AstraZeneca

Clinical Pharmacology Study Protocol

Drug Substance	AZD6140
Study Code	D5130C00028
Edition Number	1.0
Date	

An Exploratory, Double-Blind, Randomized, Placebo-Controlled, 2-Period Crossover, 3 Cohort Pharmacokinetic/Pharmacodynamic, Single Center Study to Assess the Effect of AZD6140 on Respiratory Parameters in Healthy Subjects, Mild Asthmatics and COPD Patients Age 55 to 75 Years

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

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

ASTRAZENECA PROCEDURES IN CASE OF EMERGENCY

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

Role in the study	Name	Address and Telephone number
Clinical Study Team Leader		
Clinical Study Team Physician		

For further clarifications regarding:

- Procedures in case of medical emergency see Section 8.2
- Procedures in case of overdose see Section 8.3.
- Procedures in case of pregnancy see Section 8.4 (not applicable)

PROTOCOL SYNOPSIS

An Exploratory, Double Blind, Randomized, Placebo-Controlled, 2-Period Crossover, 3 Cohort Pharmacokinetic/Pharmacodynamic, Single Center Study to Assess the Effect of AZD6140 on Respiratory Parameters in Healthy Subjects, Mild Asthmatics and COPD Patients Age 55 to 75 Years

Investigator

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

Approximately 24 subjects (male or female of non-child bearing potential) including 8 healthy volunteers, 8 mild asthmatic patients and 8 mild chronic obstructive pulmonary disease(COPD) patients aged 55 to 75 years, inclusive, will be randomized at a single center.

Study period	**Phase of development**
Estimated date of first subject enrolled	Clinical Pharmacology (I)
Estimated date of last subject completed	

Objectives

The primary objective of the study is to:

- Assess the effect of AZD6140 on respiratory rate and minute ventilation

The secondary objectives of the study are to:

- Assess the effect of AZD6140 on other respiratory parameters;
- Evaluate the pharmacokinetic/pharmacodynamic (PK/PD) relationship between AZD6140/AR-C124910XX concentrations and respiratory parameters in healthy volunteers, mild asthma patients, and mild COPD patients;

- Compare respiratory parameters between the healthy volunteers, mild asthma patients, or COPD patients;
- Compare the pharmacokinetics (PK) of AZD6140 and AR-C124910XX in healthy volunteers, mild asthma patients, or COPD patients.
- Examine the safety and tolerability of AZD6140 in healthy volunteers compared with mild asthma patients or mild COPD patients.

Study design

This will be a randomized, double blind, placebo-controlled, 3 cohort PK/PD, single center study to assess the effect of AZD6140 on respiratory parameters in healthy volunteers, mild asthmatic and COPD patients age 55 to 75 years.

Investigational product, dosage and mode of administration

Each subject will receive each of the following treatments according to the randomization schedule:

Treatment A: A single, oral dose of 450 mg AZD6140 (5 x 90 mg immediate release tablets) will be administered in the AM of Study Day 1; followed by a single, oral dose of 180 mg AZD6140 (2 x 90 mg immediate release tablets) in the PM (12 hours after the first dose); thereafter, 180 mg AZD6140 (2 x 90 mg immediate release tablet) will be administered bid (every 12 hours) on Study Days 2 and 3 and in the AM only on Study Day 4.

Treatment B: A single, oral dose of AZD6140 matching placebo (5 placebo tablets) will be administered in the AM of Study Day 1; followed by a single, oral dose of AZD6140 matching placebo (2 placebo tablets) in the PM (12 hours after the first dose); thereafter, AZD6140 matching placebo (2 placebo tablets) will be administered bid (every 12 hours) on Study Days 2 and 3 and in the AM only on Study Day 4.

Duration of treatment

The duration of subject participation will be up to approximately 45 days, including:

- **Screening period**: within 21 days before Day -1, Period I admission
- **Periods I/II**: will each consist of a 6 day/5 night inpatient stay
- **Washout Period**: at least 7 outpatient days between Study Day 5 of Period I and Study Day 1 of Period II.
- **Follow-up visit**: within 4 to 7 days of last inpatient day of Period II

Each subject will receive 1 dose of 450 mg AZD6140, 6 doses of 180 mg AZD6140 and 7 doses of AZD6140 matching placebo tablets according to the randomization schedule.

Variables

- **Pharmacokinetic**

  The following pharmacokinetic parameters will be assessed:

  Day 1: C_{max}, t_{max}, $AUC_{(0-12)}$,for AZD6140 and AR-C124910XX and the metabolite to parent C_{max} and AUC ratios.

  Day 4: C_{max}, t_{max} and AUC_{τ}, for AZD6140 and AR-C124910XX, AZD6140 CL/F and the metabolite to parent C_{max} and AUC ratios.

- **Pharmacodynamic**

  Cardiopulmonary exercise test

  Minute ventilation

  Spirometry (FEV_1, FVC)

  Respiratory Rate

  Modified Borg Scale

  Bidirectional Dyspnea Index

- **Safety**

  Safety and tolerability will be assessed by collection of adverse events, 12-lead ECG (electrocardiogram), blood pressure, heart rate, physical examination results and laboratory assessments (chemistry, hematology, urinalysis), use of albuterol MDI.

- **Genetics**

  Genetic analysis of the genes that are involved in absorption and disposition of, and response to AZD6140 such as the MDR-1 gene may be performed for those volunteers providing separate informed consent. The genetic samples collected from this study may be pooled with those from other studies involving AZD6140.

- **Statistical methods**

  The respiratory parameters, and changes from baseline, will be summarized at each scheduled time point by treatment and visit for each of the cohorts using descriptive statistics. These will also be supported with graphical displays in order to ease making comparisons.

  The pharmacokinetic parameters will also be descriptively summarized at each scheduled time point and treatment for the cohorts. Furthermore, graphical plots of the respiratory parameter and concentration of AZD6140 will also be explored for each of the three cohorts by visit.

Safety data (adverse events, vital signs, 12-lead ECGs, laboratory data, physical examination data) will be summarized at each scheduled time point by treatment for the three cohorts using descriptive statistics.

The results of genetic analysis, if any, will be exploratory and will not be included in the clinical study report of this study.

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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
ABG	Arterial blood gas
ACS	Acute Coronary Syndrome
ADP	Adenosine diphosphate
AE	Adverse event
ALT	Alanine transaminase
ANOVA	Analysis of variance
Assessment	An observation made on a variable involving a subjective judgment
ASA	Acetylsalicylic acid
AST	Aspartate transaminase
AUC_{τ}	Area under the plasma concentration-time curve in a dosing interval
Bid	Bis in die, twice a day
BMI	Body Mass Index
BP	Blood pressure
C_{max}	Maximum (peak) plasma concentration
CGG	Clinical genotyping group
CL/F	Apparent oral clearance
COPD	Chronic obstructive pulmonary disease
CRF	Case report form
CSR	Clinical Study Report
DQF	Data query form
ECG	Electrocardiogram
EDTA	Ethylene diamine tetra-acetic acid
FEV	Forced Expiratory Volume
FSH	Follicle Stimulating Hormone
FVC	Forced vital capacity
GCP	Good Clinical Practice
GGT	Gamma glutamyl transferase
HbsAg	Hepatitis B surface antigen

Abbreviation or special term	Explanation
HIPPA	Health Insurance Portability and Accountability Act
HIV	Human immunodeficiency virus
HR	Heart rate
IC	Inspiratory capacity
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IPA	Percent inhibition of ADP-induced platelet aggregation
IRB	Institutional Review Board
Kg	Kilogram
Measurement	An observation made on a variable using a measurement device
Mg	Milligram
ML	Milliliter
OAE	Other significant adverse event (See Appendix B)
Od	Once daily
OTC	Over-the-counter
Outcome variable	A, usually derived, variable specifically defined to be used in the analysis of a study objective
PCP	Phencyclidine
PCRF	Paper Case Report form
PD	Pharmacodynamics
PGx	Pharmacogenetics
PK	Pharmacokinetics
Parameter	A quantity (usually unknown) that characterizes the distribution of a variable in a population of subjects
Principal investigator	A person responsible for the conduct of a clinical study at a study site. Every study center has a principal investigator.
RPM	Revolutions per minute
SAE	Serious adverse event
SaO_2	Oxygen saturation
SAP	Statistical analyses plan
TBD	To be determined
THC	Tetrahydrocannabinol
$t_{½}$	Terminal phase half-life

Abbreviation or special term	Explanation
t_{max}	Time to reach maximum (peak) concentration
ULN	Upper limit of Normal
Variable	A characteristic or a property of a subject that may vary e.g., from time to time or between subject
V_E	Minute ventilation
VO_2	Oxygen uptake
V_T	Tidal volume
Wcap	Work capacity
WHO	World Health Organization

1. INTRODUCTION

1.1 Background

Adenosine diphosphate (ADP) is an important mediator of platelet activation and aggregation through its binding to at least 2 distinct subtypes of purinoceptor, designated $P2Y_1$ and $P2Y_{12}$, found on platelets. Two ADP receptor antagonists, thienopyridine pro-drugs, clopidogrel and ticlopidine have shown clear benefits for the reduction of clinical thromboembolic events in patients with atherosclerosis due to their ability to block the $P2Y_{12}$-receptor. However, this blockade is irreversible and usually incomplete. In addition, there are some safety concerns for the use of ticlopidine. Therefore, the search continues for agents which can further improve the clinical outcomes of these patients through improved efficacy and/or safety.

AZD6140 is a potent, reversible, $P2Y_{12}$-receptor antagonist (antiplatelet agent) being developed to reduce thromboembolic events in patients with atherosclerosis. It is orally active and does not require metabolic activation, different from clopidogrel, for which only the metabolites are active. Unlike clopidogrel and ticlopidine, which incompletely block the $P2Y_{12}$-receptor response in humans, pre-clinical studies indicate that AZD6140 can produce long-lasting and complete inhibition of ADP-induced platelet aggregation *ex vivo* following oral dosing.

Twelve phase I studies conducted primarily in Caucasian subjects and two phase I trials conducted in Japanese and Caucasian subjects have completed dosing as of the end of June 2004. The data from these studies demonstrate a well tolerated safety profile for AZD6140 over the dose range of 0.1 to 600 mg (for periods up to 20 days). There is a positive relationship between the plasma concentration of AZD6140 and the degree of inhibition of platelet aggregation (IPA), with all volunteers achieving a high level of inhibition of 20 μmol ADP-induced aggregation.

Study SC-532-5239 evaluated the safety and tolerability of multiple ascending doses (tablets) of AZD6140 in healthy male and female subjects in Europe. Once and twice daily dosing regimens of AZD6140 with total daily doses ranging from 50 mg to 600 mg administered for 5 days at each dose level (a total duration of 15 or 20 days) were studied, compared with 75 mg clopidogrel. Multiple doses of AZD6140 were well tolerated. The pharmacokinetics (PK) of AZD6140 following multiple oral dosing was approximately linear over 50 mg to 600 mg. Maximum plasma concentrations (C_{max}) were reached within 1.5 to 3 hours after dose intake, and the mean terminal half-life ($t_{½}$) of AZD6140 ranged from 6 to 13 hours. The metabolite area under the plasma concentration-time curve (AUC) and C_{max} were about 35% of the corresponding parameters for AZD6140 and were approximately linear over 50 mg to 600 mg dosing of AZD6140. Greater than 80% inhibition of platelet aggregation was observed at all doses studied. In terms of inhibition of platelet aggregation, twice-daily doses were superior to the equivalent total daily dose given every 24 hours. All total daily doses of AZD6140 above 200 mg were superior to once-daily doses of 75 mg clopidogrel in terms of pharmacodynamics response. In addition, in this study, the effect of food on the PK and PD of AZD6140 was studied in an exploratory way (comparison between the data under fasting

condition on Day 15 and under fed condition on Day 16). Plasma concentrations and AUC values for AZD6140 were slightly higher following administration of AZD6140 under fed condition. There did not appear to be any effect on these for the metabolite. Due to the study design, however, the exact magnitude of the effect of food could not be estimated from these data. There was no obvious effect of food on PD response.

