks on a weekly basis (Visit 3: Day 28 to Day 29; Visit 4: Day 35 to Day 36; and Visit 5: Day 42 to Day 43) to provide additional excreta samples as well as urine and plasma samples following discharge from the study centre.

Volunteers will return on Day 84 (Visit 6) for a final 24-hour visit which will include poststudy medical assessment. A window of ±2 days (with a minimum of 5 days between consecutive visits) may be applied for Visits 3 through 6.

The total duration of the study including screening, treatment and follow-up visits will be approximately 16 weeks. Depending on emerging data and quantification of recovery, study timings may be shortened.

Outcome variables:

Primary endpoint

- Amount and percentage of radioactive dose of [^{14}C] radiolabelled AZD9291 recovered in urine, faeces, and in total will be calculated.

Secondary endpoints

- Amount of [^{14}C] radioactivity in plasma and whole blood and the resulting area under the concentration-time curve from time zero to infinity (AUC), area under plasma concentration-time curve from time zero to the last quantifiable concentration ($AUC_{(0-t)}$),

area under the concentration-time curve from time zero to 72 hours postdose ($AUC_{(0-72)}$), area under the concentration-time curve from time zero to 24 hours postdose ($AUC_{(0-24)}$), maximum concentration (C_{max}), time of C_{max} (t_{max}), lag time before observation of quantifiable analyte concentrations (t_{lag}), terminal half-life ($t_{1/2,\lambda z}$), elimination rate constant (λ_z), apparent oral clearance (CL/F), and apparent volume of distribution (V_z/F) will be determined.

- Whole blood to plasma radioactivity ratios for concentrations and selected pharmacokinetic parameters (AUC and C_{max}).

- Estimates for the distribution of radioactivity into red blood cells.

- Plasma AZD9291, AZ7550, and AZ5104 to plasma [^{14}C] radioactivity ratios for concentrations and selected pharmacokinetic parameters (AUC [or $AUC_{(0-t)}$ if AUC cannot be determined] and C_{max}) will be determined.

- AZD9291, AZ7550, and AZ5104 plasma concentrations and the resulting AUC, $AUC_{(0-t)}$, $AUC_{(0-72)}$, $AUC_{(0-24)}$, C_{max}, t_{max}, t_{lag}, $t_{1/2,\lambda z}$, λ_z, CL/F (AZD9291 only) and V_z/F (AZD9291 only).

- Plasma metabolite (AZ7550 or AZ5104) to parent (AZD9291) ratios for AUC and C_{max}, plasma AZD9291, AZ7550, and AZ5104 to plasma radioactivity ratios for AUC and C_{max}, and the whole blood radioactivity to plasma radioactivity ratio for AUC. (If AUC is not reportable in most volunteers, $AUC_{(0-t)}$ will be used rather than AUC).

- Cumulative amount recovered (Ae_u) and cumulative percent (or fraction, fe_u) of administered radioactivity recovered in urine; Ae_u, fe_u, and renal clearance (CL_R) for AZD9291, AZ7550, and AZ5104.

Safety endpoints

Safety parameters include adverse events, vital signs, physical examinations, ophthalmologic examination, electrocardiograms, and clinical laboratory assessments.

Exploratory endpoints

Characterisation and quantification of metabolites in plasma and excreta which will be reported separately from the Clinical Study Report.

Statistical methods

Given the exploratory nature, no formal statistical hypothesis testing will be performed in this study. The statistical analysis will be descriptive and consist of subject listings, graphs, and summary statistics comprising geometric mean, coefficient of variation (CV%), arithmetic mean, standard deviation (SD), median, minimum (min), and maximum (max) values as appropriate. Categorical variables will be summarised in frequency tables (frequency and proportion of volunteers in the analysis set).

TABLE OF CONTENTS PAGE

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

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

Abbreviation or special term	Explanation
λ_z	Elimination rate constant
$\%AUC_{ex}$	Percentage of AUC obtained by extrapolation
ADME	Absorption, distribution, metabolism, and elimination
AE	Adverse event (see definition in Section 6.3.1)
Ae_f	Amount recovered in faeces during each collection interval and overall
Ae_u	Amount recovered in urine during each collection interval and overall
ALP	Alkaline phophatase
ALT	Alanine aminotransferase
AMS	Accelerator mass spectrometry
ARSAC	Administration of Radioactive Substances Advisory Committee
AST	Aspartate aminotransferase
AUC	Area under the concentration-time curve from time zero extrapolated to infinity
$AUC_{(0-72)}$	Area under the concentration-time curve from time zero to 72 hours postdose
$AUC_{(0-24)}$	Area under the concentration-time curve from time zero to 24 hours postdose
$AUC_{(0-t)}$	Area under plasma concentration-time curve from time zero to the last quantifiable concentration
BLQ	Below the limit of quantification
BMI	Body mass index
CIOMS	Council for International Organisations of Medical Sciences
CL/F	Apparent oral clearance
CL_R	Renal Clearance
C_{max}	Maximum observed concentration
C_p	Concentration in plasma
CPA	Clinical Pharmacology Alliance
CrCL	Creatinine clearance
CSA	Clinical Study Agreement

Abbreviation or special term	Explanation
CSP	Clinical Study Protocol
CSR	Clinical Study Report
CTC	Common Terminology Criteria
CTCAE	Common Terminology Criteria for Adverse Events
Cum Ae_f	Cumulative radioactive amount recovered in faeces
Cum Ae_u	Cumulative amount recovered in urine
Cum fe_f	Cumulative percent (or fraction) of actually administered radioactivity recovered in faeces
Cum fe_u	Cumulative percent (or fraction) of actually administered dose/radioactivity recovered in urine
CV%	Coefficient of variation
C_{wb}	Concentration in whole blood
CYP	Cytochrome P450
DAE	Discontinuation of Investigational Product due to Adverse Event
DMPK	Drugs, metabolism and pharmacokinetic
DNA	Deoxyribonucleic acid
DUS	Disease under Study
EC	Ethics Committee, synonymous to Institutional Review Board (IRB) and Independent Ethics Committee (IEC)
ECG	Electrocardiogram
ED	Effective dose
EDC	Electronic data capture
EGFR	Epidermal growth factor receptor
fe_f	Percent (or fraction) of actually administered radioactivity recovered in faeces during each collection interval and overall
fe_u	Percent (or fraction) of actually administered radioactivity recovered in urine during each collection interval and overall
GCP	Good Clinical Practice
GCV%	Geometric coefficient of variation
GMP	Good Manufacturing Practice
HIV	Human immunodeficiency virus
Ht	Haematocrit
IATA	International Airline Transportation Association

Abbreviation or special term	Explanation
IB	Investigator's Brochure
ICF	Informed consent form
ICH	International Conference on Harmonisation
ICRP	International Commission on Radiological Protection
IRB	Institutional Review Board
LIMS	Laboratory Information Management System
LLOQ	Lower Limit of Quantification
LSC	Liquid scintillation counting
LSLV	Last Subject Last Visit
M/P_{AUC}	Metabolite to AZD9291 AUC ratio
M/P_{Cmax}	Metabolite to AZD9291 C_{max} ratio
Max	Maximum
MedDRA	Medical Dictionary for Regulatory Activities
Min	Minimum
NA	Not applicable
ND	Not determined
NMT	Not More Than
NOEL	No observed effect level
NSCLC	Non-small cell lung cancer
OAE	Other Significant Adverse Event (see definition in Section 11.1.2)
pCRF	Paper Case Report Form (paper)
PGx	Pharmacogenetic research
PK	Pharmacokinetic
PL	Plasma AZD9291 or plasma AZ7550 or plasma AZ5104
PR	Plasma radioactivity
QTc	Corrected QT interval
RBC	Red blood cells
Rsq	Goodness of fit statistic for calculation of λ_z (regression coefficient)
SAE	Serious adverse event (see definition in Section 6.3.2).
SD	Standard deviation
SI	Système International
SUSAR	Suspected unexpected serious adverse reactions

Abbreviation or special term	Explanation
$t_{1/2,\lambda z}$	Elimination half-life
t_{lag}	Lag time before observation of quantifiable analyte concentrations
t_{max}	Time to C_{max}
TKIs	Tyrosine kinase inhibitors
ULN	Upper limit of normal
V_z/F	Apparent volume of distribution
Wb	Whole blood
WBDC	Web Based Data Capture
WBR	Whole blood radioactivity
WHO	World Health Organisation

1. INTRODUCTION

1.1 Background

Lung cancer has been the most common cancer in the world for several decades, and by 2008, there were an estimated 1.61 million new cases, representing 12.7% of all new cancers. It was also the most common cause of death from cancer, with 1.38 million deaths (18.2% of the total) (GLOBOCAN 2008). Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) are now the established first line therapy in patients with non-small cell lung cancer (NSCLC) known to have activating mutations in EGFR (EGFRm+) (NCCN 2012). Selective inhibition of EGFR tyrosine kinase has demonstrated clinical benefit in approximately 70% of patients with advanced NSCLC harbouring the sensitivity mutations (the most common of which are L858R and deletions in exon 19 [Ex19del]). The tumours initially respond to EGFR TKIs, but subsequently develop resistance to therapy, with a median time to progression of 9 months. In at least 50% of these initially EGFR TKI-responsive patients, disease progression is associated with the emergence of a secondary EGFR mutation, T790M in exon 20 of EGFR that confers resistance to therapy (Pao et al 2005).

AZD9291 is a potent irreversible inhibitor of both the single EGFRm+ (TKI-sensitivity conferring mutation) and dual EGFRm+/T790M+ (TKI-resistance conferring mutation) receptor forms of EGFR. AZD9291 commenced its first time in man study in March 2013. Phase II studies are due to commence in Q2 2014.

Preliminary pharmacokinetic (PK) data from the ongoing clinical study with AZD9291 in patients with advanced NSCLC who have received at least 1 prior regimen of an EGFR TKI agent (Study D5160C00001) are available following 20 mg (N=6), 40 mg (N=6), 80 mg (N=6), and 160 mg (N=6) single and once daily multiple dosing. Following a single dose, all AZD9291 PK profiles showed a lag time; time to maximum concentration (t_{max}) ranged from 3 to 24 hours; half-life ranged from 30 to 110 hours (median 50 hours). However, half-life was not well characterised due to the PK sampling scheme utilised in the majority of patients in these cohorts (a modified PK sampling scheme was used in the 160 mg cohort and will be used in later cohorts). Accumulation was observed for AZD9291, AZ5104, and AZ7550 after multiple dosing, as would be expected based on the single dose data. Approximately dose-proportional PK for AZD9291, AZ5104 and AZ7550 was observed across the 20 mg to 160 mg dose range.

1.2 Summary of relevant preclinical and clinical information

Investigations into the in vitro metabolism of AZD9291 have shown metabolites present in rat, dog and human hepatocytes. Of the 16 metabolites characterised, 7 were present in human hepatocytes. Two of these, AZ7550 and AZ5104, are active and have been monitored in toxicology studies and in the ongoing first-time-in-man patient study (D5160C00001) and healthy volunteer formulation comparison study (D5160C00005).

Experiments performed with recombinant expressed cytochrome P450 (CYP) isozymes suggested that AZD9291 was predominantly metabolised by CYP3A4 and also to a lesser extent by CYPs 2C8, 1A2, 2C9 and 2C19.

Similarly, the metabolite AZ5104 was also predominantly metabolised by CYPs 3A4 and 3A5 and, to a lesser extent, by CYPs 2C8, 2C19, 1A2, 2D6 and 2C9. The metabolite AZ7550 was almost exclusively metabolised by CYP3A4 with a minor contribution from CYP2C8.

In an intact rat excretion study, the major route of excretion was via the faeces (>80%). Excretion was rapid with the majority of dose recovered within 48 hours of dosing. Urinary elimination was minor.

To date >150 patients with NSCLC have received at least 1 dose of AZD9291 at dose levels of 20 mg, 40 mg, 80 mg, 160 mg, and 240 mg once daily in a clinical study (D5160C00001). In addition, 16 male healthy volunteers have received a single dose of 20 mg AZD9291 in a separate study (D5160C00005). Clinical experience with AZD9291 as monotherapy is described in the current version of the AZD9291 Investigator's Brochure (IB).

