Clinical Pharmacology Study Protocol

Drug Substance	AZD3199
Study Code	D0570C00001
Date	[redacted]

A phase I, placebo-controlled, first time into man study of single ascending doses of inhaled AZD3199, Part A double-blind and randomised, to investigate the safety, tolerability and pharmacokinetics in healthy male volunteers and Part B single-blind, non-randomised and crossover ascending dose scheme to investigate the efficacy, safety, tolerability and pharmacokinetics in patients with asthma

Sponsor:

AstraZeneca AB, [redacted]

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

Amendment Number	Date of Amendment

Administrative Change Number	Date of Administrative Change

AstraZeneca

PROTOCOL SYNOPSIS

A phase I, placebo-controlled, first time into man study of single ascending doses of inhaled AZD3199, Part A double-blind and randomised, to investigate the safety, tolerability and pharmacokinetics in healthy male volunteers and Part B single-blind, non-randomised and crossover ascending dose scheme to investigate the efficacy, safety, tolerability and pharmacokinetics in patients with asthma

Investigator

[redacted]

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

This study is planned to be carried out at one or more centres in Sweden.

At least 64 healthy men are planned to be included in Part A, and 16 men with asthma in Part B.

Study period	Phase of development
Estimated date of first subject enrolled: [redacted]	Clinical Pharmacology (I)
Estimated date of last subject completed: [redacted]	

End of study is defined as database lock, which is the time point after which no subject will be exposed to study related activities.

Objectives

Part A

The primary objective of Part A of the study is to investigate the safety and tolerability of single ascending doses of AZD3199 delivered as a solution for nebulisation by assessment of:

- incidence and nature of adverse events (AEs)
- clinically significant abnormalities in ECG parameters, blood pressure (BP), pulse, lung function, body temperature and laboratory variables (clinical chemistry, haematology and urinalysis)

The secondary objectives of Part A of the study are:

1. to investigate the pharmacokinetics (PK) of single ascending doses of AZD3199 by assessment of drug concentration in plasma and urine and calculated PK parameters, with particular attention to dose linearity
2. to investigate extrapulmonary β_2- adrenoreceptor mediated effects of single ascending doses of AZD3199 by assessment of potassium and lactate concentrations, tremor and palpitations, heart rate, QTc, and blood pressure
3. to obtain material for possible exploratory analysis of metabolites of AZD3199 in urine and plasma (exploratory objective)

Part B

The primary objective of Part B is to investigate the efficacy of single ascending doses of AZD3199 by assessment of magnitude and duration of action on lung function (FEV_1)

The secondary objectives of Part B of the study are:

1. to investigate the safety and tolerability of single ascending doses of AZD3199 by assessment of incidence and nature of AEs, clinically significant abnormalities in ECG parameters, blood pressure (BP), pulse, lung function and laboratory variables (clinical chemistry, haematology and urinalysis)
2. to investigate the pharmacokinetics (PK) of single ascending doses of AZD3199 by assessment of drug concentrations in plasma and calculated PK parameters, with particular attention to dose linearity
3. to investigate extrapulmonary β_2- adrenoreceptor mediated effects of single ascending doses of AZD3199 by assessment of potassium and lactate concentrations, tremor and palpitations, heart rate, QTc, and blood pressure

Study design

Part A will be a double-blind, randomised, placebo-controlled study with single ascending dose levels of inhaled AZD3199, to investigate the tolerability, safety and PK in healthy men aged 18-45 years.

Part B will be a single-blind, non-randomised, placebo-controlled, crossover study with single ascending dose levels of inhaled AZD3199 to investigate the efficacy, tolerability, safety and PK in men with asthma aged 18-50 years.

Investigational product, dosage and mode of administration

AZD3199 (as AZD3199 dihydrobromide) is supplied as powder for solution for nebulisation. The drug substance (30 to 50 mg, free base equivalent) is supplied in 10 mL vials. The powder is constituted with commercially available Sodium Chloride Solution for Injection, 9 mg/mL (Ph Eur) to achieve a stock solution of up to 5 mg/mL AZD3199.
Sodium Chloride Solution for Injection, 9 mg/mL (Ph Eur) is used as a placebo.

Single ascending doses will be administered as a solution for nebulisation for inhalation via Spira nebuliser.

Planned doses for Part A:

Table S1 Planned lung deposited doses

Dose level	1	2	3	4	5	6	7	8
Lung deposited dose (µg)	2	10	40	160	640	1500	3000	4500

Doses are expressed as AZD3199 free base

These doses may be adjusted during the course of the study, after evaluation of Safety Review Committee, depending on safety, pharmacodynamic and pharmacokinetic results obtained. Pre-study calculations of inhaled aerosol disposition will be considered when deciding the actual dose delivered to healthy subjects.

Planned doses for Part B: Part B will comprise a maximum of 4 dose levels and will employ 2 cohorts of 8 patients. These cohorts will be administered one placebo dose, one low and one high dose each. The starting dose will not exceed half the dose showing systemic pharmacological activity in the healthy subjects. If no systemic pharmacological effects are seen after Dose 5 in Part A, Part B in patients will be initiated with a starting dose of at most half of Dose 5.

Duration of treatment

Part A: A single dose will be administered to each subject.

Part B: One placebo dose and 2 single doses (a lower and a higher dose) of AZD3199 will be administered to each patient with a wash-out of at least 1 week in between the active doses.

Variables

- Pharmacokinetic

Standard pharmacokinetic parameters calculated from AZD3199 concentrations in plasma and urine (urine, Part A only).

- Pharmacodynamic

FEV_1 (Part B only), potassium and lactate concentrations, tremor and palpitations, heart rate, QTcB (Part B only), and blood pressure

- Safety

Incidence and nature of AEs, effects on ECG parameters, blood pressure (BP), pulse, lung function, body temperature (Part A only) and laboratory variables (clinical chemistry, haematology and urinalysis)

- Statistical methods

Safety/tolerability aspects of the study will be evaluated mainly by using descriptive statistics. The evaluation of pharmacodynamic data will be focused on signals of response versus placebo in Part A and on dose-response patterns of the drug in Part B. Pairwise comparisons between active drug and placebo, and between different doses of active drug will be made using analysis of variance models. For dose-response, if appropriate, an E_{max} model will be fitted to data using a mixed effects model.

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

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

Abbreviation or special term	Explanation
AE	Adverse event
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
Assessment	An observation made on a variable involving a subjective judgement
AST	Aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical classification system
AUC	Area under the plasma concentration - time curve, from time zero to infinity
BMI	Body Mass Index
CEFTS	Central ECG Files Transfer and Storage; the AZ central dECG repository
CPU	Clinical Pharmacology Unit
CRF	Case Report Form
dECG	digital ECG
DQF	Data query form
ECG	Electrocardiogram
EClysis©	User-interactive computer-based system for analysis of digital ECGs and measurement of ECG intervals and wave amplitudes.
eCRF	electronic Case Report Form
EDC	Electronic data capture
EMEA	European Medicines Agency
ERS	European Respiratory Society
FEV_1	Forced expiratory volume during 1 second
GAME	Global Arrhythmia Monitoring and ECG; system for secure and compliant dECG data transfer from AZ CPUs to the central server.
GCP	Good Clinical Practice

Abbreviation or special term	Explanation
HPLC/MS/MS	High Performance Liquid Chromatography/Tandem Mass Spectrometry
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IPS	Investigational Products
IRB	Institutional Review Board
LABA	Long-acting β_2-agonist
LEFTS	Local ECG Files Transfer and Storage; the AZ CPU local dECG repository
MABEL	Minimal Anticipated Biological Effect level
OAE	Other Significant Adverse Event (ie, adverse events of particular clinical importance, other than SAEs and those AEs leading to discontinuation of the subject from study treatment; see definition in Section 4.6.1.1).
pCRF	paper Case Report Form
PGx	Pharmacogenetics
PK	Pharmacokinetics
PR (PQ)	ECG interval measured from the beginning of the P wave to the beginning of the Q wave or beginning of 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.
Principal investigator	A person responsible for the conduct of a clinical study at a study site. Every study centre has a principal investigator.
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 activation and recovery
QTcB	QT interval corrected for heart rate using Bazett formula.
QTcF	QT interval corrected for heart rate using Fredericia formula.

Abbreviation or special term	Explanation
QTcX	QT interval corrected for heart rate using a specific individualised factor.
QT_{tang}	QT interval measured by EClysis from the beginning of the Q wave or the R wave if Q is missing, to the intercept between the isoelectric line and the regression line, derived on the T-wave downstroke from values between 80 and 20 % of the T-top amplitude.
RR interval	The time between corresponding points on 2 consecutive R waves on ECG; the interval from one ventricular depolarisation to the next. In EClysis, the variable is named RR before.
SAE	Serious adverse event
SRC	Safety Review Committee
TELC	Treatment emergent laboratory changes
ULN	Upper limit of normal reference interval
WHO	World Health Organisation

This study consists of 2 parts; Part A in healthy volunteers, Sections 1-9 and Part B in patients with asthma, Sections 10-18.

1. PART A - INTRODUCTION

1.1 Background

Pharmacologically mediated bronchodilatation is an important element in the management of asthma and COPD. The effect is primarily obtained with β_2-agonists, normally administered by inhalation, the reason being more rapidly attained onset of effect and improved therapeutic index. The second aspect, signifying selectively improved potency for the desired local pulmonary effect, implies that a higher dose of a β_2-agonist can be administered to the site of action by inhalation than by oral administration, which in principle means that the duration of bronchodilatation can be maximized by inhalation (Löfdahl et al 1989). A prerequisite for this approach to sustain the effect, however, is that the β_2-agonist has a high affinity for lung tissues (or is slowly released and absorbed from the airway lumen), so that the drug somehow is retained in the lung after inhalation. Otherwise, the effect will rapidly disappear as the concentration difference between airways and the systemic circulation decreases with time towards the ratio obtained after oral therapy. Formoterol (OXIS® [1] and FORADIL®, Novartis) and salmeterol (SEREVENT™, GlaxoSmithKline) are lipophilic drugs with high affinity for airway tissues. Maintained high lung concentrations with positive gradients vis-à-vis the systemic circulation of these drugs are important for fairly selectively prolonged bronchodilatation after inhalation. The bronchodilatating effect of inhaled salmeterol and formoterol lasts for at least 12 h (Palmqvist M et al 1997), and is prolonged with the concomitant administration of fluticasone and budesonide, respectively (Masoli et al 2005, Masoli et al 2006). Indacaterol, a new β_2-agonist under development for the treatment of asthma and COPD, has extended the concept and provides a duration of action in man of at least 24 h after a single dose of 200 to 400 µg by inhalation (Beeh et al 2007).

The AZD3199 project objective is to develop a well tolerated inhaled once daily, long-acting β_2-agonist (LABA) with fast onset of bronchodilatation, a systemic side effect profile similar to or better than that of inhaled formoterol at comparable peak bronchodilatating effects, and effect maintenance in parity with that of for example Indacaterol, ie, at least 24 hour duration of action. AZD3199 is intended to be developed in combination with another drug, eg, a long-acting muscarinic antagonist at COPD or an anti-inflammatory drug at asthma, either administered once daily or by flexible dosing. Treatment with AZD3199 could then significantly improve the pharmacological treatment of obstructive pulmonary diseases.

[1] OXIS® is a trademark of the AstraZeneca group of companies.

1.2 Rationale

This is the first study in man with AZD3199. Relevant pre-clinical studies, eg, pharmacology, toxicology, safety pharmacology and pharmacokinetic studies have been performed and are summarized in the Investigator's Brochure (IB).

Part A of the study is designed to assess the safety and tolerability of AZD3199 in healthy men after single doses of solution inhaled via nebuliser and to obtain the first information regarding pharmacokinetics. Part A will serve as a basis for dosing of Part B of the study in patients with asthma.

2. PART A - STUDY OBJECTIVES

2.1 Primary objective

The primary objective of Part A of the study is to investigate the safety and tolerability of single ascending doses of AZD3199 delivered as a solution for nebulisation by assessment of:

- incidence and nature of adverse events (AEs)
- clinically significant abnormalities in ECG parameters, blood pressure (BP), pulse, lung function, body temperature and laboratory variables (clinical chemistry, haematology and urinalysis)

2.2 Secondary objective(s)

The secondary objectives of Part A of the study are:

1. to investigate the pharmacokinetics (PK) of single ascending doses of AZD3199 by assessment of drug concentration in plasma and urine and calculated PK parameters, with particular attention to dose linearity
2. to investigate extrapulmonary β_2- adrenoreceptor mediated effects of single ascending doses of AZD3199 by assessment of potassium and lactate concentrations, tremor and palpitations, heart rate, QTc, and blood pressure
3. to obtain material for possible exploratory analysis of metabolites of AZD3199 in urine and plasma (exploratory objective)

An optional objective in this study is to collect pharmacogenetic samples for possible retrospective exploratory analysis, investigating the influence of genotype on pharmacokinetics (and pharmacodynamic response where appropriate). This genetic part is described in detail in Appendix D.

3. PART A - 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.

Part A will be a double-blind, randomised, placebo-controlled study with single ascending dose levels of inhaled AZD3199 to investigate the tolerability, safety and PK in healthy men aged 18-45 years. This part of the study will be carried out at 1 or more centres.

Part A will comprise a maximum of 8 dose levels and each dose escalation will employ a new cohort of subjects. At each dose level, 8 subjects will be dosed of which 6 will receive AZD3199 and 2 will receive placebo. In total 64 subjects will be randomised.

Dose escalation will only proceed after a safety review of data generated at the previous dose level(s). The first lung deposited dose to man of AZD3199 will be 2µg (1/10 of the minimum dose anticipated to be clinically relevant). At the end of each dose level, an interim data assessment will be made by a Safety Review Committee (SRC) to determine the next dose. Adjustment of doses are guided by criteria for dose escalation pace, see Section 3.4.2.1 and stopping criteria, see Section 3.1.1. Dose escalation may continue until the defined maximal exposure (AUC or C_{max}) of AZD3199 is reached, or predicted to be reached, unless the maximum tolerated dose has been reached before. The SRC will decide which dose to give next, see Section 6.6.

The subjects will be resident at the clinic from the evening before dosing until 48 hours (h) following each dose. The length of the residence period at the clinic, assessments and the follow up period may be adjusted if justified. In addition, the two last dose groups will be resident at the clinic for 36 h before dosing, to allow for a dummy day with ECG measurements.

Subjects will be fasting from 22:00 the evening before dosing until 4 hours post dose. Standardised lunch and dinner avoiding potassium rich food will be served at 4 and 8-10 hours after drug administration.

Part A of this study comprises 4 visits (see Figure1 and Table 1); 2 screening visits, a treatment visit and a follow-up visit.

Visits 1 and 2 are screening visits. At Visit 1 informed consent will be obtained before any study related procedures are undertaken. Visits 1 and 2 will be performed within 30 days prior to randomisation and intake of study drug.

Visit 3 is the treatment visit when the healthy volunteers will receive a single dose of AZD3199 or placebo inhaled via nebuliser. Study measurements will be performed pre-dose and repeatedly after administration of study drug up to 48 hours as scheduled in Table 2. However, the number of samples and time points for measurements may be adjusted if justified from the emerging PK and safety profile of AZD3199.

Visit 4, the follow-up visit, will be performed within 10 ± 3 days after administration of study drug. The length of the follow up period may be adjusted if felt necessary.

