Clinical Pharmacology Study Protocol

Drug Substance	AZD9668
Study Code	D0520C00004
Date	[redacted]

A Randomised, Double-blind, Placebo-controlled, Parallel Group, Phase 1 Study to Assess the Safety, Tolerability and Pharmacokinetics of Single and Multiple Ascending Oral Doses of AZD9668 in Healthy Japanese and Caucasian Volunteers

Sponsor:

AstraZeneca AB, [redacted]

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

Amendment No.	**Date of Amendment**
Administrative Change No.	**Date of Administrative Change**

ASTRAZENECA PROCEDURES IN CASE OF EMERGENCY, OVERDOSE OR PREGNANCY

In the case of a medical emergency you may contact the Study Delivery Team Leader. If the Study Delivery Team Leader is not available, contact the Study Delivery Team Physician at the AstraZeneca Research and Development site shown below.

Role in the study	Name	Address and Telephone number
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Study Delivery Team Physician responsible for the protocol at central R&D site	[redacted]	AstraZeneca R&D Charnwood [redacted] Tel: [redacted]

For further clarifications regarding:

- Procedures in case of medical emergency see Section 8.2.
- Procedures in case of overdose see Section 8.3.
- Procedures in case of pregnancy see Section 8.4.

PROTOCOL SYNOPSIS

A Randomised, Double-blind, Placebo-controlled, Parallel Group, Phase 1 Study to Assess the Safety, Tolerability and Pharmacokinetics of Single and Multiple Ascending Oral Doses of AZD9668 in Healthy Japanese and Caucasian Volunteers

Investigator

[redacted]

Study centre, type and number of subjects planned

[redacted]

16 healthy Japanese and 16 healthy Caucasian volunteers

Study period		Phase of development
Estimated date of first volunteer enrolled	[redacted]	Clinical Pharmacology I
Estimated date of last volunteer completed	[redacted]	

Objectives

Primary

To investigate the safety, tolerability and pharmacokinetics (PK) of single and multiple ascending doses of AZD9668 administered orally to healthy Japanese and Caucasian volunteers.

Secondary

1. To measure renal clearance of AZD9668 following multiple oral doses.

2. To assess the effects of AZD9668 on *ex vivo* zymosan stimulated neutrophil elastase (NE) activity in plasma.

Exploratory

1. To collect pharmacogenetic samples for possible retrospective exploratory analysis, which may be pooled and analysed with other pharmacogenetic samples collected in other AZD9668 studies to investigate the influence of genetic variation on drug response (PK, pharmacodynamics [PD], tolerability and safety). The results of this objective will not be reported in the clinical study report (CSR).

2. To obtain plasma and urine samples for possible exploratory analysis of metabolites. The results of this objective will not be reported in the CSR.

Study design

This is a double blind, randomised, placebo controlled, parallel group, Phase 1 study. Up to four single dose levels, and two multiple dose levels will be studied in two Japanese and two Caucasian cohorts, each comprising of eight healthy volunteers: In each cohort, two single dose levels, and one multiple dose level will be studied, each separated by a washout, with the intention that parallel cohorts of Japanese and Caucasian healthy volunteers (age and sex matched) are studied concurrently.

Investigational product, dosage and mode of administration

AZD9668 tablets, or placebo equivalent, will be administered orally. Each volunteer will receive two ascending single doses once daily, on Days 1 and 7, followed by multiple doses (twice daily each dose administered 12 hours apart) for 6 days (Days 13-18), and a final single dose on Day 19 (ie, a total of 15 doses).

The proposed single dose levels will be 30 mg, 60 mg, 120 mg and 150 mg, although they may change with emerging data from this study. The top dose will be no higher than 150 mg as this is the maximum dose that was administered to healthy volunteers in the AstraZeneca Global SAD/MAD Study (AstraZeneca Protocol Number: D0520C00001 "A Phase I, Randomised, Double Blind, Placebo Controlled, 2-part Study to Assess the Safety, Tolerability and Pharmacokinetics of Single and Multiple Oral Doses of AZD9668 in Healthy Volunteers" Date: [redacted]) and was found to be safe. In addition, modelled PK data from the current study will ensure that the predicted geometric mean C_{max} and $AUC_{(0-24)}$ at the top dose will not exceed 2.5 µM or 18 µM.h, respectively. The multiple doses for both cohorts 1 and 2 will be selected following a safety review meetings using emerging data from the current study and the AstraZeneca Global SAD/MAD Study The maximum multiple dose will be capped at a total daily dose of 140 mg. In addition, the emerging PK data from the current study will be modelled to ensure that for the highest dose given, the predicted geometric mean exposures (both $AUC_{(0-24),ss}$ and $C_{max,ss}$) will not exceed 2.5 µM or 18 µM.h, respectively.

Duration of treatment

For each cohort of volunteers, the study will last a minimum of four weeks.

Variables

- **Pharmacokinetic**

  Plasma concentration of AZD9668 is a primary outcome variable and concentration of AZD9668 in urine is a secondary outcome variable.

  From these data, the following PK parameters will be derived:

  Single dose - Day 1 and 7: C_{max}; t_{max}; $AUC_{(0-t)}$; AUC; $AUC_{(0-12)}$; $AUC_{(0-24)}$; $t_{1/2}$; V_z/F; CL/F; CL_R; A_e; F_e

  Multiple dose - Day 13: C_{max}; t_{max}; $AUC_{(0-12)}$; C_{12}; A_e; F_e. The A_e and F_e will be estimated using data from 0-12 hours

  Day 19: $C_{min,ss}$; $C_{max,ss}$; $t_{max,ss}$; $AUC_{(0-t),ss}$; $AUC_{(0-12),ss}$; $AUC_{(0-24),ss}$; $AUC_{,ss}$; $V_z/F_{,ss}$; $CL/F_{,ss}$; $t_{1/2,ss}$; CL_R; $A_{e,ss}$; $F_{e,ss}$; R_{ac}; $C_{12,ss}$

- **Pharmacodynamic**

  The PD outcome variables for AZD9668 are as follows:

  Single and multiple dosing – Inhibition of NE activity following zymosan stimulation *ex vivo*.

- **Safety**

  The safety outcome variables for AZD9668 are primary variable, and are as follows:

  Single and multiple dosing – Adverse events; clinical chemistry; haematology; urinalysis; 12-lead ECG; digital ECG (dECG) (if required*); 24-hour telemetry monitoring; sitting blood pressure; sitting pulse.

  ** The need to perform dECG will be based on the outcome of the ongoing AstraZeneca Global SAD/MAD Study*

- **Genetics**

  Under separate written informed consent a blood sample will be taken for possible retrospective exploratory analysis to investigate the influence of genotype on drug response (safety, tolerability, PK and PD response where appropriate).

- **Statistical methods**

  Descriptive statistics will be used for all parameters in the study.

  No statistical hypothesis will be tested.

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LIST OF SUPPLEMENTS

Supplement N/A

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

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

Abbreviation or special term	Explanation
α1-AT	Alpha 1 – antitrypsin
ACM	Ambulatory cardiac monitoring
A_e	Cumulative amount of unchanged drug excreted into urine
$A_{e, ss}$	A_e at steady state
AE	Adverse event
ALT	Alanine aminotransferase
AMC	7-amino 4-methyl coumarin
AST	Aspartate aminotransferase
ATC	Anatomical therapeutic chemical classification of drug
AUC	Area under the plasma concentration time curve from time zero to infinity
$AUC_{,ss}$	AUC at steady state
$AUC_{(0-t)}$	AUC from time zero to a specified time
$AUC_{(0-t),ss}$	$AUC_{(0-t)}$ at steady state
AZDD	AstraZeneca drug dictionary
BAL	Bronchoalveolar lavage
BMI	Body mass index
C_{12}	Concentration at 12 hours post dose
$C_{12,ss}$	C_{12} at steady state
[redacted]	[redacted]
CEFTS	Central ECG files transfer and storage; the AstraZeneca central dECG repository
CGG	Clinical genotyping group
CL/F	Apparent plasma clearance following oral drug administration
$CL/F_{,ss}$	CL/F at steady state
CL_R	Renal clearance of drug from plasma
C_{max}	Observed peak or maximum plasma concentration following drug administration
$C_{max,ss}$	C_{max} at steady state

Abbreviation or special term	Explanation
$C_{min,ss}$	Minimum plasma (trough) drug concentration after repetitive dosing after steady state is achieved, prior to the first dose of the study day
COPD	Chronic obstructive pulmonary disease
CPK	Creatinine phosphokinase
CPMP	Committee for Proprietary Medicinal Products
CPU	Clinical pharmacology unit
CRF	Case report form
CSR	Clinical study report
DMPK	Drug metabolism and pharmacokinetics
DNA	Deoxyribonucleic acid
[redacted]	[redacted]
dECG	Digital ECG
ECG	Electrocardiogram
Ecode	Enrolment code
EDTA	Ethylenediamine tetra-acetic acid
Ethics Committee	Synonymous to Institutional Review Board and Independent Ethics Committee
EU	European Union
F_e	Fraction of dose excreted unchanged in the urine
$F_{e, ss}$	F_e at steady state
FSH	Follicle stimulating hormone
GCP	Good clinical practice
GGT	Gamma glutamyltransferase
GMP	Good manufacturing practice
HDPE	High-density polyethylene
HELC	Human Exposure Limits Committee
hERG	Human-ether-a-go-go gene
HIV	Human immunodeficiency virus
HR	Heart rate
HRT	Hormone replacement therapy
hsCRP	High sensitivity C-reactive protein
ICH	International Conference on Harmonisation

Abbreviation or special term	Explanation
IC_{50}	Concentration giving 50% of the drug-induced inhibitory effect
IL-1β	Interleukin-1 beta
IL-8	Interleukin-8
IPS	Investigational product supplies
Ka	Absorption rate constant
LC-MS/MS	Liquid chromatography/mass spectrometry/mass spectrometry
LDH	Lactate dehydrogenase
LEFTS	Local ECG files transfer and storage; the local AZ CPU dECG repository
LFT	Liver function tests
LIMS	Laboratory information management system
LLN	Lower limit of normal
LLOQ	Lower limit of quantification
LTB4	Leukotriene B4
MAD	Multiple ascending dose
MCH	Mean cell haemoglobin
MCHC	Mean cell haemoglobin concentration
MCV	Mean cell volume
MedDRA	Medical Dictionary for Regulatory Activities
MPO	Myeloperoxidase
MTD	Maximum tolerated dose
NCS	Not clinically significant
NE	Neutrophil elastase
NOAEL	No observed adverse effect level
OAE	Other significant adverse event (ie, AEs of particular clinical importance, other than SAEs and those AEs leading to discontinuation of the volunteer from study treatment; see definition in Section 4.7.1.1).
OTC	Over the counter
PCP	Phencyclidine
pCRF	Paper case report form
PD	Pharmacodynamic(s)
PGx	Pharmacogenetic(s)
PK	Pharmacokinetic(s)