Following a single 20 mg capsule fasted dose in Study D5160C00001, the AZD9291 mean (minimum to maximum) area under the concentration-time curve from zero to 72 hours postdose ($AUC_{(0-72)}$) and maximum observed concentration (C_{max}) were 2177 nM*h (953 to 6890 nM*h) and 48 nM (25 to 133 nM), respectively (N=6). For AZ5104, the $AUC_{(0-72)}$ and C_{max} were 84 nM*h (55 to 167 nM*h) and 1.6 nM (0.88 to 3.6 nM), respectively. For AZ7550, the $AUC_{(0-72)}$ and C_{max} were 51 nM*h (32 to 3 nM*h) and 0.93 nM (0.54 to 1.5 nM), respectively.

One patient in the 20 mg cohort showed a higher plasma exposure (C_{max}, and $AUC_{(0-72)}$), with a longer lag time and later t_{max} than expected based on data from other patients in the 20 mg, 40 mg, and 80 mg cohorts. The reason for this is currently unclear and there may be confounding factors. This patient received a gastric pH modifying concomitant medication, which is likely to have reduced the dissolution rate, based on the results of in vitro dissolution studies; this is in agreement with the longer lag time and later t_{max} observed.

Preliminary PK data is available from 16 healthy volunteers administered a single AZD9291 20 mg dose orally as either the capsule, oral solution and tablet formulation in the relative bioavailability and fed/fasted study, D5160C00005. Pharmacokinetic analysis has been performed on the unblinded plasma concentration data provided using non-compartmental analysis and nominal sampling times. The pharmacokinetics profiles were very similar regardless of the formulation dosed. After administration of 20 mg of AZD9291 as the solution formulation the median t_{max} for AZD9291 was 6 hours postdose (range 6 to 10 hours). Geometric mean C_{max} was 15.2 ng/mL. Geometric mean $AUC_{(0-72)}$ was 482 ng*h/mL or approximately 964 nM*h. The highest individual value for $AUC_{(0-72)}$ was 737 ng*h/mL or approximately 1474 nM*h.

1.3 Rationale for conducting this study

The primary objective of this study is to determine the rates and routes of excretion of [^{14}C]-AZD9291 in healthy volunteers by assessment of concentrations of total ^{14}C radioactivity of AZD9291 and its metabolites in whole blood, plasma, and urine as well as percentage recovery of the radioactive dose in urine and faeces.

Human drug metabolism studies are essential in the support of drug development as they provide information related to major routes of drug absorption, distribution, metabolism, and excretion (ADME) as well as inter-subject variability. Definitive metabolite characterisation work carried out on samples from this study will provide a basis for comparison of the metabolism of AZD9291 across species. It is beneficial to conduct these studies so that ADME data may also be assessed for impact on aspects of both ongoing and future clinical studies, as well as being used to inform the design of further clinical pharmacology studies to investigate potential drug-drug interactions.

This study will use an oral solution with a nominal dose of 1 μCi (0.037 MBq) [^{14}C]-AZD9291 to characterise the routes and rates of excretion of [^{14}C]-AZD9291 and its radiolabelled metabolites in whole blood, plasma, urine and faeces.

Samples from this study will be used in subsequent studies in order to quantify and characterise metabolites in plasma and excreta. This will provide a basis for the validation of the species used in toxicological studies, a comparison of the metabolism of AZD9291 across species, and also provide insight into the enzymes responsible for the clearance of AZD9291 and thus inform of potential drug-drug interaction risk.

1.4 Benefit/risk and ethical assessment

1.4.1 Potential benefits

Healthy male volunteers participating in this study will not gain any therapeutic benefit from administration of AZD9291.

1.4.2 Potential risks identified from nonclinical toxicology studies

Key findings from safety pharmacology, secondary pharmacology, and toxicology studies were as follows:

- AZD9291 was negative in the in vitro genetic toxicology tests (Ames test and mouse lymphoma assay) and in vivo in the rat micronucleus test and is therefore considered not to represent a risk of genetic toxicity in humans. AZD9291 absorbs light in the ultraviolet visible range, but was not phototoxic when tested in an in vitro 3T3 assay.

- During the 1 month rat study, repeated administration of AZD9291 was associated with dose-related atrophic, inflammatory, and/or degenerative changes affecting the skin, eye, tongue, and female reproductive system. There were also histopathological findings in the male reproductive system. Histopathological

changes were present in the eye at all doses, but the low dose (4 mg/kg/day) was the no observed effect level (NOEL) for all of the other findings. All findings showed evidence of reversibility. During the 1 month dog study, repeated administration of AZD9291 was associated with dose-related atrophic changes affecting the skin, eye, tongue, and intestine. There were also histopathological findings in the male reproductive system. The low dose (2 mg/kg/day) was the NOEL for all histopathological changes with the exception of the findings in the male reproductive system. All findings showed evidence of reversibility.

- The findings in the male reproductive system comprised seminiferous tubular atrophy (rat and dog) and spermatid retention (rat) in the testes with secondary changes in the epididymides. These findings were generally of a low severity (minimal to mild with the exception of 1/10 high-dose rats with moderate tubular atrophy), are considered unlikely to be seen on single dosing (no testicular pathology seen in limited dimension, non Good Laboratory Practice dose range finding studies of up to 14 days duration), and would be expected to recover. The mechanism underlying these testicular findings is unknown at present.

- There was some evidence for an increase in QT interval and decrease in heart rate following administration of AZD9291 to guinea pigs and dogs. However, the changes seen in the dog telemetry study were marginal, transient, and not dose-related and were considered to be of limited biological significance. Increases in blood pressure were observed in the rat and guinea pig. Increases in blood pressure were not seen in the dog telemetry or 1 month dog studies.

Further details are provided in the AZD9291 IB.

1.4.3 Emerging safety profile with AZD9291

The below listed events are presented in Section 5.4 of the AZD9291 IB:

- Diarrhoea: Occurs at a Council for International Organisations of Medical Sciences (CIOMS) frequency of very common (>10%). A considerable majority of diarrhoea cases are anticipated to be Common Terminology Criteria for Adverse Events (CTCAE) grade 1.

- Rash and Acne: Occurs at a CIOMS frequency of common (>1% and <10%) to very common (>10%) across dose levels. A considerable majority of rash and acne cases are anticipated to be CTCAE grade 1.

- Dry Skin: Occurs at a CIOMS frequency of common (>1% and <10%) to very common (>10%) across dose levels. A considerable majority of dry skin cases are anticipated to be CTCAE grade 1.

1.4.4 Potential adverse events

A summary of adverse events (AEs) reported during the first week of treatment either with a single dose of 20 mg or multiple doses of 20 mg AZD9291 monotherapy treatment in patients with advanced NSCLC is presented in Table 1, up to a data cut of date of 20 November 2013. Adverse event data is available from study D5160C00001 (21 patients in total at the 20 mg dose level).

Data is preliminary and unvalidated and therefore subject to change.

Table 1 Number and percentage of advanced NSCLC patients with adverse events within 7 days of treatment with 20 mg AZD9291, as of 20 November 2013

MeDRA Preferred Term	Total N=21		Maximum CTC grade
	n	%	
Pruritis	2	9.5	1
Urticaria	2	9.5	1
Abdominal pain upper	1	4.3	1
Breath sounds abnormal	1	4.3	1
Conjunctival hyperaemia	1	4.3	1
Decreased appetite	1	4.3	2
Diarrhoea	1	4.3	1
Fatigue	1	4.3	3
Laryngitis	1	4.3	1
Rash	1	4.3	1
Vomiting	1	4.3	2
Weight decreased	1	4.3	1

CTC: Common Terminology Criteria; MedDRA: Medical Dictionary for Regulatory Activities

In a Phase I (Study D5160C0001) study conducted in patients with advanced NSCLC AZD9291 was well tolerated with no clinically important trends in haematology, biochemistry or urinalysis parameters or in vital signs or electrocardiogram (ECG) parameters.

A Phase I study is currently being conducted in healthy volunteers to compare the PK profiles of AZD9291 from different formulations (Study D5160C0005), where volunteers will receive single doses of 20 mg AZD9291 as a capsule formulation, a solution, and a tablet on one occasion each. The interim safety report showed that AZD9291 was safe and well tolerated.

Two AEs of lichen striatus and upper respiratory tract illness was reported by 2 volunteers in this study (Study D5160C0005), which was not considered to be related to AZD9291.

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

Further information on the investigational product can be found in the AZD9291 IB.

AE profile observed in advanced cancer patients:

- Diarrhoea, nausea, and vomiting
- Rashes and acne

Most, seen to date, have been Common Terminology Criteria (CTC) grade 1 and of short duration. Intensity and duration descriptions as well as the complete overview of all available safety data with AZD9291 as monotherapy are described in the current version of the AZD9291 IB.

2. STUDY OBJECTIVES

2.1 Primary objective

To determine the mass balance, rates and routes of elimination of [^{14}C]-AZD9291 in healthy volunteers by assessment of concentrations of total [^{14}C] radioactivity of AZD9291 and its metabolites (AZ5104 and AZ7550) in whole blood, plasma, urine, and faeces as well as percentage recovery of the radioactive dose in urine and faeces after oral dosing.

2.2 Secondary objectives

1. To calculate the PK parameters of AZD9291, as well as AZ5104 and AZ7550 metabolites, in plasma and urine, and the PK parameters of total whole blood and plasma radioactivity.

2. To compare disposition of drug-related total radioactivity in plasma to whole blood.

3. To provide additional information on the safety and tolerability of a single dose of AZD9291 in healthy male volunteers.

2.3 Exploratory objectives

1. To provide samples for subsequent studies to allow the characterisation of the metabolism of [^{14}C]-AZD9291 through the assessment of metabolic profiles of selected plasma and excreta samples.

2. To collect and store an optional pharmacogenetic blood sample from consenting healthy volunteers for future exploratory research into genes/genetic variation that

may influence response (ie, distribution, safety, tolerability and efficacy) to AZD9291.

These results will be reported separately from the CSR.

3. STUDY PLAN AND PROCEDURES

3.1 Overall study design and flow chart

This will be a Phase I, open-label, single centre study to determine the rates and routes of absorption and elimination of a single dose of [^{14}C]-AZD9291 in healthy male volunteers, by assessment of concentrations of total [^{14}C] radioactivity in whole blood and plasma, AZD9291 (and metabolites AZ5104 and AZ7550) in plasma and urine, and percent recovery of radioactive dose in urine and faeces.

Healthy male volunteers, aged 30 to 65 years (inclusive), will be recruited at one study centre. It is expected that 8 volunteers will be sufficient to provide a reliable estimate of rates and routes of absorption and elimination and to ensure sufficient data.

Volunteers will be screened over a period of 28 days (prior to investigational product administration) for eligibility (Visit 1). Screening assessments include evaluation of clinical chemistry, haematology, urinalysis, a physical examination, vital signs, a full ophthalmological examination and 12-lead paper ECG. Study-related procedures will only be performed after signing of the informed consent form (ICF).

Eligible volunteers will be admitted to the study centre on Day -1 for assessment of AEs, clinical chemistry, haematology, urinalysis, ECG, and vital signs including weight (Visit 2) and will remain in the study centre for 21 days.

On the morning of Day 1 each volunteer will receive a single dose of 20 mg [^{14}C]-AZD9291 administered as an oral solution (free base equivalent) containing a nominal dose of 1 μCi (0.037 MBq) activity to determine the concentrations of total ^{14}C radioactivity, AZD9291 and metabolites (AZ5104 and AZ7550) in plasma and urine as well as measurements of total radioactivity of [^{14}C]-AZD9291 in blood, faeces and urine. Additionally [^{14}C]-AZD9291 metabolism in plasma, urine and faeces will be characterised, as appropriate.