No serious adverse events were observed in the 14 Phase I studies completed to date. Approximately 388 healthy volunteers were entered into these trials, and 337 healthy volunteers received at least 1 dose of AZD6140. The most common adverse events reported to date in the Phase I studies include: increase in liver function tests, tachycardia, orthostatic hypotension, rash, and bleeding events including skin bruising, epistaxis, and gingival bleeding. However, the causal relationship of these adverse events to AZD6140 is uncertain. There were no electrocardiogram (ECG), Holter monitor or vital sign findings of concern during any of the studies.

It is well known that drug response can be affected by genetic variables. In vitro studies have shown that AZD6140 is a substrate of cytochrome P450 enzyme CYP3A and P-glycoprotein, a transmembrane transporter encoded by MDR-1 gene. Functional polymorphisms in the genes encoding both CYP3A and the transporter have been identified and have the potential to impact pharmacokinetics of AZD6140. It is also possible that information on other genes influencing the disposition of and response to AZD6140 will become available in the future. It will therefore be important to consider the possibility of investigating additional genes in the future.

1.2 Rationale

This study is to be conducted to evaluate the respiratory symptoms and physiological parameters that may be associated with the sensation of dyspnea in subjects receiving AZD6140. Dyspnea was reported in association with AZD6140 administration in DISPERSE, a phase IIa study in stable outpatients with documented atherosclerosis, but not healthy volunteers. One pre-clinical study in rats demonstrated an increase in respiratory rate at concentrations ofAZD6140 that are clinically relevant. Non-clinical studies have demonstrated that, at relatively high concentrations, AZD6140 has an affinity for the adenosine transporter in erythrocytes. This is a potential mechanism for the observed effect. Adenosine administration has been associated with dyspnea, flushing, and chest discomfort. Dyspnea has also been observed clinically with dipyridamole, which inhibits the erythrocyte adenosine transporter. Respiratory symptoms invoked by adenosine are thought to be due to activation of arterial chemoreceptors. The target population of patients with ACS are likely to include those with asthma and COPD. Therefore healthy volunteers, as well as patients with mild asthma and COPD will be included to assess whether the sensitivity of patients with respiratory disease is greater than in healthy volunteers. The pharmacodynamic effect of AZD6140 on respiratory parameters will be assessed by cardiopulmonary exercise test, respiratory rate, minute ventilation, spirometry (FVC, FEV_1), modified Borg Scale[1], and Bidirectional Dyspnea Index[2]. Pharmacokinetics of AZD6140 and its active metabolite AR-C124910XX will be assessed to correlate the PD effect on respiratory parameters with plasma concentrations of AZD6140 and AR-C124910XX.

A retrospective analysis of the polymorphisms of genes that are important to the absorption and disposition of, and response to AZD6140 may be performed. The genetic data from this study may be pooled with genetic results from other studies on AZD6140 to generate hypotheses to be tested in future studies. The results of the genetic analysis, if any, will not form part of the clinical study report.

2. STUDY OBJECTIVES

2.1 Primary objective

The primary objective of the study is to:

- Assess the effect of AZD6140 on respiratory rate and minute ventilation.

2.2 Secondary objective(s)

The secondary objectives of the study are to:

- Assess the effect of AZD6140 on other respiratory parameters;
- Evaluate the pharmacokinetic/pharmacodynamic (PK/PD) relationship between AZD6140/AR-C124910XX concentrations and respiratory parameters in healthy volunteers, mild asthma patients, and mild COPD patients;
- Compare respiratory parameters between the healthy volunteers, mild asthma patients, or COPD patients;
- Compare PK of AZD6140 and AR-C124910XX in healthy volunteers, mild asthma patients, or COPD patients.
- Examine the safety and tolerability of AZD6140 in healthy volunteers compared with mild asthma patients, or mild COPD patients.

3. STUDY PLAN AND PROCEDURES

3.1 Overall study design

This will be a randomized, double blind, placebo-controlled, 3 cohort PK/PD, single center study to assess the effect of AZD6140 on respiratory parameters in healthy volunteers, mild asthmatic and COPD patients between 55 to 75 years of age.

Up to 12 men or women (non childbearing potential) will be randomized in each cohort to ensure the completion of at least 8 subjects within each cohort.

Cohort	Volunteer Type	Respiratory criteria	Number subjects
A	Healthy	N/A	8
B	Mild Asthmatic	FEV_1 > 70%; no exacerbation in past 8 weeks; reversibility demonstrated within the past year (≥ 12% or 200 mL)	8
C	Mild COPD	FEV_1 ≥ 60%; no exacerbations in past 8 weeks; history of tobacco smoking > 10 pack years	8

On Study Day 1 of Period I, each subject will receive one of the following treatments according to the randomization schedule:

Treatment A: A single, oral dose of 450 mg AZD6140 (5 x 90 mg immediate release tablets) will be administered in the AM of Study Day 1; followed by a single, oral dose of 180 mg AZD6140 (2 x 90 mg immediate release tablet) in the PM (12 hours after the first dose); thereafter, 180 mg AZD6140 (2 x 90 mg immediate release tablet) will be administered bid (every 12 hours) on Study Days 2 and 3 and in the AM only on Study Day 4.

Treatment B: A single, oral dose of AZD6140 matching placebo (5 placebo tablets) will be administered in the AM of Study Day 1; followed by a single, oral dose of AZD6140 matching placebo (2 placebo tablets) in the PM (12 hours after the first dose); thereafter, AZD6140 matching placebo (2 placebo tablets) will be administered bid (every 12 hours) on Study Days 2 and 3 and in the AM only on Study Day 4.

At the end of Period I, all subjects will crossover to receive the alternate treatment following a 7 – 10 day washout period. During each treatment period, pharmacodynamic measurements and safety variables will be assessed. Blood samples will be collected for analysis of AZD6140 and its metabolite, AR-C124910XX.

All healthy volunteers (Cohort A) will be dosed and evaluated initially. ***A blinded review of the safety data (adverse events, spirometry, respiratory rate) for the healthy volunteers will be performed by the sponsor at the completion of Cohort A to assess any issues for safety prior to dosing Cohorts B and C. The sponsor will give written approval to proceed with dosing of the next 2 cohorts.*** Dosing of Cohorts B and C can be completed simultaneously.

3.1.1 Screening/Visit 1

In order to establish eligibility to participate in the study, potential subjects will undergo all screening procedures and assessments within 21 days before Day 1 of Period I, after giving written informed consent. Please refer to Table 1 and Section 4.1.1 for procedures and assessments to be completed at the screening visit.

3.1.2 Period I/Visit 2

Each day will be based on a 24-hour clock beginning at midnight (00:00).

Subjects will be admitted to the study center on Day –1 and will reside in the study center from Study Day-1 until completion of all protocol-driven procedures and assessments on Study Day 5 of Period I. The assessments on Study Day –1 of Period I, will reconfirm the subject eligibility to participate in the study. The results of the assessments must be confirmed before the subject is randomized and receives study medication on Day 1 of Period I.

All subjects will be required to begin an overnight fast on the evening of Study Day –1. On Study Day 1 of Period I, each volunteer will receive one of the following treatments according to the randomization schedule:

Treatment A: A single, oral dose of 450 mg AZD6140 (5 x 90 mg immediate release tablets) will be administered in the AM of Study Day 1; followed by a single, oral dose of 180 mg AZD6140 (2 x 90 mg immediate release tablet) in the PM (12 hours after the first dose); thereafter, 180 mg AZD6140 (2 x 90 mg immediate release tablet) will be administered bid (every 12 hours) on Study Days 2 and 3 and in the AM only on Study Day 4.

Treatment B: A single, oral dose of AZD6140 matching placebo (5 placebo tablets) will be administered in the AM of Study Day 1; followed by a single, oral dose of AZD6140 matching placebo (2 placebo tablets) in the PM (12 hours after the first dose); thereafter, AZD6140 matching placebo(2 placebo tablets) will be administered bid (every 12 hours) on Study Days 2 and 3 and in the AM only on Study Day 4.

The optional genetic sampling will be taken from subjects who have signed a separate consent form for genetic analysis on Study Day 1 prior to dose administration (Period I only). Blood samples for the determination of AZD6140, and its metabolite AR-C124910XX, in plasma will be collected on Study Days 1, 2, 3 and 4 in each treatment. Refer to Table 2 , Table 3 and Sections 4.3 and 4.4 for the timing and detailed descriptions of all the study procedures and assessments to be performed in each treatment.

If a subject experiences a spontaneous dyspnea episode any time throughout the inpatient study period (beginning with first dose administration), specific study procedures need to be completed sequentially as described in Table 3. These procedures should be completed at the onset of each dyspnea episode with the exception of an occurrence during cycle exercise testing.

If a dyspnea episode manifests intermittent symptoms within short time intervals (hours), generally if symptoms are similar and no initial abnormalities are observed on assessments, repeat assessments may be performed as needed at the investigator's judgement to monitor the subject's condition. Other diagnostic studies, such as echocardiogram or chest x-ray, may be performed to evaluate dyspnea at the investigator's discretion.

If a dyspnea episode subjectively worsens, assessments should be repeated at the investigator's discretion. The subject's dyspnea should resolve, or return to subject's baseline, before subject proceeds on to Period II (see Section 3.3.5.1).

Subjects will be discharged from the study center on Study Day 5 , after the completion of all protocol-driven procedures and assessments and at the discretion of the investigator. At discharge, subjects will be reminded of the study restrictions listed in Section 3.3.4 and instructed when to return for admission to Period II.

3.1.3 Washout Period

There will be an outpatient washout period of 7 - 10 days between Periods I and II. The washout period will be counted starting from Study Day 5 of Period I.

3.1.4 Period II/Visit 3

Each day will be based on a 24-hour clock beginning at midnight (00:00).

Subjects will be admitted to the study center on Study Day –1 and will reside in the study center from Study Day-1 until completion of all protocol-driven procedures and assessments on Day 5 of Period II. The assessments on Day –1 of Period II, will reconfirm subject eligibility to participate in the study.

Subjects will crossover to receive the alternate treatment (Treatment A or B) given in Period I as described in the previous Section 3.1.2.

Subjects will be discharged from the study center on Study Day 5, after the completion of all protocol-driven procedures and assessments and at the discretion of the investigator. At discharge, subjects will be reminded of the study restrictions listed in Section 3.3.4 and instructed when to return for the follow-up visit.

3.1.5 Follow-up/Visit 4

Subjects return to the study center for the Follow-up Visit within 4 - 7 days of Study Day 5 of Period II. Please refer to Section 4.1.2 and Table 1 for the procedures and assessments to be completed at the Follow-up Visit.