Abbreviation or special term	Explanation
Principal Investigator	A person responsible for the conduct of a clinical study at a study site. Every study centre has a Principal Investigator.
PR (PQ)	ECG interval measured from the beginning of the P wave to the beginning of the Q wave or beginning of the R wave in the absence of a Q wave. PR (PQ) represents the time interval from start of atrial depolarisation to the start of ventricular depolarisation.
QRS	ECG interval measured from the beginning of the Q wave (or the R wave if Q is missing) to the J point; the time interval of ventricular depolarisation
QT	ECG interval measured from the beginning of the Q wave (or the R wave if Q is missing) to the end of the T wave; the time interval of ventricular depolarisation and repolarisation
QTc	Corrected QT
QTcB	QT interval corrected for heart rate using Bazzett formula
QTcF	QT interval corrected for heart rate using Fridericia formula
QTcX	QT interval corrected for heart rate using a specific individualised factor
QT tang	QT interval measured from the beginning of the Q wave or the R wave if Q is missing, to the intercept between isoelectric line and the regression line, derived on the T-wave downstroke from values between 8- and 20% of the T-top amplitude
R_{ac}	Accumulation ratio (index)
RBC	Red blood cell
RR	The time between corresponding points on 2 consecutive R waves on ECG; the interval from one ventricular depolarisation to the next.
SAD	Single ascending dose
SAE	Serious adverse event
SAP	Statistical analysis plan
SRC	Safety review committee
TCA	Tricyclic antidepressants
THC	Tetrahydrocannabinoids
t_{max}	Time to reach observed peak or maximum concentration following drug administration
$t_{max,ss}$	t_{max} at steady state
$t_{1/2}$	Terminal half-life of drug in plasma
$t_{1/2,ss}$	$t_{1/2}$ at steady state

Abbreviation or special term	Explanation
UK	United Kingdom
ULN	Upper limit of normal
V_z/F	Apparent terminal volume of distribution following extra-vascular dosing
$V_z/F_{,ss}$	V_z/F at steady state
WHO	World Health Organisation

1. INTRODUCTION

1.1 Background

Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of morbidity and mortality in the USA (US Department of Health and Human Services 1998) and worldwide is projected to rank fifth in burden of disease in 2020 (Lopez 1998). The prevalence of physiologically defined COPD in adults, aged 40 years or over, is around 9-10% (Halbert 2006). A significant proportion of these patients suffer from severe disability, and this subset of patients contributes largely to the health care costs of COPD (Rutten van Molken 1999).

COPD is a chronic lung disease, characterized by airflow limitation that is not fully reversible, and this is associated with an abnormal chronic inflammatory response in the respiratory system to noxious particles or gases (GOLD 2005). Currently, the mainstay treatment of airflow limitation is bronchodilators. Despite a prominent inflammatory response in the airways in COPD, in contrast to asthma, anti-inflammatory therapy with corticosteroids is relatively ineffective. Therefore, there is a need to explore the possibilities of developing other therapies for COPD.

Neutrophil elastase (NE) is a serine protease found in high levels in neutrophils. NE is able to degrade extracellular matrix and proteins leading to long-term destruction of the lung parenchyma. Inhibition of NE has the potential to inhibit this proteolytic destruction raising the possibility of disease modification. If, as predicted from *in vivo* animal studies, NE inhibition can affect not only lung destruction but also epithelial metaplasia, goblet cell hyperplasia and the loss of cilia caused by exposure to cigarette smoke, as well as infiltration of inflammatory cells, shorter-term symptomatic benefits are also possible with this approach.

There are a number of clinical and experimental observations supporting a role for NE in COPD. In α1-antitrypsin (α1-AT) deficiency, an established genetic risk factor, the development of emphysema is believed to be caused by the unchecked action of proteases on lung tissue. Cigarette smoking, a major cause of COPD, induces a functional antiprotease deficiency in the lower respiratory tract of humans. There is a strong relationship between the severity of emphysematous change in human lung and the amount of NE in lung interstitium. NE in bronchoalveolar lavage (BAL) fluid correlates directly, and anti-elastase capacity inversely, with emphysema severity. Desmosine excretion, a biological marker of lung destruction, is significantly increased in patients with COPD as compared to healthy smokers.

Airway inflammation plays an important role in the pathogenesis of COPD. Neutrophils are recognized as major cellular mediators of inflammation and play a central role in many of the features of COPD. Sputum from COPD patients has significantly greater numbers of neutrophils and the neutrophil specific marker myeloperoxidase (MPO) in sputum correlates with the NE concentrations. The activation of neutrophils results in the release of LTB4 and IL-8, which further contributes to a self-perpetuating inflammatory response in COPD.

In the guinea pig, the NE inhibitor ZD0892 inhibited lung neutrophilic inflammation and emphysema development, due to chronic inhalation of cigarette smoke. Data from experimental studies suggest that NE knock-out mice are partially (59%) protected against development of emphysema in the smoking model. Transgenic mice that express extremely low levels of α1-AT were partially (63%) protected against emphysema development in a 6 month study when treated with human α1-AT. Studies in rat models have shown that oral administration of NE inhibitor ONO-6818, attenuated lung haemorrhage and accumulation of neutrophils in the lung induced by human NE, and long-term administration of ONO-6818 prevented human BE-induced lung emphysematous changes. In addition, there are a number of publications supporting a key role for NE in experimental models of mucus hypersecretion of COPD.

AZD9668, a pyridine of molecular weight of 545.5 g/mol, is a potent, orally active, selective, reversible inhibitor of human NE. It is being developed as a possible therapeutic agent both for symptomatic treatment as well as disease modification in COPD. Orally administered AZD9668 produced a dose-dependent inhibition of human BE-induced lung haemorrhage in the mouse. Mice exposed to cigarette smoke and treated with AZD9668 showed a significant reduction in total number of cells and neutrophils and IL 1-β, compared to vehicle treated smoke exposed mice, indicating the possibility of inhibiting endogenously released NE with this drug.

AZD9668 was well tolerated in rat and dog toxicity studies. In a dog 28-day toxicity study minor haematological abnormalities were shown at high doses. There was no bone-marrow pathology. Electrophysiological studies showed a hERG IC50 is 69 μM, and *in vivo* a slight QT-prolongation (9%) at a high dose in a dog telemetry study (11 μM free C_{max} representing a 204-fold margin to free therapeutic concentration estimates in man). Mutagenicity tests indicated that AZD9668 does not pose a significant genotoxicity risk.

Further details of the preclinical toxicology and pharmacology can be found in the Investigator's Brochure (AZD9668 – COPD (Edition 1). Date: [redacted].)

1.2 Rationale

The objective is to evaluate the overall safety, tolerability and PK of single and multiple ascending doses of AZD9668 administered orally as a tablet formulation in both healthy Japanese and Caucasian volunteers. It is anticipated that pharmacologically active exposures will be reached. The pharmacodynamic effect of AZD9668 will be assessed using an *ex vivo* assay measuring the effect of AZD9668 on *ex vivo* zymosan stimulated NE activity in plasma.

This study is not a first time in human study as there is an ongoing AstraZeneca Global SAD/MAD Studyin healthy volunteers (see Section 3.2.1).

2. STUDY OBJECTIVES

2.1 Primary objective

The primary objective of the study is to investigate the safety, tolerability and PK of single and multiple ascending doses of AZD9668 administered orally to healthy Japanese and Caucasian volunteers.

2.2 Secondary objectives

The secondary objectives of the study are:

1. To measure renal clearance of AZD9668 following multiple oral doses.

2. To assess the effects of AZD9668 on *ex vivo* zymosan stimulated NE activity in plasma.

2.3 Exploratory Objectives

The exploratory objectives of the study are:

1. To collect pharmacogenetic samples for possible retrospective exploratory analysis, which may be pooled and analysed with other pharmacogenetic samples collected in other AZD9668 studies to investigate the influence of genetic variation on drug response (PK, pharmacodynamics [PD], tolerability and safety). These data will not form part of the clinical study report (CSR).

2. To obtain urine and plasma samples for possible exploratory analysis of metabolites. These data will not form part of the CSR.

3. STUDY PLAN AND PROCEDURES

3.1 Overall study design

This Clinical Study Protocol has been subjected to a peer review according to AstraZeneca standard procedures.

This is double blind, randomised, placebo controlled, parallel group, Phase 1 study. Up to four single dose levels, and two multiple dose levels will be studied in two Japanese and two Caucasian cohorts, each comprising of eight healthy volunteers: In each cohort, two single dose levels, and one multiple dose level will be studied, each separated by a washout period (minimum of 7 days), with the intention that parallel cohorts of Japanese and Caucasian healthy volunteers (age and sex matched) are studied concurrently.

Each volunteer will receive two ascending single doses once daily (on Days 1 and 7), followed by multiple doses (twice daily each dose administered 12 hours apart) for 6 days (Days 13-18), and a final single dose on the morning of Day 19 (ie, a total of 15 doses).

Cohorts of Japanese and Caucasian volunteer will receive the same dose of AZD9668, and will be run in parallel as far as is possible.

The intention is that volunteers remain resident in the [redacted] from Day -1 until approximately 72 hours after each of the single doses and after the last dose in Visit 4 (ie, from Day -1 to Day 4; Day 6 to Day 10; and Day 12 to Day 22). The volunteers will be allowed home in between the single doses or may stay in the unit at the Investigators discretion. If the dosing schedule is separated further for practical reasons, volunteers will be allowed home, from approximately 72 hours after the last dose in each treatment period. (Note - Further separation of the dosing periods will not affect the 'study day' nomenclature).

Figure 1 shows the study flow chart and Table 1 outlines the assessments at each visit.

Visit 1a (to provide informed consent only) and Visit 1b (enrolment and screening) will take place up to 21 days prior to Visit 2.

At Visit 2, volunteers will arrive at the [redacted] on the evening of Day -1 (the day before dosing). Volunteers will be randomised to receive a single dose of AZD9668 or placebo on Day 1 (Visit 2) in a ratio of 6:2 (AZD9668:placebo) in each ethnic group. Volunteers will remain in the [redacted] for assessments until approximately 72 hours post dose (Day 4) and then can be discharged. Volunteers will return to the [redacted] on the evening of Day 6. On Day 7 (Visit 3), each volunteer will receive a second single dose. Volunteers will remain in the [redacted] for assessments until approximately 72 hours post dose (Day 10) and then can be discharged. Volunteers will return to the [redacted] on the evening of Day 12. Multiple doses will be administered twice daily on Days 13 to 18 (Visit 4), each dose administered 12 hours apart, and a final, single dose on the morning of Day 19. Volunteers will remain in the [redacted] for assessments until approximately 72 hours after this last dose (Day 22), when they can be discharged (at the Investigator's discretion). Volunteers will attend for a follow-up (Visit 5) at least 7 days (+2 days) after discharge from Visit 4.

Within each cohort, dose escalation will only proceed once dosing has been completed at the previous dose level, however, it is possible that multiple dose (cohort 1) and single dose 1 (cohort 2) may run in parallel.

Investigator /SRC's will review available data as described below for each cohort and as shown in Figure 1. For cohort 1, following completion of the first single dose level, the Investigator will review available safety data (blinded) to confirm that it is safe to proceed with the next single dose level. After the second single dose, a blinded Safety Review Committee (SRC) will meet to review the available safety data from single doses 1 and 2 to determine whether it is safe to proceed with the multiple dose in these individuals and the single dose 1 for cohort 2. The SRC will also review the PK data (up to 24 hours post dose as a minimum) from the single dose 1, and emerging data from the AstraZeneca Global SAD/MAD Study to confirm dose selection. Following the completion of the multiple dose, the SRC will review the available safety data from the multiple dose (cohort 1) and the PK data (up to 24 hours post dose as a minimum) from the first two single doses.