Volunteers will be resident at the study centre for 21 days following administration of the investigational product. During residency samples of blood, urine and faeces as well as safety assessments will be collected. Volunteers will be discharged on Day 21 after completion of all assessments. Volunteers will return to the study centre for a 24-hour residency on a weekly basis for a further 3 weeks (up to Day 42) for further sample collection of blood, urine, and faeces as well as safety assessments (Visit 3: Day 28 to Day 29; Visit 4: Day 35 to Day 36; and Visit 5: Day 42 to Day 43). A final 24 hour study visit will be conducted at Day 84 to Day 85 (Visit 6) which will include collection of additional blood, urine, and faecal samples as well as all final poststudy medical assessments. If at any time during return visits the subject is unable to produce a faecal sample, home collections (outpatient basis) of faeces

may be requested at the discretion of the investigator and scientific lead for individual subjects. The home collections of faeces should be brought to the study centre. Volunteers will be discharged from the study on Day 85.

A window of ±2 days for Visits 3 through 6 can be applied, provided there is a minimum of 5 days between consecutive visits. The total duration of the study including screening, treatment and follow-up visits will be approximately 16 weeks. Depending on emerging data and quantification of recovery, study timings may be shortened.

To try and ensure a faecal sample is produced in every collection procedure lactulose will be available at the study centre and will be administered as follows:

1. If following dosing a subject does not produce a sample in a 24 hour period a single dose of 15 mL of lactulose will be given at the start of the next collection period. This will be given up to 3 times per day until samples are produced in 2 consecutive 24 hour collection periods. The lactulose will then either be discontinued or continued based on investigator judgement.

2. At admission for the return collection visits the subjects will be asked when they last opened their bowels. If this was more than 24 hours prior to the admission, lactulose 15 ml will be given. This will be given up to 3 times per day until a sample is produced. The lactulose will then either be discontinued or continued based on investigator judgement.

At any time the lactulose may be discontinued based on the judgement of the investigator (eg, if the subject experiences intolerable abdominal pains).

A study flow chart is shown in Figure 1. The overall study plan and schedule of assessments are presented in Table 2 and Table 3.

Figure 1 Study flow chart

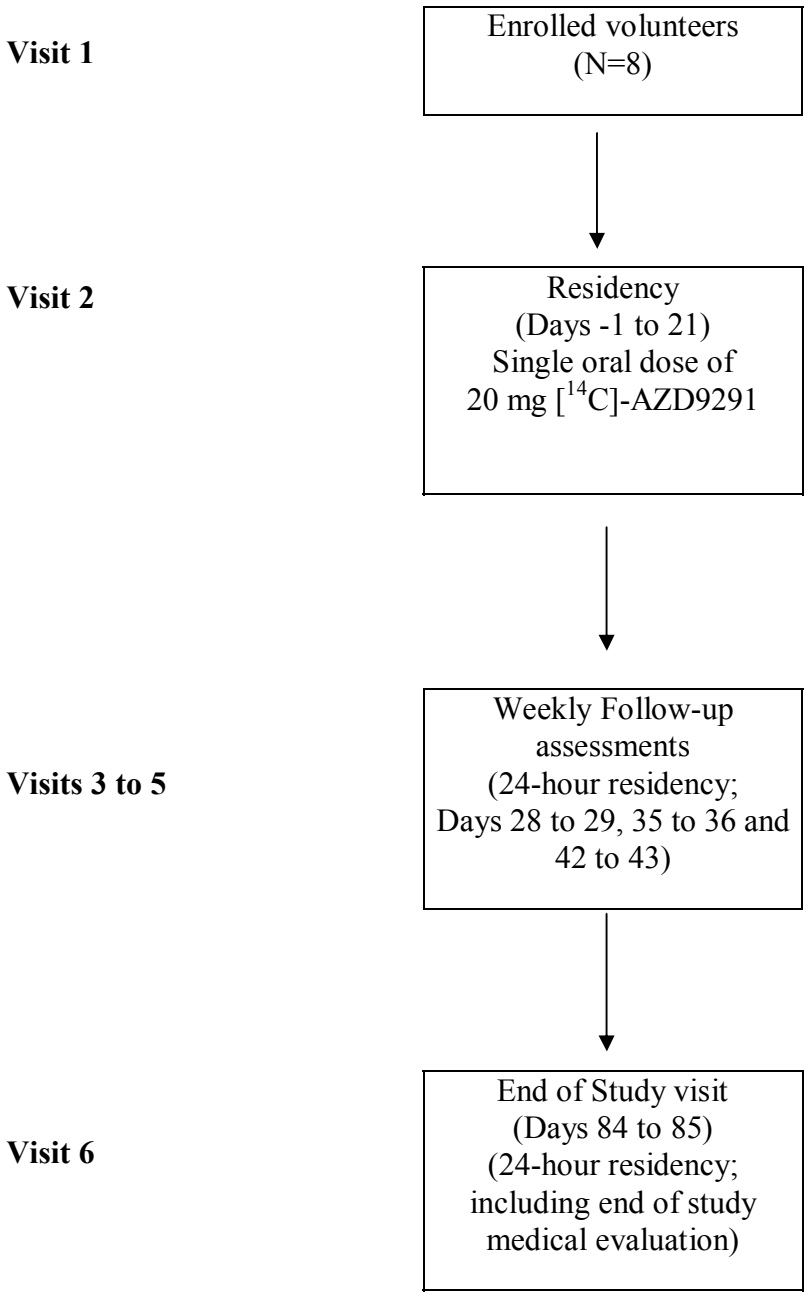

Table 2 Overall study plan

Assessment	Screening	Admission	Dosing day and sampling period			End of Study visit including poststudy physical examination
			Residential period		Weekly 24-hour residency	
Visit	**1**	**2**			**3 through 5**	**6**
Days	**-28 to -2**	**-1**	**1**	**2 to 21**	**(28 to 29, 35 to 36, 42 to 43)[a]**	**(84 to 85)[b] 24-hour residency**
Informed consent	X					
Optional informed consent for genotyping[c]		X[c]				
Pharmacogenetic blood sample collection			X[c]			
Inclusion/exclusion	X	X				
Demography	X					
Height/weight/BMI[d]	X	X[d]				X[d]
Medical/surgical history	X					
Prior/concomitant medication	X	X	X	X	X	X
Physical examination	X				X	X
Brief physical examination[e]		X	X			
Clinical chemistry, haematology[f]	X		X	X	X[f]	X
Serology (hepatitis B surface antigen, hepatitis C antibody, and HIV)	X					
Estimate creatinine clearance[g]	X					
Ophthalmic examination	X[h]					
Urinalysis/urine microscopy[i]	X		X			X

Table 2 Overall study plan

Assessment	Screening	Admission	Dosing day and sampling period			End of Study visit including poststudy physical examination
			Residential period		Weekly 24-hour residency	
Visit	**1**	**2**			**3 through 5**	**6**
Days	**-28 to -2**	**-1**	**1**	**2 to 21**	**(28 to 29, 35 to 36, 42 to 43)[a]**	**(84 to 85)[b] 24-hour residency**
Alcohol/urine drug screen/breath carbon monoxide test[j]	X	X				
Vital signs (supine blood pressure and pulse rate)	X		X[k]	X	X	X
12-Lead ECG[l]	X		X	X		X
Investigational product administration[m]			X			
PK blood/plasma collection[n]			X[n]	X[n]	X[n]	X
Urine collection[n]			X[n]	X[n]	X[n]	X[n]
Faeces collection[n]			X[n]	X[n]	X[n]	X[n]
Discharge				X		X[o]
Record AEs		X	X	X	X	X
Record SAEs[p]	X[p]	X[p]	X	X	X	X

AE: adverse event; BMI: body mass index; ECG: electrocardiogram; HIV: human immunodeficiency virus; PK: pharmacokinetics; SAE: serious adverse event.

a A visit window of ±2 days could be applied from Day 28 onwards.

b Poststudy physical evaluations will be performed at the time of the last PK blood sample collection (Days 84 to 85) or at the point at which the volunteer discontinues from the study.

c Blood sampling for genotyping is optional and will be collected if the volunteer provides additional informed consent on Day -1.

d Height/weight will be evaluated at the screening visit. Only weight will be evaluated on Day-1 and Day 84 (End of Study visit).

e A brief physical examination (including general appearance, skin, abdomen, cardiovascular system, and lungs) performed at admission or predose.

f The volunteers will fast from overnight prior to clinical laboratory evaluations at screening. Postdose time points will be Days 7, 14, 28 and End of Study visit (not fasted).

g Creatinine clearance will be estimated using the Cockroft-Gault formula at screening.

h Ophthalmic examination (best corrected visual acuity and slit-lamp fundoscopy and intraocular pressure measurement) will be conducted at screening (will be considered the baseline value; no need to repeat) and for cause (on occurrence of AE only).

i A sample for urinalysis to be collected together with the other clinical laboratory evaluations. Microscopy will only be performed if abnormal urinalysis results are observed.

j Alcohol will be assessed by an alcohol breath test. Smoking will be assessed by performing a breath carbon monoxide test. A positive test will constitute a value of greater than 10 ppm.

k Supine blood pressure and pulse rate will be evaluated after the volunteer has rested in a supine position for at least 10 minutes. If possible, the same arm and equipment should be used for each evaluation (refer to Table 3 for time points).

l Refer to Table 3 for ECG time points.

m Volunteers will receive a single administration of the investigational product on Day 1.

n Blood, plasma, urine and faeces samples will be collected (refer to Table 3 for time points).

o Discharge on Day 85.

p Only SAEs will be collected from screening until the End of Study visit whereas AEs will be collected from Day -1 through to the End of Study Visit.

Table 3 Schedule of assessments

Time (Day/hours)	12-lead ECG	P & BP[a]	Safety blood & urine[c]	24-hour urine collection[b]	Faecal collection[b]	PK and Met-ID Blood/plasma	Food and Fluid Restrictions[c]	Physical examination	Residency
Screening[d]	X	X	X					X	
Day -1[d,e]							F[f]	X[j]	┬
Predose	X[g]	X	X	┬	┬	X	D[h]	X[j]	│
Dose (Day 1)				│	│		D		│
0.5				│	│	X			│
1	X	X		│	│	X	D[h]		│
1.5				│	│	X			│
2	X	X		│	│	X			│
3				│	│	X			│
4	X	X		│	│	X	F[f], M		│
6				│	│	X	F		│
8	X	X		│	│	X			│
10				│	│	X			│
12	X	X		│	│	X			│
18				│	│	X			│
Day 1 (24)	X[g]	X		│	│	X			│
Day 2 (36)				│	│	X			│
48				│	│	X			│
Day 3 (72)				│	│	X			│
Day 4 (96)				│	│	X			│
Day 5 (120)				│	│	X			│

Table 3 Schedule of assessments

Time (Day/hours)	12-lead ECG	P & BP[a]	Safety blood & urine[c]	24-hour urine collection[b]	Faecal collection[b]	PK and Met-ID Blood/plasma	Food and Fluid Restrictions[c]	Physical examination	Residency
Day 6 (144)				|	|	X			|
Day 7 (168)	X	X	X	|	|	X			|
Day 8				|	|				|
Day 9				|	|				|
Day 10				|	|				|
Day 11				|	|				|
Day 12				|	|				|
Day 13				|	|				|
Day 14 (336)	X	X	X	|	|	X			|
Day 15				|	|				|
Day 16				|	|				|
Day 17				|	|				|
Day 18				|	|				|
Day 19				|	|				|
Day 20				|	|				|
Day 21 (504)	X	X	X	⊥	⊥	X			⊥
Day 28 (672)			X	X	X	X			24 h
Day 29									
Day 35 (840)				X	X	X			24 h
Day 36									
Day 42 (1008)				X	X	X		X[i]	24 h
Day 43									

Table 3 Schedule of assessments

Time (Day/hours)	12-lead ECG	P & BP[a]	Safety blood & urine[c]	24-hour urine collection[b]	Faecal collection[b]	PK and Met-ID Blood/plasma	Food and Fluid Restrictions[c]	Physical examination	Residency
End of Study visit (Day 84)			X			X			24 h
Discharge (Day 85)	X	X		X	X			X	

BP: blood pressure; D: drink; ECG: electrocardiogram; F: free access to food & fluid; M: meal; Met-ID: metabolite identification; P: pulse rate; PK: pharmacokinetic

a Only supine BP is to be measured. BP will be recorded after the volunteer has been resting for 10 minutes.
b Urine and faeces will be collected using separate containers as specified in the Laboratory Manual.
c The volunteers will fast from overnight prior to clinical laboratory evaluations at screening. Postdose time points will be Days 7, 14, 28 and End of Study visit (not fasted).
d Drugs of abuse testing will be performed at screening and Day -1.
e A 10 mL blood sample will be taken prior to receiving the first dose of AZD9291 for pharmacogenetic analysis provided the subject has provided additional informed consent on Day -1.
f Meals to be consumed at the same times as planned in the 24 to 72 hours period after investigational product administration – as per the study centre standard procedures. Volunteers are to be provided with 240 mL of water 2 hours postdose. Volunteers will remain fasted until 4 hours postdose.
g Volunteers will have 3 12-lead ECG traces recorded at 10 minute intervals.
h Water is allowed up to 1 hour predose and again starting at 1 hour postdose.
i Physical examination on Day 42 will include weight.
j Brief physical examination conducted on Day -1 or predose.