Figure 1 Study Flow Chart

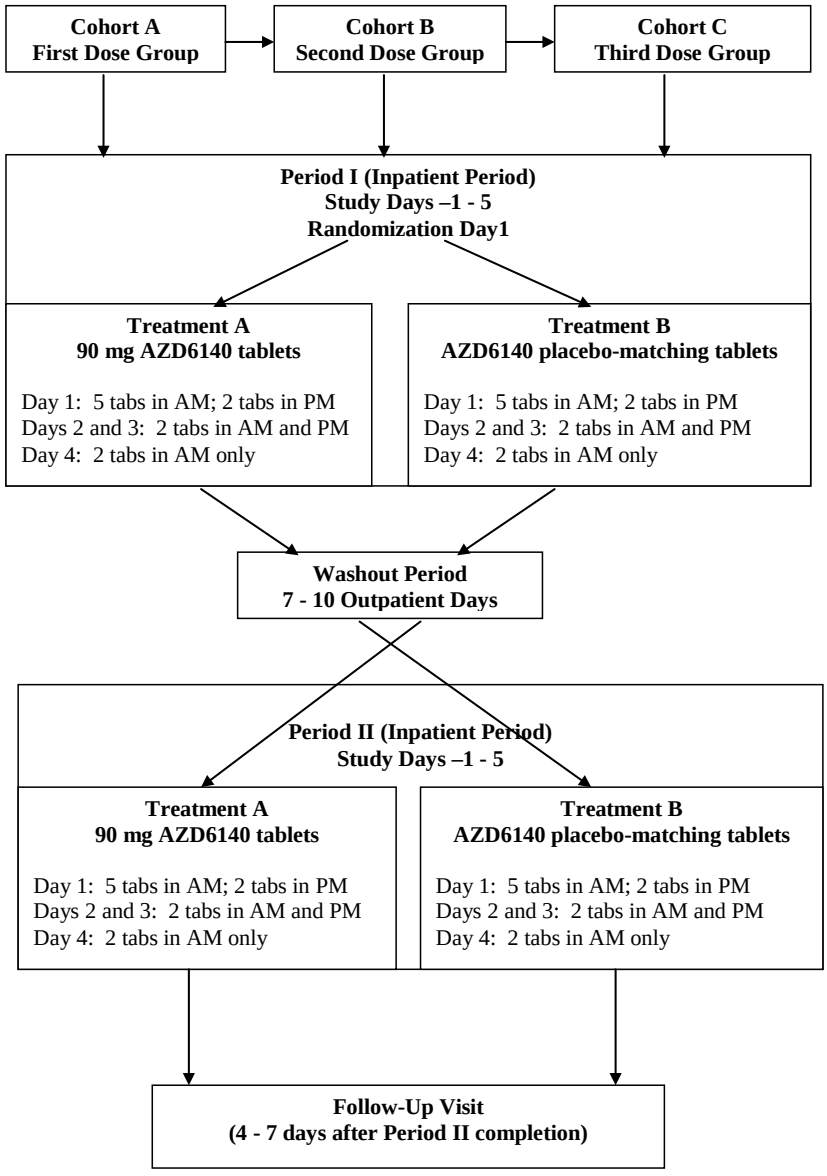

Table 1 Overall Study Plan

Study Assessment	Visit 1[a] Screening	Visit 2 Period I	Visit 3[b] Period II	Visit 4[c] Follow-up
Informed consent[d]	X			
Demographics	X			
Inclusion/exclusion criteria	X	X	X	
Medical/surgical history	X			
Clinical laboratory assessments	X	X	X	X
Urine drug screen	X	X	X	
HIV antibody & hepatitis B and C screens	X			
Breath ethanol test		X	X	
Oral temperature	X	X		X
BP and pulse	X	X	X	X
Height and weight	X			X[e]
Complete physical examination	X			X
Brief physical examination		X	X	
12-lead ECG	X	X	X	X
Genetic blood sample[d, f]		X		
Inpatient stay		X	X	
Randomization[f]		X		
AZD6140 or placebo administration		X	X	
AZD6140/AR-C124910XX PK blood samples		X	X	
Modified Borg Scale/Bidirectional Dyspnea Index[g]	X	X	X	
Minute ventilation (including respiratory rate)[g]	X	X	X	
Spirometry(FEV_1, FVC)[g]	X	X	X	
Cardiopulmonary exercise test[g]		X	X	
Arterial blood gas[h]		X	X	
Pulse oximetry		X	X	
Concomitant medication monitoring	X	X	X	X
Adverse event monitoring[i]	X	X	X	X

a. Within 21 days of Day 1, Period I.
b. There will be a 7–10 day washout period between Period I and Period II.
c. Within 4 to 7 days of Period II(Day 5).
d. Separate consent required for genetic sampling.
e. Weight only.
f. Prior to study drug administration in Period I.
g. Subjects will be shown the basic assessments at the screening visit to ensure they can comply with the procedures required of the study. A full cardiopulmonary exercise test is not performed at screening; however, subjects will be asked to demonstrate that they can utilize the equipment for the test and will be familiarized with the procedure.
h. Arterial blood gas will **only** be assessed if a subject experiences an acute episode of dyspnea.
i. SAEs will be collected from the time of informed consent obtained through the Follow-up visit. Non-SAEs will be collected from the time of 1st dose on Day 1, Period I through the Follow-up visit.

Table 2 Study Plan Periods I and II

Period I (Visit 2) and Period II (Visit 3)[a]																						
Study Day	**-1**	**1**							**2**			**3**			**4**							**5**
Study hour relative to dose		**PreDose**	**0**	**2**	**3**	**4**	**8**	**12**	**0**	**3**	**12**	**0**	**3**	**12**	**0**	**2**	**3**	**4**	**6**	**8**	**12**	**0**
Admission/inpatient stay	X----------																					X
Inclusion/exclusion criteria	X																					
Medical history update	X																					
Clinical laboratory assessments	X											X										X
Brief physical examination	X																					X
Breath ethanol test	X																					
Urine drug screen	X																					
12-lead ECG	X																					
Oral temperature	X																					X
BP and heart rate	X	X																				X
Randomization (AZD6140 or placebo)[b]			X																			
Pharmacogenetic sample[b]		X																				
AZD6140/placebo administration			X					X	X		X	X		X	X							
AZD6140/AR-C124910XX PK sample[c]		X		X	X	X	X	X	X	X		X	X		X	X	X	X		X	X	X
Modified Borg Scale /Bidirectional Dyspnea Index		X		X	X	X	X	X	X	X		X	X		X	X	X	X		X	X	X
Minute ventilation including respiratory rate		X		X	X	X	X	X	X	X		X	X		X	X	X	X		X	X	X
Spirometry (FEV_1, FVC)		X		X	X	X	X	X	X	X		X	X		X	X	X	X		X	X	X
Pulse oximetry		X		X	X	X	X	X	X	X		X	X		X	X	X	X		X	X	X

Period I (Visit 2) and Period II (Visit 3)[a]																						
Study Day	**-1**	**1**							**2**			**3**			**4**							**5**
Study hour relative to dose		**PreDose**	**0**	**2**	**3**	**4**	**8**	**12**	**0**	**3**	**12**	**0**	**3**	**12**	**0**	**2**	**3**	**4**	**6**	**8**	**12**	**0**
Cardiopulmonary exercise test																			X			
Concomitant medication monitoring	X----------X																					
Adverse event monitoring	X----------X																					
Discharge from study center																						X[d]

a. Separated by 7 – 10 day washout period.
b. Prior to dose administration on Study Day 1 of Period I only.
c. PK sample to obtained prior to dose administration .
d. After completion of all study related procedures.

Table 3 Procedures for Spontaneous Dyspnea Episode

Period I (Visit 2) and Period II (Visit 3)	
Procedure[a]	**Sequence**
Borg Dyspnea Index/Bidirectional Dyspnea Index	1
Minute ventilation including respiratory rate	2
Pulse oximetry	3
Arterial blood gas(ABG)	4
Spirometry(FEV_1, FVC)	5
AZD6140/AR-C124910XX PK sample	6

a. The first time a volunteer reports experiencing dyspnea, and for subsequent episodes therefter, these procedures are to be completed sequentially. Additionally, at the investigator's discretion, other diagnostic tests such as echocardiography or chest x-ray may be performed to evaluate dyspnea

3.2 Rationale for study design, doses and control groups

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

The objective of this study is to evaluate the effect of AZD6140 on the respiratory symptoms and physiological parameters that may be associated with the sensation of dyspnea in healthy volunteers, patients with mild asthma, and patients with mild COPD. Dyspnea was reported in association with AZD6140 administration in DISPERSE, a phase IIa study in outpatients with documented atherosclerosis, but not in healthy volunteers in the Phase I studies completed. The DISPERSE study assessed pharmacodynamic effects of AZD6140 at doses of 50 mg twice daily, 100 mg twice daily, 200 mg twice daily, and 400 mg once daily in the presence of acetyl salicylic acid (ASA) compared to clopidogrel plus ASA in subjects with documented atherosclerotic disease by evaluation of ADP-induced platelet aggregation and bleeding time. Two hundred subjects were enrolled with 163 exposed to AZD6140. Approximately 20% patients reported dyspnea at the 400 mg dose level, with 5 patients in that group reporting dyspnea within the first 3 days of receiving an AZD6140 dose. Most reports were mild and resolved with continued dosing. One patient reported symptoms of moderate intensity and discontinued the study. Of approximately 200 healthy volunteers that have received multiple doses of AZD6140 in Phase I studies, there has been only one report of dyspnea (unrelated to study drug).

Mechanistically, AZD6140 may affect the adenosine A3 receptor, and pre-clinical observations include increased respiratory rate in rats. The hypothesis of this observation is that AZD6140 may affect respiratory drive through adenosine receptors, with differing sensitivity to the sensation of dyspnea evoked by change in respiratory drive between healthy volunteers and patients. Therefore, this study will assess the effect of AZD6140 on respiratory parameters, and will also provide safety data on the effect of AZD6140 on

respiratory drive in mild asthma and COPD patients which will be of utility to later clinical studies.

This will be a double-blind, randomized, placebo-controlled, 2-period crossover, 3 cohort, pharmacodynamic study. Healthy volunteers are included as well as patient population (mild asthma and COPD patients) so that qualification of the differences in physiologic response and subjective experience of dyspnea can be compared to a healthy population.

Asthma and COPD patients are included primarily to ascertain if the physiologic response differs from healthy volunteers as this observation was only observed in a patient population and not in healthy volunteers in Phase I studies. Additionally, examining any dyspnea effects in COPD and asthma patients as compared to healthy volunteers will help to establish safety parameters. Healthy volunteers will be studied first to increase safety. The age range encompasses that of the Phase II study where dyspnea was first observed. Mild disease patients are chosen to minimize impact on any underlying disease state.

The primary variable will be respiratory rate and minute ventilation, based on the hypothesis of affecting adenosine receptors and preclinical observations. Cardiopulmonary exercise testing is included because provocation of the system may elicit measurable changes in physiological parameters that are not otherwise observed. Exercise testing also provides an opportunity to simulate the conditions that could contribute to dyspnea during daily activities.

A loading dose of 450 mg is chosen to maximize the potential of eliciting dyspnea. This dose will establish safety margins for a clinical loading dose that may be up to 270 mg. Dosing to 3 days will allow for attainment of steady-state plasma concentrations. Again, this will provide for assessment of dyspnea under clinically relevant conditions. A placebo crossover will be performed because dyspnea is a highly subjective sensation, and therefore comparison to a placebo baseline is desirable.