Figure 1 Part A - Study design

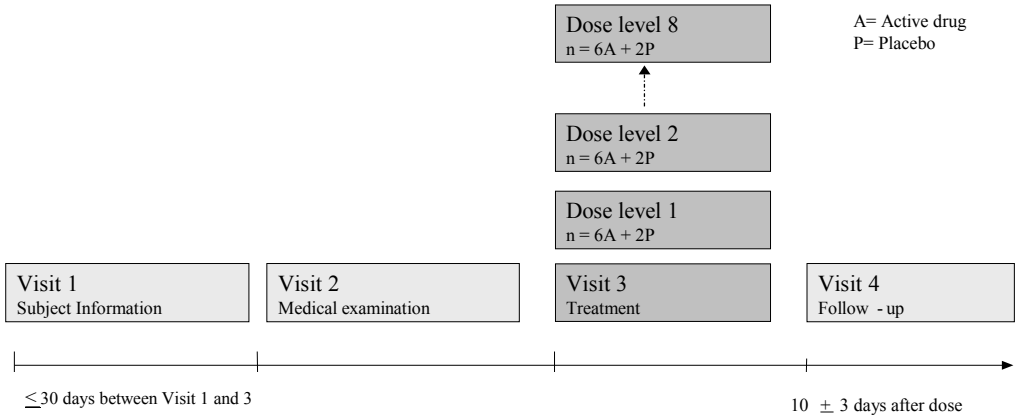

Table 1 Part A - Study plan

Event	Visit 1	Visit 2	Visit 3	Visit 4
	Screening 1	**Screening 2**	**Treatment**	**Follow-up**
Informed consent/Allocation of enrolment code	X			
Demographics	X			
Height and weight	X			
Medical/surgical history		X		

(Continued)

Table 1 Part A - Study plan

Event	Visit 1	Visit 2	Visit 3	Visit 4
	Screening 1	**Screening 2**	**Treatment**	**Follow-up**
Inclusion/exclusion criteria	X	X	X	
Physical examination		X		X
Spirometry		X	X	X
Concomitant medication		X	X	X
Laboratory safety assessments	X		X	X
HIV and hepatitis B and C tests	X			
Drugs of abuse test	X		X	
Alcohol breath test		X	X	
Blood pressure, pulse		X	X	X
12-lead ECG		X		X
12-lead dECG			X	
Telemetry monitoring			X	
Inhalation training		X	X	
Allocation of randomisation code			X	
Blood sampling for PGx			X[a]	
Administration of study drug			X	
Body temperature			X	
Blood sampling for PK and metabolite identification/characterisation			X	
Urine sampling for PK and metabolite identification/characterisation			X	
Blood sampling for potassium and lactate concentrations			X	
Assessment of tremor and palpitations			X	
Adverse Events questioning			X	X

a This sample can also be taken at any other Visit after Visit 3.

During Visit 3, assessments will be made according to the following time plan:

Table 2 Part A - Study Time Schedule (Visit 3)

			Time (minutes)								Time (hours)									
Assessment	**Day -1**	**Pre**	**0**	**5**	**10**	**20**	**30**	**40**	**60**	**90**	**2**	**3**	**4**	**6**	**8**	**10**	**12**	**24**	**36**	**48**
Drug Administration			x																	
12-lead dECG[ac]		x					x		x	x	x	x	x	x			x	x		
Telemetry ECG monitoring		→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→
Blood pressure and Pulse		x			x		x	x	x	x	x	x	x	x	x		x	x		x
Body Temperature	x	x															x	x	x	
Laboratory safety assessments		x															x	x		x
Spirometry for safety assessment		x			x				x					x						
Assessment of tremor and palpitations		x			x				x		x		x							
Blood sampling for lactate		x			x				x		x		x							
Blood sampling for potassium (K^+)	x	x			x	x		x	x	x	x	x	x	x	x		x	x		
Blood sampling - AZD3199		x		x[b]	x	x		x	x		x		x	x	x		x	x	x	x
Urine sampling		x	Urine collected 0-6, 6-12, 12-24, and 24-48 hours after dose																	
Adverse Events questioning	x	x															x	x		x

a ECG recorded during 10 min predose (- 0.5h), and during 5 min at all other timepoints

b The first blood sample to be taken as soon as possible after the end of inhalation

c Dose groups 7 and 8 will start with a ECG dummy day at Visit 3 before dosing is started

3.1.1 Stopping criteria for dose escalation

A Safety Review Committee (see Section 6.6) will review safety and tolerability results after each dose before proceeding to the next dose.

Any safety concerns will be evaluated using the following guidance, which is based on preclinical studies with AZD3199 and knowledge from clinical experience with LABA:s:

Stopping guidance for dose escalation

- A difference of 30% in the incidence of specific criteria (as described below) between placebo and AZD3199 groups. For example, if 0 of 2 subjects on placebo and 1 of 6 subjects on active treatment are affected dose escalation would likely continue. If 0 of 2 subjects on placebo and 2 of 6 subjects on active treatment are affected stopping dose escalation would be considered.

Cardiovascular system:

- A QTcF > 500 ms (QT interval corrected for heart rate by Fredericia method) or a QTcF prolongation > 60 ms on average – compared to baseline - sustained for more than 30 minutes, or on 2 contiguous time points.
- A persistent (>60 min) heart rate >120 beats/min related to the study drug according to the Investigator
- A clinically significant supraventricular or ventricular tachyarrhythmia of any kind, except isolated ventricular extrasystoles according to the Investigator
- A persistent (>30 min) diastolic blood pressure <45 mm Hg related to the study drug according to the Investigator

Potassium concentrations:

- A persistent (>30 min) plasma potassium concentration <2.8 mmol/L

Liver:

- Increase in AST or ALT≥ 3 x upper limit of normal reference interval (ULN) or bilirubin >2 x ULN or an increase in bilirubin ≥1.5 x ULN and AST or ALT> 2 x ULN.

- Any other clinically relevant changes and significant changes in the safety parameters (e.g. ECG parameters, blood pressure, pulse, spirometry, body temperature, laboratory assessments and AEs) making the continuation of dosing unjustified.
- Dose escalation will be stopped if the mean total plasma AUC or C_{max} of AZD3199 is higher than 111 nmol/L*h or 63 nmol/L, respectively. (Section 3.2.1)

3.2 Rationale and risk/benefit assessment

3.2.1 Rationale for study design, doses and control groups

This is the first clinical study with AZD3199. Part A of this study is designed to assess the safety, tolerability and pharmacokinetic (PK) profile of inhaled AZD3199 when administered to healthy male volunteers as a single dose. Part A will help the selection of appropriate doses of AZD3199 for Part B.

A double-blind design regarding treatment (active or placebo) within each dose group was selected in order to avoid bias from the subjects' knowledge of treatment. The placebo control will provide a reference to aid interpretation of safety and tolerability data. One single dose will be administered to each subject to avoid repeated exposure of the study drug to one individual. Food/water intake will be standardised/restricted to keep the conditions similar between subjects and to minimize variability on the ECG monitoring and potassium concentrations levels.

Subjects in *the two last* dose groups will be resident at the clinic an extra day, to perform an ECG dummy day before dosing starts. This will be done to obtain individual correction factors to be used in the calculations of QTc for each subject after administration of AZD3199.

Healthy non-nicotine using men aged 18-45 years will be included in order to minimize the effects of concomitant disease states or medications on study measurements. The study is restricted to male subjects, since sufficient reproduction toxicology data in female animals is not yet available. The inclusion and exclusion criteria for Part A are chosen in order to select subjects who are known to be free from any significant illness.

The beta adrenergic receptor is a well known target for pharmacological intervention and inhaled LABAs have been in clinical use for more than 10 years, thus it would not be categorized as high risk as defined in the EMEA guideline for First Time in Man studies. The starting dose of AZD3199 will thus consider although not be strictly conditioned by the Minimal Anticipated Biological Effect level (MABEL) concept in this study. When deciding the starting dose in this study the FDA guidance "Estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers 2005" and the predicted pharmacologically active human dose have been taken into consideration. This is further described in the Investigator´s Brochure (IB).

A first lung deposited dose of 1/10 of the dose anticipated to be minimally clinically relevant, is aimed for in the first time into man study giving a rounded off value of 2 µg/60 kg. The maximum lung deposited dose will be <200 µg/kg, ie, <12000 µg/60 kg (limits set by NOAEL in the rat, and gradually increasing systemically mediated class effects in the dog above NOAEL) or the dose giving a mean C_{max} of >63.0 nmol/L or a mean AUC of

>111 nmol/L*h (total mean plasma exposures at NOAEL in the rat). The incremental dosing will consider observed local and systemic effects and the expected clinically relevant level. Markers of systemic pharmacological activity in the AZD3199 SAD study are a decrease in plasma potassium, tremor and an increase in heart rate. If these are noted the dose escalation pace will be slowed down (Section 3.4.2.1).

Adrenaline is the non-selective endogenous prototype for synthetic non-selective β- or selective β_2- adrenoreceptor stimulants. Tremor is likely to be mediated via stimulation of the β_2-adrenoceptor on the plasma membrane of skeletal muscles. Positive chronotropic and inotropic effect on the heart is primarily mediated via stimulation of the β_1-adrenoceptor, but selective β_2-agonists still have such cardiovascular effects via the less frequently abundant β_2-adrenoreceptor. Furthermore, stimulation of β_2-adrenoceptors can increase the cellular uptake of potassium, induce peripheral vasodilatation, and have a calorigenic effect. Thus, systemically mediated dose-dependent effects of inhaled AZD3199 could be increased incidences of reported tremor or tachycardia/palpitations, increased systolic blood pressure, decreased extracellular concentration of potassium, decreased diastolic blood pressure, or increased concentration of glucose and lactate (the latter because the supply of oxygen to muscles and capacity for glycogenesis are limited). These well known potential side-effects upon β-adrenoreceptor stimulation will be monitored.

The number of samples and time points for measurements may be adjusted if justified from the emerging PK and safety profile of AZD3199.

3.2.2 Risk/benefit and ethical assessment

The healthy volunteers will have no individual benefits. Potential risks have been identified through preclinical animal studies with AZD3199 and by reviewing literature. By incorporating relevant exposure margins to animal toxicology findings and by using close monitoring in a controlled setting, the risks to the subjects have been minimized as much as possible.

There are several inhaled LABAs in clinical use and under development for the treatment of asthma and COPD. The most cited adverse effects are palpitation, headache and tremor. At higher doses tachycardia, hyperglycemia, hypokalemia and an increased QTc interval may be seen.

The most important adverse effects observed in the non-clinical studies with AZD3199 with potential relevance for humans are: signs of irritation in the nose and larynx in the rat and heart rate increase, QTc prolongation and decrease in plasma potassium in the dog. These effects can easily be monitored in the clinical study and are not considered to pose any safety concerns.

For an overall risk benefit assessment see the IB.

3.3 Selection of study population

3.3.1 Study selection record

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

Healthy volunteers will be recruited from the Volunteer Panel at CPU, AstraZeneca R&D, Sweden.

3.3.2 Inclusion criteria

For inclusion in Part A of the study subjects must fulfil all of the following criteria:

1. Provision of informed consent prior to any study specific procedures

2. Healthy men aged 18 to 45 years inclusive

3. Body mass index (BMI) between 19 and 30 kg/m^2 and a body weight between 60 and 100 kg.

4. Be a non-smoker or ex-smoker who has stopped smoking (or using other nicotine products) for >6 months prior to study start.

5. Be able to inhale from the Spira nebuliser according to given instructions

3.3.3 Exclusion criteria

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

1. Any clinically significant disease or disorder (e.g. cardiovascular, pulmonary, gastrointestinal, liver, renal, neurological, musculoskeletal, endocrine, metabolic, malignant, psychiatric, major physical impairment) which, in the opinion of the investigator, may either put the subject at risk because of participation in the study, or influence the result of the study, or the subject´s ability to participate in the study.

2. Any clinically relevant abnormal findings in physical examination, clinical chemistry, haematology, urinalysis, vital signs, ECG or lung function at baseline, which, in the opinion of the investigator, may put the subject at risk because of his participation in the study.

3. QTcF > 450 ms or QT > 500 ms or other abnormality making interpretation more difficult, as judged by the investigator.

4. Use of any medication, herbal preparations, vitamins or nutritional supplements within 2 weeks prior to Visit 3, except for occasional intake of paracetamol.

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

6. Donation of blood within 3 months or donation of plasma within 14 days prior to Visit 1

7. History of or current alcohol or drug abuse, as judged by the investigator

8. A suspected/manifested infection according to WHO risk classification 2 , 3 or 4 (See Appendix C).

9. Positive results on screening tests for hepatitis B and/or C and/or HIV.

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

11. Participation in another investigational drug study within 3 months before Visit 3, or participation in a method development study (no drug) 1 month prior to Visit 3 (Note: participation is identified as the completion of a treatment-related visit)

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

13. Subjects who, in the opinion of the investigator should not, participate in the study.

3.3.4 Restrictions

Subjects will be required to:

1. abstain from alcohol for 72 hours prior to and during Visits 3

2. abstain from strenuous physical activity that is not within the subject's normal weekly routine 5 days before and during Visits 3-4

3. abstain from drugs of abuse during the entire study. In addition, poppy seeds (eg, on bread rolls) can give a positive signal for opiates and should not be ingested during the study

4. abstain from food and drink (except water) for at least 3 hours prior to safety laboratory measurement at Visit 4

5. abstain from liquorice containing products 24 hours prior to and during Visit 3

6. abstain from caffeine containing beverages and food, eg, coffee, tea, chocolate and soft drinks (eg, Red Bull) for 10 hours before drug administration. Intake of caffeine will be restricted to the equivalent of 1 cup of coffee (approx. 200 mL) at lunch and a maximum of 3 cups in total per 24 hours (Visit 3)

7. follow the additional dietary restrictions described in Section 3.3.4.1

Subjects will be recommended to:

1. abstain from donation of blood or plasma during the study and 3 months after last AZD3199 dose

2. avoid sperm donation or having procreative sex until 3 months after administration of AZD3199

3.3.4.1 Dietary restrictions during study sessions

The following applies together with the requirements specified in Section 3.3.4

Subjects will be served standardised meals during Visit 3 according to normal procedures at the study site, and no other foods will be permitted. Water will be allowed except during ECG recordings. The subjects will be fasting from 22:00 Day -1 until 4 hours post dose. Standardised lunch and dinner avoiding potassium rich food will be served at 4 and 8-10 hours and a snack will be served at 12-14 hours post dosing. Meals are served after all required study measurements have been collected at the relevant time point.

3.3.5 Discontinuation of subjects from treatment or assessment

3.3.5.1 Criteria for discontinuation

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

- Voluntary discontinuation by the subject, who is at any time free to discontinue his 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 inclusion ie, the subject does not meet the required inclusion/exclusion criteria for the study.
- Subject lost to follow-up

- A suspected/manifested infection according to WHO risk classification 2 , 3 or 4 (See Appendix C).

3.3.5.2 Procedures for discontinuation

Subjects who discontinue should always be asked about the reason(s) for their discontinuation and the presence of any adverse events. If possible, they should be seen and assessed by an investigator. Adverse events should be followed up.

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

3.4 Treatment(s)

3.4.1 Investigational product(s)

3.4.1.1 Identity of investigational product and comparator

The drug product is supplied as AZD3199 powder for solution for nebulisation. The drug is the dihydrobromide salt of AZD3199.

AZD3199 drug substance (30 to 50 mg, free base equivalent) is supplied in 10 mL clear glass injection vial with grey rubber stopper for injection and tear-off aluminium cap. The powder is constituted with commercially available Sodium Chloride Solution for Injection, 9 mg/mL (Ph Eur) (NATRIUMKLORID FRESENIUS KABI®, 9 mg/mL Solution for Injection, Fresenius Kabi, Sweden, or equivalent) purchased locally to achieve a stock solution of up to 5 mg/mL AZD3199.

Sodium Chloride Solution for Injection, 9 mg/mL (Ph Eur) is used as a placebo.