3.2 Rationale for study design, doses and control groups

Healthy male volunteers are considered appropriate because the PK data generated will not be influenced by any disease process or concomitant medication. It is expected that 8 volunteers will be sufficient to provide a reliable estimate of rates and routes of absorption and elimination and to ensure sufficient data. The number of volunteers is based on the desire to gain adequate information on the primary endpoints of PK, metabolism, and safety profile whilst exposing as few volunteers as possible to radioisotopes and study procedures.

A 20 mg AZD9291 dose incorporating a nominal dose 1 μCi (0.037 MBq) of [^{14}C]-AZD9291 will be administered as a single dose. This provides an effective dose (ED) of approximately 0.038 mSv which falls within the radiolabelled Category I dose, World Health Organisation (WHO) 1977 and Category I, ICRP 1992 guidelines.

A single dose of 20 mg [^{14}C]-AZD9291 administered as a solution (prepared in a citrate buffer) will be used in this study. The solution formulation is currently being used in a healthy volunteer study (D5160C00005). Although the solution has not been used in patient studies it produced similar PK in Study D5160C00005 to the capsule and tablet formulations. In Study D5160C00001, the exposures of AZD9291 were dose-proportional across the range 20 to 240 mg, therefore it is expected to provide representative exposure of a tablet formulation and information on the routes and rate of elimination of [^{14}C]-AZD9291 and/or its radiolabelled metabolites in whole blood, plasma, urine, and faeces. Data from study D5160C00005 has demonstrated similar PK profiles between the solution and tablet formulation.

The dose level of 20 mg is considered to be in the therapeutic dose range and is high enough to characterise fully the single oral dose pharmacokinetics of parent compound and to enable metabolite quantification and characterisation as part of a separate study.

Based on the preliminary patient PK data available to date, the terminal half-life ($t_{1/2,\lambda z}$) of AZD9291 in human plasma is approximately 30 to 110 hours (median 50 hours, based on PK samples collected up to 72 hours following single dose administration in the majority of patients [N=19 out of 24], samples collected to 168 hours in N=5 patients). In addition, preliminary data on the half-lives of the AZ5104 and AZ7550 metabolites have suggested these to be approximately 70 and 120 hours, respectively (median values based on N=4). Based on this, a period of 21 days for continuous excreta collection is thought to be sufficient to account for the majority of [^{14}C]-AZD9291 radioactivity excreted. Weekly sampling of [^{14}C]-AZD9291 radioactivity in urine and faeces over an additional 3-week interval will aid the characterization of the elimination rate for [^{14}C]-AZD9291. In Study D5160C0001 where sampling was performed for longer, based on preliminary data the observed apparent mean (min-max) terminal half-life was 48.91 (31.1 – 65.2) and 73.21 (52.4 – 105) hours for AZ5104 and AZ7550, respectively.

4. SUBJECT SELECTION CRITERIA

The Principal Investigator should keep a record, the subject screening log, of all volunteers who entered screening.

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

4.1 Inclusion criteria

For inclusion in the study volunteers should fulfil the following criteria:

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

2. Regular bowel movements (ie, on average production of at least 1 stool per day).

3. Healthy male volunteers aged 30 to 65 years (inclusive) with suitable veins for cannulation or repeated venipunctures. (Healthy as determined by medical history, physical examination, laboratory parameters, ECG, and eye examination performed before first administration of investigational product.).

4. Have a body mass index (BMI) between 19 and 32 kg/m2 inclusive and weigh at least 50 kg and no more than 100 kg, inclusive.

5. At screening, calculated creatinine clearance (CrCL) ≥50 mL/min using Cockcroft-Gault formula.

6. Male volunteers must be willing to use reliable methods of contraception (condom and spermicide), even if their partners are postmenopausal, surgically sterile, or using an effective hormonal method of contraception or intrauterine coil. In addition, volunteers must agree to continue to take similar contraceptive precautions until 6 months after the last administration of AZD9291 and avoid procreative sex as well as sperm donation for 6 months.

7. Be willing and able to comply with the study procedures, restrictions and requirements.

4.2 Exclusion criteria

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

1. Involvement in the planning and/or conduct of the study (applies to AstraZeneca, staff).

2. History of any clinically significant disease or disorder which, in the opinion of the Principal 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.

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

4. Any clinically significant abnormalities in physical examination, vital signs (supine blood pressure >150 mmHg systolic, >90 mmHg diastolic, or pulse rate ≤35 or ≥100 beats per minute), or clinical laboratory assessment as judged by the Principal Investigator.

5. Acute illness, surgical procedures, or trauma from within 2 weeks before screening until the first administration of investigational product.

6. Volunteers who have received live or live-attenuated vaccine in the 2 weeks prior to investigational product administration.

7. Volunteers with active malignancy or neoplastic disease in the previous 12 months.

8. A suspected/manifested infection according to International Airline Transportation Association (IATA) Categories A and B infectious substances.

9. Positive results on screening tests for serum hepatitis B surface antigen, hepatitis C antibody, or human immunodeficiency virus (HIV).

10. Any clinically important abnormalities in rhythm, conduction, or morphology of resting 12-lead ECG, QT interval >470 ms, as judged by the Principal Investigator.

11. Known or suspected history of significant drug abuse as judged by the Principal Investigator.

12. Positive screen for drugs of abuse or carbon monoxide (>10 ppm) at screening or positive screen for alcohol, drugs of abuse, or carbon monoxide (>10 ppm) on admission to the study centre prior to the first administration of investigational product.

13. History of alcohol abuse or excessive intake of alcohol, defined as regular weekly intake of more than 21 units of alcohol in men (Note: 1 unit=25 mL spirits, 125 mL wine, or 250 mL beer or lager).

14. History of severe allergy/hypersensitivity or ongoing allergy/hypersensitivity, as judged by the Principal Investigator, or history of hypersensitivity to AZD9291, its excipients, or drugs with a similar chemical structure or class.

15. Use of any prescribed or non-prescribed medication, including drugs during the 4 weeks (or longer depending on the medication's half-life) prior to the

administration of AZD9291 is not permitted. Occasional use of paracetamol and nonsteroidal nasal decongestant is permitted at the discretion of the Principal Investigator. Exceptions may apply on a case by case basis as agreed by the Principal Investigator and sponsor's medical monitor if considered not to interfere with the aims of the study.

16. Use of drugs with enzyme inducing properties such as St John's Wort within 4 weeks prior to the investigational product administration.

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

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

19. Volunteers who received another new chemical entity (defined as a compound which has not been approved for marketing) or participated in any other clinical study (including methodology studies where no drugs were given) within 3 months of the first administration of investigational product in this study are not eligible.

20. Judgment by the Principal Investigator that the volunteer should not participate in the study if the volunteer is considered unlikely to comply with study procedures, restrictions, and requirements.

21. Ongoing or planned inpatient surgery, dental procedure, or hospitalisation during the study.

22. Radiation exposure from clinical studies, including that from the present study, excluding background radiation but including diagnostic X-rays and other medical exposures, exceeding 5 mSv in the last 12 months or 10 mSv in the last 5 years. No occupationally exposed worker, as defined in the Ionising Radiation Regulations 1999, shall participate in the study.

23. Volunteers who have been administered any amount of a [^{14}C]-labelled compound within the last 12 months.

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

25. Judgment by the Principal Investigator that the volunteer would not be able to understand or cooperate with the requirements of the study.

Procedures for withdrawal of incorrectly enrolled volunteers see Section 5.2.

5. STUDY CONDUCT

5.1 Restrictions during the study

- Volunteers should be fasted overnight for 10 hours before investigational product administration (water is allowed up to 1 hour predose) and volunteers remain fasted for 4 hours after investigational product administration. Water is allowed 1 hour postdose.

- The volunteers will be fasted for at least 4 hours prior to any clinical laboratory evaluations. Postdose time points will be Days 7, 14, 28 and End of Study visit (not fasted).

- Volunteers with sexual partners who are pregnant or who could become pregnant (ie, women of child-bearing potential), are postmenopausal, surgically sterile, or using an effective hormonal method of contraception or intrauterine coil should use barrier methods of contraception for at least 6 months after [^{14}C]-AZD9291 administration to avoid pregnancy and/or potential adverse effects on the developing embryo. Reliable methods of contraception should be used consistently and correctly. Two or more of the following methods are acceptable and must include at least one barrier method:

  Acceptable methods for volunteers' partners include:

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

  Acceptable methods for volunteers include:

  – Volunteers will be required to use reliable methods of contraception (condom and spermicide) for the duration of the study until 6 months after the investigational product administration. If a man has had a vasectomy this should be considered an appropriate method of contraception (along with a condom).

  Alternatively, true abstinence is acceptable when it is in line with the volunteer's preferred and usual lifestyle. If a volunteer is usually not sexually active but becomes active, they, with their partner, should use 2 of the contraceptive methods listed above.

- Volunteers must avoid fathering a child during the study and for 6 months after [^{14}C]-AZD9291 administration.

- Volunteers should abstain from sperm donation for 6 months after [^{14}C]-AZD9291 administration.

- Volunteers should abstain from donating blood during the study and for at least 3 months after the End of Study visit.

- Volunteers should abstain from taking drugs of abuse from 4 weeks prior to [^{14}C]-AZD9291 administration and until 3 months afterwards.

- Volunteers should abstain from taking any prescribed medication, over-the-counter remedies, herbal medications, high-dose or 'mega' vitamins, mineral supplements, or medicines purchased via the Internet beginning 4 weeks (or longer depending on the medication's half-life) before investigational product administration and continuing until 3 months after investigational product administration. Paracetamol 1 g, every 6 hours, to a maximum daily dose of 4 g is permitted; however, the Principal Investigator should be informed so this can be recorded.

- Volunteers should not consume grapefruit, grapefruit juice, Seville oranges, Seville orange marmalade, or other products containing grapefruit or Seville oranges from 7 days prior to [^{14}C]-AZD9291 administration until after the End of Study visit.

- Volunteers who wear contact lenses should discontinue wearing their lenses if they have any mild to moderate eye symptoms following exposure to the investigational product for at least 1 week after symptoms have resolved.

- Volunteers should not use any eye drops or ointment for treatment of eye symptoms, unless agreed by a study doctor, at any time during the study up to 1 week after [^{14}C]-AZD9291 administration.

- Volunteers should abstain from taking caffeine-containing drinks or foods (eg, coffee, tea, cocoa, chocolate, and cola) while in-patient during the study. During the outpatient period, volunteers should avoid excessive intake of caffeine-containing drinks or food, eg, coffee, tea, chocolate, caffeine-containing energy drinks (Red Bull), and cola (more than 5 cups of coffee or equivalent, 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.

- Volunteers should abstain from drinking alcohol beginning 72 hours before admission to the study centre and continuing until 24 hours after leaving the study centre and also beginning 72 hours prior to blood tests or the End of Study visit.

- Volunteers should abstain from consuming poppy seeds from enrolment until after the End of Study visit.