A retrospective genetic analysis to explore the genetic variables in genes that are important to absorption and disposition of, and response to AZD6140 may be carried out. Genetic sampling will be optional to all subjects enrolled. Subjects will receive the same treatment and care in the main study whether or not subjects donate a blood sample for genetic research. The genetic data from this study may be pooled with genetic results from other studies on AZD6140 to generate hypotheses to be tested in future studies.

3.3 Selection of study population

3.3.1 Study selection record

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

3.3.2 Inclusion criteria

For inclusion in the study subjects must fulfill all of the following criteria:

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

2. Generally healthy male or female ages 55 to 75 years, inclusive; within the following 3 cohorts:

Cohort	Volunteer Type	Respiratory criteria
A	Healthy	N/A
B	Mild Asthmatic	Clinical diagnosis > 1 year; $FEV_1 \geq 70\%$ predicted; no exacerbation in past 8 weeks; reversibility demonstrated within the past year ($\geq$ 12% or 200 mL)
C	Mild COPD	Clinical diagnosis > 1 year; $FEV_1 \geq 60\%$ predicted; no exacerbation in past 8 weeks; FEV_1/FVC ratio < 70%; history of tobacco smoking > 10 pack year

3. Weight at least 50 kg and have a BMI between 18 to 30 kg/m^2 inclusive unless approved by the sponsor and investigator. (BMI will be calculated as weight in kg/height in m^2 and will not be reported on the case report form.);

4. Females must be post-menopausal (cessation of regular menses for 12 months) or surgically sterile;

5. Have normal physical examination, laboratory values and vital signs, unless the investigator considers an abnormality to not be clinically significant or a symptom of mild COPD or mild asthma (Cohort B and Cohort C patients);

6. Subjects are able to communicate with the investigator, and to understand and comply with all study requirements.

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

1. Provision of informed consent for genetic sampling and analyses

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

3.3.3 Exclusion criteria

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

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

2. Use of prescription medication for a chronic medical condition within 2 months of Day 1 of Period I (Refer to Appendix C: Restricted Medications) unless approved by the investigator and sponsor;

3. Use of prescription medication for an acute medical condition within 4 weeks of Day 1 of Period I unless approved by the investigator and sponsor;

4. Use of aspirin, ibuprofen, or any other drug known to increase the propensity for bleeding within 2 weeks prior to Day 1 of Period I;

5. Use of leukotriene antagonists within 7 days of Day 1, Period I;

6. Use of over-the counter preparations including herbal remedies such as Cordyceps sinensis, dan shen, feverfew, Ganoderma lucidum, ephedra, echinacea, St. John's Wort, and garlic, [aged extract taken on an ongoing basis], ginseng, ginkgo, and vitamin preparations within 7 days of Day 1 of Period I unless approved by the investigator and sponsor;

7. A history or presence of neurological, hematological, psychiatric, gastrointestinal, hepatic, or renal disease, or other condition known to interfere with the absorption, distribution, metabolism or excretion of drugs unless approved by the investigator and sponsor;

8. A personal history of vascular abnormalities including aneurysms; a personal history of severe hemorrhage, hematemesis, melena, hemoptysis, severe epistaxis, or intracranial hemorrhage; rectal bleeding within 3 months prior to screening;

9. History suggestive of peptic ulcer disease or bleeding diatheses;

10. History of diabetes, claudication, CHF (congestive heart failure) or myocardial infarction in the past year;

11. History of anxiety disorder;

12. History of intubation/mechanical ventilation due to respiratory disease or bronchospasms;

~~10.~~13. Platelet count <100,000/mm^3 at screening;

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~~11.~~14. A significant history of alcohol or substance abuse within the past year;

~~12.~~15. Surgery or significant trauma within 3 months prior to Day 1 of Period I;

~~13.~~16. Current tobacco use or history of tobacco or nicotine containing products use in the past 6 months;

~~14.~~17. Positive test results for HIV, (human immunodeficiency virus) HBsAg, (hepatitis B surface antigen) or hepatitis C antibody (anti-HCV);

~~15.~~18. Positive urine drug screen unless resulting from declared and confirmed medication;

~~16.~~19. Receipt of an investigational drug within 60 days prior to Day 1 of Period I;

~~17.~~20. Previous participation in an AstraZeneca AZD6140 study;

~~18.~~21. Consumption of Seville oranges (e.g. orange marmalade) or grapefruit-containing products, alcohol, medicines or nutritional supplements within 1 week before Day 1 of Period I unless approved by the sponsor and investigator;

22. Consumption of caffeine-containing or methylxanthine products within 48 hours prior to Day 1 of each study period;

~~19.~~23. Blood donation within 90 days before Day 1 of Period I;

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

25. A suspected/manifested infection according to WHO risk categories 2, 3 and 4 (Refer to Appendix D);

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

3.3.4 Restrictions

Subjects will be required to adhere to the following restrictions:

1. No alcohol consumption will be allowed from 1 week prior to Day 1 of Period I, and through completion of the Follow-up Visit;

2. No consumption of caffeine-containing or methylxanthine products will be allowed within 48 hours prior to Day 1 of each study period through inpatient discharge on Day 5;

3. Seville oranges and grapefruit containing products will be restricted for 1 week prior to Day 1 of Period I, and through completion of the Follow-up Visit;

4. Subjects will be required to refrain from strenuous physical exercise within 48 hours of Day 1 of Period I, and through completion of the Follow-up Visit with the exception of the study required cardiopulmonary exercise test on Study Day 4 of Period I and II;

5. Subjects should refrain from over-the-counter preparations including herbal remedies such as Cordyceps sinensis, dan shen, feverfew, Ganoderma lucidum, ephedra, echinacea, St. John's Wort, and garlic, [aged extract taken on an ongoing basis], ginseng, ginkgo, and vitamin preparations within 7 days prior to Day 1 of Period I, and through completion of the Follow-up Visit unless approved by the investigator and sponsor;

6. Refrain from taking aspirin, ibuprofen, or any other drug known to increase the propensity for bleeding for 2 weeks before Day 1 of Period I, and through completion of the Follow-up Visit;

7. Scheduled surgery, including dental surgery, at anytime following the screening visit, and through completion of the Follow-up Visit.

8. No use of tobacco or other nicotine-containing products from screening and throughout the Follow-up Visit.

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 are at any time free to discontinue their participation in the study without prejudice to further treatment
- Safety reasons as judged by the investigator and/or AstraZeneca including moderate to severe dyspnea that does not resolve or return to subject's baseline within 2 weeks.
- Severe non-compliance to protocol as judged by the investigator and/or AstraZeneca.
- Incorrect enrollment (ie, the subject does not meet the required inclusion/exclusion criteria) or randomization (ie, the subject is not allocated study drug as described in the protocol) of the subject.
- Subject lost to follow-up
- A suspected/manifested infection according to WHO risk categories 2, 3, and 4 (Refer to Appendix D).

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(s). 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.

3.3.5.3 Procedures for Discontinuation from Genetic aspects of the study

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

- agrees to the genetic sample and any DNA extracted from the sample being kept for genetic analyses in the future.
- withdraws consent for the sample to be kept for genetic analysis in the future and wishes the sample to be destroyed. Destruction of the sample (or the DNA extracted from the sample) will only be possible so long as the particular sample is traceable. In the event that DNA analysis has already been performed, AstraZeneca will retain the results and associated data for regulatory reasons but these will not be used in any subsequent analyses.

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

The appropriate process will be that the investigator contacts the study site monitor in the first instance, and then the study the monitor will forward this notification at the earliest possible opportunity to the head of the Clinical Genotyping Group (CGG). The CGG group will provide a written confirmation of the actions taken. The address of the CGG group is: Clinical Genotyping Group (CGG), Block 17, Mereside, Alderley Park, Macclesfield, UK, SK10 4TG, Tel: +44 (0) 1625 230959, Fax: +44 (0) 1625 230958.

3.4 Treatment(s)

AstraZeneca R & D Charnwood will supply all AZD6140 tablets and AZD6140 placebo-matching tablets. The investigator or institution has the responsibility to establish a system for handling study treatments, including investigational medicinal products, so as to ensure that:

1. Deliveries of such products from AstraZeneca Investigational Product (IPS) are correctly received by the investigator or his designee.
2. Such deliveries are recorded on the drug log.
3. Study treatments are handled and stored safely and properly.
4. Study treatments are only dispensed to study subjects in accordance with the protocol

5. Unused products are accounted for and returned to designated facility or AstraZeneca for destruction

3.4.1 Investigational Product(s)

3.4.1.1 Identity of investigational product

Table 4 Identity of Investigational Product

Investigational product	Dosage form and strength	Manufacturer	Formulation Number	Ingredients
AZD6140	90 mg immediate release tablets	AstraZeneca R & D Charnwood, UK	307	AZD6140, Mannitol, Dibasic calcium phosphate, Povidone K30 (PVP K30), Croscarmellose sodium, Magnesium stearate. Film coat: Hydroxypropyl methylcellulose 2910, titanium dioxide, lactose monohydrate, polyethylene glycol 3000, triacetin, iron oxide red, iron oxide yellow, iron oxide black
AZD6140 Placebo	Tablet, containing zero active therapy (identical in appearance to active tablets)	AstraZeneca R & D Charnwood, UK	234	Lactose monohydrate, Microcrystalline cellulose, Magnesium stearate Film coat: Hydroxypropyl methylcellulose 2910, titanium dioxide, lactose monohydrate, polyethylene glycol 3000, triacetin, iron oxide red, iron oxide yellow, iron oxide black

3.4.1.2 Labelling

AZD6140 and placebo-matching AZD6140 will be packed in individual wallet cards and labelled with randomization number and period for dosing. Each wallet card will contain a blister pack containing 21 tablets (overage included) of AZD6140 or placebo with a detachable tear-off label. Wallet cards labeled for Period I and Period II will be packed into a carton labelled with a single panel label.

The supplies will be labelled with the following information in accordance with Good Manufacturing Practice (GMP) and local regulatory requirements:

- **name of sponsor and address**
- **randomization number**

- **product name, dosage form, and quantity of dosage units**
- **Period I or II**
- **directions for use**
- **storage conditions**
- **lot number**
- **Keep out of reach of children**
- **Caution: New Drug - Limited by Federal (or USA) Law to Investigational Use**

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 label and investigator brochure.

3.4.1.4 Accountability

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

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

3.4.2 Doses and treatment regimens

Each subject will receive each of the following treatments in one of two treatment sequences (AB or BA) according to the randomization schedule:

Treatment A: A single, oral dose of 450 mg AZD6140 (5 x 90 mg immediate release tablets) will be administered in the AM of Study Day 1; followed by a single, oral dose of 180 mg AZD6140 (2 x 90 mg immediate release tablet) in the PM (12 hours after the first dose); thereafter, 180 mg AZD6140 (2 x 90 mg immediate release tablet) will be administered bid (every 12 hours) on Study Days 2 and 3 and in the AM only on Study Day 4.

Treatment B: A single, oral dose of AZD6140 matching placebo (5 placebo tablets) will be administered in the AM of Study Day 1; followed by a single, oral dose of AZD6140 matching placebo (2 placebo tablets) in the PM (12 hours after the first dose); thereafter, AZD6140 matching placebo (2 placebo tablets) will be administered bid (every 12 hours) on Study Days 2 and 3 and in the AM only on Study Day 4.