Table 3 Part A - Identity of investigational product

Investigational product	Dosage form and strength	Manufacturer
AZD3199 dihydrobromide	Powder for solution for nebulisation, 30 to 50 mg, free base equivalent	AstraZeneca
Placebo	Sodium Chloride solution for injection, 9 mg/mL	Fresenius Kabi

3.4.1.2 Labelling

The packaging and labelling of bulk drugs will be performed by Investigational Products (IPS), AstraZeneca R&D. Labelling will be in accordance with GMP and local regulatory guidelines. The label will fulfil GMP Annex 13 requirement for labelling.

The investigational product will be supplied as bulk supply to the hospital pharmacy. Each vial containing drug substance will be labelled with a tear-off label. The vials will be packed in boxes.

Individual dosing containers with dispensed solution for nebulisation will be labelled with study specific information. The label will have a tear-off label to be inserted in a separate administration record. Individual dosing containers must be labelled AZD3199/placebo to keep the study blind.

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 bottle label.

3.4.1.4 Accountability

The medication provided for this study is for use only as directed in the clinical study protocol. At completion of the study unused investigational products will be returned to Investigational Products (IPS), AstraZeneca R&D. The Pharmacy personnel will account for all drugs dispensed and returned. Certificates of delivery, destruction and return must be signed.

3.4.2 Doses and treatment regimens

Table 4 Planned lung deposited doses

Dose level	1	2	3	4	5	6	7	8
Lung deposited dose (µg)	2	10	40	160	640	1500	3000	4500

Doses are expressed as AZD3199 free base

Single doses of AZD3199 and placebo will be administered as a solution for nebulisation for inhalation. Table 4 shows the planned lung deposited doses. These doses may be changed during the course of the study, see Section 3.4.2.1. Pre-study calculations of inhaled aerosol disposition will be considered when deciding the actual dose delivered to healthy subjects.

3.4.2.1 Adjustment of dose levels

The incremental dosing will consider observed local and systemic effects and the expected clinically relevant level. The planned dose levels may be changed, postponed or repeated during the course of the study depending on safety, pharmacodynamic and pharmacokinetic results obtained. The SRC will make a safety evaluation between each dose level and change the dose escalation, if necessary, see Section 6.6. The SRC makes all decisions regarding modification of dose levels.

If, on a certain dose level a systemic pharmacological effect of AZD3199 is seen, ie,

- a mean decrease of potassium C_{min} of more than 0.25 mmol/L compared with placebo related to the study drug

or

- a mean increase of maximum resting pulse of more than 15 beats/min compared with placebo related to the study drug

the suggested dose escalation factor for the future doses will be re-evaluated and lowered to a maximum of 3.

After each Safety Committee evaluation personnel dispensing investigational product at the hospital pharmacy and the personnel at IPS, AstraZeneca R&D will be informed about the decision for the next dose escalation and IPS will issue a corresponding dosing schedule.

3.4.2.2 Preparation of solution for nebulisation

Dose preparation will take place at the hospital pharmacy according to separate handling instructions and dose confirmation. The powder for solution will be constituted with Sodium chloride for Injection to a stock solution of 3 to 5 mg/mL. For doses requiring a lower concentration the stock solution can be diluted with sodium chloride for injection. The doses, both active drug and placebo, will be dispensed in individual dosing containers according to the randomisation scheme. Before drug administration personnel at the clinic will transfer the solution from the individual dosing container to the nebuliser cup.

3.4.2.3 Administration of dose

The subjects will practice inhalation technique on Visit 2 and also on Visit 3, before the dose will be inhaled.

Administration of dose at the clinic including inhalation procedure will be performed according to detailed separate instructions.

In order to avoid contamination particularly of plasma samples intended for drug analysis the following precautions will be taken: Dosing will take place in a separate inhalation room. Furthermore, subjects and personnel involved in the study drug administration must wear protective gloves and clothing. Personnel involved with drug administration should not be involved with blood/urine sampling.

The study drug will be administered in the morning. The subject will inhale the drug/placebo solution via Spira Electro 2 dosimeter in a sitting upright position. The number of inhalations from the dosimeter required to administer the planned dose will be written on the label of the individual dosing containers. After inhalation the subjects will take off the protective gloves and clothing and wash the outside and around their mouth and their hands before leaving the inhalation room. As soon as possible after this a PK sample will be drawn.

After inhalation, aerosol from the dosimeter may be withdrawn to a collection filter for subsequent analysis and calculation of the actual output from the dosimeter.

3.4.3 Method of assigning subjects to treatment groups

Informed consent will be obtained before enrolment and the subjects in Part A will be identified with an enrolment number starting with E0001001. Subjects fulfilling the eligibility criteria will be assigned randomisation codes (subject numbers) starting with number 101 for dose group 1, number 201 for dose group 2 etc.

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

3.4.4 Blinding and procedures for unblinding the study

3.4.4.1 Methods for ensuring blinding

This part of the study is double-blind with regard to treatment (AZD3199/placebo) at each dose level, not to dose escalation *per se*.

At each dose level, both the active and the placebo dose will be of the same volume and the same number of inhalations from the solution for nebulisation will be performed to ensure the blinding.

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 hospital pharmacy

- the personnel analysing the PK samples

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

3.4.4.2 Methods for unblinding the study

Individual treatment codes, indicating the treatment randomisation for each randomised subject, will be available to the investigator(s) and to the chairman of the SRC.

At the study site, the individual treatment codes must not be broken except in medical emergencies when the appropriate management of the subject necessitates knowledge of the treatment randomisation. The investigator(s) must document and report to AstraZeneca any breaking of the treatment code. AstraZeneca retains the right to break the code for Serious Adverse Events (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 final analyses of data until all decisions on the evaluability of the data from each individual subject have been made and documented. However, the SRC will have access to unblinded data at the SRC meeting in between each dose cohort.

3.4.5 Concomitant medication

In Part A of the study no concomitant prescribed or non-prescription medication (including vitamins, herbal remedies eg, St John's wort and mineral supplements) will be allowed from 2 weeks prior to Visit 3 and during the study except for occasional use of paracetamol. The subjects must be instructed that no additional medication will be allowed without the prior consent of the investigator.

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

3.4.6 Treatment compliance

Compliance will be assured by supervised administration of the investigational product by the investigator or his delegate. Details of dosing (time, date) will be captured electronically. Tear-off labels will be inserted in separate administration records.

4. PART A - MEASUREMENT OF STUDY VARIABLES

The following study measurements will be obtained. The timing of these measurements is detailed in Table 1 and in Table 2. The following 'priority order' will be in effect when more than one assessment is required at a particular time point occurs:

1. ECG recordings
2. Blood pressure, pulse and body temperature
3. Blood sampling
   (a) Blood sampling for potassium and lactate concentrations
   (b) Blood sampling for safety laboratory assessments
   (c) Blood sampling for PK (except blood samples directly after inhalation which may be taken as soon as possible, before any other assessments)
4. Assessment of palpitations and tremor
5. Spirometry
6. Urine sampling for PK

4.1 Medical examination and demographic measurements

4.1.1 Enrolment medical examination and demographic measurements

Each subject will undergo an enrolment medical examination at Visit 1 and 2 within 30 days prior to the treatment visit. This will consist of:

- Recording of demographic data - date of birth, sex, height, weight, race
- A standard medical/surgical history and a physical examination including the cardiovascular and respiratory systems
- A resting 12-lead ECG
- A resting blood pressure and pulse
- Laboratory assessments (clinical chemistry, haematology and urinalysis)
- A blood sample for HIV, Hepatitis B and C test

- Drugs of abuse screen and alcohol breath test
- Review of inclusion and exclusion criteria
- Spirometry

4.1.2 Post-study medical examination

A follow-up medical examination will be performed at Visit 4 within 10 ± 3 days after the administration of study drug. This will consist of:

- A physical examination
- A resting 12-lead ECG
- A resting blood pressure and pulse
- Laboratory assessments (clinical chemistry, haematology and urinalysis)
- Spirometry
- Collection of adverse events

4.2 Pharmacokinetic measurements

4.2.1 Determination of drug concentration in biological samples

Samples for measurement of drug concentration will be analysed by a method using HPLC/MS/MS. Details of the methods used will be referred to in the clinical study report.

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 sampling for AZD3199 plasma concentration and metabolite(s)

Blood samples (6 mL) for determination of AZD3199 in plasma will be taken at the times presented in Table 2. The plasma derived from the 6 mL of blood will be divided into 2 aliquots, 1 for PK measurement and 1 for potential metabolite identification. PK analysis during the study may indicate that the sampling schedule is sub-optimal and therefore additional samples may be taken, samples may be omitted, and/or the times of sampling may be adjusted. Blood samples will be collected, labelled and shipped according to separate instructions from the laboratory. The date and time of collection will be recorded in the CRF.

Samples should be stored at -20°C or below and analysed within the time frame 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 disposal of samples will be decided within 1 month after the clinical study report has been finalised.

4.2.2.2 Urine sampling for AZD3199 and metabolite(s)

Pre-dose urine samples (blank urine) of 10 mL will be collected from each subject. Prior to dosing, the subjects will empty their bladders.

After dosing all urine will be collected over intervals of 0-6, 6-12, 12-24 and 24-48 hours in tared bottles for each subject. The urine bottles should be stored in a refrigerator when not used during the collection periods. Approximately 15 minutes before the end of each collection interval the subject will be asked to void their bladders. At the end of each collection period the urine pool will be thoroughly mixed and the total weight of urine will be measured. One 10 mL aliquot of the well-mixed urine pool from each collection interval will be transferred into polypropylene tubes and frozen immediately at or below -20°C until transport on dry ice to DvD&B, AstraZeneca R&D Charnwood, England.

Separate instructions on collection, labelling and shipment will be provided by DvD&B, AstraZeneca R&D Charnwood, England. The date, time and weight of collection will be recorded in the CRF.

The disposal of samples will be decided within 1 month after the clinical study report has been finalised.

4.3 Pharmacodynamic measurements

4.3.1 Assessment of tremor and palpitations

Tremor and palpitations will be assessed by a scoring question. The subject will be asked to estimate the tremor and palpitations by using a 4-grade scale; no, mild, moderate or severe. For time points of assessments see Table 2

4.3.2 Potassium concentrations

Blood will be drawn in order to determine potassium concentrations and sent to the local laboratory at the hospital for analysis. Blood must be sampled without stasis and handled gently according to separate instructions in order to avoid haemolysis. Samples will be collected at the times given in Table 2.

4.3.3 Lactate concentrations

Blood will be drawn in order to determine lactate concentrations and will be sent to the local laboratory at the hospital for analysis. Blood must be sampled without stasis and handled gently according to separate instructions in order to avoid haemolysis. Samples should be put on ice prior to being processed. Samples will be collected at the times given in Table 2.

4.3.4 Heart rate and QTc

QT and heart rate will be extracted from the 12-lead dECG measurements. For time points of assessments see Table 2.

4.3.5 Blood pressure and pulse

Supine blood pressure (BP) and pulse will be measured using an semiautomatic blood pressure recording device with an appropriate cuff size. The equipment and method of obtaining BP and pulse shall be consistent for all subjects in the study. BP and pulse will be measured after the subject has rested in bed for at least 10 minutes, at the times given in Table 2.

4.4 Safety measurements

4.4.1 Laboratory safety measurements

Blood and urine samples for determination of clinical chemistry, haematology and urinalysis parameters will be taken at the times given in Table 1 and Table 2. The date and time of collection will be recorded on the appropriate CRF (at Visit 1, 2 and 4 only date).

Subjects should rest at least 3 minutes in the supine position before blood sampling. Samples will be collected in tubes according to standard routines. See Section 4.5 for information on the volumes of blood to be taken.

The following laboratory variables will be measured:

Clinical chemistry (serum or plasma)	**Haematology (whole blood)**
S/P-Creatinine	B-Haemoglobin (Hb)
S/P-Bilirubin (total) (Bil, tot)	B-Platelet count
S/P-Alkaline phosphatase (ALP)	B-Leucocyte count (LPC)
S/P-Aspartate aminotransferase (AST)	B-Leucocyte differential count
S/P-Alanine aminotransferase (ALT)	
S/P-Albumin	
S/P-Potassium (K)	**Urinalysis**

S/P-Calcium (total) (Ca)	U-Protein/Albumin
S/P-Sodium (Na)	U-Hb/Erythrocytes/Blood
S/P-Glucose	U-Glucose
S/P Thyroxine free (T4)	
S/P Thyroid-stimulating hormone (TSH)	
S/P-C-Reactive Protein (CRP)	

All samples will be handled and analysed by a local hospital laboratory, except for the urinalysis (dipstick) that will be analysed at the study site according to local procedures.

4.4.1.1 HIV, Hepatitis B and C test

For safety of the study site staff, all subjects will be tested for antibodies to HIV, hepatitis B surface antigen and for antibodies to hepatitis C virus at Visit 1. If a subject is found positive to any of these tests, he will be referred for further examination and treatment and will not be included in the study. The samples will be handled and analysed at a local hospital laboratory.

Results will not be recorded in the CRF.

4.4.1.2 Drugs of abuse and alcohol testing

A drug screen (cannabis, methamphetamines, opiates, cocaine, methadone, benzodiazepines, barbiturates, tricyclic anti depressants, phencyclidine (PCP), and amphetamines) in the urine will be done at Visit 1 and at Visit 3 in order to exclude or withdraw subjects with drug abuse. The samples will be analysed at the clinic according to local routines.

A breath alcohol test will be performed at Visits 2 and 3.

If a subject tests positive in the drugs of abuse test, a confirming test will be sent to a local hospital laboratory. If the drug is illegal, counselling and advice will be offered and the subject will be removed from the AstraZeneca Volunteer Panel.

Results will not be recorded in the CRF.

4.4.2 Electrocardiographic measurements

4.4.2.1 Resting 12-lead ECG

Paper ECGs

At enrolment and follow-up visit (see Table 1), 12-lead ECG recordings will be obtained after 10 minutes supine rest. A print out at paper speed 50 mm/sec with an overall evaluation, normal / abnormal (specified) will be recorded in the CRF.

Digital ECGs

At Visit 3 dECG will be recorded at the time points indicated in Table 2. The subjects will have to be in bed (in a semirecumbent position, with the same maximum 30 degree hip flexion throughout the measurement) 10 minutes before each ECG registration. The procedure of recording continuous dECG is described in Section 5.7.4. Baseline will be the recording at predose.

Subjects in the two last dose groups will be resident at the clinic an extra day, to perform an ECG dummy day before dosing starts. During this day dECGs will be collected at the same time points as during the treatment day, see Table 2. There will be no other measurements and no study drug/placebo administration during this day. The dummy day will be done to obtain individual correction factors to be used in the calculations of QTc for each subject after administration of AZD3199.

4.4.2.2 Real-time ECG display (telemetry)

A real-time ECG will be displayed for at least 48 hours at Visit 3 starting within 1 hour before the intake of investigational product, according to local routines at the clinic, and will be monitored by the investigator or his/her delegate. The time might be adjusted once more information about the compound characteristics are available.

Date and time for start and end of measurement will be collected in the CRF. Clinically relevant findings should be documented as AEs in the CRF, at the discretion of the investigator or his/her delegate.

Short time periods off telemetry are permitted at the discretion of the investigator.

4.4.3 Vital signs

4.4.3.1 Blood pressure and pulse

For timing of individual measurements refer to Table 1 and Table 2.

Supine blood pressure and pulse will be measured after the subject has rested in bed for at least 10 minutes. Blood pressure and heart rate (HR) will be measured using a

semi-automatic blood pressure recording device with an appropriate cuff size. The equipment and method of obtaining BP and pulse shall be consistent for all subjects in the study. At Visit 3, all data will be captured electronically.

4.4.3.2 Body temperature

For timing of individual measurements refer to Table 2.

Body temperature will be measured according to routines at the clinic.