- Volunteers should refrain from strenuous physical activity that is not within the volunteer's normal weekly routine, beginning 5 days before each visit to the study centre and continuing until after the End of Study visit.

- Volunteers should refrain from actively trying to lose weight from the prestudy physical examination until after the End of Study visit.

5.2 Subject enrolment and initiation of investigational product

The Principal Investigator will:

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

2. Assign potential volunteer a unique enrolment number, beginning with 'E10001001'.

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

4. Volunteers will be assigned a unique subject number at the time of dosing in the format of 1001 to 1008

If a volunteer withdraws from participation in the study, then his enrolment code cannot be reused. Enrolment codes will be assigned strictly sequentially as volunteers become eligible for enrolment.

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

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

Where volunteers that do not meet the inclusion and/or exclusion criteria, are enrolled in error, or 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 Principal Investigator regarding whether to continue or discontinue the volunteer from the study.

The AstraZeneca CPA Physician is to ensure all such decisions are appropriately documented. In situations where an agreement cannot be reached, the volunteer should be discontinued from the study.

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

5.5 Treatments

5.5.1 Identity of investigational product

Table 4 Formulation of Investigational Product

Investigational product	Dosage form and strength	Manufacturer
AZD9291	20 mg Solution formulation containing a nominal 1 µCi activity[a,b] ([^{14}C]-labelled and unlabelled should add up to this quantity based on desired activity)	AstraZeneca
Solution buffer	0.01M citrate buffer pH 3.5	

[a] The container must be kept for quantification of residual radioactivity.
[b] Once the volunteer has consumed the initial preparation of 100 mL, a further 100 mL of purified water must be used to rinse the container twice (2x50 mL) and administered to the volunteer. The total fluid volume administered will be 240 mL of fluids (buffer and water) inclusive of the volume of solution administered and any rinses.

5.5.2 Doses and treatment regimens

Each volunteer will receive a single dose of 20 mg [^{14}C]-AZD9291 oral solution (free base equivalent) containing a nominal dose 1 µCi (0.037 MBq) activity as a single administration on Day 1. [^{14}C]-AZD9291 will be administered with 240 mL of fluids (buffer and water) inclusive of the volume of solution administered and any rinses.

Volunteers should be fasted overnight for 10 hours prior to investigational product administration (water is allowed up to 1 hour prior to investigational product administration). Volunteers must remain fasted for 4 hours after investigational product administration. Water is allowed 1 hour after investigational product administration.

5.5.3 Labelling

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

5.5.4 Storage

All investigational products should be kept in a secure place under appropriate storage conditions. The investigational product label specifies the appropriate storage.

5.6 Concomitant and poststudy treatment(s)

Volunteers should abstain from taking any prescribed medication, over-the-counter remedies, herbal medications, high-dose or 'mega' vitamins, mineral supplements, or medicines purchased via the Internet beginning 4 weeks (or longer depending on the medication's

half-life) before investigational product administration and continuing until 3 months after investigational product administration. Paracetamol 1 g, every 6 hours, to a maximum daily dose of 4 g is permitted; however, the Principal Investigator should be informed so this can be recorded in the volunteer's paper Case Report Form (pCRF).

Other medication, which is considered necessary for the volunteer's safety and well being, may be administered at the discretion of the Principal Investigator and recorded in the appropriate sections of the pCRF.

5.7 Treatment compliance

The date and time of administration of the investigational product should be recorded in the appropriate sections of the pCRF. In order to ensure treatment compliance, the administration of the investigational product will be performed under the supervision of the study personnel.

5.7.1 Accountability

The investigational product provided for this study will be used only as directed in this CSP. The investigational product will be administered under supervision.

Study centre personnel will account for all investigational product received, all unused investigational product, and for appropriate destruction of unused investigational product. Destruction must not take place unless the responsible person at AstraZeneca has approved it. Certificates of delivery, destruction, and return should be signed.

5.8 Discontinuation of investigational product (Not applicable)

5.9 Withdrawal from study

Volunteers are at any time free to withdraw from the study (investigational product and assessments), without prejudice to further treatment (withdrawal of consent). These 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 Principal Investigator. Adverse events will be followed up (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 and Table 3. Additional assessments may be performed if the Principal Investigator considers them necessary for volunteer safety.

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 ECG.

2. Vital signs (blood pressure and pulse rate).

3. PK blood sample.

4. Safety laboratory assessment blood sample.

5. PK urine sample.

Apart from the predose PK blood sampling, which should occur within 30 minutes prior to investigational product administration, other predose assessments may be performed up to 60 minutes prior to administration of the investigational product.

For occasions when more than one assessment is required at a particular time point, PK blood samples should be prioritised. Additional assessments may be performed if the Principal Investigator considers them necessary for volunteer safety.

6.1 Recording of data

The Principal Investigator will ensure that data are recorded on the pCRF as specified in the study protocol and in accordance with the instructions provided.

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

6.2 Data collection at enrolment and follow-up

Following a signed informed consent, volunteers will be screened for eligibility to be enrolled in the study.

6.2.1 Enrolment procedures

At enrolment 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, and alcohol consumption.

Each volunteer will undergo screening in the 28 days prior to investigational product administration. This will consist of:

- Obtaining written informed consent prior to starting any study-specific procedures
- Recording demographic data – date of birth, gender, race, and ethnicity
- Height, weight, and calculation of BMI
- A standard medical, medication, and surgical history with review of the inclusion and exclusion criteria with the volunteer
- A complete physical examination
- Habits of nicotine and alcohol use
- Vital signs measurements (supine pulse rate and blood pressure)
- Recording of 12-lead paper ECG
- A blood sample for routine clinical chemistry and haematology as well as a screen for hepatitis B surface antigen, antibodies to hepatitis C virus, and antibodies to HIV
- A urine sample for routine urinalysis and drugs of abuse screen (including carbon monoxide and alcohol)
- Assessment of any SAEs
- Assessment of any prior and/or concomitant medication use
- Ophthalmic examination

6.2.2 Follow-up procedures

The final medical assessment on Day 84 or on the last day of urine/faecal collection (whichever is later) will include the assessment of concomitant medication, complete physical examination, clinical chemistry, haematology, PK blood collection, urinalysis, urine and faeces collection, supine blood pressure and pulse rate, weight, 12-lead ECG, and recording of AEs/SAEs.

6.3 Safety

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

6.3.2 Definitions of serious adverse event

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

- Results in death
- Is immediately life-threatening
- Requires inpatient hospitalisation or prolongation of existing hospitalisation
- 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 jeopardise the volunteer or may require medical intervention to prevent one 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

Only SAEs will be collected from screening until the End of Study visit (Day 85) whereas AEs will be collected from Day -1 through to the End of Study visit (Day 85).

Follow-up of unresolved adverse events

Any AEs that are unresolved at the volunteer's End of Study visit, should be followed up by the Principal Investigator for as long as medically indicated, but without further recording in the pCRF. 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 collect for each AE:

- AE (verbatim)

- The date and time when the AE started and stopped
- Maximum intensity, rated according to the following scale:
  - Mild (awareness of sign or symptom, but easily tolerated)
  - Moderate (discomfort sufficient to cause interference with normal activities)
  - Severe (incapacitating, with inability to perform normal activities)
- Whether the AE is serious or not
- Principal Investigator causality rating against the investigational product (yes or no)
- Action taken with regard to investigational product
- Whether AE 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 Principal Investigator became aware of SAE
- AE is serious due to
- Date of hospitalisation
- 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
- Description of AE

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria in Section 6.3.2. 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 Principal Investigator will assess causal relationship between investigational product and each AE, and answer 'yes' or 'no' to the question 'Do you consider that there is a reasonable possibility that the event may have been caused by the investigational product?'

For SAEs, causal relationship will also be assessed for other medication and study procedures. 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 pCRF. When collecting AEs, the recording of diagnoses is preferred (when possible) to recording a list of signs and symptoms. However, if a diagnosis is known and there are other signs or symptoms that are not generally part of the diagnosis, the diagnosis and each sign or symptom will be recorded separately.

Adverse Events based on examinations and tests

The results from protocol-mandated laboratory tests and vital signs will be summarised in the CSR. Deterioration as compared to baseline in protocol-mandated laboratory values, vital signs, or other safety variables should therefore only be reported as AEs if they fulfil any of the SAE criteria or are the reason for discontinuation of treatment with the investigational product.

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

NB. Cases where a volunteer shows an aspartate aminotransferase (AST) **or** alanine aminotransferase (ALT) ≥3 times the upper limit of normal (ULN) **or** total bilirubin ≥2 times the ULN may need to be reported as SAEs, please refer to Appendix D 'Actions required in

cases of combined increase of aminotransferase and total bilirubin – Hy's Law', for further instructions.

6.3.4 Reporting of serious adverse events

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

If any SAE occurs in the course of the study, then the Principal Investigator or other study centre personnel will inform the 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 Principal 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 Principal Investigator or other study centre personnel inform AstraZeneca representatives of any follow-up information on a previously reported SAE immediately, or **no later than 24 hours** of when he or she becomes aware of it.

The reference document for definition of expectedness/listedness is the AZD9291 IB.

6.3.5 Laboratory safety assessment

Blood and urine samples for determination of clinical chemistry, haematology, and urinalysis will be collected at the time points indicated in the study plan (see Table 2 and Table 3).

The laboratory variables to be measured are provided in Table 5.

Table 5 Laboratory safety variables

Haematology[a]	Clinical Chemistry[a]	Virology[c]	Urinalysis[b]	Drugs of Abuse
Basophils	Alanine aminotransferase (ALT)	Hepatitis B surface antigen	Bilirubin	Amphetamines
Eosinophils	Albumin	Hepatitis C antibody	Blood	Barbiturates
Haematocrit (packed cell volume- PCV)	Alkaline phosphatase Lactate dehydrogenase	Human immunodeficiency virus (HIV) antibody	Glucose	Benzodiazepines
Haemoglobin	Aspartate aminotransferase (AST)		Ketones	Cocaine

Haematology[a]	Clinical Chemistry[a]	Virology[c]	Urinalysis[b]	Drugs of Abuse
Lymphocytes	Bicarbonate		Leukocytes	Marijuana/cannabis.
Mean cell haemoglobin (MCH)	Bilirubin (total)		Nitrites	Methadone
Mean cell haemoglobin concentration (MCHC)	Bilirubin (direct) (only if total is elevated)		pH	Methamphetamine
Mean cell volume (MCV)	Calcium		Protein	Ecstasy
	Chloride			
Monocytes	Lipase		Specific gravity	Morphine/opiates
Neutrophils	Creatinine		Urobilinogen	Phencyclidine
Platelet count	Gamma glutamyl transpeptidase (GGT)			Tricyclic antidepressants
Red blood cell (RBC) count	Glucose (fasting)			
White blood cell (WBC) count	Potassium			
	Phosphate (inorganic)			
	Protein (total)			
	Amylase			
	Sodium			
	Urea			

a Volunteers will fast from overnight prior to clinical laboratory evaluations at screening. Postdose time points will be Days 7, 14, 28 and End of Study visit (not fasted).
b Urinalysis to be performed by standard dipstick analysis. Microscopy should only be performed if the urinalysis shows a positive result.
c Only at screening.

At screening a blood sample will be collected to screen for hepatitis B surface antigen, hepatitis C antibodies, and HIV. Creatinine clearance will be calculated at screening using the Cockcroft-Gault formula:

$$\textbf{Estimated CrCL (mL/min)} = \frac{(140\text{-age [years]}) \times (\text{body weight [kg]})}{\text{Serum creatinine (mg/dL)} \times 72}$$

Urine will be tested at screening and on Day -1 for drugs of abuse. Urine will be collected at the study centre. Alcohol will be assessed by an alcohol breath test. Smoking will be assessed by performing a breath carbon monoxide test. 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 Principal Investigator.

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 normalisation or for as long as the Principal Investigator considers necessary. Additional laboratory variables may be performed for safety reasons if judged appropriate by the Principal Investigator. Further details are provided in the Laboratory Manual.

The safety laboratory samples will be analysed using routine methods at

.

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

For blood volume see Section 7.1.