All doses given in each treatment will be administered with 240 mL of room temperature water while the subject is sitting in an upright or in a semi-recumbent position. Subjects must remain either sitting or semi-recumbent for at least 2 hours after each dose intake.

On Study Days 1 and 4 of each treatment, the subjects will receive the AM study medication following an overnight (10 hour) fast and will continue to fast for 4 hours post dose. The PM

dose on Study Days 1, 2 and 3 will be given either 1 hour before or 1 hour after food intake. On Study Day 2 and 3, breakfast will be served 1 hour after the AM study medication.

3.4.3 Method of assigning subjects to treatment groups

Written informed consent will be obtained before enrollment and the volunteers identified with an enrollment number starting with E0001001. Subjects fulfilling the eligibility criteria will be assigned subject numbers starting with either number **101** (Cohort A), **201** (Cohort B) or **301** (Cohort C).

Using the AZ Global Randomization system (Grand) by AstraZeneca LP, volunteers treatment will be allocated as generated by the system.

Cohort	Volunteer Type	Randomization Number
A	Healthy Volunteers	101, 102, 103, 104, etc.
B	Mild Asthma	201, 202, 203, 204, etc.
C	Mild COPD	301, 302, 303, 304, etc.

Subjects will be randomized strictly sequentially within their Cohort as subjects are eligible for randomization on Day 1 of Period I before study drug administration. If a subject should be incorrectly randomized, randomization should continue with no attempt to correct the error. If a subject discontinues from the study, the randomization number will not be re-used and the subject will not be allowed to re-enter the study. Subjects who withdraw after having received study medication may be replaced by a subject who will be assigned to the same treatment sequence.

Each treatment group will consist of the same combination of matching active and placebo tablets. There will be four additional individual drug packages supplied for each cohort in the event of misrandomization, early withdrawal, etc. These will be used only as back-up and not for over enrolling subjects.

3.4.4 Blinding and procedures for unblinding the study

3.4.4.1 Methods for ensuring blinding

The treatment allocation in this study will be double-blind. The active study medication or placebo assignment will be blinded to both the subjects and to the investigator. To ensure the blinding of the treatments, matching AZD6140 placebo will be provided. Packaging, labelling and preparation of investigational products will be performed in a way that will ensure the blinding throughout the study. Neither the sponsor's representative responsible for monitoring the study, the study personnel, nor the investigator will know whether study drug or placebo has been allocated.

3.4.4.2 Methods for unblinding study

Individual treatment codes, indicating the treatment randomization for each randomized volunteer, will be available to the investigator or pharmacist at the study center.

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

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

The decision to dose the next cohort will be made following the review of the safety variables and without breaking of the study blind by the investigator and study team.

3.4.5 Concomitant medication

All subjects should refrain from taking any of the following medications:

1. prescribed medication from 4 weeks prior to the 1st dose of study drug until completion of the assessments and procedures scheduled during the Follow-up Visit that are known to inhibit or induce CYP3A4 isoenzymes and medications; (Refer to Appendix C: Restricted Medications). Other medications that are permitted with the approval of the investigator and sponsor include medications the patient has been stable on for at least 2 months prior to Study Day –1 of Period I and may include, but are not limited to: hormone replacement therapy, inhaled corticosteroids, thiazide diuretics, ACE inhibitors, and angiotensin II inhibitors.

2. OTC preparations that include herbal remedies and vitamin preparations from 1 week prior to the 1st dose of study drug until completion of the assessments and procedures scheduled during the Follow-up Visit unless approved by the investigator and sponsor.

3. aspirin, ibuprofen, or any other drug known to increase the propensity for bleeding are specifically prohibited from 2 weeks prior to the 1st dose of study drug until completion of the assessments and procedures scheduled during the Follow-up Visit, unless approved by the investigator and sponsor

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 paper case report form (pCRF).

3.4.5.1 Concomitant medication restrictions for Cohort B and Cohort C patients

Cohort B and Cohort C patients should refrain from taking any of the following medications in addition to those listed in Section 3.4.5:

1. Tiotropium within 7 days of Study Day 1 of Period I until completion of the assessments and procedures scheduled on Study Day 5 of Period II;

2. Atrovent within 12 hours of Study Day 1 until completion of the assessments and procedures scheduled on Study Day 5 (Periods I and II);

3. Long-acting β-agonists within 72 hours of Study Day 1 until completion of the assessments and procedures scheduled on Study Day 5 (Periods I and II);

4. Short-acting β-agonists (i.e., albuterol) within 8 hours of Study Day 1 until completion of the assessments and procedures scheduled on Study Day 5 (Period I and II). These may be allowed on as needed basis with the investigator approval. However if required during an acute dyspnea episode, it is preferred that respiratory assessments as defined in Table 3 be completed first.

5. Leukotriene antagonists within 7 days of Study Day 1 of Period I until completion of the assessments and procedures on study Day 5 of Period II.

6. OTC bronchodilators (i.e., Primatine Mist) within 7 days of Study Day 1 of Period I until completion of the assessments and procedures on study Day 5 of Period II.

3.4.6 Treatment compliance

Compliance will be assured by supervised administration of the investigational product by the investigator and/or his or her designee.

4. MEASUREMENT OF STUDY VARIABLES

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

- Modified Borg Scale/Bidirectional Dyspnea Index
- Minute ventilation including respiratory rate
- Pulse oximetry
- ABG (will only be performed if a subject experiences an acute episode of dyspnea)
- Spirometry

- Blood pressure/heart rate
- ECG
- PK sample

Note: In order to collect PK sampling at the precise scheduled time, other assessments may be initiated prior to the time point to ensure that the PK sample is collected at the scheduled time. The exact time for PK and PD samples will be recorded in the CRF.

4.1 Medical examination and demographic measurements

4.1.1 Enrollment medical examination and demographic measurements

Each subject will undergo an enrollment medical examination within 21 days before Day 1, Period I. This will consist of the following: (Please refer to Section 4.4 for detailed descriptions of the assessments.)

- A written and approved informed consent form must be signed and dated before screening procedures are performed (the informed consent form must be approved by AstraZeneca and the investigator's Institutional Review Board [IRB])
- Review of inclusion and exclusion criteria
- A standard medical and surgical history and drug history
- Recording of demographic data - date of birth, sex, height, weight, race
- Complete physical examination
- 12-lead ECG
- A blood sample for human immunodeficiency virus (HIV) antibodies, hepatitis B surface antigen (HBsAg), and hepatitis C antibodies (anti-HCV)
- Breath ethanol test
- A fasting blood sample for standard clinical chemistry and hematology assessments, and a mid-stream urine sample for urinalysis and drugs of abuse screen
- Blood pressure and heart rate, height (cm), weight (kg), and oral temperature in °C
- Adverse event and concomitant medication monitoring.

4.1.2 Post-study medical examination

Subjects will return to the study center within 4 to 7 days after Study Day 5 of Period II for the Follow-up visit. At this visit the following procedures and assessments will be performed: (Refer to Section 4.4 for detailed descriptions of the assessments.)

- Complete physical examination
- 12-lead ECG
- Fasting blood sample for standard clinical chemistry and hematology assessments, and a mid-stream urine sample urinalysis
- Blood pressure and heart rate, weight (kg), and oral temperature (°C)
- Adverse event questioning and concomitant medication monitoring

At the completion of the Follow-up visit (Visit 4) procedures and assessments, volunteers may be discharged from the study at the discretion of the investigator.

4.2 Pharmacokinetic measurements

For timing of individual PK samples refer to Table 2 and Table 5.

4.2.1 AZD6140 and AR-C124910XX

4.2.1.1 Determination of drug concentration in biological samples

Samples for measurement of drug concentration of AZD6140 and its metabolite, AR-C124910XX, in plasma will be analysed by York Bioanalytical Solutions, Upper Poppleton, York, UK using fully validated bioanalytical methods. Details of the methods used will be provided in the clinical study report (CSR). Samples will be disposed of after the CSR has been finalized.

4.2.1.2 Collection and processing of biological samples for determination of AZD6140/AR-124910XX in plasma

Venous blood samples (2 mL) for determination of AZD6140 and AR-C124910XX concentrations in plasma will be taken at the times presented in Table 2, Table 3 and Table 5. Blood samples will be collected, labelled and shipped as detailed below. The date and time of collection will be recorded on the appropriate CRF.

Blood will be collected according to site procedure. Disposable needles and disposable and lithium heparinized tubes shall be used. Individual venipunctures for each time point may be performed or an indwelling catheter may be used. Between two consecutive blood samples, the IV cannula of the catheter will be closed using a single-use stylet to avoid the use of saline and from discarding any blood. Blood samples (2 mL) will be collected into a lithium-heparinized tube. The heparin and blood will be carefully mixed. The sample will be placed on ice until centrifugation, which will begin within 30 minutes after the sample is obtained.

The sample will be centrifuged for 10 minutes at 4°C at a relative centrifugal force of 1500g. The resulting plasma will be transferred to a 2.0 mL conical polypropylene tube with screw cap and immediately frozen upright at -20°C or below in a non frost-free freezer and kept frozen at this temperature before, during and after transport to the designated laboratory.

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

4.2.1.3 Labelling of AZD6140 and/AR-C124910XX plasma samples for shipment to AstraZeneca

The labels supplied by AstraZeneca R & D Wilmington, DE must be applied to the plasma sample tubes. The labels should include the following information for the ***scheduled PK sample***s in Periods I and II:

Study number: D5130C00028

Subject number:

Tube number:

Period I or II/Study day:

Scheduled time:

Analyte: AZD6140/AR-C124910XX

Matrix: PLASMA

Table 5 Schedule of AZD6140/AR-C124910XX PK blood sampling and tube numbers

Periods I and II			
Study Day	**Analyte**	**Scheduled Time Relative to Dose (Hours)**	**Tube Number**
Day 1	AZD6140/AR-C124910XX	Predose*	1
	AZD6140/AR-C124910XX	2	2
	AZD6140/AR-C124910XX	3	3
	AZD6140/AR-C124910XX	4	4
	AZD6140/AR-C124910XX	8	5
	AZD6140/AR-C124910XX	12*	6
Day 2	AZD6140/AR-C124910XX	0*	7
	AZD6140/AR-C124910XX	3	8
Day 3	AZD6140/AR-C124910XX	0*	9
	AZD6140/AR-C124910XX	3	10
Day 4	AZD6140/AR-C124910XX	0*	11

Periods I and II			
Study Day	**Analyte**	**Scheduled Time Relative to Dose (Hours)**	**Tube Number**
	AZD6140/AR-C124910XX	2	12
	AZD6140/AR-C124910XX	3	13
	AZD6140/AR-C124910XX	4	14
	AZD6140/AR-C124910XX	8	15
	AZD6140/AR-C124910XX	12	16
Day 5	AZD6140/AR-C124910XX	0	17

- Blood sample must be drawn prior to dose administration.