4.4.4 Other safety measurements

4.4.4.1 Lung function tests - Spirometry

For safety reasons, spirometry for determination of FEV_1 and FVC will be performed at enrolment and follow-up and at some timepoints during the treatment visit (see Table 1 and Table 2). Lung function testing will be performed according to European Respiratory Society (ERS) recommendations (Quanjer et al 1993). The highest FEV_1 and FVC out of 3 measurements will be recorded. The highest and the second highest values must not differ by more than 5%. If the difference is larger than 5% the subject may perform up to 8 measurements. If the variation is still above these limits, the largest value will be recorded with a comment.

The spirometer should be calibrated according to local practice.

4.4.4.2 Physical examination

The physical examination will include: general appearance, skin, mouth, teeth and throat, lymph nodes, thyroid, musculoskeletal/extremities, cardiovascular, lungs, abdomen and neurological. The examination will be carried out at enrollment and follow-up visit (see Table 1) according to the normal routines at the study site.

4.5 Volume of blood sampling

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

Table 5 Part A - Volume of blood to be drawn from each subject

Assessment		Sample volume (mL)	n of samples	Total volume (mL)
Safety	clinical chemistry	3	6	18
	Glucose	2	6	12
	haematology	2	6	12

(Continued)

Table 5 Part A - Volume of blood to be drawn from each subject

Assessment		Sample volume (mL)	n of samples	Total volume (mL)
Pharmacokinetics (AZD3199)[a]		6	14	84
Pharmacodynamics[a]	Potassium	3	14	42
	Lactate	2	5	10
Pharmacogenetics[b]		10	1	10
HIV/Hepatitis B and C		7	1	7
Total				195

a The number of samples may be modified during the course of the study, but the total blood volume taken in the study will not exceed 450 mL.

b The pharmacogenetics part is described in detail in Appendix D

4.6 Adverse Events

The methods for collecting adverse events are described below.

4.6.1 Adverse Events

4.6.1.1 Definitions

The definitions of AEs, 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 subject or may require medical intervention to prevent 1 of the outcomes listed above.

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

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

Other Significant Adverse Events (OAE)

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

4.6.1.2 Recording of adverse events

AEs including SAEs will be collected from time of signed informed consent until the end of study. However, the first AE questioning will be done at Visit 3.

Symptoms

AEs will be collected by means of 3 standard questions. The questions will be put to each subject in Swedish. The question “Have you had any health problems since the first visit?” will be put to each subject when they arrive at the clinic at Visit 3. The question “Have you

had any health problems since you were last asked?" will be put to each subject at set times after study drug administration at Visit 3 (see Table 2). Furthermore, the question "Have you had any health problems since the previous visit?" will be put to each subject at arrival to the clinic at Visit 4. The timing of these questions may be changed during the study, to reflect the safety and pharmacokinetic data produced during the study. The subject's response to these questions and spontaneously reported and/or observed AEs will be recorded on the AE form with information about seriousness, causality, action taken, date (and time, if starting during a clinic visit) of onset, date (and time, if ends at a visit) of recovery (or marked as ongoing), intensity and outcome.

Causality

The causality of all AEs (ie, the relationship to study treatment) will be assessed by the investigator(s), who in completing the relevant case report form must answer "yes" or "no" to the question "Do you consider that there is a reasonable possibility that the event may have been caused by the investigational product?" A guide to the interpretation of the causality question is found in Appendix B.

Intensity

The subjects will be asked to assess the intensity of the reported AEs according to the following scale:

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

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

Assessment of tremor and palpitations

In order to evaluate the expected tremor and palpitations after inhalation of β_2-agonists, questions will be put to the subjects at specified timepoints (see Table 2). The presence and, if applicable intensity, of the symptom will be recorded according to the following scale: No, Mild, Moderate or Severe.

Symptoms emerging from the above specified active questioning are not to be recorded as AEs on the AE form unless:

- the symptom is serious according to definitions, see Section 4.6.1.1

or

- the subject discontinues the study due to the symptom

Abnormal findings

During the study, abnormal laboratory results, pulse, blood pressure, FEV_1 and FVC , and ECG are not to be recorded as AEs in the CRF unless they result in discontinuation or represent SAEs, or the investigator judges them to be of such clinical importance as to merit recording as AEs.

The investigator will review results from physical examination. All new and/or aggravated findings (at follow-up) as compared with baseline measurement (at screening visit) must be identified and recorded on the AE form. These will be considered AEs.

Concomitant medication

All changes in the subject's medication, eg, dose change or addition of new medication, must be reported in the medication log. Reasons for changes in medication, which reflect an AE, must be recorded on the AE form.

Unresolved AEs

If an AE is unresolved when the study is terminated, its subsequent course must be followed until the AE subsides or until the investigator decides that no further follow-up is necessary. However, more information about such AEs may be requested.

Overdose

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.

Coding of AEs

AstraZeneca will code AEs by using the Medical Dictionary for Regulatory Activities (MedDRA).

MedDRA includes 5 levels: lowest level terms, preferred terms, high level terms, high level group terms, and system organ class terms. Lowest level terms are used on the input side

(data entry) to reflect as closely as possible the term used by the investigator or subject to describe the event. Preferred terms are mainly used on the output side (data presentation) to group terms that are synonymous or closely related. High level terms and high level group terms are also used on the output side. System organ class terms group AEs pertaining to the same body system.

4.6.1.3 Reporting of serious adverse events

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

The AstraZeneca representative will work with the investigator to compile all the necessary information and ensure that the appropriate AstraZeneca Drug Safety Department receives a report by Day 1 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 1 day as described above. For a non-serious AE that become serious but which is not fatal or life-threatening a report should be received within 5 days.

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

5. PART A - STUDY MANAGEMENT

5.1 Monitoring

5.1.1 Study monitoring

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

5.1.2 Data verification

It is a prerequisite of this study that the study monitor has direct access to source data for data verification. This will be done by comparing data from the CRFs with those in the subject’s medical notes (permission from the subject will be sought as part of the consent process).

Such verification is an essential element of quality control, as it allows the rectification of transcription errors and omissions.

Data for which the CRF will be regarded as source data will be described in a separate document.

Monitoring including source data verification should routinely be performed prior to the transfer of data to Data Management. However, data will not necessarily be monitored prior to Safety Review Committee meetings.

5.2 Audits and inspections

Authorised representatives of AstraZeneca, a regulatory authority, 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 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.

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 the Ethics Committee, and also the local regulatory authority, before implementation. Local requirements must be followed.

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

AstraZeneca will distribute amendments and new versions of the protocol to the principal investigator(s) 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 by Regulatory Affairs at AstraZeneca.

5.5 Study agreements

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

5.6 Study timetable and end of study

Part A of the study is expected to start in October 2007 and to be completed by April 2008

End of study is defined as database lock, which is the time point after which no subject will be exposed to study related activities.

5.7 Data management

Data entry, editing, clean file and analysis will be performed by AstraZeneca R&D Lund, Sweden.

Medical and surgical history and AEs will be coded using the standardized dictionary MedDRA. Medications will be coded using the AZ approved Drug Dictionary (AZDD) including Anatomical Therapeutic Chemical classification system (ATC) and route of administration will be coded using Committee for Medicinal Products for Human Use (CHMP) dictionary.

Clean file will be declared after a final quality check has been performed on a sample of the data. Before declaring Clean File, the possible influence of protocol deviations on the statistical analysis will be discussed.

Results from the PK assessments will be electronically transferred into the study database at the time of Clean File. After the database is locked, all data will be transferred to the statistician at AstraZeneca R&D Lund, Sweden, for statistical analysis. After locking the database, editing in the database will not be allowed without proper documentation.

Procedures for verification and validation of data will be described in a separate document.

5.7.1 Case report forms

Paper CRFs (pCRFs) can be used to record 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 AstraZeneca 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 onto AstraZeneca's clinical study database and validated. Procedures for verification and validation of data will be described in the Data Management Plan. Any missing, impossible or inconsistent recordings in the pCRFs will be referred back to the Investigator using a data query form (DQF), and be documented for each individual subject before clean file status is declared.

5.7.2 Electronic data capture (Immediate data entry)

Electronic CRFs (eCRF) can be used to record data not captured electronically at bedside.

The data entry screens used will be designed according to the AstraZeneca CRF Standard.

Data entry will be done by study site personnel. In cases where immediate data entry is not possible, data will first be recorded on a pCRF page, and thereafter entered into the database. The investigator will ensure that the recorded data are correct.

5.7.3 Electronic data capture at bedside

During the study days all data, except for medication, AEs, lung function, 12-lead ECG, laboratory results and tremor/palpitations scoring will be captured electronically at bedside.

The Investigator will ensure that the captured data are correct before transferred to the AstraZeneca Clinical Study Database for final checking. Any changes made during validation will be documented with a full audit trail within the Electronic Data Capture application.

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

5.7.4 Electronic capture and analysis of 12 lead continuous digital ECGs (Schiller CS-200 recorder)

At protocol indicated time points (see Table 2), 12-lead continuous digital ECGs will be recorded according to AstraZeneca Working Instructions (GAME WI 004, WI 005 and WI 006), using the "Continuous ECG function" of the Schiller Cardiovit CS-200 recorders (Schiller AG, Baar, Switzerland). The same recorder will be used for each subject during all study visits, if possible. Date and time settings must be checked at the start of each study day and aligned with an official timekeeper for all machines used in the study.

Skin preparation and electrode positions must be according to AstraZeneca´s Working Instruction WI 005. Electrode positions will be marked with an indelible pen at the start of the study days to ensure exact reposition. Permanent electrodes will be applied at least 30 minutes before first study recording and left in place for the duration of each relevant study day. Subjects must rest for at least 10 minutes before start of each recording.

In this study, beside the final analysis, the ECG Centre will deliver, at agreed upon dates, a safety evaluation of the digital ECG data to be submitted to the Safety Review Committee for evaluation. Upon completion of ECG recordings for each dose group, the digital files are transferred at specified time points from the CS 200 to the local server (LEFTS) at CPU Astra Zeneca R&D Lund. Accredited users can correct metadata on the LEFTS, using the GAME File Viewer and also monitors success of transfer. A printout from GAME File Viewer must be checked, signed and stored at the CPU for monitoring purposes. From LEFTS, the digital ECG files are transferred with checksum-protection to the central server (CEFTS). Transfer logs are kept and signed by the local GAME administrator.

From the CEFTS, the source ECGs will be imported into the EClysis©system. From the continuous ECG recording, 10-second ECGs are retrieved twice per minute and analysed by the dedicated ECG Scientific Advisor at the ECG Centre, who will perform a preliminary analysis in lead V2 with main focus on QT changes, wave morphology changes and dysrhythmia. The ECG Centre Cardiologist will perform the ECG evaluation and interpretation of findings and will provide a Safety report.

On study completion, the ECG Scientific Advisor will ensure that all protocol-defined ECGs have been imported into EClysis© databases and will then perform the final analysis with any required corrections of the ECG annotations provided by EClysis©. An external cardiologist will conduct the final review and adjustments before locking the EClysis© database into a read only state containing all results, with all actions logged into the audit trail and Event log in a user- and time- attributable manner. The results from the locked database will then be exported in secure, checksum-protected files, which are accessible on the CEFTS to the accredited Programmer for conversion into SAS files for statistical analysis.

In this study the following data from lead V2 will be analysed and reported as primary: RR, PQ (PR), QRS, QTtang. 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 visit.

6. PART A - PHARMACOKINETIC, PHARMACODYNAMIC, SAFETY AND STATISTICAL METHODOLOGY

6.1 Pharmacokinetic / pharmacodynamic evaluation

6.1.1 Calculation or derivation of pharmacokinetic variables

The pharmacokinetic analyses will be performed by the Department of Clinical Information Science, AstraZeneca R&D Lund, Sweden.

From the plasma concentrations of AZD3199 collected following a single dose, pharmacokinetic parameters will be calculated using standard non-parametrical methods. The pharmacokinetic parameters will include: the maximum plasma drug concentration (C_{max}), the time to maximum plasma drug concentration (t_{max}), the area under the (plasma drug concentration-time) curve from time zero to infinity (AUC), terminal half-life of drug in plasma ($t_{1/2}$), apparent plasma clearance (CL/F).

From the urine volumes collected and the concentrations of AZD3199 therein, the following additional parameters will be computed: the total amount unchanged drug excreted in urine (Ae), the fraction of administered dose excreted unchanged in urine, and the renal clearance (CL_R).

6.1.2 Calculation or derivation of pharmacodynamic variables

Pharmacodynamic variables in this study will include potassium and lactate concentrations, blood pressure, heart rate, tremor and palpitations. For each variable the maximum effect during the post-dose measurement interval (the minimum value, E_{min}, for potassium and diastolic blood pressure, and the maximum value, E_{max}, for other variables) and the average effect, E_{av} (AUC/length of interval), will be computed and used as variables of systemic and local effects.

6.2 Safety evaluation

All adverse events will be analysed at AstraZeneca R&D Lund, Sweden, by means of descriptive statistics and qualitative analysis. AEs will be listed for each subject and summarised by body system and preferred term assigned to event by using MedDRA. Adverse events will also by summarised by intensity and causality to study drug/study procedures (as judged by the investigator).

Safety laboratory data, vital signs and ECG data will be listed for each subject and summarised by treatment groups. These data will be presented using descriptive statistics for each visit and assessment time within the framework of the standard laboratory safety evaluation at AstraZeneca R&D Lund, Sweden. For clinical chemistry and haematology, the minimum ways to present the results are: mean values over time, individual values

over time, shift tables, and when appropriate also shift plots, and presentation of individual clinically important abnormalities at any time during treatment (ie, outside the defined extended reference ranges) . AstraZeneca extended reference ranges should be used for the identification of individual clinically important abnormalities (TELCs). For urinalysis the minimum way to present data are shift tables presenting the number of patients with categorical changes from baseline versus on-treatment values. Vital signs data (ie, body temperature, pulse, systolic blood pressure, and diastolic blood pressure) are to be presented in the same way as for clinical laboratory data. The minimum ways to present numeric ECG variables (ie, heart rate, PR interval, QRS duration, QT and QTc intervals) are the same as for the clinical laboratory data. In addition, shift plots and additional graphical presentations can be included. For variables like sinus rhythm, overall evaluation, etc, the minimum way to present data are shift tables presenting the number of patients with categorical changes from baseline versus on-treatment values.

All safety data will be compared between the treatments at each dose level and placebo.

An AstraZeneca expert will review all laboratory results and data for vital signs outside the AstraZeneca extended reference limits, during the safety evaluation and Other Significant AEs (OAEs) will then be identified, as described in Section 4.6.1.1, Definitions. Follow-up information about such findings may be requested.

6.3 Statistical methods and determination of sample size

6.3.1 Statistical evaluation

The statistical analyses will be performed by AstraZeneca R&D Lund, Sweden. Safety data will primarily be comprehensively described in terms of figures, descriptive statistics and listings, in order to facilitate safety and tolerability assessments of different doses and the pharmacokinetic properties of the drug. The evaluation of pharmacodynamic data will be focused on signals of response versus placebo and, if appropriate, dose-response patterns of the drug.

6.3.2 Description of variables in relation to hypotheses

See Section 6.3.1.

6.3.3 Description of analysis sets

The full analysis set will consist of all subjects with data collected after intake of study drug. The safety data set will have the same definition as the full analysis set. Subjects enrolled in the study but who never took any study drug, will not be included in the analysis.

6.3.4 Methods of statistical analyses

All data will be graphically illustrated and presented by descriptive statistics for each treatment group. Safety and pharmacodynamic data will be compared between active treatment and placebo at each dose level. Placebo subjects from different dose-levels will be pooled for the comparisons. For pharmacodynamic parameters comparisons will be made using analysis of variance model with fixed factor treatment and using baseline as a covariate. Dose-response is considered an exploratory part of the evaluation, and the final model will be data driven. AEs will be analysed by means of descriptive statistics and qualitative analyses.