For cohort 2 after completion of the first single dose level the SRC will meet to review safety data from the single dose 1 (cohort 2) and the PK data (up to 24 hours post dose) from the single doses in cohort 1 as a minimum. The SRC will then determine whether it is safe to proceed to single dose 2 (cohort 2) and use available PK data, and emerging data from the AstraZeneca Global SAD/MAD Study to confirm the dose. After single dose 2 (cohort 2) a SRC will meet to review the available safety data from single doses 1 and 2 (cohort 2) to determine whether it is safe to proceed to multiple dose (cohort 2) and use the PK data available from single dose 1 (cohort 2) and cohort 1 (both single and multiple doses), and emerging data from the AstraZeneca Global SAD/MAD Study to confirm the dose. The SRC will also meet following the completion of the multiple dose (cohort 2) to review safety data, and PK data if available, for internal information.

Note: All safety and PK data from the current study will be reviewed blinded.

Figure 1 Study flow chart

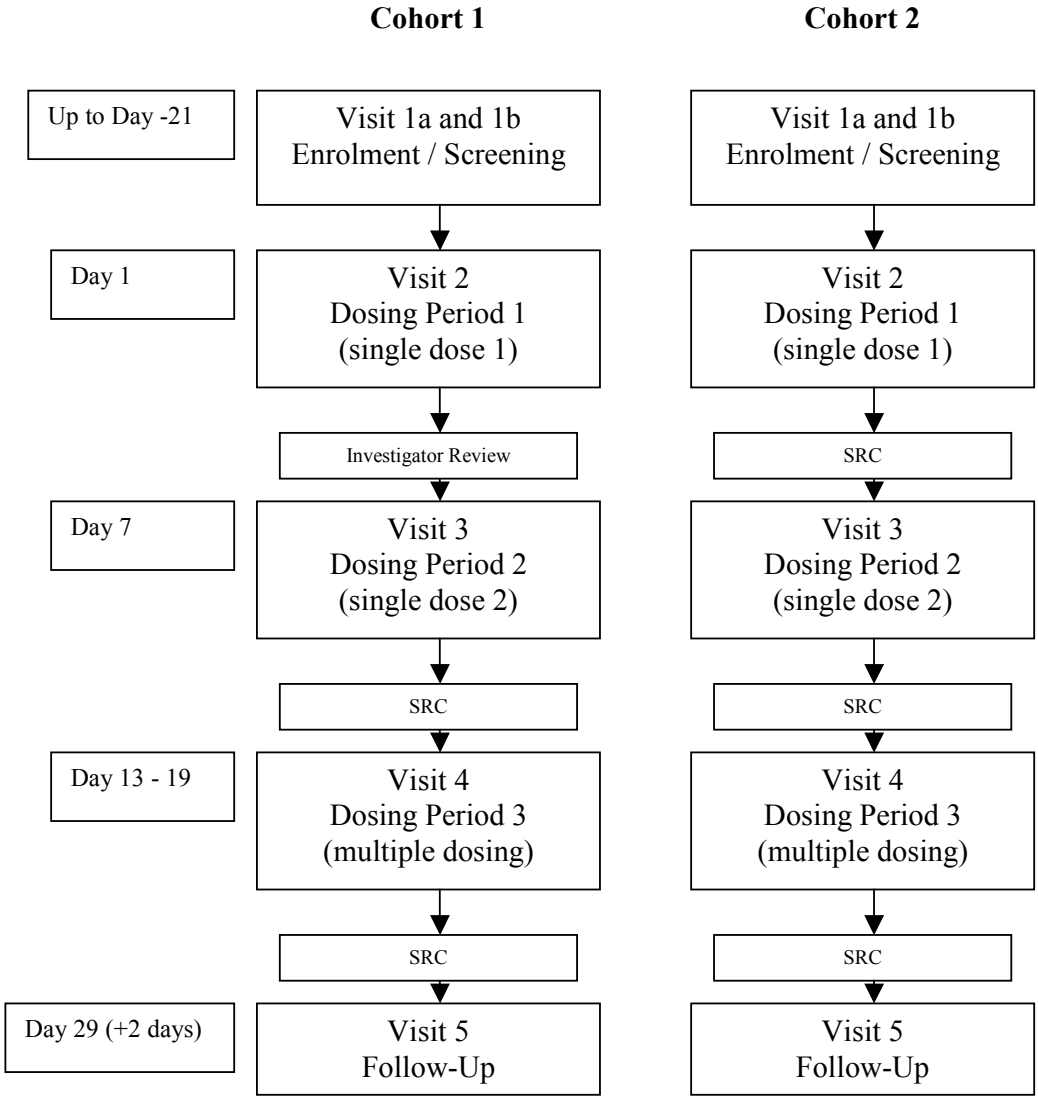

Table 1 Study plan

Assessment	Visit 1a	Visit 1b Day -21 to -1	Visit 2 Day 1	Visit 3 Day 7	Visit 4 Day 13 to 19	Visit 5 Day 29 (+2 days)
Informed consent / enrolment	X					
Genetic consent	X					
Inclusion/Exclusion criteria		X	X			
Demographics		X				
Medical and surgical history		X				
Concomitant medication		X	X	X	X	X
Physical examination (inc. height and weight)		X	X [1, 2]			X [2]
Alcohol and drugs of abuse screen		X	X	X [3]	X [3]	X [3]
Hepatitis B&C and HIV screen		X				
Safety bloods and urinalysis		X	X [4]	X [4]	X [4]	X
12-Lead ECG (paper)		X	X	X	X [5]	X
12-Lead ECG (digital)			X [6]	X [6]	X [6]	
Telemetry			X [7]	X [7]	X [7]	
Sitting blood pressure and pulse		X	X [8]	X [8]	X [8]	X
Pregnancy test		X [9]				
Adverse events		X	X	X	X	X
Randomisation			X			
AZD9668 or placebo administration			X	X	X	
Plasma PK profile			X [10]	X [10]	X [10]	
Full urine collection (PK)			X [11]	X [11]	X [11]	
PD blood sample			X [12]	X [12]	X [12]	
PGx blood sample			X [13]			

[1] Abbreviated physical examination only.
[2] Weight only will be measured at this visit.
[3] To be performed when volunteer returns to the [redacted] between study visits.
[4] Pre-dose in each dosing period.
[5] Paper ECGs recorded at the timepoints shown in Table 2, and at 48 and 72 hrs post dose at Visits 2, 3 and 4. If dECGs are to be performed each should be preceded by a paper ECG recording.
[6] Digital ECGs (if required) will be performed at the times shown in Table 2.
[7] Telemetry from -1 to 24 hrs post-dose at Visits 2, 3 and 4, and from -1 to 24 hrs post dose on Day 19 (Visit 4).
[8] Vital signs will be measured at the timepoints shown in Table 2.
[9] Female volunteers only.
[10] PK sampling times are detailed in Table 3.
[11] Urine collections: Pre-dose on Days 1, 7 and 13 and post dose at 0-4, 4-8, 8-12, 12-24, 24-36, 36-48 hrs on Days 1, 7 and 19. On Day 13 at 0-4, 4-8, 8-12 hrs post dose.
[12] Pharmacodynamic (PD) blood sampling times are shown in Table 4.

[13] Pharmacogenetic (PGx) blood sample to be obtained at any suitable timepoint post dose.

Table 2 ECG and vital signs assessment schedule – Visits 2, 3 and 4

Time (Relative to dosing)
-01:00
00:00
00:30
01:00
01:30
02:00
03:00
04:00
06:00
08:00
12:00
24:00

Paper ECGs, dECGs (if required), and vital signs (ie, sitting blood pressure and pulse) will be performed at the above times at Visits 2 and 3 (single doses) and after the first and last multiple doses (ie on Days 13 and 19 [Visit 4]). Paper ECGs will also be measured at 48 and 72 hours post dose at Visits 2, 3 and 4.

Table 3 Pharmacokinetic sampling schedule

Day	Time (hours - relative to dosing)
1	Pre-dose, 0.25, 0.5, 1, 2, 3, 4, 5, 7, 9, 12, 24, 36, 48, 72 post-dose
7	Pre-dose, 0.25, 0.5, 1, 2, 3, 4, 5, 7, 9, 12, 24, 36, 48, 72 post-dose
13	Pre-dose, 0.25, 0.5, 1, 2, 3, 4, 5, 7, 9, 12 (pre-dose 2), 13, 15 post-dose
14 and 15	Pre-dose (dose 1)
16 and 17	Pre-dose (dose 1), 1 post-dose
18	Pre-dose (dose 1)
19	Pre-dose, 0.25, 0.5, 1, 2, 3, 4, 5, 7, 9, 12, 24, 36, 48, 72 post-dose

Table 4 Pharmacodynamic sampling schedule

	Time (hours - relative to dosing)	
Day	Cohort 1	Cohort 2
1	Pre-dose, 1, 24 post-dose	Pre-dose, 1, 48 post-dose
7	Pre-dose, 1, 48 post-dose	Pre-dose, 1, 48 post-dose
13	Pre-dose (dose 1), 1 post-dose	Pre-dose (dose 1), 1 post-dose

	Time (hours - relative to dosing)	
19	Pre-dose, 1, 24 post-dose	Pre-dose, 1, 48 post-dose

Total of 11 blood samples per volunteer in each cohort.

The proposed single dose levels are 30 mg, 60 mg, 120 mg and 150 mg, although they may change with emerging data from this study. The top dose will be no higher than 150 mg as this is the maximum dose that was administered to healthy volunteers in the AstraZeneca Global SAD/MAD Study and was found to be safe. In addition, modelled PK data from the current study will ensure that the predicted geometric mean C_{max} and $AUC_{(0-24)}$ at the top dose will not exceed 2.5 μM or 18 μM.h, respectively. The multiple doses for both cohorts 1 and 2 will be selected following a safety review meetings using emerging data from the current study and the AstraZeneca Global SAD/MAD Study The maximum multiple dose will be capped at a total daily dose of 140 mg. In addition, the emerging PK data from the current study will be modelled to ensure that for the highest dose given, the predicted geometric mean exposures (both $AUC_{(0-24),ss}$ and $C_{max,ss}$) will not exceed 2.5 μM or 18 μM.h, respectively.

3.1.1 Stopping criteria for dose escalation

3.1.1.1 Safety Review Committee

Within each cohort dose escalation will only proceed once dosing has been completed at the previous dose level, however, it is possible that multiple dose (cohort 1) and single dose 1 (cohort 2) may run in parallel. For cohort 1, following completion of the first single dose level, the Investigator will review available safety data (blinded) to confirm that it is safe to proceed with the next single dose level. After the second single dose, a blinded Safety Review Committee (SRC) will meet to review the available safety data from single doses 1 and 2 to determine whether it is safe to proceed with the multiple dose in these individuals and the single dose 1 for cohort 2. The SRC will also review the PK data (up to 24 hours post dose as a minimum) from the single dose 1, and emerging data from the AstraZeneca Global SAD/MAD Study to confirm dose selection. Following the completion of the multiple dose, the SRC will review the available safety data from the multiple dose and the PK data (up to 24 hours post dose as a minimum) from the first two single doses.

For cohort 2 after completion of the first single dose level the SRC will meet to review safety data from the single dose 1 (cohort 2) and the PK data (up to 24 hours post dose) from the single doses in cohort 1 as a minimum. The SRC will then determine whether it is safe to proceed to single dose 2 (cohort 2) and use available PK data, and emerging data from the AstraZeneca Global SAD/MAD Study to confirm the dose. After single dose 2 (cohort 2) a SRC will meet to review the available safety data from single doses 1 and 2 (cohort 2) to determine whether it is safe to proceed to multiple dose (cohort 2) and use the PK data available from single dose 1 (cohort 2) and cohort 1 (both single and multiple doses), and emerging data from the AstraZeneca Global SAD/MAD Study to confirm the dose. The SRC will also meet following the completion of the multiple dose (cohort 2) to review safety data, and PK data if available, for internal information.