The labels for ***unscheduled PK samples*** collected during a spontaneous dyspnea episode will include the following information:

Study number: D5130C00028

Subject number:

Period I or II/Study Day:

Collection time:

Analyte: AZD6140/AR-C124910XX

Matrix: PLASMA

4.2.1.4 Shipment of AZD6140/AR-C124910XX plasma samples to AstraZeneca

All PK plasma samples accompanied by the specimen shipment logs will be shipped via an agreed upon overnight courier (World Courier). The frozen samples must be packed securely to avoid breakage during transit, should be double bagged to contain leaks and packed with a sufficient quantity of dry ice to ensure they remain frozen for at least 72 hours to allow for delays in shipment. The samples from each subject will be placed in separate bags and labelled as instructed in Section 4.2.1.2. All applicable shipping regulations must be followed. Documentation sufficient to identify each sample must be included in the shipment. **The primary contact, , and the designated laboratory identified below must be notified by e-mail and fax at the time samples are shipped. The fax notification should include a copy of the specimen shipment log.**

Samples should only be shipped on Monday through Wednesday. Do not ship on or within two days prior to a legal holiday.

Plasma samples should be shipped to:

Notification to DMPK Sweden:

4.3 Pharmacodynamic measurements

4.3.1 Modified Borg Scale

The modified Borg CR-10 scale will be used to assess the subject's perception of dyspnea at the times pre-specified in the study plan, as well as during the cycle exercise test. Numeric measurement will be recorded on the pCRF and the scale will be kept in the appropriate subject's source document file~~file~~s. (Refer to Appendix E.)

The subject should be given the following instructions:

"This is a scale for rating breathlessness. The number "0" represents no breathlessness. The number 10 represents the strongest or greatest breathlessness you have ever experienced. Prior to completing the minute ventilation test and spirometry tests, you will be asked to point to a number with your finger, which represents your perceived level of breathlessness at that time. Use the written description to the right of the number to help guide your selection. I will say the number out loud in order to confirm your choice. If you have an even stronger or greater intensity of breathlessness than you have ever previously experienced, you should then point to the word "maximal" if the severity is greater than 10."

The subject should be given the following additional instructions during the cycle exercise test:

"Each minute during the exercise test you will be asked to complete this assessment. During the cycle exercise you may have an even stronger or greater intensity of breathlessness than you have ever previously experienced. You should then point to the word "maximal", if the severity is greater than 10. You can tell us this number after the mouthpiece has been removed."

The numeric value the subject reports should be recorded on the pCRF.

4.3.2 Bidirectional Dyspnea Index

The Bidirectional Dyspnea Index is a category scale in which words describing varying degrees of ease or difficulty of breathing are linked to number (+5 to –5). Thus, subjects categorize their sense of breathing effort during study time points as being unchanged (zero), slightly (-1), moderately (-2 to –3), or markedly (-4 to –5) easier; or slightly (+1), moderately (+2 to +3), or markedly (+4 to + 5) harder than during the immediately preceding period. (Refer to Appendix F.)

The numeric value the subject reports should be recorded on the pCRF.

4.3.3 Respiratory rate

Respiratory rate should be assessed after the subject has been quietly at rest for at least 10 minutes. Respiratory rate should be assessed by counting the number of breaths for a full minute by direct observation or palpation of the subject's chest wall. A numeric value of the number of breaths per minute as well as the quality of the breaths, (i.e., normal, shallow, labored) should be recorded on the pCRF for each time point observed.

4.3.4 Minute ventilation

The total volume of gas that enters or leaves the lungs each minute is referred to as the minute ventilation. It is the product of tidal volume and respiratory rate. Minute ventilation (VE), tidal volume (V_T), and breathing frequency (*f*) will be determined with Crapo flowmeter. Volume, barometric pressure, and temperature calibrations will be completed before each test period.

V_E (L/min), V_T (mL/min) and *f* (b/min) will be recorded on the pCRF for each time point measured.

4.3.5 Spirometry

Spirometry will be performed for asthma and COPD patients at screening to establish entry criteria. Additionally, healthy volunteers will be given the opportunity to attempt spirometry at screening visit to ensure that they can adequately perform the testing during study assessments.

Spirometry will be measured in the seated position using the KoKo testing system. The highest values for forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1) selected from a minimum of three FVC maneuvers will be recorded. Subjects should be allowed time to recover before starting the next test in the series of three; however, the time between tests should not exceed 3 minutes. Predicted values for spirometry were taken from Crapo[3].

FVC, FEV_1 and FEV_1/FVC ratio should be recorded on the pCRF.

4.3.6 Arterial blood gas

An arterial blood gas sample will be drawn from a subject's radial artery using standard technique as per the study center's Standard Operating Procedure if a subject experiences an acute dyspnea episode (Refer to Table 3).

PCO_2, PO_2, and pH should be recorded on the pCRF.

4.3.7 Cycle exercise testing

Cycle exercise testing will be performed using methodology described by O'Donnell et al. (O'Donnell, 1998; O'Donnell, 1993)[4,5]. Minute ventilation (V_E), tidal volume (V_T), oxygen saturation(SaO_2) by pulse oximetry, and oxygen uptake (VO_2) will be monitored throughout exercise testing and during exercise recovery.

On Study Day 4 of Periods I and II, an incremental cycle exercise test will be performed to a symptom-limited maximum (maximum work capacity). Refer to Table 2 for specific time.

4.3.7.1 Testing equipment

Exercise testing will be conducted using a cardiopulmonary exercise testing system and an electronically braked cycle ergometer, with a constant pedaling frequency of 50-70 revolutions per minute (rpm). Subjects will breath through a mouthpiece attached to a low resistance flow transducer. This mouthpiece and valve setup will be adjusted to a fixed height for each subject, thus ensuring a relatively fixed position of the torso. Oxygen saturation by pulse oximetry and electrocardiographic monitoring will also be carried out continuously throughout testing.

The cycle ergometer should have a range of at least 0-600W with an accuracy of 2% or 3W above 25W. Calibration of the cycle ergometer should be performed within 2 weeks prior to the study. In addition, calibration of the cycle ergometer should be performed if the cycle is moved or when unusual response profiles raise concerns about the equipment.

4.3.7.2 Subject preparation

Subjects should not eat for at least 2hours before any exercise challenge. Subjects should dress appropriately for the exercise challenge. On arrival in the laboratory, a detailed explanation of the testing procedure and equipment should be given to the subject, outlining the risks and potential complications.

Before exercise, ECG electrodes will be carefully placed and secured after preparing the skin to ensure good recordings (if necessary, the area of the electrode placement should be shaved). Just prior to exercise, the subject will be informed that it is acceptable to swallow with the mouthpiece in place and that he/she must signal any unexpected difficulty, i.e., the subject is advised to point to the site of discomfort if chest or leg pain is experienced. A signal, such as a wave, should also be used when the subject wishes to terminate the test. Good communication with the subject throughout the whole procedure will increase the subject's confidence and helps to ensure a good effort.

Seat height should be adjusted so that the subject's legs are almost completely extended when the pedals are at the lowest point and the cycling rhythm practiced. When the subject is seated comfortably at the desired seat height, the distance between the seat and the floor will be measured and recorded. The same seat height will be used in Periods I and II for the subject.

4.3.7.3 Symptom-limited incremental cycle exercise test

Before exercise, while seated comfortably on the cycle ergometer, the subject will breathe for 3 minutes through the mouthpiece with nose clips in place. V_E, V_T, and VO_2 will be measured throughout the exercise test. Oxygen saturation by pulse oximetry and electrocardiographic monitoring of heart rate (HR) will be carried out throughout the testing. All variables will be recorded as 30 second averages at each work load and values at end-exercise (last 30 seconds of exercise).

The initial work rate will be set at 0 watts (i.e., loadless pedaling). The subject will perform 1minutes of loadless pedaling prior to increasing the work rate. The subject will begin to pedal at 50-70 rpm and will be encouraged to maintain this pedaling frequency throughout the exercise challenge. The subject is encouraged to continue exercise for as long as possible (i.e., to exhaustion or maximal exercise).

At the end of each minute, the work rate will be increased in increments of 15 watts.

The subject will exercise until:

- Limited by symptoms (i.e., is unwilling to continue exercising because of discomfort associated with the exercise); OR
- Unable to maintain a pedaling frequency of at least 40 rpm despite continued encouragement to increase the frequency to 50-70 rpm; OR
- Unable to continue safely (in the opinion of the supervising technician).

At the end of the exercise, the time of exercise is recorded (minutes and seconds): time from the start of loaded pedalling to the end of exercise. Work capacity (Wcap) is defined as the highest work rate that is maintained for at least 30 seconds. During recovery, the subject is told to continue with no external workload (i.e., loadless pedaling) for at least 2 minutes to prevent fainting and to accelerate lactate removal.

Immediately upon completion of the exercise test, subjects will rate the discomfort with breathing at maximal exercise using the modified Borg Scale. Subjects will also perform an IC (inspiratory capacity) maneuver within 15 seconds of the end of exercise. ECG monitoring will continue for 5-10 minutes during the recovery period. Heart rate will be recorded from the ECG at various intervals (i.e., 1, 2, 3, 5, 7 and 10 minutes) during recovery.

Reasons for stopping the test for safety reason must be clearly established and known by all personnel involved in testing. These include:

Symptoms such as:

- acute chest pain;
- sudden pallor;
- loss of coordination;
- mental confusion;
- extreme dyspnea;

and signs such as:

- clinically significant abnormalities in ECG recordings during exercise, i.e., ST segment depression of > 0.1 mV, T-wave inversion, sustained ventricular tachycardia, polymorphic and/or frequent premature ventricular beats;
- fall in systolic pressure below resting value or about 20 mmHg below its highest value during exercise;
- hypertension > 200 mmHg systolic or > 100 mmHg diastolic

If the exercise has been stopped for one of these reasons, the subject should be monitored in the laboratory until signs/symptoms/ECG modifications have completely resolved. Full CPR equipment should be available in the laboratory.

4.3.7.4 Additional procedures for exercise testing

The following procedures for exercise testing should be observed to reduce exercise performance variability.

Pre-exercise diet: Subjects will fast 4 hours following study drug administration on Study Day 4. Water will be allowed to beginning 2 hours after study drug administration and volunteers should be encouraged to drink water in order to maintain an adequate hydration state. Immediately upon completion of the fast, subjects will be served lunch. Subjects will not be allowed to eat again until after completion of the cycle exercise test. Fluids are to be encouraged during this time.

Previous exercise: Subjects should be encouraged to stay well-rested and to refrain from any strenuous, fatiguing or exhausting activities (i.e., walking up flights of stairs) on the morning of the exercise test. Very strenuous, heavy type of activities, especially activities to which the volunteer is unaccustomed, can lead to muscle soreness 24-48 hours after the activity. Subjects should be encouraged to refrain from any heavy lifting, exhaustive digging in the garden, etc. for 2-3 days prior to each clinic visit, especially if the subject has not performed these activities recently.

Psychological – external motivational cues: The exercise tests performed in this study are symptom-limited, i.e., the subject will stop exercising when no longer able to tolerate the discomfort associated with the exercise. As such motivational cues provided to the subject can have profound effects on exercise performance. It is imperative that external motivational cues are controlled across subjects.

Time of exercise: No visual cues regarding the time of exercise should be provided to the volunteer. The subject's watch should be removed prior to exercise, and all other timekeeping devices (i.e., study staff watches, wall clocks, stopwatch, etc.) should be kept away from the volunteer's view.