6.3.6 Physical examination

Physical examinations will be performed at the time points indicated in Table 2. A complete physical examination will be performed at screening, Days 28, 35, 42, and End of Study visit that includes an assessment of the following: general appearance, respiratory, cardiovascular, abdomen 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 (including general appearance, skin, abdomen, cardiovascular system, and lungs) to be performed at admission or predose.

6.3.7 Resting 12-lead electrocardiogram

Twelve-lead ECGs will be recorded at the time points indicated in the study plan (Table 2 and Table 3).

Electrocardiograms will be recorded in the supine position after the volunteer has rested in this position for at least 10 minutes. A total of 3 12-lead ECG will be recorded at 10 minute intervals at predose and 24-hours postdose.

Only the overall evaluation (normal/abnormal) will be captured in the pCRF. Any abnormalities (including corrected QT interval [QTc] values) should be reviewed by an appropriately qualified person.

The original ECG printouts with variables must be signed and dated and stored in the volunteer's source workbook as source data.

6.3.8 Vital signs

Vital signs assessment will be performed at the time indicated in Table 2 and Table 3.

6.3.8.1 Pulse rate and supine blood pressure

Supine blood pressure and pulse rate will be measured using standard equipment after 10 minutes rest on a bed. Additional blood pressure/pulse rate assessments may be taken for safety at the discretion of the Principal Investigator or delegate.

6.3.8.2 Height and weight

Height (cm) and weight (kg) will be evaluated at screening and BMI (kg/m^2) will be calculated. Only weight will be evaluated at Day -1 and on Day 84. The volunteers will be required to remove their shoes and wear light indoor clothing for these measurements. When requested or judged necessary for the program, body weight and/or BMI can be measured at additional time points.

6.3.9 Ophthalmology

A full ophthalmologic examination including a slit-lamp fundoscopy, best corrected visual acuity, and intraocular pressure measurement must be performed at screening for all volunteers.

The same ophthalmic expert will perform ophthalmic assessments on each occasion where possible.

An ophthalmological examination should be performed if the volunteer reports eye symptoms such as dry eyes, grittiness, or irritation during the study. In case of clinically relevant ophthalmological abnormalities, an additional full examination will be performed. Any corneal changes must be monitored frequently, with therapeutic intervention as appropriate until resolution. Any abnormalities elicited will be recorded as an AE.

6.4 Pharmacokinetics

6.4.1 Collection of samples

Venous blood samples (approximately 13 mL of whole blood) for the determination of concentrations of AZD9291 and metabolites in plasma, blood and plasma [^{14}C] radioactivity, and metabolite characterisation will be taken at the times presented in Table 2 and Table 3. The actual date and time of collection will also be recorded.

Samples will be collected, labelled, stored, and shipped as detailed in the Clinical Sample Processing Manual. Amber tubes should be used to protect against the possibility of light-induced degradation.

At each time point when a PK sample is required, a 13 mL sample of whole blood will be taken, to be divided as follows:

1 x 1 mL blood	for total [^{14}C] measurement ( )
2 x 0.5 mL plasma	for total [^{14}C] measurement ( )

2 x 0.75 mL plasma	for AZD9291, AZ5104, and AZ7550 concentration measurement ( )
2 x 1 mL plasma	for circulating metabolite profiling characterisation (and then AZ)

Collection of Urine

Each volunteer will pass all urine into specially provided containers during the intervals given in the study plan (Table 3). The start and stop date and time of the interval will be recorded. Samples will be collected, labelled, stored, and shipped as detailed in the Clinical Sample Processing Manual.

Volunteers will void all urine before dosing, and a 10 mL predose urine sample will be retained for drugs, metabolism and pharmacokinetic (DMPK) and further metabolite characterisation studies. Following receipt of the radioactive dose, each volunteer's urine will be collected as voided into pre-weighed screw top plastic containers, using separate containers for each specified (24-hour) collection period.

Urine samples will be collected and pooled from each volunteer over the following time periods: predose, 0 to 4, 4 to 8, 8 to 12, 12 to 24, 24 to 48, 48 to 72, 72 to 96, 96 to 120, 120 to 144, 144 to 168 hours, then continuing for each 24 hour period during residency up to and including Day 21 after dosing, and then nominally 648 to 672 (Day 28), 816 to 840 (Day 35), 984 to 1008 (Day 42) and 1992 to 2016 (Day 84).

Samples collected must be kept refrigerated (approximately 4^{o}C) during each collection period. The total urine volume will be calculated (weight x density [1 g/cm^{3}]) for each urine collection and will be recorded along with the actual start and stop dates and times of the pooled collection.

From each sample collection period when a urine sample is taken, in it will be divided as follows:

2 x 5 mL urine	for total [^{14}C] measurement ( )
2 x 5 mL urine	for AZD9291, AZ5104 and AZ7550 concentration measurement ( )
2 x 10 mL urine	for circulating metabolite profiling characterisation (and then AZ)

From the remainder of each sample, 25 mL is to be kept in case re-analysis is required for any part above. The above urine volume for AZD9291, AZ5104 and AZ7550 concentration measurement ( ) may be modified to accommodate assay requirements; the final sample collection and handling requirements will be identified in the Laboratory Manual.

All urine sub-samples will be stored at a temperature defined in the Clinical Sample Processing Manual when not required for analysis.

Collection of Faeces

Each volunteer will pass all faeces into specially provided containers during the interval given in the study plan (see Table 3). The actual time and date each stool sample is produced will be recorded.

Following receipt of the radioactive dose, each volunteer's faeces will be collected into pre-weighed rigid polypropylene containers (with lids), contained in bags. Individual containers will be used for each stool sample. All tissue wipes will be collected and put into the relevant faecal bag (tissue wipes are to be kept separate from the actual faecal samples). The weight of each stool sample collected must be recorded. Samples collected will be stored at -80°C.

To facilitate stool collection, softeners (eg, lactulose) may be used as per standard study centre practice (see Section 3.1). These must be documented as concomitant medication.

At each time point when samples are collected, it will be processed as follows:

Samples will be shipped for homogenisation within 24 hours of collection.

Stool samples will be collected by the volunteer over the following time periods: 0 to 24, 24 to 48, 48 to 72, 72 to 96, 96 to 120, 120 to 144, 144 to 168, 168 to 216, 216 to 264, 264 to 312, 312 to 360, 360 to 408, 408 to 456, 456 to 504, 648 to 672 (Day 28), 816 to 840 (Day 35), 984 to 1008 (Day 42) and 1992 to 2016 hours (Day 84). Each pooled sample will be homogenised with an appropriate volume of water (typically ca 1:1, w/v) and the homogenate will be sub-sampled as follows:

2 x 50 g of homogenate	For metabolite profiling characterisation (and then AstraZeneca).
2 x 20 g of homogenate	Required to produce 2 g of freeze-dried sample for total [^{14}C] measurement.

One or more aliquots from the homogenate will be retained as backup sample(s); the final sample collection and handling requirements will be identified in the Clinical Sample Processing Manual.

Collection of Vomitus

Should a volunteer vomit within 12 hours of dosing all vomitus will be collected into pre-weighed rigid polypropylene containers (with lids), contained in bags, including all tissue wipes used. Instances of vomiting will be recorded as an AE, and date and time of vomiting will be recorded on the appropriate AE CRF, in addition to on the container label. Samples collected must be weighed and will be stored frozen at -80°C.

The analysis of radioactivity in vomitus will be performed by by AMS (working assumption) methodology. Full details will be included in the CSR.

For blood volume see Section 7.1.

6.4.2 Determination of drug concentration

Samples for determination of AZD9291, AZ5104 and AZ7550 concentration in plasma will be analysed by , using an appropriate bioanalytical method. Samples for determination of AZD9291, AZ5104 and AZ7550 in urine will be analysed by using an appropriate bioanalytical method. Full details of the analytical methods used will be described in a separate bioanalytical report. All samples still within the known stability of the analytes of interest at time of receipt by the bioanalytical laboratory will be analysed.

The analysis of radioactivity in whole blood, plasma, urine, faeces and vomitus will be performed by by liquid scintillation counting (LSC) or accelerator mass spectrometry (AMS) methodology. The mass balance output will be provided as a separate report from which will be appended to the CSR. Full details of the analytical methods used in the determination of mass balance will be detailed in the CSR.

As part of a further study performed by AstraZeneca Oncology iMed, Alderley Park, profiling and characterisation of the metabolites in plasma, urine, faecal components will be carried out using appropriate analytical techniques. These results will be reported separately from the CSR.

6.5 Pharmacogenetics

6.5.1 Collection of pharmacogenetic samples

The blood sample for genetic research will be obtained from the volunteers at Day -1 after the optional informed consent has been signed. 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 at admission, 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, labelled, stored, and shipped as detailed in the Laboratory Manual.

For blood volume see Section 7.1.

7. BIOLOGICAL SAMPLING PROCEDURES

7.1 Volume of blood

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

Table 6 Volume of blood to be drawn from each volunteer

Assessment		Sample volume (mL)	No. of samples	Total volume (mL)
Safety	Clinical chemistry	5	7	35
	Haematology	2	7	14
Pharmacokinetics		13	26	338[a]
Pharmacogenetics (optional)		10	1	10
Total				**397**

a If an in-dwelling catheter is used, an additional 0.5 mL of blood will be collected to flush the catheter prior to collecting each sample.

The number of samples collected, as well as the volume required for each analysis, may be changed during the study (ie, if additional samples are drawn for repeated safety assessments). However, the maximum volume to be drawn from each volunteer during the study will not exceed 550 mL. To put this in context, a single withdrawal of 470 mL occurs 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 on behalf of AstraZeneca for a maximum of 15 years following the last volunteer's last visit in the study. The results from future analysis will not be reported in the CSR but separately in a scientific report.

7.2.1 Pharmacokinetic samples

Pharmacokinetic samples received by will be disposed of after each entity produces a final Bioanalytical Report or 6 months after issuance of a draft Bioanalytical Report (whichever is earlier), unless requested for future analysis.

Pharmacokinetic samples may be disposed of or destroyed and anonymised by pooling. Additional analyses may be conducted on the anonymised, pooled PK samples to further evaluate and validate the analytical method. Any results from such analyses may be reported separately from the CSR.

Selected PK samples may be used and/or pooled for metabolite characterisation and/or quantification. These samples will be retained by Drug Metabolism and Pharmacokinetics, on

behalf of AstraZeneca, for a maximum of 1 year following the finalisation of the CSR. The results from this additional work will not be reported in the CSR.

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

7.2.2 Pharmacogenetic samples

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 15 years, from the date of the last volunteer's last visit, after which they will be destroyed. Deoxyribonucleic acid (DNA) is a finite resource that may be used up during analyses. Samples will be stored and used until no further analyses are possible or the maximum storage time has been reached.

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

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

7.3 Labelling and shipment of biohazard samples

The Principal Investigator will ensure that samples are labelled and shipped in accordance with the Laboratory Manual and the Biological Substance, Category B Regulations (materials containing or suspected to contain infectious substances that do not meet Category A criteria), see Appendix C 'IATA 6.2 Guidance Document'.

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 labelling, shipment, and containment provisions are approved.

7.4 Chain of custody of biological samples

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

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

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

AstraZeneca keeps oversight of the entire life cycle through internal procedures, monitoring of study sites, 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 analysed, AstraZeneca is not obliged to destroy the results of this research.

As collection of the biological samples is an integral part of the study, then the volunteer is withdrawn from further study participation.

The Principal Investigator:

- Ensures volunteers' 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 site, 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 centre.
- Ensures that the volunteer and AstraZeneca are informed about the sample disposal.

AstraZeneca ensures the central laboratory(ies) holding the samples is/are informed about the withdrawn consent immediately and that samples are disposed of/destroyed and the action documented and returned to the study centre.

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 Harmonisation

(ICH)/Good Clinical Practice (GCP), applicable regulatory requirements and the AstraZeneca policy on Bioethics and Human Biological Samples.