Note: All safety and PK data from the current study will be reviewed blinded.

The SRC will be chaired by an AstraZeneca Physician and will comprise the following individuals, as a minimum:

- AstraZeneca study team physician, or nominated deputy.
- AstraZeneca drug safety physician, or nominated deputy.
- AstraZeneca study team pharmacokineticist, or nominated deputy.
- Principal Investigator, or nominated deputy.
- [redacted] medical monitor, or nominated deputy
- [redacted] study team statistician, or nominated deputy.

The safety review will lead to one of five possible outcomes:

- Escalate to a higher dose.
- Repeat dose level.
- Stop escalation and investigate lower dose(s).
- End overall study.
- End part of study.

Volunteers may be withdrawn from the study at any time at the discretion of the Principal Investigator. Specific reasons for discontinuing a volunteer from the study are listed in Section 3.3.5.

Where there is no consensus between the Principal Investigator and the SRC on dose escalation, the Principal Investigator will take the final decision in the interest of safety of the volunteers.

3.1.1.2 Stopping criteria for dose escalation during the single dose phase

Escalation to the next dose level will not occur if any of the following criteria are met with the previous dose levels:

- Either an alanine aminotransferase (ALT) / aspartate aminotransferase (AST) level ≥3 x upper limit of normal (ULN) at two consecutive time points, or any pattern of liver function test (LFT) abnormalities giving cause for concern, in two or more volunteers on active treatment.

- Evidence of haemolysis as evidenced by the following in two or more volunteers on active treatment.

  - Reticulocyte count of over 5%, and,

At least two of the following:

  - A drop in haemoglobin below 10.5 g/dL.

  - Decrease of 25% in haptoglobin. (The value post-dose to be compared with the average of two pre-dose values. The value should also be 15% below the lower limit of normal [LLN]).

  - Increase of 25% in unconjugated bilirubin or lactate dehydrogenase (LDH). (The value post-dose to be compared with the average of two pre-dose values. The value should also be 15% above ULN).

- A platelet count of below 80,000 $x10^9$/L confirmed by a repeat test (in the absence of obvious platelet clumping in a peripheral blood film) in two or more volunteers on active treatment.

- A QTcF >500 ms in two or more volunteers on active substance (QT interval corrected for heart rate by Fridericia method) or a QTcF prolongation >60 ms on average, sustained for more than 30 minutes, ie, present on two contiguous timepoints, in two or more volunteers on active substance based on the results of the continuous readings compared to baseline.

- Any general safety finding that in the opinion of the Investigator gives cause for concern.

- Data indicate that at the next proposed dose, the geometric mean plasma C_{max} would be expected to exceed 2.5 μM or the geometric mean $AUC_{(0-24)}$ would be expected to exceed 18 μM.h. If $AUC_{(0-24)}$ cannot be calculated $AUC_{(0-t)}$ will be used.

3.1.1.3 Stopping criteria for dose escalation during the multiple dose phase

Escalation to the next dose level will not occur if any of the following criteria are met with the previous dose levels:

- Two or more volunteers on active treatment are discontinued because of either an ALT/AST level ≥3 x ULN or any pattern of LFT abnormalities giving cause for concern.

- Evidence of haemolysis as evidenced by the following in two or more volunteers on active treatment.

  - Reticulocyte count of over 5%, and,

At least two of the following:

- A drop in haemoglobin below 10.5 g/dL.
- Decrease of 25% in haptoglobin. (The value post-dose to be compared with the average of two pre-dose values. The value should also be 15% below LLN).
- Increase of 25% in unconjugated bilirubin or LDH. (The value post-dose to be compared with the average of two pre-dose values. The value should also be 15% above ULN).

- A platelet count of below 80,000 $x10^9$/L confirmed by a repeat test (in the absence of obvious platelet clumping in a peripheral blood film) in two or more volunteers on active treatment.
- A QTcF >500 ms in two or more volunteers on active substance (QT interval corrected for heart rate by Fridericia method) or a QTcF prolongation >60 ms on average, sustained for more than 30 minutes, ie, present on two contiguous timepoints, in two or more volunteers on active substance based on the results of the continuous readings compared to baseline.
- Any general safety finding that in the opinion of the Investigator gives cause for concern.
- Data indicate that at the next proposed dose the geometric mean steady state exposure ($C_{max,ss}$ or $AUC_{(0-24),ss}$) will exceed 18 μM.h and 2.5 μM, respectively.

Dosing in an individual volunteer may be discontinued in the multiple dose phase of the study (see Section 3.3.5.1 for criteria).

The stopping criteria refer to all volunteers at a specific dose level. In the event of the need to unblind the data for a given volunteer, or a given cohort, the study design will revert to a single blind study for that volunteer or cohort.

3.2 Rationale and risk/benefit assessment

3.2.1 Rationale for study design, doses and control groups

A first administration to man, Phase I, randomised, double blind, placebo controlled, two-part AstraZeneca Global SAD/MAD Study to assess the safety and tolerability and PK of single and multiple oral doses of AZD9668 in healthy volunteers is ongoing. Up to [redacted], AZD9668 has been administered in single ascending doses of 2 mg, 10 mg, 30 mg, 60 mg, 120 mg and 150 mg with 36 subjects receiving the active drug and 12 subjects receiving the placebo. No serious adverse events (SAEs) occurred. There were 46 non-serious adverse events (AEs) reported by 24 subjects. The severity for the majority of the events was mild (37/46 AEs); few were reported as moderate (7/46 AEs), and for one subject, two events were reported as severe (syncope associated with a symptomatic

bradycardia, 4 hours 37 minutes after a 2 mg dose). These last two events resolved with supportive care within 3 minutes. The most frequently reported AE was headache (18/10 subjects), followed by musculo-skeletal pain/myalgia/backpain (5/4 subjects) and light headedness (3/2 subjects). None of the AEs were considered by the Investigator to be causally related to the investigational drug. The multiple ascending dose part of this study has commenced on the [redacted], at 30 mg daily for 8 days, with no safety or tolerability issues identified so far.

The present study will be conducted in healthy volunteers, in a double blind, placebo controlled manner, and volunteers will be randomised to receive either AZD9668 or placebo

Healthy volunteers will be used in this study in order to optimise compliance with study procedures and to avoid interference with the results from disease processes and other drugs. The inclusion/exclusion criteria are defined such that the volunteers selected will be known to be free of any significant illness when included in the study. This study is being performed double blind in order to minimise both potential selection bias in the allocation of volunteers to specific treatments and bias to the volunteer's data as a result of the volunteer or study personnel being aware of whether a volunteer is receiving AZD9668 or placebo. The placebo control will provide a reference to aid interpretation of safety and tolerability data.

Healthy, male or female (non-childbearing potential), Japanese or Caucasian volunteers will be included; safety, tolerability and PK data will be compared between these populations to enable inclusion of Japanese volunteers in the subsequent clinical development programme for AZD9668. To be considered as 'Japanese', both of the volunteer's parents and both sets of grandparents must be Japanese, the volunteer must have been born in Japan, have a valid Japanese passport, and must not have lived outside Japan for more than 8 years. These criteria ensure representative study population by excluding mixed race or naturalised Japanese living in the West.

The starting dose for cohort 1 will be 30 mg. This dose has been selected as a low dose that was found to be safe and well tolerated based upon the emerging draft data from the AstraZeneca Global SAD/MAD Study It is proposed that the subsequent single doses will be 60 mg, 120 mg and 150 mg. These are proposed doses only and may change with emerging data from this study. The top dose will be no higher than 150 mg as this is the maximum dose that was administered to healthy volunteers in the AstraZeneca Global SAD/MAD Study and was found to be safe. In addition, modelled PK data from the current study will ensure that the predicted geometric mean C_{max} and $AUC_{(0-24)}$ at the top dose will not exceed 2.5 μM or 18 μM.h, respectively. These exposure limits have been determined by the AstraZeneca Human Exposure Limits Committee (HELC) following a review of the data obtained in the 1-month toxicology studies in the rat and dog. The limits have been set based upon the exposures at the no observed adverse effect level (NOAEL) in rat (370 mg/kg), corrected for the differences in plasma protein binding between the species. The NOAEL in the rat has been used because the free exposures at this dose are lower than those obtained in the dog at the NOEL dose.

The multiple doses for both cohorts 1 and 2 will be selected following a safety review meetings using emerging data from the current study and the AstraZeneca Global SAD/MAD Study The maximum multiple dose will be capped at a total daily dose of 140 mg. In addition, the emerging PK data from the current study will be modelled to ensure that for the highest dose given, the predicted geometric mean exposures (both $AUC_{(0-24),ss}$ and $C_{max,ss}$) will not exceed 2.5 μM or 18 μM.h, respectively.

Once a day dosing will be used for the single doses to enable investigation of single dose pharmacokinetics, and the twice daily (bid) dosing regimen will be used for the multiple doses because this is the dosing regimen that is planned to be used in patient intervention studies with AZD9668. If bid dosing is not tolerated then once a day dosing will be considered.

3.2.2 Risk/benefit and ethical assessment

The volunteers will have no individual benefit from participation in the study. The early development studies are to enable further investigations to evaluate AZD9668 as a treatment of COPD.

The current knowledge of the possible adverse effects of AZD9668 is based on the pre-clinical safety assessments. The main findings from these, which could be of relevance in human studies, include haematological changes at high doses (90 mg/kg) in a preliminary 28-day toxicity study in dogs; a slight QT prolongation (9%) at a high dose in a dog telemetry study; and slightly elevated plasma creatinine at high doses in repeat-dose studies in dogs and in rat renal function in a single dose study. In the current healthy volunteer study, host defence issues are not expected to be a problem.

For an overall risk benefit assessment of developing a NE inhibitor in COPD, see the Investigator's Brochure

3.3 Selection of study population

3.3.1 Study selection record

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

Replacements will be considered for all cases of volunteer drop-out, except for reasons of AE. This will all be at the Investigator's discretion

3.3.2 Inclusion criteria

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

1. Provision of signed, written informed consent.

2. Healthy Japanese or Caucasian volunteers. To be considered as 'Japanese', both of the volunteer's parents, and both sets of grandparents must be Japanese. The

volunteer must have been born in Japan, have a valid Japanese passport and must not have lived outside Japan for more than 8 years.

3. Male or female aged between 20-45 years (inclusive).

4. Weight 45-90 kg and Body Mass Index (BMI=weight/height^2) 18-30 kg/m^2 as measured at Visit 1b.

5. Females must be post menopausal (defined as amenorrhoeic for 12 months and follicle-stimulating hormone (FSH) plasma concentration within the postmenopausal range as defined by the laboratory) or surgically sterile (defined as having undergone bilateral oophrectomy and/or hysterectomy; tubal ligation on its own is not adequate).

6. Have normal physical examination, laboratory values and vital signs (blood pressure and pulse) as measured at Visit 1b, unless the Investigator considers an abnormality to be clinically irrelevant.

7. Have a normal resting ECG at Visit 1b, as judged by the Investigator, with normal QTc interval (<450 ms for both males and females), unless the Investigator considers an abnormality to be clinically irrelevant.

8. Have negative screens for Hepatitis B surface antigen, Hepatitis C antibodies and human immunodeficiency virus (HIV) 1 and 2 antibodies.

For inclusion in the genetic component of the study, volunteers must fulfil the following criterion:

1. Provision of signed, separate written informed consent for genetic research.

If a volunteer declines to participate in the genetic component of the study, there will be no penalty or loss of benefit to the volunteer. The volunteer will not be excluded from other aspects of the study described in this clinical study protocol, so long as they consent.