Verbal encouragement: The wording and intensity of verbal encouragement provided to the subject will be standardized. Verbal encouragement will only be provided by one member of the study team, who is blinded to the results of the lung function tests. The tone of the encouragement should be enthusiastic and supportive, but not overbearing, overly loud or coercive in nature. Examples would be: "good work", "well done", "excellent job so far", "keep up the good work". Reinforcement could also consist of "that's a good pedaling rate" or if the pedaling rate is dropping you could try a positive reminder "keep up the pedaling rate – that's great". You may reinforce a little regarding the IC maneuver as well, i.e., "that was a good one" or use constructive criticism if it wasn't, i.e., "that was good, but maybe next time try to…".

Comfort with equipment: Subjects should be instructed to bring appropriate dress for exercise (i.e., shorts or track pants, gym shoes, T-shirt or sweat shirt) when reporting in to the study center on Study Day –1 of Period I and II. This attire should be worn for the exercise test, and study staff should ensure that the volunteer is comfortable with the mouthpiece and the positioning of other equipment prior to starting the exercise.

Familiarity with test: Before starting exercise, a member of the study team should make sure that the subject is completely familiar with the type of exercise that is to be performed; for the incremental test, subjects should be told that the test will feel like he/she is cycling up a hill and that each minute the slope of the hill will increase; for the constant work rate test, subjects should be told that the test will feel like he/she is cycling up a hill with a constant slope. It is important that the subject understands the difference between the incremental test and constant work rate test

Understanding "exercise for as long as you can": It is extremely important that the subject understands that there is no specific time limit to any of the exercise tests, and that the test will continue as long as the subject is able to. Stress to the subjects that the study team will not stop the test unless there is concern for the subject's safety, or the subject is unable to maintain the required pedaling frequency.

The cycle exercise test will be captured on the pCRF in four categories: predicted, baseline, maximum exercise and % predicted. Each category will include (as appropriate): work load (watts), VO_2 (L/min), VCO_2 (L/min), RQ, HR (BPM) O_2 pulse (mL/beat), systolic BP

(mmHg), diastolic BP (mmHg), VE-Norm Pred, VE-BTPS (L/min), RR (BPM) VD/VT-ABG(%), V_t (Liters), FIO_2(%), SPO_2(% and SaO_2(%).

4.4 Safety measurements

Please refer to the study plans in Table 1 through Table 3 for the precise timing of safety measurements.

4.4.1 Demographics and informed consent

The subject's signed and dated informed consent form must be obtained before conducting any procedure specifically for the study (Refer to Section 7.3). Demographics (date of birth, sex, race, date of signed informed consent) for each subject will be obtained and collected on the appropriate CRF, whether or not they are entered into the study.

4.4.2 Inclusion and exclusion criteria

The inclusion and exclusion criteria must be assessed and reviewed with each subject at the screening visit, Day -1 of Periods I and II in order to establish and confirm their eligibility to participate in the study.

4.4.3 Medical history

A detailed medical history including surgical and medication histories will be recorded for each subject at the initial screening visit and updated at admission on Day -1 of Periods I and II. Significant medical conditions that have occurred within the past 2 years or conditions that are ongoing (i.e., headache, backache, indigestion) are to be recorded in the appropriate pCRF.

The medication history must identify any known drug allergies, presence or history of drug abuse and use of chronic medications. All medications and over-the-counter (OTC) products (including vitamins and herbal products) taken within 2 weeks before Day -1 of Period I are to be recorded on the pCRF.

4.4.4 Laboratory safety measurements

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

The following laboratory variables will be measured:

Periods I and II

Clinical chemistry	**Hematology**	**Urinalysis**
Calcium	Hemoglobin	Specific gravity
Glucose	Hematocrit	PH
Total bilirubin	Erythrocytes (RBCs)	Glucose
Alkaline phosphatase	Leukocytes (WBCs)	Ketones
Creatinine	Platelet count	Protein
Total protein	Red cell distribution width	Bilirubin
Albumin	Mean corpuscular volume	Occult blood
Aspartate aminotransferase	Mean corpuscular hemoglobin concentration (MCHC)	Microscopic analysis of formed elements[a]
Alanine aminotransferase	Differential leukocytes including:	
Sodium	Lymphocytes	
Potassium	Basophils	
Magnesium	Monocytes	
Phosphate	Neutrophils	
Blood urea nitrogen	Eosinophils	
Chloride		
Uric acid		
Gamma glutamyl transpeptidase		
Creatine kinase (CK)		

[a]If a urine sample is positive for protein or blood, a microscopic examination of the urine sediment will be performed.

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

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

4.4.5 Urine drug screen

Urine will be tested for the following drugs of abuse: benzodiazepines, cocaine and/or metabolites, amphetamines, tetrahydrocannabinol (THC), opiates, methamphetamines (including ecstasy), phencyclidine (PCP), and barbiturates.

If a subject tests positive for drugs of abuse they will be excluded from entering, or continuing in, the study. If the drug is illegal, advice will be offered and the subject will be removed from the AstraZeneca Volunteer Panel.

4.4.6 HIV and hepatitis screens

Testing for the HIV antibody, HBsAg, and Hepatitis C antibody is to be performed on all subjects at screening only. If a test result is positive, the subject will not be allowed to proceed in the study.

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

4.4.7 Breath ethanol testing

Breath ethanol will be measured at the times specified in Table 1 and Table 2 If the result is positive, the subject will not be permitted to proceed in the study.

4.4.8 Electrocardiographic measurements

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

4.4.8.1 Resting 12-lead ECG

Twelve-lead ECGs will be obtained after the subject has been lying down for 5 minutes in each case.

4.4.9 Vital signs

Vital signs assessments in addition to those discussed below can be made at the discretion of the investigator in order to follow the subject's clinical condition. These assessments should be entered as unscheduled assessments in the appropriate sections of the pCRF.

4.4.9.1 Blood pressure and heart rate

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

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

4.4.9.2 Respiratory rate

One minute respiratory rate will be assessed after the subject has been sitting for 5 minutes. Refer to Table 1 through Table 3 for the timing of individual measurements.

4.4.9.3 Oral temperature

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

4.4.10 Height and weight

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

4.4.11 Physical examination

4.4.11.1 Complete physical examination

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

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

4.4.11.2 Brief Physical Examination

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

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

4.5 Genetic measurements and co-variables

4.5.1 Collection of samples for genetic testing

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

A single venous blood sample (9 mL) will be collected into a polypropylene tube containing ethylenediamine tetra-acetic acid (EDTA) and gently inverted a minimum of five times to mix thoroughly. Tubes will be labeled with the protocol study number, centre number, enrollment code and/or randomization number and date of sample collection. No personal identifiers (subject name, initials or date of birth) will be placed on the tube or accompanying documentation. A record of the date of the subject consent to the genetic research and the date of the blood sample collection will be recorded in the appropriate section of the CRF.

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

4.5.1.1 Sample processing and shipping

Samples will be frozen (-20°C or below) and transported to the relevant DNA extraction laboratory within one month of collection and must remain frozen at all times.

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

4.5.1.2 Storage and coding of DNA samples

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

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

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

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

Samples will be stored for a maximum of 20 years, from the date of completion of the study, after which they will be destroyed.

DNA is a finite resource that is used up during analysis. Samples will be stored and used until no further analyses are possible. Further samples will not be acquired from subjects.

4.5.1.3 Summary of genetic assessments and analysis

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

4.6 Volume of blood sampling

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

Table 6 Volume of blood to be drawn from each subject

Assessment		Sample volume (mL)	n of samples	Total volume (mL)
AZD6140 PK samples		2 mL	17	34 mL
Arterial blood gas[a]		1 mL	1	1 mL
Blood Sample for genotyping[b]		9 mL	1	9 mL
Safety	Clinical chemistry	10 mL	7	70 mL
	Hematology	5 mL	7	35 mL
Screening	Clinical chemistry	10 mL	1	10 mL
	Hematology	5 mL	1	5 mL
	HIV/HBsAg, hepatitis C	10 mL	1	10 mL
Total				**174 ML[c]**

a. To be collected only if a subject experiences a spontaneous dyspnea episode
b. A separate informed consent required.
c. Total blood volume may vary if subject experiences a spontaneous dyspnea episode, which requires both an ABG (1 mL) and PK (2 ML) sample(s) to be drawn.

4.7 Adverse Events

The methods for collecting adverse events are described below.

4.7.1 Adverse Events

4.7.1.1 Definitions

The definitions of adverse events (AEs), serious adverse events (SAEs) and other significant adverse events (OAEs) are given below. It is of the utmost importance that all staff involved

in the study are familiar with the content of this section. The principal investigator is responsible for ensuring this.

Adverse event

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

Serious adverse event

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

- results in death
- is immediately life-threatening
- requires in-patient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability or incapacity
- is a congenital abnormality or birth defect
- is an important medical event that may jeopardise the subject or may require medical intervention to prevent one of the outcomes listed above.

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

Other Significant Adverse Events (OAE)

OAEs will be identified by the Drug Safety Physician and if applicable also by the Clinical Study Team 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.

4.7.1.2 Recording of adverse events

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

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

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

The following variables will be recorded for each AE noted:

- Onset, resolution
- Intensity (mild/ moderate/ severe)
- Action(s) taken
- Outcome of the AE
- Causality of the AE (yes or no)
- Whether it constitutes an SAE or not

The intensity rating is defined as:

1 = mild (awareness of sign or symptom, but easily tolerated)

2 = moderate (discomfort sufficient to cause interference with normal activities)

3 = severe (incapacitating, with inability to perform normal activities)

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

4.7.1.3 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 one for all fatal and life-threatening cases and by day five for all other SAEs.

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

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

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

5. STUDY MANAGEMENT

5.1 Monitoring

5.1.1 Study monitoring

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

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

5.1.2 Data verification

It is a 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 pCRFs 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.

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

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

5.1.3 Archiving of study documentation

AstraZeneca will retain all documentation pertaining to this study in AstraZenca for central file for as long as AZD6140 is available for human consumption..

The investigator will retain all documentation pertaining to this study for at least 15 years.

5.2 Audits and inspections

Authorized representatives of AstraZeneca, a regulatory authority, an Independent Ethics Committee (IEC) or an Institutional Review Board (IRB) may visit the centre to perform audits or inspections, including source data verification. The purpose of an AstraZeneca audit or inspection is to systematically and independently examine all study related activities and documents to determine whether these activities were conducted, and data were recorded, analysed, and accurately reported according to the protocol, GCP guidelines of the ICH and any applicable regulatory requirements. The investigator should contact AstraZeneca immediately if contacted by a regulatory agency about an inspection at his or her centre.

5.3 Training of staff

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

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

5.4 Changes to the protocol

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

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

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

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

5.5 Study agreements

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

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

5.6 Study timetable and end of study

The study is expected to start in October 2004 and to be completed by December 2004.

5.7 Data management

5.7.1 Case report forms

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

The AstraZeneca Monitor will check data at the monitoring visits to the investigational 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 under the direction of the Data Manager. 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 Pharmacogenetic data

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

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

However, some or all of the clinical study dataset may be duplicated within the AstraZeneca LIMS database or other appropriate system for exploratory analysis.

5.8 Reporting of Genotypic Results

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

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

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

6.1 Pharmacokinetic / pharmacodynamic evaluation

6.1.1 Calculation or derivation of pharmacokinetic variables

The pharmacokinetic analyses will be performed by Clinical Pharmacokinetics, Experimental Medicine, AstraZeneca , Wilmington, DE. To be followed by a description of each pharmacokinetic variable to be included in the analysis and the means by which each is calculated.