6.3.5 Determination of sample size

This is an initial safety and tolerability study and the number of subjects are therefore based on the desire to gain adequate safety information to support further clinical studies. Thus, the number of subjects is not based on statistical considerations. Up to 64 subjects will be randomised.

6.4 Interim analyses

No formal interim analysis will be performed. However, prior to each dose escalation, a Safety review Committee will review the findings of the preceding dose level(s) in order to decide whether it is appropriate to continue the study or not. For details about the Safety Review Committee see Section 6.6.

6.5 Data presentation

Safety data will be described as listings, individual graphs and statistical descriptive summaries by dose level of AZD3199.

6.6 Safety Review committee

Safety and tolerability will be evaluated by a Safety Review Committee (SRC).

The study will be blinded at the dosing occasion. The SRC will receive blinded data prior to the meeting, but will have access to unblinded data at the SRC meeting in between each dose cohort.

The SRC will consist of medical expertise. The chairman of the committee will be the Clinical Study Team Physician. The other experts will be the Principal Investigator, the Medical Science Director and the Global Drug Safety Physician. Other relevant expertise (eg, medical, statistic, pharmacokinetic, drug safety) will be consulted if deemed necessary. The members of the committee will review interim study data and decide upon the progression of dosing from one dose level of AZD3199 to the next. Safety and tolerability data (ECG parameters, blood pressure, pulse, body temperature, lung function, safety laboratory variables, and adverse events) and potassium and lactate concentrations from the

first 24 hours after dose and available PK results will be evaluated prior to progression to the next dose level. Data obtained more than 24 hours after the dosing (adverse event reports and laboratory safety variables) must be reviewed by the Investigator and communicated with the SRC. The SRC will decide whether to:

- continue to next planned dose
- escalate or de-escalate the next dose level
- repeat a previous dose level
- postpone the next dose level
- terminate the study

The decision of the SRC will have to be taken in consensus. The decisions and decision-making will be noted by the chairman and a written recommendation will be provided to the study site and IPS AstraZeneca R&D, prior to the next scheduled dose group. The SRC and/or the principal investigator will have the full authority to terminate the study at any time during the course of the study.

7. PART A - 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 can enrol any subject 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 subjects for the study.

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 Good Clinical Practice, applicable regulatory requirements and the AstraZeneca policy on Bioethics.

7.3 Informed Consent

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

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

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

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

7.4 Subject data protection

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

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

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 subjects' medical history.

8. PART A - PROCEDURES IN CASE OF EMERGENCY, OVERDOSE OR PREGNANCY

8.1 AstraZeneca emergency contact procedure

See Supplement 1: Study Delivery Team Contacts in Event of Emergency

8.2 Procedures in case of medical emergency

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

The treatment code may not be broken unless in an emergency situation when the appropriate management of the patient necessitates knowledge of the treatment allocation. In such an emergency, the investigator will, if time and circumstances permit, contact the monitor/ Clinical Study Team Leader prior to breaking the treatment code. If the code is broken, the date, time and reason should be recorded and the investigator should sign the record.

8.3 Procedures in case of overdose

Serious intolerability resulting from drug administration can not be excluded. In the case of known or suspected intolerability or overdose, symptomatic treatment as well as monitoring of vital functions should be performed, based on the judgment of the investigator.

If symptomatic tachycardia is seen, non-asthmatic subjects may be considered for treatment with metoprolol or propranolol, at the discretion of the investigator, see Appendix E.

For the purpose of this study, an accidental or deliberate intake of blinded treatment of higher doses than planned according to the protocol is defined as an overdose and must be reported as such as described below.

- Use of study medication in doses in excess of that specified in the protocol should not be recorded in the CRF as an AE of ‘Overdose’ unless there are associated symptoms or signs.
- An Overdose with associated SAEs should be recorded as the SAE diagnosis/symptoms on the relevant AE forms in the CRF.
- An Overdose with associated non-serious AEs should be recorded as the AE diagnosis/symptoms on the relevant AE forms in the CRF. In addition, the Overdose should be reported on the separate AZ “Clinical Study Overdose Report Form.”
- An Overdose without associated symptoms should not be recorded as an AE in the CRF. The Overdose should be reported on the separate AZ “Clinical Study Overdose Report Form”.

8.4 Procedures in case of pregnancy

8.4.1 Maternal exposure (Not applicable)

8.4.2 Paternal exposure

Pregnancy of the subject's partner is not considered to be an AE. However, the outcome of all pregnancies reported to the investigator (spontaneous miscarriage, elective termination, normal birth or congenital abnormality) must be followed up and documented. The outcomes of any conception, reported to the investigator, occurring from the date of the first dose until 3 months after the last dose must be followed up and documented.

All outcomes of these pregnancies must be reported to AstraZeneca on the pregnancy outcomes report form. Part I of this form must be completed in full and returned to AstraZeneca within 30 days. Part II of the form must be completed when the outcome of the pregnancy is known. Reports of normal outcomes should be sent within 30 days.

9. PART A - REFERENCES

Beeh KM, Derom E, Kannies F, Cameron R, Higgins M, van As A. Indacaterol, a novel inhaled β_2-agonist , provides sustained 24-h bronchodilation in asthma. Eur Respir J 2007; 29: 871-8

Löfdahl C, Svedmyr N. Formoterol fumarate, a new beta$_2$-adrenoceptor agonist. Acute studies of selectivity and duration of effect after inhaled and oral administration. Allergy 1989;44:264-71

Masoli M, Weatherall M, Ayling J, Williams M, Beasley R. The 24 h duration of bronchodilator action of the salmeterol/fluticasone combination inhaler. Respir Med 2005;99(5):545-52

Masoli M, Williams M, Weatherall M, Beasley R. The 24 h duration of bronchodilator action of the budesonide/formoterol combination inhaler. Respir Med 2006;100(1):20-25

Palmqvist M, Persson G, Lazer L, Rosenborg J, Larsson P, Lötvall J. Inhaled dry-powder formoterol and salmeterol in asthmatic patients: onset of action, duration of effect and potency. Eur Respir J 1997;10:2484-89

Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault J-C. Lung volumes and forced ventilatory flows. Report Working Party Standardisation of Lung Function tests, European Community for Steel and Coal. Official statement of the European Respiratory Society. Eur Respir J 1993;6 (suppl 16):5-40.

10. PART B - INTRODUCTION

10.1 Background

Pharmacologically mediated bronchodilatation is an important element in the management of asthma and COPD. The effect is primarily obtained with β_2-agonists, normally administered by inhalation, the reason being more rapidly attained onset of effect and improved therapeutic index. The second aspect, signifying selectively improved potency for the desired local pulmonary effect, implies that a higher dose of a β_2-agonist can be administered to the site of action by inhalation than by oral administration, which in principle means that the duration of bronchodilatation can be maximized by inhalation (Löfdahl et al 1989). A prerequisite for this approach to sustain the effect, however, is that the β_2-agonist has a high affinity for lung tissues (or is slowly released and absorbed from the airway lumen), so that the drug somehow is retained in the lung after inhalation. Otherwise, the effect will rapidly disappear as the concentration difference between airways and the systemic circulation decreases with time towards the ratio obtained after oral therapy. Formoterol (OXIS® [2] and FORADIL®, Novartis) and salmeterol (SEREVENT™, GlaxoSmithKline) are lipophilic drugs with high affinity for airway tissues. The maintained high lung concentrations with positive gradients vis-à-vis the systemic circulation of these drugs are important for fairly selectively prolonged bronchodilatation after inhalation. The bronchodilatating effect of inhaled salmeterol and formoterol lasts for at least 12 hours (h) (Palmqvist M et al 1997), and is prolonged with the concomitant administration of fluticasone and budesonide, respectively (Masoli et al 2005, Masoli et al 2006). Indacaterol, a new β_2-agonist under development for the treatment of asthma and COPD, has extended the concept and provides a duration of action in man of at least 24 h after a single dose of 200 to 400 µg by inhalation (Beeh et al 2007).

The AZD3199 project objective is to develop a well tolerated inhaled once daily, long-acting β_2-agonist with a fast onset of bronchodilatation, a systemic side effect profile similar to or better than that of inhaled formoterol at comparable peak bronchodilatating effects, and effect maintenance in parity with that of for example Indacaterol, ie, at least 24 hour duration of action. AZD3199 is intended to be developed in combination with another drug, eg, a long-acting muscarinic antagonist at COPD or an anti-inflammatory drug at asthma, either administered once daily or by flexible dosing. Treatment with AZD3199 could then significantly improve the pharmacological treatment of obstructive pulmonary diseases.

10.2 Rationale

Part B of the study is designed to assess the efficacy (magnitude and duration) of AZD3199 in patients with asthma after single doses of solution inhaled via nebuliser.

[2] OXIS® is a trademark of the AstraZeneca group of companies.

The reason for studying patients with asthma is to test whether the locally mediated bronchodilating effect of AZD3199 is of higher magnitude and particularly of longer duration than systemically mediated side effects on e.g. potassium concentrations and heart rate.

Before inhaled doses of AZD3199 are administered to patients with asthma, the same or higher doses have been evaluated in healthy volunteers.

11. PART B - STUDY OBJECTIVES

11.1 Primary objective

The primary objective of Part B is to investigate the efficacy of single ascending doses of AZD3199 by assessment of magnitude and duration of action on lung function (FEV_1)

11.2 Secondary objective(s)

The secondary objectives of Part B of the study are:

1. to investigate the safety and tolerability of single ascending doses of AZD3199 by assessment of incidence and nature of adverse events (AEs), clinically significant abnormalities in ECG parameters, blood pressure (BP), pulse, lung function and laboratory variables (clinical chemistry, haematology and urinalysis)

2. to investigate the pharmacokinetics (PK) of single ascending doses of AZD3199 by assessment of drug concentrations in plasma and calculated PK parameters, with particular attention to dose linearity

3. to investigate extrapulmonary β_2- adrenoreceptor mediated effects of single ascending doses of AZD3199 by assessment of potassium and lactate concentrations, tremor and palpitations, heart rate, QTc, and blood pressure

An optional objective in this study is to collect pharmacogenetic samples for possible retrospective exploratory analysis, investigating the influence of genotype on pharmacokinetics (and pharmacodynamic response where appropriate). This genetic part is described in detail in Appendix D.

12. PART B - STUDY PLAN AND PROCEDURES

12.1 Overall study design

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

Part B will be a single-blind, non-randomised, placebo-controlled, crossover study with single ascending dose levels of inhaled AZD3199 investigating the efficacy, tolerability, safety and PK in patients with asthma aged 18-50 years. This part of the study will be carried out at 1 or more centres.

Part B will comprise a maximum of 4 dose levels and will employ 2 cohorts of 8 patients. Each cohort will receive one placebo treatment and 2 different doses. No doses will exceed the highest doses given to the healthy subjects.

The administration of AZD3199 in Part B will start when clinical and/or laboratory data from the healthy volunteers in Part A indicate systemic effects, or if no systemic effects are seen, when 5 dose levels have been administered in Part A. Markers of systemic pharmacological activity are primarily a decrease in plasma potassium and an increase in heart rate.

The following criteria will be used:

- a mean decrease of potassium C_{min} of more than 0.25 mmol/L compared with placebo related to the study drug

or

- a mean increase of maximum resting pulse of more than 15 beats/min compared with placebo related to the study drug

Dose escalation will only proceed after a safety review of data generated at the previous dose level(s). At the end of each dose level, an interim data assessment will be made by a Safety Review Committee (SRC) to determine the next dose. This evaluation will be based both on efficacy (up to 26 hours), safety and tolerability (data from at least the first 12 hours after dose) and available PK results. The SRC will decide which dose to give next, see Section 15.6.

The study will be single-blind, ie, open to the study site personnel and SRC, but the patients will not be informed about the dosing order.

The patients will be resident at the clinic from the evening before dosing until 48 hours (h) following each dose. The length of the residence period at the clinic, assessments and the follow up period may be adjusted if felt necessary.

Patients will be fasting from 22:00 the day before dosing until 4 hours post dose. Standardised lunch and dinner avoiding potassium rich food will be served at 4 and 8-10 hours after drug administration.

Part B of the study comprises 6 visits (see Figure 2 and Table 6); 2 screening visits, 1 placebo visit, 2 treatment visits and a follow-up visit.

Visits 1 and 2 are screening visits. At Visit 1 informed consent will be obtained before any study related procedures are undertaken. At Visit 2, a stepwise reversibility test to check eligibility will be done. Visits 1 and 2 will be performed within 30 days prior to Visit 3.

Visits 3-5 are the treatment visits when the patients will receive placebo and single doses of AZD3199 inhaled via nebuliser. Study measurements will be taken pre-dose and repeatedly after administration of study drug up to 48 hours as scheduled in Table 7. However, time points for measurements may be adjusted if felt necessary. Visit 3 should be performed at least 72 h after Visit 2.

Visit 6, the follow-up visit, will be performed within 10 ±3 days after last administration of study drug. The length of the follow up period may be adjusted if felt necessary. On this visit also reversibility after 9 µg formoterol will be tested.

Figure 2 Part B - Study design

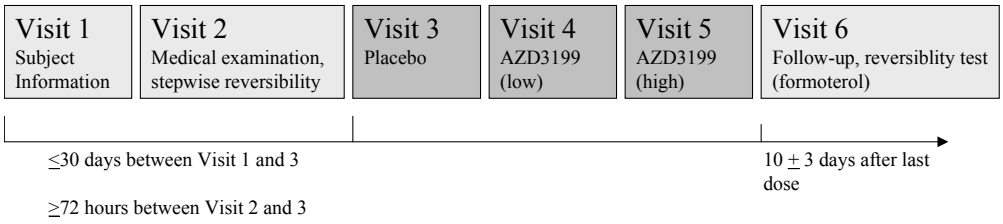

Table 6 Part B - Study plan

Event	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6
	Screen-ing 1	Screen-ing 2	Placebo	AZD3199 low dose	AZD3199 high dose	Fol-low-up
Informed consent/Allocation of enrolment code	X					
Demographics	X					
Height and weight	X					
Medical/surgical history		X				

(Continued)

Table 6 Part B - Study plan

Event	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6
	Screening 1	**Screening 2**	**Placebo**	**AZD3199 low dose**	**AZD3199 high dose**	**Follow-up**
Inclusion/exclusion criteria	X	X	X			
Physical examination		X				X
Spirometry[a]		X	X	X	X	X
Reversibility test with formoterol						X
Stepwise Reversibility Test		X[b]				
Concomitant medication		X	X	X	X	X
Laboratory safety assessments	X		X	X	X	X
HIV and hepatitis B and C tests	X					
Drugs of abuse test[c]	X		X			
Alcohol breath test[c]		X	X			
Blood pressure, pulse		X	X	X	X	X
12-lead ECG		X	X	X	X	X
Inhalation training		X	X	X	X	
Allocation of randomisation code			X			
Blood sampling for PGx			X[d]			
Administration of study drug			X	X	X	
Telemetry monitoring (48 h)			X	X	X	
Blood sampling for PK				X	X	
Blood sampling for potassium and lactate concentrations			X	X	X	
Assessment of tremor and palpitations			X	X	X	
Adverse events questioning			X	X	X	X

a FEV_1 and FVC at Visit 2 and 6. During V3-5 FEV_1 only

(Continued)

Table 6 Part B - Study plan

b The stepwise reversibility test can be repeated once

c Drugs of abuse and alcohol breath tests to be taken at screening and at random once during Visits 3-5.

d The PGx sample can also be taken at any other Visit after Visit 3.