3.3.3 Exclusion criteria

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

1. Habitual smokers (the volunteer may be an occasional smoker providing that he/she is able to avoid smoking during the periods in the [redacted] Volunteers who smoke >10 cigarettes a day will be excluded from the study.

2. Symptoms of any clinically significant illness within two weeks prior to Visit 2.

3. Use of any prescribed medication (other than hormone replacement therapy [HRT]) or use of any non-prescribed preparations (other than paracetamol 1 g up to a

maximum daily dose of 4 g, permitted for occasional use) within two weeks prior to Visit 2.

4. Use of any herbal preparations and/or vitamins in the 7 days prior to Visit 1b, at the Investigator's discretion.

5. A history, or presence, of conditions known to interfere with the absorption, distribution, metabolism or excretion of drugs, eg, haematological, gastrointestinal, hepatic or renal disease, etc.

6. A definite, or suspected, personal history of intolerance or hypersensitivity to drugs and/or their excipients, judged to be clinically relevant by the Investigator.

7. Surgery or significant trauma within 90 days of Visit 2.

8. Involvement in the planning and conduct of the study (applies to both AstraZeneca and CRO staff).

9. Participation in a clinical study involving administration of another investigational medicinal product, or a new formulation of a marketed drug, the last follow-up visit of which is within 90 days prior to Visit 2 in this study (Note: participation is defined as having received at least one dose of investigational product).

   or,

   Participation in a method development study, where no drugs were given, but invasive procedures were used, the last follow-up visit of which is within 30 days prior to Visit 2 in this study.

10. If participation in the study would result in the donation of more than 1200 mL of blood in the year prior to the planned final study visit, or donation of blood in total >500 mL within the 90 days before the end of the study.

11. A significant history of alcohol abuse or consumption of greater than 28 units/week in males or 21 units/week in females (1 unit is equivalent to half a pint [285 mL] of beer, 1 glass of wine [125 mL] or 1 measure pf spirits [25 mL]).

12. A significant history of drug abuse (including benzodiazepines) or positive drugs of abuse test at Visit 1b.

13. Anticipated difficulty with venous access.

14. Volunteers who, in the opinion of the Investigator, should not participate in the study.

15. Planned in-patient surgery, dental procedure or hospitalisation during the study.

If a volunteer fails the inclusion/exclusion criteria, the assessment may be repeated once only at discretion of the Principal Investigator.

3.3.4 Restrictions

Volunteers will be required to adhere to the dietary and activity restrictions as outlined below:

Dietary restrictions

1. To avoid false positive drugs of abuse test, volunteers should abstain from consuming poppy seeds (as found on speciality breads) from Visit 1a until the last assessment at Visit 5.

2. Volunteers should abstain from the consumption of energy drinks containing glucuronolactone or taurine (eg, Red Bull) from Visit 1a until the last assessment at Visit 5.

3. Volunteers should abstain from drinking alcohol from 48 hours prior to Visit 1b and until the last assessment at each visit.

4. Volunteers should refrain from consuming grapefruit, grapefruit juice or other grapefruit containing products from 7 days prior to Visit 2 until the last assessment at Visit 5.

5. Volunteers should restrict the consumption of caffeine-containing drinks (eg, coffee, tea, cocoa, chocolate, cola, and "sports" drinks containing caffeine) to 4 cups per day, and caffeine-containing foods should be restricted from 72 hours prior to Visit 2 until the last assessment at Visit 5.

6. On waking on the morning of each dosing day volunteers will consume 150 mL of water. This must be a minimum of 1 hour before dosing. No further liquids will be allowed before dosing and for 2 hours after dosing. During the multiple dosing, volunteers will also not be allowed fluids for 2 hours before and 2 hours after their evening dose.

7. For the single doses, volunteers will be required to fast from midnight the night before dosing day (except water for thirst) prior to safety samples being taken. No food is allowed until 4 hours after dosing.

   For the multiple dosing, volunteers will be required to fast from midnight the night before dosing (except water for thirst). On Days 13 and 19, no food is allowed until 4 hours after the morning dose and for 2 hours before and 2 hours after their evening dose. On Days 14-18, volunteers will have the same restrictions except that they will be allowed a light breakfast at 2 hours post the morning dose.

Activity restrictions

The following activity restrictions are required during both the single and multiple dose phases of the study:

1. Volunteers should refrain from strenuous physical activity, which is not in the volunteer's normal daily routine, from 72 hours prior to Visit 2 until discharge from the study.

2. Volunteers should abstain from taking any prescribed medication (apart from HRT) from two weeks prior to Visit 2 until completion of Visit 5. However, this should not obviate necessary medical treatment.

3. Volunteers should abstain from taking non-prescribed medication (including over-the-counter [OTC] drugs, drugs bought over the internet, herbal remedies and vitamin supplements) from two weeks prior to Visit 2 until completion of Visit 5. Paracetamol 1 g, a minimum of 4 hours between doses, up to a maximum daily dose of 4 g is permitted for occasional use, however the Investigator should be informed so that this can be recorded.

4. Volunteers should not donate blood from the enrolment visit and for 3 months following dosing.

5. Volunteers should not participate in another study whilst participating in this study.

6. Volunteers should not get out of bed for 4 hours post morning dose on Days 1, 7, 13, and 19.

7. Male volunteers should abstain from unprotected sex and sperm donation from date of consent until 4 months after last dosing.

8. Volunteers should remain resident in the [redacted] from the day before dosing until discharged by the [redacted] physician.

3.3.5 Discontinuation of subjects from treatment or assessment

3.3.5.1 Criteria for discontinuation

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

- Voluntary discontinuation by the volunteer, who is at any time free to discontinue his/her participation in the study without prejudice to further treatment.

- Safety reasons as judged by the Investigator and/or AstraZeneca.

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

- Incorrect enrolment ie, the volunteer does not meet the required inclusion/exclusion criteria for the study.

- Volunteer lost to follow-up.

Dosing will be discontinued in the multiple dosing period (ie, during Visit 4) if the criteria below are met:

- An ALT/AST level ≥3 x ULN or any pattern of LFT abnormalities giving cause for concern.

- Evidence of haemolysis as evidenced by the following in two or more volunteers on active treatment.

  – Reticulocyte count of over 5%, and,

At least two of the following:

  – A drop in haemoglobin below 10.5 g/dL.

  – Decrease of 25% in haptoglobin. (The value post-dose to be compared with the average of two pre-dose values. The value should also be 15% below LLN).

  – Increase of 25% in unconjugated bilirubin or LDH. (The value post dose to be compared with the average of two pre dose values. The value should also be 15% above ULN).

- A platelet count of below 80,000 $x10^9$/L confirmed by a repeat test (in the absence of obvious platelet clumping in a peripheral blood film) in two or more volunteers on active treatment.

- A QTcF >500 ms in two or more volunteers on active substance (QT interval corrected for heart rate by Fridericia method) or a QTcF prolongation >60 ms on average, sustained for more than 30 minutes, ie, present on two contiguous timepoints, in two or more volunteers on active substance based on the results of the continuous readings compared to baseline.

- Any general safety finding that in the opinion of the Investigator or SRC gives cause for concern.

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

- Withdrawal of consent for genetic research. A volunteer may withdraw from this genetic research at any time, independent of any decision concerning participation

in other aspects of the main study described in this protocol. Voluntary discontinuation by the volunteer will not prejudice further treatment.

3.3.5.2 Procedures for discontinuation

Volunteers who discontinue should always be asked about the reason(s) for their discontinuation and the presence of any AEs. If possible, they should be seen and assessed by an Investigator. Adverse events should be followed up and investigational products should be returned by the volunteer.

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

If a volunteer withdraws consent to continued study participation, it must be specifically determined whether or not they also withdraw consent to future analysis of their biological samples collected during the study (in this case, blood and urine samples for future biomarker analysis) as this may entail destruction of the volunteer's stored samples.

Samples that have already been used for the intended research cannot be destroyed, and the results obtained will continue to be used for their intended research purposes. If consent is withdrawn to further analysis of stored samples before the samples are analysed, these will be traced as quickly as possible, and destroyed, or returned to the study site if required by local regulations.

3.3.5.3 Procedures for handling incorrect enrolled subjects

Volunteers not meeting the inclusion/exclusion criteria for a study should, under no circumstances, be enrolled into that study - there can be no exceptions to this rule.

Where volunteers not meeting the study criteria are enrolled in error, incorrectly randomised, or where volunteers subsequently fail to meet the criteria for the study post enrolment, procedures to be followed for the discontinuation of such volunteers are given in Section 3.3.5.2.

3.3.5.4 Procedures for discontinuation from genetic aspects of the study

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

- Agrees to the genetic sample and any deoxyribonucleic acid (DNA) extracted from the sample being kept for genetic analyses in the future.
- Withdraws consent for the sample to be kept for genetic analysis in the future and wishes the sample to be destroyed. Destruction of the sample (or the DNA extracted from the sample) will only be possible so long as the particular sample is

traceable. In the event that genetic research has already been performed, AstraZeneca will retain the results and associated data for regulatory reasons but these will not be used in any subsequent analyses.

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

Requests for sample destruction should be forwarded to the head of the Clinical Genotyping Group (CGG) at [redacted], UK. Requests should include copies of the relevant documentation detailing study protocol number and volunteer Ecode. The clinical study team and the Investigator will receive written confirmation from CGG that the sample has been destroyed.

3.4 Treatment(s)

3.4.1 Investigational product(s)

3.4.1.1 Identity of investigational product

AZD9668 will be provided as a tablet for oral administration and with a matching placebo. Tablets of AZD9668 will be provided in the strengths 10 mg and 30 mg (Table 5).

Table 5 Identity of investigational product

Investigational product	Dosage form and strength	Manufacturer	Formulation number	Batch number
AZD9668	Tablet 10 mg Tablet 30 mg	AstraZeneca R&D, [redacted]	1756-4 1756-1	Will be detailed in the CSR
Placebo AZD9668	Tablet	AstraZeneca R&D, [redacted]	P1756-0	Will be detailed in the CSR

The composition of the tablet is 10 mg and 30 mg AZD9668 (corresponding to 13.2 mg and 39.5 mg AZD9668 tosylate).

Excipients: cellulose microcrystalline, calcium hydrogen phosphate dihydrate, crospovidone, sodium laurilsulfate, sodium stearyl fumarate.

The drug product is a white to off-white, plain oblong, biconvex tablet. The different tablet strengths are size matched. The nominal tablet weight is 400 mg.

Placebo tablets match the AZD9668 tablets in appearance and contain the following excipients: cellulose microcrystalline and sodium stearyl fumarate.

3.4.1.2 Labelling

The investigational product will be supplied as bulk supply. Study site dispensary staff will dispense the investigational product according to the random scheme. Individual volunteer containers will be labelled with a detachable tear off label. The AZD9668, or matching placebo, tablets will be filled in high-density polyethylene (HDPE) bottles with a child restraint cap.

The packaging and labelling will be performed by Investigational Product Supplies (IPS), AstraZeneca R&D [redacted]. All supplies and labels will be prepared in accordance with Good Manufacturing Practice (GMP) and local regulatory guidelines. The labels will fulfil the GMP Annex 13 requirement for labelling.

Each dosing container will be labelled with a one-part detachable tear-off label, which will be affixed to the case report form (CRF) at the time of administration.

3.4.1.3 Storage

All investigational products must be kept in a secure place under appropriate storage conditions. A description of the appropriate storage and shipment conditions are specified on the investigational product container label and in the Investigator's Brochure The storage location will be locked and only accessible to authorised site personnel.