8.2 Subject data protection

The ICF 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 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 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 the Principal Investigator might know a volunteer's identity and also have access to his or her own 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

Approval to administer the chosen dose of radioactivity will be sought from the Department of health (United Kingdom) Administration of Radioactive Substances Advisory Committee (ARSAC) prior to commencing the study. As part of the CSP review process, the CSP will be reviewed and approved by the ARSAC certificate holder.

An Ethics Committee (EC) should approve the final CSP, including the final version of the ICF and any other written information and/or materials to be provided to the volunteers. The Principal Investigator will ensure the distribution of these documents to the applicable EC, and to the study centre staff.

The opinion of the EC should be given in writing. The Principal Investigator should submit the written approval to AstraZeneca before enrolment of any volunteer into the study.

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

AstraZeneca should approve any modifications to the ICF that are needed to meet local requirements.

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

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

The Principal Investigator is responsible for providing the EC and ARSAC certificate holder with reports of any serious and unexpected adverse drug reactions from any other study conducted with the investigational product. AstraZeneca will provide this information to the Principal Investigator so that he/she can meet these reporting requirements.

8.4 Informed consent

The Principal Investigator at the centre 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 before conducting any procedure specifically for the study. A separate ICF will be provided for genotyping.
- Ensure the original, signed ICF(s) is/are stored in the Investigator's Study File.
- Ensure a copy of the signed ICF is 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 ICF that is approved by an EC.

8.5 Changes to the protocol and informed consent form

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

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

The amendment is to be approved by the relevant EC, the ARSAC certificate holder, and if applicable, also the national regulatory authority, 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 Principal Investigator. For distribution to the EC see Section 8.3.

If a protocol amendment requires a change to a study centre's ICF, AstraZeneca and the study centre's EC are to approve the revised ICF before the revised form is used.

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

8.6 Audits and inspections

Authorised representatives of AstraZeneca, a regulatory authority, or an EC may perform audits or inspections at the centre, 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, analysed, and accurately reported according to the CSP, GCP, guidelines of the ICH, and any applicable regulatory requirements. The Principal Investigator will contact AstraZeneca immediately if contacted by a regulatory agency about an inspection at the centre.

9. STUDY MANAGEMENT

9.1 Prestudy activities

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

- Determine the adequacy of the facilities
- Determine availability of appropriate volunteers for the study
- Discuss with the Principal Investigator (and other personnel involved with the study) their responsibilities with regard to protocol adherence, and the responsibilities of AstraZeneca or its representatives. This will be documented in a CSA between AstraZeneca and the Principal 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 study centre staff and also train them in any study-specific procedures and system(s) utilised.

The Principal 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 Principal 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 site, including visits to:

- Provide information and support to the Principal Investigator.
- Confirm that facilities remain acceptable.
- Confirm that the investigational team is adhering to the protocol, that data are being accurately and timely recorded in the CRFs, 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 CRFs 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, study centre 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 Principal Investigator or other staff at the study centre needs information and advice about the study conduct.

Source data

Refer to the CSA for location of source data.

9.4 Study agreements

The Principal Investigator at the study centre 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 Principal Investigator should be in place before any study-related procedures can take place, or volunteers are enrolled.

9.4.1 Archiving of study documents

The Principal 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 Q2 2014 and to end by Q3 2014.

The study may be terminated at the study centre 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 AZD9291.

10. DATA MANAGEMENT

Data management will be performed by .

When the completed CRFs have been scanned and indexed, the data are entered into the study database and proofread.

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 of the Medical Dictionary for Regulatory Activities (MedDRA). Medications will be classified according to the AstraZeneca Drug Dictionary. All coding will be performed by .

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

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

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

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

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

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 post-treatment value minus the value at baseline. The baseline values will be as follows:

- Clinical laboratory tests: Day -1
- 12-Lead ECG:
  - Time-matched, Day -1 for time points through 24 hours postdose
  - Day 1 predose for all time points after 24 hours postdose
- Vital signs: Blood pressure and pulse rate
  - Time-matched, Day -1 for time points through 24 hours postdose
  - Day 1 predose for all time points after 24 hours postdose
- All other vital signs: Day 1 predose

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 discontinuation of IP due to AE (DAEs). Based on the expert's judgement, significant AEs of particular clinical importance may, after consultation with the Global Safety Physician, be considered OAEs and reported as such in the CSR. A similar review of laboratory/vital signs/ECG data will be performed for identification of OAEs.

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

11.2 Calculation or derivation of pharmacokinetic variables

The PK analyses of [^{14}C] radioactivity concentration data in whole blood, plasma, urine, and faeces and AZD9291, AZ7550 and AZ5104 concentration data in plasma and urine will be performed at . Pharmacokinetic analyses will

be conducted according to Standard Operating Procedures unless otherwise specified.

Total [^{14}C] radioactivity in whole blood and plasma will be converted to concentration equivalents of AZD9291 based on the actual specific activity of the dose.

Pharmacokinetic parameters will be derived using noncompartmental methods with Phoenix® WinNonlin® version 6.3 or higher (Pharsight Corp., Mountain View, California, United States). All PK computations will be performed using Phoenix® WinNonlin® version 6.3 or higher or SAS® version 9.2 or higher (SAS Institute, Inc., Cary, North Carolina, United States). Graphics will be prepared with SAS version 9.2 or higher, SigmaPlot® version 12.3 or higher (Systat Software, Inc., San Jose, California, United States) or Phoenix® WinNonlin® version 6.3 or higher.

Actual elapsed time from dosing will be used for final blood and plasma based PK parameter calculations. Pharmacokinetic parameter units will be consistent with the concentration units specified in the bioanalytical data. For units with a mass element, both absolute weight (eg, ng) and molar weight (eg, nano mole) will be reported.

The following PK parameters will be determined for plasma AZD9291, AZ7550 and AZ5104 and whole blood and plasma [^{14}C] radioactivity:

AUC	Area under the concentration-time curve in the sampled matrix from time zero to infinity, calculated by linear up/log down trapezoidal summation
$AUC_{(0-t)}$	Area under the concentration-time curve in the sampled matrix from time zero to the time of the last quantifiable concentration, calculated by linear up/log down trapezoidal summation
$AUC_{(0-72)}$	Area under the concentration-time curve in the sampled matrix from time zero to the time of 72 hours, calculated by linear up/log down trapezoidal summation
$AUC_{(0-24)}$	Area under the concentration-time curve in the sampled matrix from time zero to the time of 24 hours, calculated by linear up/log down trapezoidal summation
C_{max}	Maximum concentration in the sampled matrix, obtained directly from the observed concentration versus time data
t_{max}	Time to C_{max}
t_{lag}	Lag time before observation of quantifiable analyte concentrations
CL/F	Apparent oral clearance (AZD9291 plasma, whole blood and plasma radioactivity only)

V_z/F	Apparent volume at distribution (AZD9291 plasma, whole blood and plasma radioactivity only)
$t_{1/2,\lambda z}$	Elimination half-life
λ_z	Elimination rate constant
M/P_{AUC}	AUC metabolite to parent ratio, AZ7550 or AZ5104 AUC/AZD9291 AUC adjusted for differences in molecular weight (AZD929 =499.62; AZ5104 and AZ7550=485.59). If AUC is not reportable in most volunteers, $AUC_{(0-t)}$ will be used rather than AUC.
M/P_{Cmax}	C_{max} metabolite to parent ratio, AZ7550 or AZ5104 C_{max} /AZD9291 C_{max} adjusted for differences in molecular weight (AZD9291=499.62; AZ5104 and AZ7550=485.59)
C_{max}(PL)/C_{max}(PR)	C_{max} ratio of plasma AZD9291, AZ7550 or AZ5104 (PL) to plasma radioactivity (PR)
C_{max}(WBR)/C_{max}(PR)	C_{max} ratio of whole blood radioactivity (WBR) to PR
AUC(PL)/AUC(PR)	AUC ratio of plasma AZD9291, AZ7550 or AZ5104 (PL) to plasma radioactivity (PR). If AUC is not reportable in most volunteers, $AUC_{(0-t)}$ will be used rather than AUC.
AUC(WBR)/AUC(PR)	AUC ratio of WBR to PR. If AUC is not reportable in most volunteers, $AUC_{(0-t)}$ will be used rather than AUC.

The following PK parameters for plasma AZD9291, AZ7550 and AZ5104 and whole blood and plasma [^{14}C] radioactivity will be calculated for diagnostic purposes and listed, but will not be summarised:

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

The ratio of plasma AZD9291, AZ7550 and AZ5104 to plasma radioactivity concentrations, and whole blood to plasma [^{14}C] radioactivity concentrations and the distribution of

radioactivity in blood cells (expressed as percentage) will be reported for each sampling time to assess the radioactivity distribution in blood based matrices over time.

The distribution of radioactivity in red blood cells will be calculated by the following equation:

$$\frac{Cwb-(1-Ht)\times Cp}{Cwb}\times 100\%$$

Where Cwb is concentration in whole blood, Cp is concentration in plasma and Ht is haematocrit (Ht will be the mean value of the laboratory Ht data obtained from Day -1, 7, 14, and 28).

The following PK parameters will be computed from the urine AZD9291, AZ7550, AZ5104, and urine total radioactivity:

Ae_u	Amount recovered in urine during each collection interval (t1 to t2), calculated as urine concentration or concentration equivalent x urine volume
Cum Ae_u	Cumulative amount recovered in urine
fe_u	Percent (or fraction) of actually administered dose/radioactivity recovered in urine during each collection interval and overall (AZD9291 and urine total radioactivity only)
Cum fe_u	Cumulative percent (or fraction) of actually administered dose/radioactivity recovered in urine (AZD9291 and urine total radioactivity only)
CL_R	Renal clearance calculated as $CumAe_u/AUC_{(0-t)}$ (AZD9291, AZ7550, and AZ5104 only)

The following parameters will be computed from the faecal total radioactivity versus time data:

Ae_f	Radioactive amount recovered in faeces during each collection interval (t1 to t2), calculated as [faecal radioactive concentration equivalent x faeces weight]
Cum Ae_f	Cumulative radioactive amount recovered in faeces
fe_f	Percent (or fraction) of actually administered radioactivity recovered in faeces during each collection interval and overall
Cum fe_f	Cumulative percent (or fraction) of actually administered radioactivity recovered in faeces

For all calculations, the faecal weights reported by the bioanalytical laboratory (the weight on which subsequent sample processing is based) will be used in calculation of the faecal radioactivity recovery.

The total recovery of radioactivity (fe_{tot}; percent of administered radioactive dose recovered in urine and faeces overall) will be computed as the sum of the cumulative excretion in urine and faeces. If a volunteer vomits, the emesis product will be collected during the 0 to 12 hour postdose period only for potential analysis of [^{14}C] radioactivity, and if appropriate, included in calculation of the total radioactivity excreted for this volunteer.

In the case of meaningful recovery in any of the 24-hour intervals on Day 28, 35, 42 and 84, the rate of excretion (ie % administered dose/h) will be plotted against time and the area under the rate curve (AURC) for the intervals during which samples were not collected will be calculated for urine and faeces.

Determination of metabolic profiling and metabolite characterisation will be performed on relevant plasma/urine/faeces samples. The relative systemic exposure of any major metabolites will be calculated, if appropriate. This may be summarised in a separate study report.

12. STATISTICAL METHODS AND SAMPLE SIZE DETERMINATION

12.1 Description of analysis sets

12.1.1 Safety analysis set

All volunteers who received 1 dose of [^{14}C]-labelled investigational product and for whom any postdose data are available will be included in the safety population.

12.1.2 Pharmacokinetic analysis set

The PK analysis set will include all volunteers who receive 1 dose of [^{14}C]-AZD9291 and have at least 1 postdose PK measurement without important protocol deviations/violations or events thought to significantly affect the PK of the investigational product (eg, volunteer vomited at or before 2 times median t_{max}, wrong dose administered, prohibited concomitant medication, etc).

12.2 Methods of statistical analyses

Statistical analysis will be performed by using Standard Operating Procedures and Work Instructions, unless otherwise specified. Statistical analysis will be carried out by using the SAS® version 9.2 or higher and where appropriate, additional validated software. Other analyses than those stated below might be performed to gain information needed for the planning and analyses of future studies.