Table 7 Part B - Study Time Schedule (Visit 3 - 5)

			Time (minutes)						Time (hours)												
Assessments	**Day -1**	**Pre**	**0**	**5**	**10**	**20**	**30**	**60**	**2**	**3**	**4**	**6**	**8**	**10**	**12**	**14**	**22**	**24**	**26**	**36**	**48**
Drug Administration			X																		
12-lead ECG		X[a]					X[a]	X[a]	X[a]		X[a]		X[a]		X[a]			X			
Telemetry ECG monitoring		→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→
Blood Pressure and Pulse		X			X		X	X	X		X		X		X			X			X
Safety laboratory sampling		X													X			X			X
Spirometry - FEV_1		X			X		X	X	X		X	X	X	X	X	X	X	X	X		
Assessment of tremor and palpitations		X			X			X	X		X										
Blood sampling -lactate		X			X			X	X		X										
Blood sampling -potassium (K^+)	X	X			X	X	X	X	X	X	X	X	X		X			X			
Blood sampling - AZD3199[b]		X		X[c]	X	X	X	X	X		X	X	X		X			X		X	X
Adverse events questioning	X	X																	X		X

a Heart rate, QT and an overall evaluation

b Blood sampling for AZD3199 only at Visit 4 and 5

c To be taken as soon as possible after the end of inhalation

12.1.1 Stopping criteria for dose escalation

A Safety Review Committee (see Section 15.6) will review safety and tolerability results after each dose before proceeding to the next dose.

Any safety concerns will be evaluated using the following guidance, which is based on preclinical studies with AZD3199 and knowledge from clinical experience with LABAs. The guidance will be used to decide if a patient can be re-exposed with AZD3199 at a higher dose.

Stopping guidance for dose escalation

- Cardiovascular system
  - A QTcF > 500 ms (QT interval corrected for heart rate by Fredericia method) or a QTcF prolongation > 60 ms on average – compared to baseline - sustained for more than 30 minutes, or on 2 contiguous time points.
  - A persistent (>60 min) heart rate >120 beats/min related to the study drug according to the Investigator
  - A clinically significant supraventricular or ventricular tachyarrhythmia of any kind, except isolated ventricular extrasystoles according to the Investigator
  - A persistent (>30 min) diastolic blood pressure <45 mm Hg related to the study drug according to the Investigator
- Potassium concentrations
  - A persistent (>30 min) plasma potassium concentration <2.8 mmol/L
- Liver
  - Increase in AST or ALT≥ 3 x upper limit of normal reference interval (ULN) or bilirubin >2 x ULN or an increase in bilirubin ≥1.5 x ULN and AST or ALT> 2 x ULN.
- Any other clinically relevant changes and significant changes in the safety parameters (e.g. ECG parameters, blood pressure, pulse, spirometry, body temperature, laboratory assessments and AEs) making the continuation of dosing unjustified.
- Dose escalation will be stopped if the mean total plasma AUC or C_{max} of AZD3199 is higher than 111 nmol/L*h or 63.0 nmol/L, respectively. (Section 12.2.1)

12.2 Rationale and risk/benefit assessment

12.2.1 Rationale for study design, doses and control groups

Part B of this study is designed to assess the efficacy, safety, tolerability and pharmacokinetic profile of inhaled AZD3199 when administered to patients with asthma in single doses. The study will help the selection of appropriate doses of AZD3199 for future studies.

Following a placebo administration, 2 single doses of AZD3199, 1 low and 1 high dose will be administered to two sub-groups of asthmatic patients in order to assess a graded dose bronchodilatation response. The non-randomised design with the fixed order of doses was chosen in order to allow for the dose given in Part B to be covered by an equal or higher dose in Part A. This design would automatically unblind the study site personnel, and therefore a single-blind study was selected in order to avoid bias from the patients' knowledge of treatment. Placebo administration was chosen to obtain reference data and to avoid bias. Food/water intake will be standardised/restricted to keep the conditions similar between patients and visits and to minimise variability on the ECG monitoring and potassium levels.

β_2-agonists are functional antagonists. Stimulation of β_2-adrenoceptors, located in the cell membrane of smooth muscles in the airways, decreases tone, irrespective of the cause of the constriction, ie, the calibre of constricted airways is increased. Forced expiratory volume during in the first second (FEV_1) is used as the bronchodilatation effect variable. Asthmatic patients with a known step wise reversibility (increase in FEV_1 upon administration of a β_2-agonist) will be included to be able to assess dose-response. The study is restricted to men, since sufficient reproduction toxicology data in female animals are not yet available. The inclusion and exclusion criteria for Part B are chosen in order to select patients who are known to be free from any significant illness other than asthma.

The particular pharmacodynamic objective in Part B is to assess the clinical potential of AZD3199 by exploring its dose-dependent bronchodilatation response and therapeutic index (relation to systemically mediated effects). To achieve this, 2 doses will be administered to reversible patients with asthma. The doses should provide dose-dependent bronchodilatation that last for at least 24 h - only the high dose should produce adverse extrapulmonary effects. In order to maximise the possibility to assess graded bronchodilatation response, previous experience with β_2-agonists suggest that the high dose increment should be at least 4 times the low. If the cardiovascular and metabolic effects are generally small or even negligible within the studied range of inhaled AZD3199 in Part A, the guiding principle would be to study wide ranges of doses covering the predicted clinically relevant interval (see Section 3.2.1).

A dose given in Part B will always be covered by an equal or higher dose studied in Part A. The starting dose administered to patients with asthma will not exceed half the dose showing systemic pharmacological activity in the healthy subjects. If no systemic pharmacological effects are seen after Dose 5 in Part A, Part B in patients will be initiated with a starting dose

of at most half of Dose 5. Based on preclinical data the therapeutic lung dose of AZD3199 has been estimated to 35 µg, ie, about the proposed Dose 3 in Part A.

12.2.2 Risk/benefit and ethical assessment

The patients will have no individual benefits. Before inhaled doses of AZD3199 are administered to patients with asthma, the same or higher doses have been evaluated in healthy volunteers.

There are several inhaled LABAs in clinical used and under development for the treatment of asthma and COPD. The most cited adverse effects are palpitation, headache and tremor. At higher doses tachycardia, hyperglycemia, hypokalemia and an increased QTc interval may be seen.

The most important adverse effects observed in the non-clinical studies with AZD3199 with potential relevance for humans are: signs of irritation in the nose and larynx in the rat and heart rate increase, QTc prolongation and decrease in plasma potassium in the dog. These effects can easily be monitored in the clinical study and are not considered to pose any safety concerns.

For an overall risk benefit assessment see the Investigator's Brochure.

12.3 Selection of study population

12.3.1 Study selection record

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

Patients for Part B will be recruited from the asthma patient population in Sweden.

12.3.2 Inclusion criteria

For inclusion in Part B of the study patients must fulfil all of the following criteria:

1. Provision of signed informed consent prior to any study specific procedures

2. Men aged 18 to 50 years (inclusive)

3. Body mass index (BMI) between 19 and 30 kg/m^2 and a body weight between 60 and 100 kg.

4. Be non-smoker or ex-smoker who has stopped smoking (or using other nicotine products) for >6 months prior to study start.

5. A history of asthma for at least 6 months. Asthma is defined according to American Thoracic Society (ATS 1987)

6. A stepwise reversible airway obstruction, tested according to routines at the clinic. A minimum of 5% units additional increase from first dose and a total of at least 15% increase in FEV_1 are required

7. Pre-bronchodilator FEV_1 >60% of predicted normal and >1.5 L at Visit 2

8. Be able to inhale from the Spira nebuliser according to given instructions

12.3.3 Exclusion criteria

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

1. Any clinically significant disease or disorder (e.g. cardiovascular, pulmonary other than asthma, gastrointestinal, liver, renal, neurological, musculoskeletal, endocrine, metabolic, malignant, psychiatric, major physical impairment,) which, in the opinion of the investgator, may either put the patient at risk because of participation in the study, or influence the result of the study, or the patient´s ability to participate in the study.

2. Any clinically relevant abnormal findings in physical examination, clinical chemistry, haematology, urinalysis, vital signs, ECG or lung function at baseline, which, in the opinion of the investigator, may put the subject at risk because of his participation in the study.

3. QTcF > 450 ms or QT > 500 ms or other abnormality making interpretation more difficult, as judged by the investigator.

4. Use of any medication, herbal preparations, vitamins or nutritional supplements within 2 weeks prior to Visit 3 except for asthma medication and occasional intake of paracetamol (see Section 12.4.5 for restrictions in asthma medication).

5. Treatment with systemic glucocorticosteroids within 30 days of Visit 3

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

7. Donation of blood within 3 months or donation of plasma within 14 days prior to Visit 1

8. History of or current alcohol or drug abuse, as judged by the investigator

9. A suspected/manifested infection according to WHO risk classification 2, 3 or 4 (See Appendix C).

10. Positive results on screening tests for hepatitis B and/or C and/or HIV.

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

12. Participation in another investigational drug study within 3 months before Visit 3, or participation in a method development study (no drug) 1 month prior to Visit 3 (Note: participation is identified as the completion of a treatment-related visit)

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

14. Patients who, in the opinion of the investigator should not, participate in the study.

12.3.4 Restrictions

Patients will be required to:

1. abstain from alcohol for 72 hours prior to and during Visits 3-5

2. abstain from strenuous physical activity that is not within the subject's normal weekly routine 5 days before and during Visits 3-6

3. abstain from drugs of abuse during the entire study. In addition, poppy seeds (eg, on bread rolls) can give a positive signal for opiates and should not be ingested during the study

4. abstain from food and drink (except water) for at least 3 hours prior to safety laboratory measurement at Visit 6

5. abstain from liquorice containing products 24 hours prior to and during Visits 3-5

6. abstain from caffeine containing beverages and food, eg, coffee, tea, chocolate and soft drinks (eg, Red Bull) for 10 hours before drug administration. Intake of caffeine will be restricted to the equivalent of 1 cup of coffee (approx. 200 mL) at lunch and a maximum of 3 cups in total per 24 hours (Visits 3-5)

7. follow the additional dietary restrictions described in Section 12.3.4.1

Patients will be recommended to:

1. abstain from donation of blood or plasma during the study and 3 months after last AZD3199 dose

2. avoid sperm donation or having procreative sex until 3 months after last AZD3199 dose

12.3.4.1 Dietary restrictions during study sessions

The following applies together with the requirements specified in Section 12.3.4

Patients will be served standardised meals during Visits 3-5 according to normal procedures at the study site, and no other foods will be permitted. Water will be allowed except during ECG recordings. The patients will be fasting from 22:00 Day -1 until 4 hours post dose. Standardised lunch and dinner avoiding potassium rich food will be served at 4 and 8-10 hours and a snack will be served at 12-14 hours post dosing. Meals are served after all required study measurements have been collected at the relevant time point.

12.3.5 Discontinuation of patients from treatment or assessment

12.3.5.1 Criteria for discontinuation

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

- Voluntary discontinuation by the patient, who is at any time free to discontinue his 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 patient does not meet the required inclusion/exclusion criteria for the study.
- Patient lost to follow-up
- A suspected/manifested infection according to WHO risk classification 2 , 3 or 4 (See Appendix C).

Cardiovascular system:

- A persistent (>60 min) heart rate >120 beats/min related to the study drug according to the Investigator

- A clinically significant supraventricular or ventricular tachyarrhythmia of any kind, according to the Investigator
- A persistent (>30 min) diastolic blood pressure <45 mm Hg related to the study drug according to the Investigator
- A QTcF > 500 ms (QT interval corrected for heart rate by Fredericia method) or a QTcF prolongation > 60 ms on average – compared to baseline - sustained for more than 30 minutes, or on 2 contiguous time points.

Potassium concentration:

- A persistent (>30 min) plasma potassium concentration < 2.8 mmol/L

Liver:

- Increase in AST/ALT ≥ 3 x upper limit of normal reference interval (ULN) or bilirubin >2 x ULN or and increase in bilirubin ≥1.5 x ULN and AST/ALT > 2 x ULN.

Any new, single unexplained serious adverse event that may be considered related to the investigational drug.

12.3.5.2 Procedures for discontinuation

Patients who discontinue should always be asked about the reason(s) for their discontinuation and the presence of any adverse events. If possible, they should be seen and assessed by an investigator(s). Adverse events should be followed up.

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

12.4 Treatment(s)

12.4.1 Study Drugs

12.4.1.1 Identity of investigational product and comparator

The drug product is supplied as AZD3199 powder for solution for nebulisation. The drug is the dihydrobromide salt of AZD3199.

AZD3199 drug substance (30 to 50 mg, free base equivalent) is supplied in 10 mL clear glass injection vial with grey rubber stopper for injection and tear-off aluminium cap. The powder is constituted with commercially available Sodium Chloride Solution for Injection, 9 mg/mL

(Ph Eur) (NATRIUMKLORID FRESENIUS KABI®, 9 mg/mL Solution for Injection, Fresenius Kabi, Sweden, or equivalent) purchased locally to achieve a stock solution of up to 5 mg/mL AZD3199.

Sodium Chloride Solution for Injection, 9 mg/mL (Ph Eur) is used as a placebo.

Table 8 Part B- Identity of investigational product

Investigational product	Dosage form and strength	Manufacturer
AZD3199 dihydrobromide	Powder for solution for nebulisation 30 to 50 mg, free base equivalent	AstraZeneca
Placebo	Sodium Chloride solution for injection, 9 mg/mL	Fresenius Kabi

12.4.1.2 Identity of additional study drug

Table 9 Active products for reversibility testing

Active substance	Dosage form and strength	Manufacturer
Salbutamol	pMDI, 100 µg/dose	GlaxoSmithKline
Formoterol	inhalation powder, 4.5 µg/dose	AstraZeneca

The Hospital Pharmacy will supply the drugs for reversibility tests.

12.4.1.3 Labelling

The packaging and labelling of bulk drugs will be performed by Investigational Products (IPS), AstraZeneca R&D. Labelling will be in accordance with GMP and local regulatory guidelines. The label will fulfil GMP Annex 13 requirement for labelling.

The investigational product will be supplied as bulk supply to the hospital pharmacy. Each vial containing drug substance will be labelled with a tear-off label. The vials will be packed in boxes.

Individual dosing containers with dispensed solution for nebulisation will be labelled with study specific information. The label will have a tear-off label to be inserted in a separate administration record.

12.4.1.4 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 bottle label.

12.4.1.5 Accountability

The medication provided for this study is for use only as directed in the clinical study protocol. At completion of the study unused investigational products will be returned to Investigational Products (IPS), AstraZeneca R&D. The Pharmacy personnel will account for all drugs dispensed and returned. Certificates of delivery, destruction and return must be signed.

12.4.2 Doses and treatment regimens

Single doses of AZD3199 and placebo will be administered as a nebulised solution for inhalation. Planned doses for Part B in this study are placebo and 1 low and 1 high dose of AZD3199 administered to two cohorts of patients with asthma. The starting dose will not exceed half the dose showing systemic pharmacological activity in the healthy subjects. If no systemic pharmacological effects are seen after Dose 5 in Part A, Part B in patients will be initiated with a starting dose of at most half of Dose 5.

12.4.2.1 Adjustment of dose levels

The planned dose levels may be changed or postponed during the course of the study depending on safety, efficacy and pharmacokinetic results obtained. The SRC will make a safety evaluation between each dose level and change the dose escalation if necessary, see Section 15.6. The SRC makes all decisions regarding modification of dose levels.

If no systemic pharmacological effects are seen after Dose 5 in Part A, Part B in patients will be initiated with a starting dose of at most half of Dose 5. Based on preclinical data the therapeutic lung dose of AZD3199 has been estimated to 35 µg, ie, about the proposed Dose 3 in Part A.

In order to maximise the possibility to assess graded dose - bronchodilatating response, previous experience suggest that the high dose increment should be at least 4 times the low.

After each SRC evaluation personnel dispensing investigational product at the hospital pharmacy and the personnel at IPS, AstraZeneca R&D will be informed about the decision for the next dose escalation and IPS will issue a corresponding dosing schedule.