3.4.1.4 Accountability

The medication provided for this study is for use only as directed in the protocol. All unused study drugs will be accounted for and destroyed appropriately by [redacted] personnel. The study personnel will account for all drugs dispensed and returned. Certificates of delivery and return must be signed.

The Investigator (or delegated staff) is responsible for maintaining study drug accountability at site.

3.4.2 Doses and treatment regimens

Tablets of AZD9668/placebo will be administered by the Investigator (or his qualified staff) on each dosing day. The study drug will be taken orally in an upright position with 240 mL of water and the volunteers must drink all the water.

Each volunteer will receive two ascending single doses (on Days 1 and 7) followed by multiple doses (twice daily each dose administered 12 hours apart) for 6 days (Days 13-18), and a final single dose on the morning of Day 19 (ie, a total of 15 doses).

The proposed single dose levels are 30 mg, 60 mg, 120 mg and 150 mg, although they may change with emerging data from this study. The top dose will be no higher than 150 mg as this is the maximum dose that was administered to healthy volunteers in the AstraZeneca Global SAD/MAD Study and was found to be safe. In addition, modelled PK data from the

current study will ensure that the predicted geometric mean C_{max} and $AUC_{(0-24)}$ at the top dose will not exceed 2.5 μM or 18 μM.h, respectively.

The multiple doses for both cohorts 1 and 2 will be selected following a safety review meetings using emerging data from the current study and the AstraZeneca Global SAD/MAD Study The maximum multiple dose will be capped at a total daily dose of 140 mg. In addition, the emerging PK data from the current study will be modelled to ensure that for the highest dose given, the predicted geometric mean exposures (both $AUC_{(0-24),ss}$ and $C_{max,ss}$) will not exceed 2.5 μM or 18 μM.h, respectively.

Cohorts of Japanese and Caucasians healthy volunteers will receive the same dose of AZD9668, and will be run in parallel as far as is possible.

For the single doses, volunteers will be fasted from midnight the night before dosing day, except water for thirst. On waking on the morning of each dosing day volunteers will consume 150 mL of water. This must be a minimum of 1 hour before dosing. No further fluids will be allowed before dosing. Two hours post dose, volunteers will be provided with 200 mL of water. No food is allowed until 4 hours after dosing. Lunch will be served at approximately 4 hours post dose, and an evening meal between 8 and 10 hours post dose. From lunchtime onwards the volunteers will be allowed fluids and snacks within the restrictions of the study.

For the multiple doses, volunteers will be fasted from midnight the night before dosing day, except water for thirst. On waking on the morning of each dosing day volunteers will consume 150 mL of water. This must be a minimum of 1 hour before dosing. No further fluids will be allowed before dosing. On Days 13 and 19, the volunteers will be provided with 200 mL of water post dose and no food is allowed until 4 hours after dosing. Lunch will be served at approximately 4 hours post dose, and an evening meal between 8 and 10 hours post morning dose. Volunteers will not be allowed food or fluids for 2 hours before and 2 hours after their evening dose, after which they are allowed free access to fluids and snacks within the restrictions of the study up to midnight (when they will fast for their next day's dose). On Days 14 –18, volunteers will have the same restrictions except that they will be allowed a light breakfast at 2 hours post morning dose.

3.4.3 Method of assigning subjects to treatment groups

Informed consent will be obtained before enrolment and the volunteers identified with an enrolment number starting with E0001001. Numbers will be allocated in ascending order.

Volunteers fulfilling the eligibility criteria will be assigned randomisation codes (unique volunteer numbers), starting with 100 (Japanese volunteers) and 200 (Caucasian volunteers). Numbers will be allocated in ascending order.

Volunteers will be assigned randomisation codes strictly sequentially as volunteers are eligible for randomisation. If a volunteer discontinues from the study the volunteer number will not be re-used and the volunteer will not be allowed to re-enter the study.

The randomisation schedule and associated individual treatment code envelopes (emergency unblinding tools) giving details of individual volunteer treatment will be produced by computer software (SAS®) that incorporates a standard procedure from generating randomisation codes.

Both Japanese and Caucasian volunteers will be allocated to either AZD9668 or placebo in a 6:2 ratio (AZD9668:placebo) per cohort. At each dose level, Japanese and Caucasian volunteers will be gender and age matched (±5 years).

3.4.4 Blinding and procedures for unblinding the study

3.4.4.1 Methods for ensuring blinding

AZD9668 and placebo tablets are manufactured to appear similar in size, shape, weight and colour.

3.4.4.2 Methods for unblinding the study

Individual treatment codes, indicating the treatment randomisation for each randomised volunteer, will be available to the Investigator at the study centre and the personnel who are independent of the study evaluation at the Drug Safety Department, AstraZeneca [redacted].

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

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

The following personnel will have access to the randomisation list:

- The personnel carrying out packaging and labelling of investigational product.
- The personnel dispensing the investigational product at the [redacted]
- The personnel analysing the PK samples.

The information in the randomisation list must be kept in a secure location until the end of the study.

3.4.5 Concomitant medication

No concomitant medication or therapy will be allowed except paracetamol (1 g, a minimum of 4 hours between doses, up to a maximum daily dose of 4 g) for occasional use and HRT. The volunteers must be instructed that no additional medication will be allowed without the prior consent of the Investigator, although this does not preclude emergency treatment. In this

situation the medication/treatment received must be reported to the Investigator as soon as possible.

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

3.4.6 Treatment compliance

Compliance will be assured by supervised administration of the investigational product by the Investigator or delegate.

4. MEASUREMENT OF STUDY VARIABLES

The following study measurements will be obtained. The timing of these measurements is detailed in the study plan (Table 1). All assessments will be performed at the scheduled timepoints (pre dose ±30 minutes; post dose ±10 minutes). The following 'priority order' will be in effect when more than one assessment is required at a particular time point:

ECG; vital signs (blood pressure and pulse); PK sample; PD sample; safety blood samples.

4.1 Medical examination and demographic measurements

4.1.1 Enrolment medical examination and demographic measurements

Volunteers will attend the [redacted] to provide written informed consent at Visit 1a. Subsequently, each eligible volunteer will undergo an enrolment medical examination at Visit 1b (up to 21 days prior to Visit 2). Volunteers should be fasted (minimum of 8 hours), with only water permitted.

Assessment at Visit 1b will include:

- Recording of demographic data - date of birth, sex, ethnic origin, BMI, smoking status (ie, number of cigarettes/cigars/etc smoked per day).
- A standard medical and surgical history assessment and review.
- Concomitant medication assessment and review.
- Physical examination (including height and weight).
- An alcohol screen (breath test).
- Virology blood sample for Hepatitis B surface antigen, Hepatitis C antibody, and HIV status.
- A 12-lead ECG (paper).

- Resting blood pressure and pulse measurement (sitting position).
- A blood sample (fasted) for standard clinical chemistry (including FSH at Visit 1b for female volunteers) and haematology assessments, and a mid-stream urine sample for urinalysis and drugs of abuse screen.
- Pregnancy test (female volunteers only).

4.1.2 Post-study medical examination (Visit 5)

Procedures at the follow-up visit (Visit 5) will consist of:

- Concomitant medication assessment and review.
- Physical examination (weight only will be measured at this visit).
- An alcohol screen (breath test).
- A 12-lead ECG (paper).
- Resting blood pressure and pulse measurement (sitting position).
- A blood sample for standard clinical chemistry and haematology assessments, and a mid-stream urine sample for urinalysis and drugs of abuse screen (only required if volunteer was allowed out of the clinical unit during the study).
- Assessment of AEs.

4.2 Pharmacokinetic measurements

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

The number and timings of PK sample collection may be revised based upon emerging data from the AstraZeneca Global SAD/MAD Study, but the total number of samples will not be increased from those listed in Table 3.

4.2.1 Determination of drug concentration in biological samples

Plasma samples for measurement of drug concentration will be analysed by Plasma samples for measurement of drug concentration will be analysed by [redacted] AstraZeneca R&D [redacted] using a validated method of liquid chromatography and mass spectrometry (LC-MS/MS). The lower limit of quantification (LLOQ) will be 1.00 nM.

Urine samples for the determination of AZD9668 concentrations will be analysed using appropriate quantitative methods (including test of stability) by Plasma samples for measurement of drug concentration will be analysed by [redacted] AstraZeneca R&D [redacted]. The methods used will be referred to in the CSR.

Both plasma and urine samples may be used in exploratory metabolite identification investigations, which in such case will be reported separately.

4.2.2 Collection of biological samples

4.2.2.1 Blood samples for PK measurements

Blood samples (4 mL K_2EDTA) for determination of AZD9668 in plasma will be taken at the times presented in Table 3.

Blood samples will be collected, labelled and shipped as detailed in the laboratory handbook. The date and time of collection will be recorded on the appropriate CRF page.

Samples should be stored at -18°C or below, and analysed within the timeframe after collection for which the stability in the samples has been validated and found acceptable. Results from analyses stored longer than the period stated will not be reported.

The methods used will be referred to in the CSR.

Samples may be kept for up to five years before destruction.

4.2.2.2 Urine samples for PK measurements

Urine will be passed into a specially provided container(s) at the times presented in the study plan (Table 1). Urine containers should be stored in the refrigerator (2-8°C) when not in use during the collection period. Samples will be collected, labelled and shipped as detailed in the laboratory handbook.

The date and time of collection will be recorded on the appropriate CRF page.

After mixing, 4 x 10 mL aliquots will be removed into polypropylene tubes (only 2 x 10 mL aliquots will be removed for the "blank" when the volunteers empty their bladders prior to dosing). Samples should be stored at -18°C or below.

The methods used will be referred to in the CSR.

Samples may be kept for up to five years before destruction.

4.3 Pharmacodynamic measurements

4.3.1 Ex-vivo zymosan stimulated neutrophil elastase (NE) activity in plasma

A blood sample (4.5 mL sodium citrate vacutainer) will be taken for the analysis of zymosan stimulated NE activity at times indicated in Table 4.

Each blood sample will be aliquoted as follows:

Pre-dose samples

(a) Unstimulated.

(b) Zymosan stimulated.

(c) Zymosan stimulated and single concentration of AZD9668 (AZD9668 will be added *ex-vivo* to act as a positive control).

Post-dose samples

(a) Unstimulated.

(b) Zymosan stimulated.

All samples will be incubated at 37°C and handled according to instructions in the laboratory handbook. Following incubation, the samples will be processed and separated before freezing. Samples should be frozen at -18°C or below and transferred frozen, on dry ice, to [redacted] AstraZeneca R&D, [redacted] for analysis.

The number and timings of sample collection may be revised based upon emerging data from the AstraZeneca Global SAD/MAD Study, however the total number if samples will not be increased from those listed.

Samples may be kept for up to five years before destruction.

Full details of sample processing and shipping can be found in the laboratory handbook.

4.3.2 Method of assessment

Plasma aliquots are analysed for NE activity in a 96-well assay format using a fluorogenic substrate that is cleaved according to NE activity. Increasing activity yields increasing amounts of the cleavage product 7-amino 4-methyl coumarin (AMC).

4.4 Safety measurements

4.4.1 Laboratory safety measurements

Blood and urine samples for the determination of clinical chemistry, haematology and urinalysis parameters will be taken at the times given in the study plan (Table 1). The date of collection will be recorded on the appropriate CRF page.

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

Clinical chemistry – 8.5 mL serum separation tube (SST) vacutainer.

Haematology – 3 mL EDTA vacutainer.

Urinalysis – 20 mL universal tube.