12.2.1 General principles

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

Given the exploratory nature, no formal statistical hypothesis testing will be performed in this study. The statistical analysis will be descriptive and consist of subject listings, graphs, and summary statistics comprising geometric mean, coefficient of variation (CV%), arithmetic mean, standard deviation (SD), median, minimum (min), and maximum (max) values as appropriate. Categorical variables (eg, gender) will be summarised in frequency tables (frequency and proportion of volunteers in the analysis set). In general, descriptive statistics will follow the rounding convention in Early Clinical Development SOPs.

Missing data will be assumed to be missing completely at random, causing a reduced sample size. Since the descriptive statistics will be presented in tables and individual data listings, no action will be taken to handle missing data.

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

Data from nonvalid volunteers (volunteers excluded from the analysis set[s]), which are recorded in the database, will only be presented in listings.

12.2.2 Pharmacokinetics

A listing of PK blood sample collection times as well as derived sampling time deviations will be provided. A listing of individual urine sample collection start and stop dates/times as well as urine weights and volumes will be provided for each urine collection. A listing of individual faecal sample collection start and stop dates/times as well as sample weights will be provided. Faecal weight will be reported by both the study centre (collected) and the bioanalytical laboratory (re-weighed for processing).

Pharmacokinetic variables will be summarised using appropriate descriptive statistics (eg, n, arithmetic mean, CV%, SD, geometric mean, geometric coefficient of variation [GCV%], min, median, and max). The geometric mean is calculated as the exponential of the arithmetic mean calculated from individual observations on a log scale. The GCV% is calculated as $100 \cdot \sqrt{(\exp(s^2)-1)}$ where s is the SD of the data on a log scale. Mean, SD, CV%, geometric mean, and GCV% will not be calculated for t_{max} or t_{lag}.

Plasma concentrations that are below the lower limit of quantification (LLOQ) 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 max value will be reported from the individual data, and the min 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.

A subject listing of all concentration-time data will be presented along with the descriptive statistics for all volunteers. Figures of geometric mean concentration-time data will be presented for plasma and whole blood [^{14}C] radioactivity concentrations and plasma AZD9291, AZ7550 and AZ5104 concentrations combined, as appropriate. Individual subject concentration-time data for the respective blood-based analyte combination will be graphically presented on linear and semi-logarithmic scales.

The ratio of plasma AZD9291, AZ7550 and AZ5104 to plasma [^{14}C] radioactivity concentrations (expressed as a percentage) for each sampling time will be listed by volunteer and summarised using descriptive statistics, to assess the contribution of unchanged drug to the total radioactivity in plasma over time.

A subject listing of whole blood/plasma [^{14}C] radioactivity concentration-time ratios (expressed as a percentage) and percent distribution of radioactivity in blood cells will be presented and the data will be summarised by scheduled time using descriptive statistics.

Blood-based PK parameters will be summarised using descriptive statistics. Individual PK parameters will be presented along with descriptive statistics for all volunteers.

Urine AZD9291, AZ7550 and AZ5104 and urine [^{14}C] radioactivity data will be summarised by scheduled collection intervals using descriptive statistics. Individual urine recovery data will be presented along with the descriptive statistics for all volunteers.

Faecal [^{14}C] radioactivity data will be summarised using descriptive statistics. Individual faecal recovery data will be presented along with the descriptive statistics for all volunteers.

The total recovery of radioactivity over the study duration will be computed as the sum of the cumulative excretion (as % of dose) in urine, faeces, and emesis (if any). Cumulative mean recovery of radioactivity-time profiles will be graphically presented for urine, faeces, and total (sum of urine plus faeces, plus emesis, if applicable). Individual cumulative recovery data

(urine, faeces, and total) will be presented graphically and in tables along with the descriptive statistics for all volunteers.

12.2.2.1 Interim analysis

The bioanalytical laboratory will calculate percent of radioactive dose recovered in urine and faeces in support of volunteer release criteria. These values will be used for interim reporting purposes only to track radioactive dose recovered on an ongoing basis. These results will be part of the radioactivity report which will be included as an appendix to the CSR. Following database lock, the percent of radioactive dose recovered in urine, faeces, and overall, and related parameters will be calculated following SOPs and rounding procedures as described above and in Section 11.2. The values calculated by will be considered the final study results and presented in the body of the CSR.

12.2.3 Safety and tolerability measurements

All safety data (scheduled and unscheduled) will be presented in the data listings.

Safety variables (eg, clinical laboratory values and vital signs) will be reported to the same precision as the source data. Derived variables will be reported using similar precision to those from which they were derived (eg, QTcF derived from QT interval).

All AEs and clinical laboratory outliers that occur following the first dose of study medication will be included in the tabulations of AEs and outlier events, including episodes that occur at unscheduled evaluations.

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

Adverse events beginning at or after the first dose of investigational product will be summarised by Preferred Term and System Organ Class using the Medical Dictionary for Regulatory Activities (MedDRA) vocabulary by treatment and across all treatments. Adverse events that begin during the washout periods will be assigned to the last treatment received prior to the onset of the AE. Furthermore, listings of SAEs and AEs that lead to withdrawal will be made and the number of volunteers who have any AEs, SAEs, AEs that lead to withdrawal, and AEs with severe intensity will be summarised.

Tabulations and listings of data for vital signs and clinical laboratory tests will be presented, as appropriate. All continuous safety data will be summarised by treatment and/or across all treatments, as appropriate, for the observed value at each scheduled assessment and for the corresponding change from baseline. For clinical laboratory tests, listings of values for each volunteer will be presented with abnormal or out-of-range values flagged. Clinical laboratory data will be reported in Système International units in the CSR.

Results from the physical examinations, ECGs, and ophthalmologic examinations will be presented in listings only.

Extra measurements (such as unscheduled or repeat assessments) will not be included in the descriptive statistics, but will be included in subject listings.

12.3 Determination of sample size

The number of volunteers is based on the desire to gain adequate information on the primary endpoints of PK, metabolism, and safety profile whilst exposing as few volunteers as possible to study procedures. Based on previous similar studies, 8 volunteers are considered to be adequate for this purpose.

13. IMPORTANT MEDICAL PROCEDURES TO BE FOLLOWED BY THE INVESTIGATOR

13.1 Medical emergencies and AstraZeneca contacts

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

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

Name	Role in the study	Address & telephone number
	AstraZeneca CPA Program Director	
	AstraZeneca CPA Physician	
Serious adverse event reporting	24-hour emergency cover at central R&D site	
	Principal Investigator	

Name	Role in the study	Address & telephone number
	Project Manager	
	Project Manager	

13.2 Overdose

A dose of AZD9291 in excess of that planned in this CSP will constitute an overdose. There is currently no known antidote to AZD9291 and treatment of an overdose should be supportive for the underlying symptoms. To date, no subject has experienced an overdose with AZD9291.

Cases of overdose will be reported as follows:

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

If an overdose on an AstraZeneca study drug occurs in the course of the study, then the Principal Investigator or other site personnel inform appropriate AstraZeneca representatives immediately, or **no later than 24 hours** of when he or she becomes aware of it.

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

For overdoses associated with SAE, standard reporting timelines apply, see Section 6.3.4. For other overdoses, reporting should be done within 30 days.

13.3 Pregnancy

All outcomes of pregnancies in partners of the male volunteers should be reported to AstraZeneca.

13.3.1 Paternal exposure

It is not known whether AZD9291 or its metabolites are excreted in human semen. Therefore, conception while on treatment must be avoided.

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 during the study and up to 6 months [^{14}C]-AZD9291 administration to avoid pregnancy and/or potential adverse effects on the developing embryo. Volunteers should avoid sperm donation during the study and up to 6 months after [^{14}C]-AZD9291 administration.

Pregnancy of the volunteer's partners is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth or congenital abnormality) occurring from the date of [^{14}C]-AZD9291 administration until 6 months after [^{14}C]-AZD9291 administration should, if possible, be followed up and documented.

14. LIST OF REFERENCES

GLOBOCAN 2008
Available from URL: http://globocan.iarc.fr/factsheets/cancers/lung.asp

NCCN 2012
National Comprehensive Cancer Network Guidelines for Treatment of Cancer by Site. 2012. Available from URL: http://www.nccn.org/professionals/physician_gls/f_guidelines.asp#site

Pao et al 2005
Pao W, Miller VA, Politi KA, Riely GJ, Somwar R, Zakowski MF et al. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. PloS Med 2005, 2(3):e73

Clinical Study Protocol Appendix A

Drug Substance	AZD9291
Study Code	D5160C00011
Edition Number	1
Date	
Protocol Dated	

Appendix A
Signatures

ASTRAZENECA SIGNATURE(S)

A Phase I, Single-centre, Non-Randomised, Open-label, Pharmacokinetic and Mass Balance Study of Orally Administered [^{14}C]-AZD9291 in Healthy Male Volunteers

---

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

I agree to the terms of this study protocol.

AstraZeneca Research and Development site representative

_____ _____

Date
(Day Month Year)

ASTRAZENECA SIGNATURE(S)

A Phase I, Single-centre, Non-Randomised, Open-label, Pharmacokinetic and Mass Balance Study of Orally Administered [^{14}C]-AZD9291 in Healthy Male Volunteers

---

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

I agree to the terms of this study protocol.

AstraZeneca Research and Development site representativ

Date
(Day Month Year)

Clinical Study Protocol Appendix A
Drug Substance AZD9291
Study Code D5160C00011
Edition Number 1
Date

SIGNATURE OF PRINCIPAL INVESTIGATOR

A Phase I, Single-centre, Non-Randomised, Open-label, Pharmacokinetic and Mass Balance Study of Orally Administered [^{14}C]-AZD9291 in Healthy Male Volunteers

---

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

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

Centre No.:

Signature:

Clinical Study Protocol Appendix B

Drug Substance	AZD9291
Study Code	D5160C00011
Edition Number	1
Date	

Appendix B
Additional Safety Information

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

Life threatening

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

Hospitalisation

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

Important medical event or medical intervention

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

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

Examples of such events are:

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

A GUIDE TO INTERPRETING THE CAUSALITY QUESTION

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

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

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

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

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

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

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

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

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

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

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

- Is there a known mechanism?

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

Clinical Study Protocol Appendix C

Drug Substance	AZD9291
Study Code	D5160C00011
Edition Number	1
Date	

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

LABELLING AND SHIPMENT OF BIOHAZARD SAMPLES

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

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

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

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

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

Exempt - all other materials with minimal risk of containing pathogens

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

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

AstraZeneca

Clinical Study Protocol Appendix D

Drug Substance	AZD9291
Study Code	D5160C00011
Edition Number	1
Date	

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

TABLE OF CONTENTS PAGE

1. INTRODUCTION

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

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

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

2. DEFINITIONS

Potential Hy's Law (PHL)

Aspartate Aminotransferase (AST) or Alanine Aminotransferase (ALT) ≥3 x Upper Limit of Normal (ULN) **and** Total Bilirubin (TBL) ≥2 x 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 (HL)

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

3. IDENTIFICATION OF POTENTIAL HY'S LAW CASES

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

- ALT ≥3 x ULN
- AST ≥3 x ULN
- TBL ≥2 x ULN

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

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

4. FOLLOW-UP

4.1 Potential Hy's Law Criteria not met

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

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

4.2 Potential Hy's Law Criteria met

If the patient does meet PHL criteria the Investigator will:

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

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

- Monitor the patient until liver biochemistry parameters and appropriate clinical symptoms and signs return to normal or baseline levels, or as long as medically indicated
- Investigate the etiology of the event and perform diagnostic investigations as discussed with the Study Physician
- Complete the three Liver CRF Modules as information becomes available
- If at any time (in consultation with the Study Physician) the PHL case meets serious criteria, report it as an SAE using standard reporting procedures

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

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

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

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

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

- If the alternative explanation is **not** an AE, record the alternative explanation on the appropriate CRF
- If the alternative explanation is an AE/SAE, record the AE /SAE in the CRF accordingly and follow the AZ standard processes

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

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

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

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

determine whether HL criteria are met. Update the SAE report according to the outcome of the review

6. REFERENCES

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

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