12.4.2.2 Preparation of solution for nebulisation

Dose preparation will take place at the hospital pharmacy according to separate handling instructions and dose confirmation. The powder for solution will be constituted with Sodium chloride for Injection to a stock solution of 3 to 5 mg/mL. For doses requiring a lower concentration the stock solution can be diluted with sodium chloride for injection. The doses, placebo and active drug, respectively , will be dispensed in individual dosing containers. Before drug administration personnel at the clinic will transfer the solution from the individual dosing container to the nebuliser cup.

12.4.2.3 Administration of dose

The subjects will practice inhalation technique on Visit 2 and also on Visit 3 - 5, before the dose will be inhaled.

Administration of dose at the clinic including inhalation procedure will be performed according to detailed separate instructions.

In order to avoid contamination particularly of plasma samples intended for drug analysis the following precautions will be taken: Dosing will take place in a separate inhalation room. Furthermore, subjects and personnel involved in the study drug administration must wear protective gloves and clothing. Personnel involved with drug administration should not be involved with blood sampling.

The study drug will be administered in the morning. The subject will inhale the drug/placebo solution via Spira Electro 2 dosimeter in a sitting upright position. The number of inhalations from the dosimeter required to administer the planned dose will be written on the label of the individual dosing containers. After inhalation the subjects will take off the protective gloves and clothing and wash the outside and around their mouth and their hands before leaving the inhalation room. As soon as possible after this a PK sample will be drawn.

After inhalation, aerosol from the dosimeter may be withdrawn to a collection filter for subsequent analysis and calculation of the actual output from the dosimeter.

12.4.3 Method of assigning patients to treatment groups

Informed consent will be obtained before enrolment and the patients in Part B will be identified with an enrolment number starting with E0002001. Patients fulfilling the eligibility criteria will be assigned patient numbers starting with number 011 for cohort 1, and number 021 for cohort 2 in Part B.

Patients will be assigned patient numbers strictly sequentially as patients are eligible for inclusion in the study. If a patient discontinues from the study the patient number will not be re-used and the patient will not be allowed to re-enter the study.

12.4.4 Blinding and procedures for unblinding the study

12.4.4.1 Methods for ensuring blinding

The study is single-blind and the patients will not know in which order they receive the placebo dose and the low and high doses of AZD3199.

The active and the placebo doses will be of the same volume, and the same number of inhalations from the solution for nebulisation will be performed to ensure the blinding. Individual dosing containers must be labelled AZD3199/placebo in order to keep the patients blinded.

AstraZeneca personnel and study site personnel will know which dose is administered.

12.4.5 Concomitant medication

The patients must be instructed that no additional medication will be allowed without the prior consent of the investigator.

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

Medication allowed prior to and during Visits 2-6:

- inhaled corticosteroids ≤1000 µg per day, nedocromil and disodium cromoglycate if the dose is kept constant the last 30 days prior to Visit 2 and during the entire study
- immunotherapy treatment if the dose is kept constant ≥90 days prior to Visit 2 and during the entire study
- leukotriene antagonists and 5-lipoxygenase inhibitor if the dose is kept constant

Medication with restricted use prior to and during Visits 2-6:

- inhaled short-acting β_2-agonists not allowed ≤8 hours prior to dose
- inhaled long-acting β_2-agonists not allowed ≤24 hours
- oral β_2-agonists, short-acting not allowed ≤12 hours
- oral β_2-agonists, short-acting sustained release not allowed ≤24 hours

- oral β_2-agonists, long-acting not allowed ≤72 hours
- inhaled anticholinergics not allowed ≤12 hours, tiotropium not allowed ≤48 hours
- Xanthine containing derivates not allowed ≤48 hours
- antihistamines not allowed ≤48 hours

Medication not allowed prior to and during Visits 2-6:

- oral and parenteral steroids are not allowed within 30 days prior to Visit 2

12.4.6 Treatment compliance

Compliance will be assured by supervised administration of the investigational product by the investigator or his delegate. Details of dosing (time, date) will be captured electronically. Tear-off labels will be inserted in separate administration records.

13. PART B - MEASUREMENT OF STUDY VARIABLES

The following study measurements will be obtained. The timing of these measurements is detailed in Table 6 and in Table7. The following 'priority order' will be in effect when more than 1 assessment is required at a particular time point occurs:

1. ECG recordings
2. Blood pressure, pulse
3. Blood sampling
   - Blood sampling for potassium and lactate concentrations
   - Blood sampling for safety laboratory assessments
   - Blood sampling for PK (except blood samples directly after inhalation which may be taken as soon as possible, before any other assessments)
4. Assessment of palpitations and tremor
5. Spirometry

13.1 Medical examination and demographic measurements

13.1.1 Enrolment medical examination and demographic measurements

Each patient will undergo an enrolment medical examination at Visit 1 and 2 within 30 days prior to the treatment visit. This will consist of:

- Recording of demographic data - date of birth, sex, height, weight, race
- A standard medical/surgical history and a physical examination including the cardiovascular and respiratory systems
- A resting 12-lead ECG
- A resting blood pressure and pulse
- Laboratory assessments (clinical chemistry, haematology and urinalysis)
- A blood sample for HIV, Hepatitis B and C test
- Drugs of abuse screen and alcohol breath test
- Review of inclusion and exclusion criteria
- Spirometry
- Stepwise reversibility test

13.1.2 Post-study medical examination

A follow-up medical examination will be performed at Visit 6 within 10 ± 3 days after the last administration of study drug. This will consist of:

- A physical examination
- A resting 12-lead ECG
- A resting blood pressure and pulse
- Laboratory assessments (clinical chemistry, haematology and urinalysis)
- Spirometry
- Reversibility test with formoterol
- Collection of adverse events

13.2 Pharmacokinetic measurements

13.2.1 Determination of drug concentration in biological samples

Samples for measurement of drug concentration will be analysed by a method using HPLC/MS/MS. Details of the methods used will be referred to in the clinical study report.

13.2.2 Collection of biological samples

13.2.2.1 Blood sampling for AZD3199 plasma concentration

Blood samples (3 mL) for determination of AZD3199 in plasma will be taken at the times presented in Table 7. PK analysis during the study may indicate that the sampling schedule is sub-optimal and therefore additional samples may be taken, samples may be omitted, and/or the times of sampling may be adjusted. Blood samples will be collected, labelled and shipped according to separate instructions from the laboratory. The date and time of collection will be recorded in the CRF.

Samples should be stored at -20°C or below and analysed within the time frame 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 disposal of samples will be decided within 1 month after the clinical study report has been finalised.

13.3 Pharmacodynamic measurements

13.3.1 Spirometry

13.3.1.1 Assessment of FEV_1

Spirometry for determination of FEV_1 and FVC will be performed at enrolment and follow-up. During treatment visits, Visits 3-5, only FEV_1 will be measured (see Table 6 and Table 7).

Lung function testing will be performed according to ERS recommendations (Quanjer et al 1993). The spirometer should be calibrated according to instructions. The highest FEV_1 and FVC out of 3 measurements will be recorded. The highest and the second highest FEV_1 values must not differ by more than 5%. If the difference is larger than 5% the patient may perform up to 8 measurements. If the variation is still above these limits, the largest value will be recorded with a comment.

13.3.1.2 Reversibility tests

Stepwise reversibility test

A stepwise reversibility in FEV_1 will be assessed at Visit 2. After baseline FEV_1 is recorded, the patient will inhale 100 µg salbutamol via *pMDI* and FEV_1 will be recorded *15 - 30* minutes later. The subject will then inhale 900 µg salbutamol and FEV_1 will be recorded *15 - 30* minutes later. An minimum of 5% additional increase from the first dose and a total of at least 15% increase in FEV_1 relative to baseline are required.

Reversibility test with formoterol

Reversibility in FEV_1 after formoterol will be assessed at Visit 6. After baseline FEV_1 is recorded the patient will inhale 2 x 4.5 µg formoterol via Turbuhaler and FEV_1 will be recorded 15 - 30 minutes later.

13.3.2 Assessment of tremor and palpitations

Tremor and palpitations will be assessed by a scoring question. The patient will be asked to estimate the tremor and palpitations by using a 4-grade scale; no, mild, moderate or severe. For timepoints of assessments see Table 7 .

13.3.3 Potassium concentrations

Blood will be drawn in order to determine potassium concentrations and sent to the local laboratory at the hospital for analysis. Blood must be sampled without stasis and handled gently according to separate instructions in order to avoid haemolysis. Samples will be collected at the times given in Table 7 .

13.3.4 Lactate concentrations

Blood will be drawn in order to determine lactate concentrations and will be sent to the local laboratory at the hospital for analysis. Blood must be sampled without stasis and handled gently according to separate instructions in order to avoid haemolysis. Samples should be put on ice prior to being processed. Samples will be collected at the times given in Table 7.

13.3.5 QTc and Heart rate

QT and heart rate will be extracted from the 12-lead ECG measurements. For timepoints of assessments see Table 7.

13.3.6 Blood pressure

Supine blood pressure (BP) will be measured using an semi automatic blood pressure recording device with an appropriate cuff size. The equipment and method of obtaining BP and pulse shall be consistent for all patients in the study. BP will be measured after the patient has rested in bed for at least 10 minutes, at the times given in Table 7.

13.4 Safety measurements

13.4.1 Laboratory safety measurements

Blood and urine samples for determination of clinical chemistry, haematology and urinalysis parameters will be taken at the times given in Table 6 and Table 7. The date and time of collection will be recorded on the appropriate CRF (at Visit 1, 2 and 6 only date).

Patients should rest at least 3 minutes in the supine position before blood sampling. Samples will be collected in tubes according to standard routines. See Section 13.5 for information on the volumes of blood to be taken.

The following laboratory variables will be measured:

Clinical chemistry (serum or plasma)	**Haematology (whole blood)**
S/P-Creatinine	B-Haemoglobin (Hb)
S/P-Bilirubin (total) (Bil, tot)	B-Platelet count
S/P-Alkaline phosphatase (ALP)	B-Leucocyte count (LPC)
S/P-Aspartate aminotransferase (AST)	B-Leucocyte differential count
S/P-Alanine aminotransferase (ALT)	
S/P-Albumin	
S/P-Potassium (K)	**Urinalysis**
S/P-Calcium (total) (Ca)	U-Protein/Albumin
S/P-Sodium (NA)	U-Hb/Erythrocytes/Blood
S/P-Glucose	U-Glucose
S/P Thyroxine free (T4)	
S/P Thyroid-stimulating hormone (TSH)	
S/P-C-Reactive Protein (CRP)	

All samples will be handled and analysed by a local hospital laboratory, except for the urinalysis (dipstick) that will be analysed at the study site according to local procedures.

13.4.1.1 HIV, Hepatitis B and C test

For safety of the study site staff, all patients will be tested for antibodies to HIV, hepatitis B surface antigen and for antibodies to hepatitis C virus at Visit 1. If a patient is found positive to any of these tests, he will be referred for further examination and treatment and will not be included in the study. The samples will be handled and analysed at a local hospital laboratory.

Results will not be recorded in the CRF.

13.4.1.2 Drugs of abuse and alcohol testing

A drug screen (cannabis, methamphetamines, opiates, cocaine, methadone, benzodiazepines, barbiturates, tricyclic anti depressants, phencyclidine (PCP), and amphetamines) in the urine will be done at Visit 1 and once randomly at any of Visits 3-5 in order to exclude or withdraw patients with drug abuse. The samples will be analysed according to local routines.

A breath alcohol test will be performed at Visit 2 and randomly at Visits 3-5.

If a patient tests positive in the drugs of abuse test, a confirming test will be sent to a local hospital laboratory. If the drug is illegal, counselling and advice will be offered.

Results will not be recorded in the CRF.

13.4.2 Electrocardiographic measurements

13.4.2.1 Resting 12-lead ECG

12-lead ECG recordings will be obtained after 10 minutes rest in the supine position, at the time points indicated in the time plans (see Table 6 and Table 7). The patient must have the same position (max 30 degrees) during all ECG registrations. A print out at paper speed 50 mm/sec will be used. Heart rate, QT and an overall evaluation, normal / abnormal (specified) will be recorded in the CRF for ECGs collected predose, and 30 minutes to 12 h post dosing. For ECGs collected at Visits 2 and 6 and the 48 h post dose ECGs at Visits 3-5 only an overall evaluation, normal/abnormal (specified) will be recorded.

13.4.2.2 Real-time display (telemetry)

A real-time ECG will be displayed for at least 48 hours at Visits 3 - 5 starting within 1 hour before the intake of investigational product, according to local routines at the clinic, and will be monitored by the investigator or his/her delegate. The time might be adjusted once more information about the compound characteristics are available.

Date and time for start and end of measurement will be collected in the CRF. Clinically relevant findings should be documented as AEs in the CRF, at the discretion of the investigator or his/her delegate.

Short time periods off telemetry are permitted at the discretion of the investigator.

13.4.3 Vital signs

13.4.3.1 Blood pressure and pulse

For timing of individual measurements refer to Table 6 and Table 7.

Supine blood pressure and pulse will be measured after the patient has rested in bed for at least 10 minutes. Blood pressure and heart rate (HR) will be measured using a semi-automatic blood pressure recording device with an appropriate cuff size. The equipment and method of obtaining blood pressure and pulse shall be consistent for all patients in the study. At Visits 3-5, all data will be captured electronically.

13.4.4 Other safety measurements

13.4.4.1 Lung function tests - Spirometry

Spirometry for determination of FEV_1 and FVC will be performed at enrolment and follow-up. During treatment visits, Visits 3-5, only FEV_1 will be measured. Spirometry measurements are described in Section 13.3.1.

13.4.4.2 Physical examination

The physical examination will include: general appearance, skin, mouth, teeth and throat, lymph nodes, thyroid, musculoskeletal/extremities, cardiovascular, lungs, abdomen and neurological. The examination will be carried out at enrollment and follow-up visit (see Table 6) according to the normal routines at the study site.

13.5 Volume of blood sampling

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

Table 10 Part B - Volume of blood to be drawn from each patient

Assessment		Sample volume (mL)	n of samples	Total volume (mL)
Safety	clinical chemistry	3	14	41
	Glucose	2	14	28
	haematology	2	14	28
Pharmacokinetics (AZD3199)[a]		3	28	84
Pharmacodynamics	Potassium	3	39	117
	Lactate	2	15	30
Pharmacogenetics[b]		10	1	10
HIV/Hepatitis B and C		7	1	7
Total				345

a The number of samples may be modified during the course of the study, see Section 13.2.2.1, but the total blood volume taken in the study will not exceed 450 mL.

b The pharmacogenetics part is described in detail in Appendix D

13.6 Adverse Events

The methods for collecting adverse events are described below.

13.6.1 Adverse Events

13.6.1.1 Definitions

The definitions of 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 1 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 patient or may require medical intervention to prevent 1 of the outcomes listed above.

The causality of SAEs (ie, their relationship to study treatment) will be assessed by the investigator(s), who in completing the relevant case report form must answer “yes” or “no” to the question “Do you consider that there is a reasonable possibility that the event may have been caused by any of the following - study medication - other medication?” For further

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

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

Other Significant Adverse Events (OAE)

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

13.6.1.2 Recording of adverse events

AEs including SAEs will be collected from time of signed informed consent until the end of study. However, the first AE questioning will be done at Visit 3.

Symptoms

AEs will be collected by means of 3 standard questions. The questions will be put to each patient in Swedish. The question “Have you had any health problems since the first visit?” will be put to each patient when they arrive at the clinic at Visit 3. The question “Have you had any health problems since you were last asked?” will be put to each patient at set times after study drug administration at Visits 3-5 (see Table 7). Furthermore, the question “Have you had any health problems since the previous visit?” will be put to each patient upon arrival to the clinic at Visits 4-6. The timing of these questions may be changed during the study, to reflect the safety and pharmacokinetic data produced during the study. The patient’s response to these questions and spontaneously reported and/or observed AEs will be recorded on the AE form with information about seriousness, causality, action taken, date (and time, if starting during a clinic visit) of onset, date (and time, if ends at a visit) of recovery (or marked as ongoing), intensity and outcome.