The following laboratory variables will be measured:

Clinical Chemistry	
Creatinine	Triglycerides
Total, conjugated, and unconjugated bilirubin	Urea
Alkaline phosphatase	Gamma Glutamyltransferase (GGT)
Aspartate aminotransferase (AST)	Creatinine phosphokinase (CPK) [a]
Alanine aminotransferase (ALT)	Lactate dehydrogenase (LDH)
Albumin	Glucose (fasting)
Potassium	Follicle stimulating hormone (FSH) [c]
Calcium, total	Haptoglobin
Sodium	
High sensitivity C-reactive protein (hsCRP)	
Cholesterol	
Haematology	**Urinalysis**
Haemoglobin	Blood
Leukocyte count	Protein
Red cell count (RBC)	Ketones
Haematocrit	Glucose
Mean cell volume (MCV)	Bilirubin
Mean cell haemoglobin (MCH)	Urobilinogen
Mean cell haemoglobin concentration (MCHC)	**Virology** [b]
Leukocyte differential count (absolute)	Hepatitis B surface antigen
Platelet count	Hepatitis C antibodies
Reticulocyte count	HIV 1 and 2 antibodies

[a] If elevated >3, P-CPK-MB will be analysed (will not be data-based).

[b] Included at Visit 1b only.

[c] Only analysed in female volunteers. Included at Visit 1b only.

Urine will be tested for the following drugs of abuse:

Methadone, benzodiazepines, cocaine, amphetamines, tetrahydrocannabinoids (THC), morphine, methamphetamines (ecstasy), barbiturates, phencyclidine (PCP) and tricyclic antidepressants (TCA).

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

Female volunteers must give a sample of the first urine passed at Visit 1b for a pregnancy test to be performed. It is important to check that volunteers are not pregnant when they enter the study but it is unnecessary to re-test during the study as they have already been defined as 'non-childbearing potential'.

4.4.2 Electrocardiographic measurements

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

4.4.2.1 Resting 12-lead ECG

Paper ECG

Paper ECGs will be recorded prior to each dECG recording (see Table 2), and at 48 and 72 hours post dose at Visits 2, 3 and 4.

Prior to the acquisition of the 12-lead ECG at each timepoint, the volunteer must be rested in bed for at least 10 minutes. The volunteer must be in the same supine (maximum 30 degrees flexion in the hip) body position at each time point at all visit and feet not in contact with footboard. The skin must be cleaned, and electrode positions marked with an indelible pen. Electrodes should be applied 30 minutes before first recording.

Digital ECG (dECG)

If required*, dECGs will be recorded for three 10-second intervals over two minutes at the timepoints (shown in Table 2) at Visits 2 and 3 (single doses) and after the first and last multiple doses (ie, on Days 13 and 19 [Visit 4]).

* The need to perform dECGs will be based on the outcome of the ongoing AstraZeneca Global SAD/MAD Study.

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

Upon completion of ECG recordings for each dose panel, the digital files are transferred from the ECG machines to an external company for over-reading by an independent cardiologist. The cardiologist will perform the analysis in a blinded manner. Each ECG will be faxed back to the site within 48 hours with the cardiologist's signature and date of review. The cardiologist will contact the site by telephone if there are any findings of concern. Beside the final analysis, at agreed dates, safety evaluation of the dECG data will be submitted to the SRC for evaluation before dose escalation.

In this study lead V2 will be analysed and reported as primary. Lead V5 will be analysed, for all visits, as backup for the individual where analysis in lead V2 is not deemed possible for

pre-dose or significant parts of whole visits or whole visits. The following variables will be reported: RR, PR (PQ), QRS, QTtang, QTcB, QTcF and QTcX will be calculated.

Additional safety paper ECGs are to be taken before each dECG recording and at the discretion of the Investigator for safety reasons. The QTc is calculated automatically by the Marquette MAC 1200 (General Electric Healthcare, UK) according to Bazzett's formula and the QT time is the same in all leads of the averaged complex. These paper ECGs will be reviewed by the Investigator and evaluated in his opinion, as "Normal" or Abnormal" or "Not Clinically Significant" (NCS) and stored with the CRF. If the QTc is considered prolonged, a repeat paper ECG and a dECG should be recorded within 30 minutes.

4.4.2.2 Telemetry

Ambulatory cardiac monitoring (ACM) will be performed using the SL2400 portable monitor (Spacelabs Healthcare, USA) from -1 hour (pre dose) to 24-hour post dose at Visits 2, 3 and 4, and from -1 hour to 24 hours post dose on Day 19 (Visit 4). The Investigator or his/her delegate will review the recordings. A print out will be signed and dated by the Investigator and filed with the CRF.

4.4.3 Vital signs

4.4.3.1 Blood pressure and heart rate

Sitting blood pressure and pulse rate will be measured at the timepoints (shown in Table 2) at Visits 2 and 3 (single doses) and after the first and last multiple doses (ie on Days 13 and 19 [Visit 4]). Vital signs will also be taken at Visit 1b (Screening) and Visit 5 (Follow-up).

On each study day, sitting blood pressure and pulse will be measured using a semi-automatic blood pressure recording device with an appropriate cuff size, after volunteers have been sitting for at least 10 minutes. These data will be entered into the CRF.

4.4.4 Other safety measurements (Not applicable)

Not applicable.

4.5 Genetic measurements and co-variables

4.5.1 Collection of samples for genetic research

Volunteers will provide a blood sample as per the inclusion criteria and visit schedule.

A single venous blood sample will be collected into a 10 mL polypropylene tube containing ethylenediamine tetra-acetic acid (EDTA) and gently inverted a minimum of five times to mix thoroughly. Tubes will be labelled with labels which details study code, volunteer ID (enrolment number and/or randomisation number), sample type, protocol timepoint, and visit number and unique barcode. No personal identifiers (volunteer name, initials or date of birth) will be placed on the tube or accompanying documentation. A record of the date of the volunteer consent to the genetic research and the date of the blood sample collection will be recorded in the appropriate section of the CRF.

Genotype is a stable parameter, therefore if for any reason the blood sample is not drawn at Visit 2 it may be taken at any visit until the last study visit. The genetic blood sample should ideally be drawn through the same cannula used to draw blood samples required for the main study.

4.5.1.1 Sample processing and shipping

Samples will be frozen (-18°C or below) and transported to the relevant DNA extraction laboratory within one month of collection and must remain frozen at all times.

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

4.5.1.2 Storage and coding of DNA samples

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

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

The blood samples and data for genetic analysis in this study will be coded. Each blood sample will be labelled with the study number and volunteer number (enrolment/randomisation code). Only the Investigator will be able to link the blood sample to the individual volunteer. The sample and data will not be labelled with a personal identifier. The link between the volunteer enrolment/randomisation code and the DNA number will be maintained.

This link file and any corresponding genetic data will be stored in a secure environment, with restricted access within the [redacted] at AstraZeneca, [redacted]. 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.

All DNA samples will be stored under secure conditions with restricted access at AstraZeneca and the contracted laboratory. The blood, DNA samples or data derived from the samples may be made available to groups or organisations working with AstraZeneca on this study or as part of the development drug project. However, the samples and any results will remain the

property of AstraZeneca at all times. AstraZeneca will not give blood, DNA samples or data derived from the samples to any other parties, except as required by law.

Samples will be stored for a maximum of 15 years, from the date of completion of the study, after which they will be destroyed.

4.5.1.3 Summary of genetic assessments and analysis

The purpose of the genetic research is to generate data for use in future retrospective analyses. Future analyses will explore genetic factors that may influence the disposition, efficacy, safety and tolerability of AZD9668. The results of the genetic research will not form part of the CSR for this study. The results may be pooled with genetic data from other studies on AZD9668 to generate hypotheses to be tested in future studies.

4.5.1.4 Derivation or calculation of genetic parameters

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

4.6 Volume of blood sampling

The total volume of blood that will be drawn from each volunteer in this study is shown in Table 6.

Table 6 Maximum volume of blood to be drawn from each volunteer

Assessment		Sample volume (mL)	n of samples	Total volume (mL)
PD analysis: zymosan stimulated NE activity		4.5	11	49.5
PK analysis		4.0	65	260.0
Pharmacogenetics [a]		10.0	1	10.0
Safety:	Clinical chemistry	8.5	5	42.5
	FSH (Visit 1b)	8.5	1	8.5
	Haematology	3.0	5	15.0
Virology:	Hepatitis B and C and HIV	8.5	1	8.5
Cannula discard (approx.)		1	65	65.0
Total				**459.0**

[a] If consent has been obtained

4.7 Adverse Events

The methods for collecting AEs are described below.

4.7.1 Adverse Events

4.7.1.1 Definitions

The definitions of adverse events (AEs), serious adverse events (SAEs) and other significant adverse events (OAEs) are given below. It is of the utmost importance that all staff involved in the study are familiar with the content of this section. The Principal Investigator is responsible for ensuring this.

Adverse event

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

Serious adverse event

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

- Results in death.
- Is immediately life-threatening.
- Requires in-patient hospitalisation or prolongation of existing hospitalisation.
- Results in persistent or significant disability or incapacity.
- Is a congenital abnormality or birth defect.
- Is an important medical event that may jeopardise the volunteer or may require medical intervention to prevent one of the outcomes listed above.

The causality of SAEs (ie, their relationship to study treatment) will be assessed by the Investigator, who in completing the relevant case report form must answer “yes” or “no” to the question “Do you consider that there is a reasonable possibility that the event may have been caused by any of the following – study medication – other medication?” For further guidance on the definition of a SAE and a guide to the interpretation of the causality question, see Appendix B to the Clinical Pharmacology Study Protocol.

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

Other Significant Adverse Events (OAE)

Other significant adverse events will be identified by the Principal Investigator in consultation with the appropriate Drug Safety Physician during the evaluation of safety data for the CSR. Significant adverse events of particular clinical importance, other than SAEs and those AEs leading to discontinuation of the volunteer from study treatment, will be classified as OAEs. Examples of these are marked haematological and other laboratory abnormalities, and certain events that lead to intervention (other than those already classified as serious), dose reduction or significant additional treatment. For each OAE, a narrative may be written and included in the CSR.

4.7.1.2 Recording of adverse events

Adverse events will be recorded in the CRF from Visit 1b until Visit 5. Any AEs remaining unresolved at Visit 5 should be recorded as "ongoing" in the CRF. Where appropriate, these should be followed as long as medically indicated but without further recording in the CRF.

The following variables will be recorded for each AE: AE description, start and stop date, intensity, action taken, outcome, Investigator's causality rating (yes or no), and whether the AE meets criteria for a SAE or not.

The following levels of intensity will be recorded:

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

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria in Section 4.7.1.1. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not a SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke but would be a SAE.

Adverse events will be collected on an ongoing basis. All AEs spontaneously reported by the volunteer or reported in response to open questions from the study personnel: "Have you had any health problems since the previous visit?" or "Have you had any health problems since you were last asked" or revealed by observation will be collected and recorded in the CRF. The first AE questioning will occur at Visit 1b.

Signs and symptoms present at Visit 1b will be recorded as 'ongoing pre-dose'. When the severity or seriousness of any pre-dose signs or symptoms changes this will be recorded as a new AE.

Adverse events and any abnormalities in laboratory tests, vital signs or physical examination of clinical relevance will be followed up until resolution. Volunteers who drop out during the study for reasons other than discontinuation of study drug will be followed up for the planned duration of the study. Abnormalities in laboratory tests and vital signs will be recorded and reported in the laboratory reports and CRFs respectively. Abnormalities in these variables will be identified, analysed and reported in the CSR. Laboratory results that constitute a SAE or lead to discontinuation of the study drug will be recorded and reported as an AE. In addition, any abnormality in laboratory tests, which meet the stopping criteria as described in Section 3.1.1, will be recorded as an AE.

Should an overdose (accidental or deliberate) occur, it must be reported in accordance with the procedures described in Section 8.3, 'Procedures in case of overdose', regardless of whether the overdose was associated with any symptom or not. All symptoms associated with the overdose should be reported as AEs.

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

4.7.1.3 Reporting of serious adverse events

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

The AstraZeneca representative will work with the Investigator to compile all the necessary information including the Investigator's causality assessment, and ensure that the appropriate AstraZeneca Drug Safety Department receives a report by day one for all fatal and life-threatening cases and by Day 5 for all other SAEs.

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

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

All SAEs have to be reported, whether or not considered causally related to the investigational product or to the study procedure(s). All SAEs will be recorded in the CRF. The Investigator and/or Sponsor are responsible for informing the Ethics Committee and/or the Regulatory

Authority of the SAE as per local requirements. For studies in countries implementing the EU Clinical Trials Directive, this will be taken care of by AstraZeneca (see Section 7.1).

5. STUDY MANAGEMENT

5.1 Monitoring

5.1.1 Study monitoring

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

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

5.1.2 Data verification

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

For this study original data recorded on the CRF and regarded as source data are as follows:

- Blood pressure, pulse, height and weight at Visit 1b (and all at Visit 5, except height and weight which are not captured).
- Adverse events.
- Concomitant medication.

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

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

5.2 Audits and inspections

Authorised representatives of AstraZeneca, a regulatory authority, and an Ethics Committee may visit the centre to perform audits or inspections, including source data verification. The purpose of an AstraZeneca audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analysed, and accurately reported according to the

protocol, GCP guidelines of the ICH and any applicable regulatory requirements. The Investigator should contact AstraZeneca immediately if contacted by a regulatory agency about an inspection at his or her centre.

5.3 Training of staff

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

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

5.4 Changes to the protocol

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

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

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

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

[redacted] will distribute amendments and new versions of the protocol to the Investigator who in turn is responsible for the distribution of these documents to his or her Ethics Committee, and to the staff at his or her centre. The distribution of these documents to the regulatory authority will be handled according to local practice.

5.5 Study agreements

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

5.6 Study timetable and end of study

The study is expected to start in [redacted] and to be completed by [redacted].

5.7 Data management

5.7.1 Case report forms

Paper CRFs (pCRFs) will be used to record all data not captured electronically. Data should be recorded legibly onto the pCRFs in blue or black ballpoint pen. Correction fluid or covering labels must not be used.

The Study Monitor will check data at the monitoring visits to the study site. The Investigator will ensure that the data in the pCRFs are accurate, complete and legible.

Data from the completed pCRFs will be entered (double data entry) onto the clinical study database and validated under the direction of the Data Manager. Any missing, impossible or inconsistent recordings in the pCRFs will be identified by electronic edit checks during data validation, and will be referred back to the Investigator using a data query form and documented for each individual volunteer until the database is declared clean prior to Quality Control checks and the final Database Lock.

5.7.2 Genetic data

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

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

5.8 Reporting of genotypic results

Results from any genetic research performed will be reported separately from the CSR. AstraZeneca will not provide individual genotype results to volunteers, their family members, any insurance company, an employer, clinical study Investigator, general physician or any other third party, unless required to do so by law. The volunteer's DNA will not be used for any purpose other than those described in the study protocol.

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

6. PHARMACOKINETIC, PHARMACODYNAMIC, SAFETY, GENETIC AND STATISTICAL METHODOLOGY

6.1 Pharmacokinetic / pharmacodynamic evaluation

6.1.1 Calculation or derivation of pharmacokinetic variables

The pharmacokinetic analyses will be performed by [redacted]

6.1.1.1 Single dosing

The PK parameters will be calculated using non-compartmental analysis. The PK parameters measured at Day 1 and Day 7 include:

Maximum plasma drug concentration (C_{max}), time to maximum plasma drug concentration (t_{max}), area under the (plasma drug contraction-time) curve from time zero to time t ($AUC_{(0-t)}$), area under the (plasma drug concentration-time) curve from time zero to infinity (AUC), AUC from time zero to 12 hours ($AUC_{(0-12)}$), AUC from time zero to 24 hours ($AUC_{(0-24)}$), terminal half-life of drug in plasma ($t_{½}$), apparent terminal volume of distribution following oral drug administration (Vz/F), apparent plasma clearance following oral drug administration (CL/F), renal clearance (CL_R), cumulative amount excreted in urine (A_e), and fraction of dose excreted unchanged in the urine (F_e).

6.1.1.2 Multiple dosing

Day 13: C_{max}; t_{max}; $AUC_{(0-12)}$; concentration at 12 hours post dose (C_{12}); A_e; F_e. The Ae and Fe will be estimated using data from 0-12 hours

Day 19: Minimum plasma (trough) drug concentration after repetitive dosing after steady state is achieved, prior to the first dose of the study day ($C_{min,ss}$), C_{max} at steady state ($C_{max,ss}$), t_{max} at steady state ($t_{max,ss}$), $AUC_{(0-12)}$ at steady state ($AUC_{(0-12),ss}$), $AUC_{(0-24)}$ at steady state ($AUC_{(0-24),ss}$), AUC at steady state ($AUC_{,ss}$), Vz/F at steady state ($V_Z/F_{,ss}$), CL/F at steady state ($CL/F_{,ss}$), $t_{½}$ at steady state ($t_{½,ss}$), CL_R, A_e at steady state ($A_{e,ss}$), F_e at steady state ($F_{e,ss}$), accumulation ration (R_{ac}), C_{12} at steady state ($C_{12,ss}$)

The R_{ac} will be calculated using the following equation:

$R_{ac} = AUC_{(0-12),ss}$ Day 19 / $AUC_{(0-12)}$ Day 13

6.1.2 Calculation or derivation of pharmacodynamic variables

The inhibition of the post-dose zymosan stimulated NE activity will be derived using the following formula:

100 – ((post dose stimulated NE activity – post dose unstimulated NE activity) / (pre dose Day 1 stimulated NE activity – pre dose Day 1 unstimulated NE activity)) * 100

6.1.3 Calculation or derivation of pharmacokinetics/pharmacodynamics

The relationship between AZD9668, and the effect of dose on the *ex vivo* zymosan stimulated NE activity in plasma will be explored graphically.

6.2 Safety evaluation

6.2.1 Calculation or derivation of safety variables

Adverse events will be analysed by means of descriptive statistics and qualitative analysis, AEs will be listed for each volunteer and summarised by System Organ Class and Preferred Term assigned to event by using MedDRA (Version 10 or higher).

Concomitant medications will be classified according to the AZDD Drug Dictionary, the ATC system and the CPMP route of administration dictionary. All concomitant medications reported at entry and recorded during the study will be listed.

Abnormalities in laboratory safety data, vital signs, ECG or physical examination will only be reported as AEs if they are accompanied by clinical symptoms, constitute SAEs or lead to discontinuation of treatment with the investigational product. For the laboratory safety data, vital signs, and ECG data change from pre dose Visit 2 will be calculated.

6.3 Genetics as a co-variate

6.3.1 Calculation or derivation of genetic variables

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

6.4 Statistical methods and determination of sample size

6.4.1 Statistical evaluation

A comprehensive SAP will be prepared before database lock and unblinding of these data in order to avoid any potential bias due to knowledge of the treatment received.

Statistical analyses will be performed by [redacted] using SAS® Version 8.1 (or older version) and WINNONLIM (for the PK analysis), and where appropriate, additional validated software. These analyses will be performed in accordance with this SAP, which will detail analyses to be performed and summaries to be produced for the CSR.

6.4.2 Description of variables in relation to hypotheses

No statistical hypotheses will be tested and only descriptive statistics will be produced.

6.4.3 Description of analysis sets

There will be one analysis set which will comprise all volunteers randomised into the study and receiving at least one dose of study medication. A second analysis set will comprise of

randomised volunteers who have receiving at least one dose of study medication and have sufficient plasma results to calculate reliable PK parameters. Volunteers will be analysed according to the treatment they actually received.

6.4.4 Methods of statistical analyses

Data will be summarised by dose administered using descriptive statistics and graphical output. In addition due to the exploratory nature of the study other statistical methods may be used where appropriate.

Summary statistics will be presented for continuous variables, by way of n, mean, standard deviation (SD), median, minimum and maximum and by way of group frequencies and percentages for categories of categorical variables. Percentages will be calculated using the total volunteers per treatment group. In addition, geometric mean, geometric coefficient of variation (CV%) and 95% confidence intervals will be presented for the PK concentrations and parameters.

No formal analysis testing is planned.

6.4.5 Determination of sample size

This study is exploratory in nature and therefore no formal analysis testing is being conducted and there is no formal powering of the study.

6.5 Interim analyses

No interim analysis is planned.

6.6 Data presentation

Data presentation will be described in the SAP.

6.7 Data monitoring committee

Data will be reviewed by a SRC. Details of the SRC can be found in Section 3.1.1.1.

7. ETHICS

7.1 Ethics review

AstraZeneca will provide Ethics Committees and Principal Investigators with safety updates/reports according to local requirements.

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

The Principal Investigator is responsible for informing the Ethics Committee of any amendment to the protocol in accordance with local requirements. In addition, the Ethics Committee must approve all advertising used to recruit volunteers for the study.

Where there is a genetic research, approval must be obtained for this genetic research and the associated genetic informed consent from the Ethics Committee. It must be clearly stated in the approval that this genetic research is approved. The Investigator must submit written approval to AstraZeneca before any volunteer participates in this genetic research.

7.2 Ethical conduct of the study

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

7.3 Informed Consent

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

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

The Principal Investigator must store the original, signed Informed Consent Form. A copy of the Informed Consent Form must be given to the volunteer.

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

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

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

7.4 Subject data protection

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

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

The Master Informed Consent Form will also explain that for data verification purposes, authorised representatives of AstraZeneca, a regulatory authority, an Ethics Committee may require direct access to parts of the hospital or practice records relevant to the study, including volunteers' medical history.

All data protection and confidentiality principles, described in the main study protocol, are applicable to this genetic research.

Reference to participation in this genetic research should not be recorded into the volunteers' general medical records. All notes should be kept within the clinical study records.

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

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

8. PROCEDURES IN CASE OF EMERGENCY, OVERDOSE OR PREGNANCY

8.1 AstraZeneca emergency contact procedure

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

- [redacted] Tel: [redacted] Mob: [redacted]

For Serious Adverse Event reporting

- Drug Safety Fax [redacted]

8.2 Procedures in case of medical emergency

The Principal Investigator is responsible for ensuring that procedures and expertise are available to cope with medical emergencies during the study. A medical emergency usually constitutes an SAE and should be reported as such, see Section 4.7.1.3.

Details on treatment code breaks can be found in Section 3.4.4.

8.3 Procedures in case of overdose

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

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

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

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

8.4 Procedures in case of pregnancy

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

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

9. REFERENCES

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Clinical Pharmacology Study Protocol: Appendix B

Drug Substance	AZD9668
Study Code	D0520C00004
Appendix Edition Number	1
Appendix Date	[redacted]

Appendix B
Additional Safety Information

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

Life threatening

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

Hospitalisation

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

Important medical event or medical intervention

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

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

Examples of such events are:

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

A GUIDE TO INTERPRETING THE CAUSALITY QUESTION

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

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

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

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

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

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

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

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

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

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

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

- Is there a known mechanism?

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