Causality

The causality of all AEs (ie, the relationship to study treatment) will be assessed by the investigator(s), who in completing the relevant case report form must answer “yes” or “no” to the question “Do you consider that there is a reasonable possibility that the event may have been caused by the investigational product?” A guide to the interpretation of the causality question is found in Appendix B.

Intensity

The patients will be asked to assess the intensity of the reported AEs according to the following scale:

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

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

Assessment of tremor and palpitations

In order to evaluate the expected tremor and palpitations after inhalation of β_2-agonists, questions will be put to the patients at specified timepoints (seeTable 7). The presence and, if applicable intensity, of the symptom will be recorded according to the following scale: No, Mild, Moderate or Severe.

Symptoms emerging from the above specified active questioning are not to be recorded as AEs on the AE form unless:

- the symptom is serious according to definitions, see Section 13.6.1.1

or

- the subject discontinues the study due to the symptom

Symptoms of the disease under study - Asthma

Asthma symptoms or signs, such as chest tightness, cough, dyspnoea, sputum increased and wheeze, will be recorded as AEs when:

- the sign or symptom is serious according to definitions, see Section 13.6.1.1

or

- the subject discontinues the study due to the sign or symptom

and/or

- the sign or symptom is new to the subject or not consistent with the subject's pre-existing asthma history (defined as within 1 year of Visit 1) as judged by the investigator

Abnormal findings

During the study, abnormal laboratory results, pulse, blood pressure, FEV_1 and FVC, and ECG are not to be recorded as AEs in the CRF unless they result in discontinuation or represent SAEs, or the investigator judges them to be of such clinical importance as to merit recording as AEs.

The investigator will review results from physical examination. All new and/or aggravated findings (at follow-up) as compared with baseline measurement (at screening visit) must be identified and recorded on the AE form. These will be considered AEs.

Concomitant medication

All changes in the patient's medication, eg, dose change or addition of new medication, must be reported in the medication log. Reasons for changes in medication, which reflect an AE, must be recorded on the AE form.

Unresolved AEs

If an AE is unresolved when the study is terminated, its subsequent course must be followed until the AE subsides or until the investigator decides that no further follow-up is necessary. However, more information about such AEs may be requested.

Overdose

Should an overdose (accidental or deliberate) occur, it must be reported in accordance with the procedures described in Section 17.3, 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.

Coding of AEs

AstraZeneca will code AEs by using the Medical Dictionary for Regulatory Activities (MedDRA).

MedDRA includes 5 levels: lowest level terms, preferred terms, high level terms, high level group terms, and system organ class terms. Lowest level terms are used on the input side (data entry) to reflect as closely as possible the term used by the investigator or patient to describe the event. Preferred terms are mainly used on the output side (data presentation) to

group terms that are synonymous or closely related. High level terms and high level group terms are also used on the output side. System organ class terms group AEs pertaining to the same body system.

13.6.1.3 Reporting of serious adverse events

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

The AstraZeneca representative will work with the investigator to compile all the necessary information and ensure that the appropriate AstraZeneca Drug Safety Department receives a report by day 1 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 1 day as described above. For a non-serious AE that becomes serious but which is not fatal or life-threatening a report should be received within 5 days.

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

14. PART B - STUDY MANAGEMENT

14.1 Monitoring

14.1.1 Study monitoring

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

14.1.2 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 patient’s medical notes (permission from the patient 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.

Data for which the CRF will be regarded as source data will be described in a separate document.

Monitoring including source data verification should routinely be performed prior to the transfer of data to Data Management. However, data will not necessarily be monitored prior to Safety Review Committee meetings.

14.2 Audits and inspections

Authorised representatives of AstraZeneca, a regulatory authority, 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.

14.3 Training of staff

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

14.4 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 the Ethics Committee, and also the local regulatory authority, before implementation. Local requirements must be followed.

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

AstraZeneca will distribute amendments and new versions of the protocol to the principal investigator(s) 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 Regulatory Affairs at AstraZeneca.

14.5 Study agreements

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

14.6 Study timetable and end of study

Part B of the study is expected to start in November 2007 (see Section 12.1) and to be completed by April 2008

End of study is defined as database lock, which is the time point after which no patient will be exposed to study related activities.

14.7 Data management

Data entry, editing, clean file and analysis will be performed by AstraZeneca R&D Lund, Sweden.

Medical and surgical history and AEs will be coded using the standardised dictionary MedDRA. Medications will be coded using the AZ approved Drug Dictionary (AZDD) including ATC and route of administration will be coded using Committee for Medicinal Products for Human Use (CHMP) dictionary.

Clean file will be declared after a final quality check has been performed on a sample of the data. Before declaring Clean File, the possible influence of protocol deviations on the statistical analysis will be discussed.

Results from the PK assessments will be electronically transferred into the study database at the time of Clean File. After the database is locked, all data will be transferred to the statistician at AstraZeneca R&D Lund, Sweden, for statistical analysis. After locking the database, editing in the database will not be allowed without proper documentation.

Procedures for verification and validation of data will be described in a separate document.

14.7.1 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 AstraZeneca 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 onto AstraZeneca's clinical study database and validated. Procedures for verification and validation of data will be described in the Data Management Plan. Any missing, impossible or inconsistent recordings in the pCRFs will be referred back to the Investigator using a data query form (DQF), and be documented for each individual patient before clean file status is declared.

14.7.2 Electronic data capture (Immediate data entry) Not applicable in Part B

14.7.3 Electronic data capture at bedside

During the treatment visits all data, except for medication, AEs, lung function, 12-lead ECG, laboratory results and tremor/palpitations scoring will be captured electronically at bedside.

The Investigator will ensure that the captured data are correct before transferred to the AstraZeneca Clinical Study Database for final checking. Any changes made during validation will be documented with a full audit trail within the Electronic Data Capture application.

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

15. PART B - PHARMACOKINETIC, PHARMACODYNAMIC, SAFETY AND STATISTICAL METHODOLOGY

15.1 Pharmacokinetic / pharmacodynamic evaluation

15.1.1 Calculation or derivation of pharmacokinetic variables

The pharmacokinetic analyses will be performed by the Department of Clinical Information Science, AstraZeneca R&D Lund, Sweden.

From the plasma concentrations of AZD3199 collected following a single dose, pharmacokinetic parameters will be calculated using standard non-parametrical methods. The pharmacokinetic parameters will include: the maximum plasma drug concentration (C_{max}), the time to maximum plasma drug concentration (t_{max}), the area under the (plasma drug concentration-time) curve from time zero to infinity (AUC), terminal half-life of drug in plasma ($t_{1/2}$), apparent plasma clearance (CL/F).

15.1.2 Calculation or derivation of pharmacodynamic variables

Pharmacodynamic variables in this study will include potassium and lactate concentrations, blood pressure, heart rate, QTcB, tremor, palpitations and FEV_1. For each variable the maximum effect during the post-dose measurement interval (the minimum value, E_{min}, for

potassium and diastolic blood pressure, and the maximum value, E_{max}, for other variables) and the average effect, E_{av} (AUC/length of interval), will be computed and used as variables of systemic and local effects. In addition, for FEV_1, the effect during 22 - 26 hours post-dose, $E_{22\text{-}26}$, will be calculated. Further, data collected at each assessment time will be used in the statistical evaluation to follow the duration of effect of the drug.

15.2 Safety evaluation

15.2.1 Calculation or derivation of safety variables

All adverse events will be analysed at AstraZeneca R&D Lund, Sweden, by means of descriptive statistics and qualitative analysis. AEs will be listed for each subject and summarised by body system and preferred term assigned to event by using MedDRA. Adverse events will also by summarised by intensity and causality to study drug/study procedures (as judged by the investigator).

Safety laboratory data, vital signs and ECG data will be listed for each subject and summarised by treatment groups. These data will be presented using descriptive statistics for each visit and assessment time within the framework of the standard laboratory safety evaluation at AstraZeneca R&D Lund, Sweden. For clinical chemistry and haematology, the minimum ways to present the results are: mean values over time, individual values over time, shift tables, and when appropriate also shift plots, and presentation of individual clinically important abnormalities at any time during treatment (ie, outside the defined extended reference ranges) . AstraZeneca extended reference ranges should be used for the identification of individual clinically important abnormalities (treatment emergent laboratory changes, TELCs). For urinalysis the minimum way to present data are shift tables presenting the number of patients with categorical changes from baseline versus on-treatment values. Vital signs data (ie, body temperature, pulse, systolic blood pressure, and diastolic blood pressure) are to be presented in the same way as for clinical laboratory data. The minimum ways to present numeric ECG variables (ie, heart rate, QTc intervals) are the same as for the clinical laboratory data. In addition, shift plots and additional graphical presentations can be included.

All safety data will be compared between the treatments at each dose level and placebo.

An AstraZeneca expert will review all laboratory results and data for vital signs outside the AstraZeneca extended reference limits, during the safety evaluation and Other Significant AEs (OAEs) will then be identified, as described in Section 4.6.1.1, Definitions. Follow-up information about such findings may be requested.

15.3 Statistical methods and determination of sample size

15.3.1 Statistical evaluation

The statistical analyses will be performed by AstraZeneca R&D Lund, Sweden. Safety data will primarily be comprehensively described in terms of figures, descriptive statistics and listings, in order to facilitate safety and tolerability assessments of different doses and the pharmacokinetic properties of the drug. The evaluation of pharmacodynamic data will be focused on dose-response patterns of the drug.

15.3.2 Description of variables in relation to hypotheses

FEV_1 and potassium will be the primary variables of local and systemic effects. FEV_1 E_{max} will be the primary variable to determine the bronchodilatory potency of the drug. FEV_1 $E_{22\text{-}26}$ will be the primary measure of the duration of the bronchodilatory effect. Potassium E_{min} will be the primary measure of the potency regarding systemically mediated side effects.

15.3.3 Description of analysis sets

The full analysis set will consist of all patients with data collected from at least 2 study periods (placebo plus 1 period with active drug). The safety data set will consist of all patients with data from at least 1 study day.

15.3.4 Methods of statistical analyses

Pairwise comparisons for pharmacodynamic parameters (between active drug and placebo, and between different doses of active drug) will be made using analysis of variance models. Depending on the outcome at the dose levels, separate models within each cohort or a combined model will be chosen. For dose-response, if appropriate, an Emax model will be fitted to data using a mixed effects model.

15.3.5 Determination of sample size

This is an initial study to evaluate the efficacy of AZD3199 in patients with asthma. The study will be performed under SAD-like conditions, and the number if patients has therefore been set with respect to the possibility to conduct the study, rather than to be able to detect a certain level of effect. From previous experience with formoterol, a within-subject coefficient of variation of 4% in FEV_1 could be expected. With 8 patients, and a 2-sided test at the 5% significance level, there will be an 80% power to detect a true pairwise difference (within cohort) of 7%. To allow for more than 2 doses of AZD3199 being tested, 2 cohorts of 8 patients each will be used, all starting with placebo, and then given 2 doses each of AZD3199 in ascending order.

15.4 Interim analyses

No formal interim analysis will be performed. However, prior to each dose escalation, data from the preceding dose level(s) will be reviewed in order to decide what the next appropriate dose would be.

15.5 Data presentation

Safety data will be described as listings, individual graphs and statistical descriptive summaries by dose level of AZD3199.

15.6 Safety Review committee

Safety and tolerability will be evaluated by a Safety Review Committee (SRC).

The SRC will consist of medical expertise. The chairman of the committee will be the Clinical Study Team Physician. The other experts will be the Principal Investigator, the Medical Science Director and the Global Drug Safety Physician. Other relevant expertise (eg, medical, statistic, pharmacokinetic, drug safety) will be consulted if deemed necessary. The members of the committee will review interim study data and decide upon the progression of dosing from 1 dose level of AZD3199 to the next. Safety and tolerability data (ECG parameters, blood pressure, pulse, body temperature, safety laboratory variables, and adverse events), from at least the first 12 hours after dose, efficacy data (lung function) up to 26 hours after dose and available PK results will be evaluated prior to progression to the next dose level. Data obtained after the stated time points must be reviewed by the Investigator and communicated with the SRC. The SRC will decide whether to:

- continue to next planned dose
- adjust the next dose level
- postpone the next dose level
- terminate the cohort or the study

The decision of the SRC will have to be taken in consensus. The decisions and decision-making will be noted by the chairman and a written recommendation will be provided to the study site and IPS, AstraZeneca R&D, prior to the next scheduled dose escalation. The SRC and/or the principal investigator will have the full authority to terminate the study at any time during the course of the study.

16. ETHICS

16.1 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 Committees as appropriate. The investigator must submit written approval to AstraZeneca before he can enrol any subject 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 patients for the study.

16.2 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 Good Clinical Practice, applicable regulatory requirements and the AstraZeneca policy on Bioethics.

16.3 Informed Consent

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

The patient'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 patient.

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

16.4 Patient 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, patients 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 and study code.

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 patients' medical history.

17. PROCEDURES IN CASE OF EMERGENCY, OVERDOSE OR PREGNANCY

17.1 AstraZeneca emergency contact procedure

See Supplement 1: Study Delivery Team Contacts in Event of Emergency

17.2 Procedures in case of medical emergency

The principal investigator(s) is responsible for ensuring that procedures and expertise are available to cope with medical emergencies during the study. **A medical emergency usually constitutes an SAE and should be reported as such, see 13.6.1.3.**

The treatment code may not be broken unless in an emergency situation when the appropriate management of the patient necessitates knowledge of the treatment allocation. In such an emergency, the investigator will, if time and circumstances permit, contact the monitor/ Clinical Study Team Leader prior to breaking the treatment code. If the code is broken, the date, time and reason should be recorded and the investigator should sign the record.

17.3 Procedures in case of overdose

Serious intolerability resulting from drug administration can not be excluded. In the case of known or suspected intolerability or overdose, symptomatic treatment as well as monitoring of vital functions should be performed, based on the judgment of the investigator.

If symptomatic tachycardia is seen, patients with asthma may be considered for treatment with verapamil, at the discretion of the investigator, see Appendix E.

For the purpose of this study, an accidental or deliberate intake of blinded treatment of higher doses than planned according to the protocol is defined as an overdose and must be reported as such as described below.

- Use of study medication in doses in excess of that specified in the protocol should not be recorded in the CRF as an AE of 'Overdose' unless there are associated symptoms or signs.
- An Overdose with associated SAEs should be recorded as the SAE diagnosis/symptoms on the relevant AE forms in the CRF.
- An Overdose with associated non-serious AEs should be recorded as the AE diagnosis/symptoms on the relevant AE forms in the CRF. In addition, the Overdose should be reported on the separate AZ "Clinical Study Overdose Report Form."
- An Overdose without associated symptoms should not be recorded as an AE in the CRF. The Overdose should be reported on the separate AZ "Clinical Study Overdose Report Form".

17.4 Procedures in case of pregnancy

17.4.1 Maternal exposure (Not applicable)

17.4.2 Paternal exposure

Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies reported to the investigator (spontaneous miscarriage, elective termination, normal birth or congenital abnormality) must be followed up and documented. The outcomes of any conception, reported to the investigator, occurring from the date of the first dose until 3 months after the last dose must be followed up and documented.

All outcomes of these pregnancies must be reported to AstraZeneca on the pregnancy outcomes report form. Part I of this form must be completed in full and returned to AstraZeneca within 30 days. Part II of the form must be completed when the outcome of the pregnancy is known. Reports of normal outcomes should be sent within 30 days.

18. PART B - REFERENCES

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