Plasma concentrations of AZD6140 and its metabolite AR-C124910XX will be listed and depicted graphically as a function of time relative to Day 1 dose for the 3 cohorts. Pharmacokinetic parameters listed below will be estimated by non-compartmental analysis.

Day 1: C_{max}, t_{max}, AUC_{0-12} of AZD6140 and AR-C124910XX, metabolite to parent C_{max} and AUC ratios

Day 4: C_{max}, t_{max}, AUC_{τ}, metabolite to parent C_{max} and AUC ratios of AZD6140 and AR-C124910XX and, AZD6140 steady state CL/F.

AZD6140 and AR-C124910XX C_{max} will be estimated as the highest measured concentration and t_{max} will be the time to maximum concentration within a dosing interval on Days 1 and 4. AUC_{0-12} and AUC_{τ} will be calculated using the linear trapezoidal method over the 12 hour dosing interval on Days 1 and 4, respectively. AZD6140 CL/F will be estimated as the ratio of AZD6140 dose and AUC_{τ} on Day 4. The ratio of AR-124910XX C_{max} to AZD6140 C_{max} ratio and, AR-124910XX AUC to AZD 6140 AUC ratio will be calculated on Days 1 and 4.

6.1.2 Calculation or derivation of pharmacodynamic variables

Changes from baseline in each period for respiratory parameters will be calculated.

6.1.3 Calculation or derivation of pharmacokinetics/pharmacodynamics

Relationship between AZD6140 concentration and PD endpoints will be explored graphically.

6.2 Safety evaluation

6.2.1 Calculation or derivation of safety variables

Changes from baseline for the safety data (adverse events, vital signs, 12-lead ECG, physical examination data, laboratory data) will be calculated.

6.3 Genetics as a co-variate (not applicable)

6.4 Statistical methods and determination of sample size

6.4.1 Statistical evaluation

A comprehensive Statistical Analysis Plan (SAP) will be prepared and finalized before database lock. The goal of this analysis is to assess the effect of AZD6140 on the respiratory parameters in healthy volunteers, mild asthma patients and mild COPD patients.

6.4.2 Description of analysis sets

6.4.2.1 Pharmacodynamic analysis set

All subjects who have evaluable respiratory measurements in both periods of the study without any protocol deviations/violations deemed to affect the pharmacodynamics of AZD6140 will be included in the summaries and listings of the pharmacodynamic data.

6.4.2.2 Pharmacokinetic analysis set

All subjects who have evaluable pharmacokinetic data without any protocol deviations/violations deemed to effect the pharmacokinetics of AZD6140 will be included in the summaries and listings of the pharmacokinetic data.

6.4.2.3 Safety analysis set

All subjects who received at least one dose of study medication will be included in the assessment of safety.

6.4.3 Methods of statistical analyses

Statistical analysis will be carried out by Biostatistics at AstraZeneca, Wilmington, Delaware using SAS (version 8.2). Graphics to be included in the text or intended for use as supplemental figures and individual profile figures will be made using SAS or other software. Pharmacokinetic analysis will be carried out by, or under the guidance of, the Pharmacokinetic Section, Experimental Medicine, at AstraZeneca, Wilmington, Delaware.

No formal statistical analysis of the pharmacodynamic, pharmacokinetic or safety data will be done.

6.4.3.1 Pharmacodynamic

The respiratory parameters will be descriptively summarized by treatment and listed by individual at each time point by treatment and visit for each of the cohorts using descriptive statistics. These will also be supported with graphical displays in order to ease making comparisons.

6.4.3.2 Pharmacokinetic

All pharmacokinetic parameters will be descriptively summarized by treatment and listed by individual at each scheduled time point for each of the cohorts. AZD6140 and AR-C124910XX plasma concentrations will be summarized by treatment, reported as descriptive statistics and listed by individual at each scheduled time point for each of the cohorts.

6.4.3.3 Pharmacodynamic/Pharmacokinetic

Relationship between AZD6140 concentration and PD endpoints will be explored graphically. Representative plots include:

- Plots of individual numeric value from Modified Borg Scale and Bidirectional Dyspnea Index versus AZD6140 concentrations
- Plots of individual and mean respiratory rate, V_E, V_T, *f*, FVC, FEV_1 versus AZD6140 concentrations
- Plots of individual and mean PCO_2, PO_2 and pH versus AZD6140 concentrations

Regressions techniques may be utilized to provide estimates of slope, along with corresponding 95% confidence intervals, for the relationships above, if distributional assumptions underlying this analysis can be confirmed.

6.4.3.4 Safety

Safety data (adverse events, vital signs, 12-lead ECGs, physical examination data, laboratory data) will be summarized at each scheduled time point by treatment for the three cohorts using descriptive statistics.

6.4.4 Determination of sample size

The sample size for each cohort was chosen based on feasibility concerns, and the need to provide adequate information to support further development. The sample size was not based on any power calculations.

6.5 Interim analyses (not applicable)

6.6 Data presentation (not applicable)

6.7 Data or safety monitoring committee (not applicable)

7. ETHICS

7.1 Ethics review

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

The Principal Investigator is responsible for informing the IRB or IEC of any amendment to the protocol in accordance with local requirements. In addition, the IRB or IEC must approve all advertising used to recruit subjects for the study. The protocol must be re-approved by the IRB or IEC annually, as local regulations require.

The Principal Investigator is also responsible for providing the IRB with reports of any serious and unexpected adverse drug reactions from any other study conducted with the investigational product. AstraZeneca will provide this information to the Principal Investigator.

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

Where there is a genetic component to the study, approval must be obtained for the genetic component of the study and the genetic informed consent process from the IRB or IEC. It must be clearly stated in the approval that the genetic component of the study is approved. The investigator must submit written approval to AstraZeneca before any subject participates in the genetic component of 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. and the IRB.

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

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

7.4 Subject data protection

The Master Informed Consent Form will incorporate (or, in some cases, be accompanied by a separate document incorporating) wording that complies with relevant data protection and privacy legislation. Pursuant to this wording, 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 randomization number/study code/initials.

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

Reference to participation in the genetic research component of the study should not be recorded into the subjects' general medical records.

8. PROCEDURES IN CASE OF EMERGENCY, OVERDOSE OR PREGNANCY

8.1 AstraZeneca emergency contact procedure

This information has been placed behind the title page of the protocol for easy reference.

For Serious Adverse event reporting

- Contact:

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 4.7.1.3.**

Please refer to Section 3.4.4.2 for instructions regarding unblinding of study medication.

8.3 Procedures in case of overdose (not applicable)

8.4 Procedures in case of pregnancy (not applicable)

9. REFERENCES

1. Mahler, DA and Horwitz, MB. "Perception of reathlessness during exercise inpatients with respiratory disease." Medicine and Science in Sports and Exercise, Vol. 26, No 9, pp. 1078-81, 1994.

2. O'Donnell DE, Sanii R, Giesbrecht G, Younes M. "Effect of continuous positive airway pressure on respiratory sensation in patients with chronic obstructive pulmonary disease during submaximal exercise." American Review of Respiratory Disease, Vol. 138, pp. 1185-1191, 1988.

3. Crapo RO, Morris AH, Gardner RM. "Reference spirometric values using techniques and equipment that meet ATS recommendations." American Review of Respiratory Disease, Vol. 123, pp. 659-64, 1981.

4. O'Donnell DE, Lam M, WebbKA. "Measurement of symptoms, lung hyperinflation and endurance during exercise in chronic obstructive pulmonary disease." American Journal of Respiratory and Critical Care Medicine. Vol. 158, pp 1557-65, 1998.

5. O'Donnell DE, WebbKA. "Exertional breathlessness in patients with chronic airflow limitation: the rate of lung hyperinflation." American Review of Respiratory Disease, Vol. 148, pp. 1351-57, 1993.

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Clinical Pharmacology Study Protocol: Appendix A	
Drug Substance	AZD6140
Study Code	D5130C00028
Appendix Edition Number	1.0
Appendix Date	

Appendix A
Signatures

ASTRAZENECA SIGNATURE(S)

An Exploratory, Double-Blind, Randomized, Placebo-Controlled, 2-Period Crossover, 3 Cohort Pharmacokinetic/Pharmacodynamic, Single Center Study to Assess the Effect of AZD6140 on Respiratory Parameters in Healthy Subjects, Mild Asthmatics and COPD Patients Age 55 to 75 Years

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

I agree to the terms of this study protocol.

AstraZeneca Research and Development site representative

_____ _____

Date
(Day Month Year)

ASTRAZENECA SIGNATURE(S)

An Exploratory, Double-Blind, Randomized, Placebo-Controlled, 2-Period Crossover, 3 Cohort Pharmacokinetic/Pharmacodynamic, Single Center Study to Assess the Effect of AZD6140 on Respiratory Parameters in Healthy Subjects, Mild Asthmatics and COPD Patients

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

I agree to the terms of this study protocol.

AstraZeneca Research and Development site representative

_____ _____

Date
(Day Month Year)

SIGNATURE OF PRINCIPAL INVESTIGATOR

An Exploratory, Double-Blind, Randomized, Placebo-Controlled, 2-Period Crossover, 3 Cohort Pharmacokinetic/Pharmacodynamic, Single Center Study to Assess the Effect of AZD6140 on Respiratory Parameters in Healthy Subjects, Mild Asthmatics and COPD Patients

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

I agree to the terms of this study protocol. I will conduct the study according to the procedures specified herein, and according to the principles of Good Clinical Practice (GCP) and local regulations.

Centre No.: 001

Signature:

_____ _____

Date
(Day Month Year)

AstraZeneca

Clinical Study Protocol: Appendix B

Drug Substance	AZD6140
Study Code	D5130C00028
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Appendix B
Additional Safety Information

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

Life threatening

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

Hospitalization

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

Important medical event or medical intervention

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

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

Examples of such events are:

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

A GUIDE TO INTERPRETING THE CAUSALITY QUESTION

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

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

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

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

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

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

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

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

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

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

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

- Is there a known mechanism

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

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Clinical Study Protocol: Appendix C

Drug Substance	AZD6140
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Appendix C
Restricted Medications

1. MEDICATIONS NOT ALLOWED DURING THE STUDY[(1)]

1.1 Potent Inhibitors and Inducers of CYP3A4

Barbituates including primidone, Carbamazepine including Oxacarbazapine, Clarithromycin, Erythromycin, Fluconazole, Griseofulvin, Hydantoins, Itraconazole, Ketoconazole, Miconazole, Nefazodone, Rifampicin, St. John's Wort, Troleandomycin, Protease Inhibitors

1.2 Calcium Channel Blockers

The full extent of CYP3A4 interaction has not been assessed at this time. Therefore the following calcium channel blockers are not allowed: Amlodipine, diltiazem, felodipine, gallopamil, isradipine, lacidipine, lercanidipine, nicardipine, nifedipine, nilvadipine, nimodipine, nisoldipine, nitrendipine, verapamil.

1.3 Statins

The full extent of CYP3A4 interaction has not been assessed at this time. Therefore the following statins are not allowed: Atorvastatin, simvastatin

1.4 Beta Blockers

Beta blockers are not permitted because of the potential to induce bronchospasm in patients susceptible to reactive airway disease.

2. ACCEPTED MEDICATIONS[(1)]

The following prescription medications may be allowed for a chronic medical condition, with the approval of the investigator and sponsor, provided the subject has been on a stabile dose for 2 months prior to Study Day 1 of Period I. Initiation of treatment or change in dose regimen is not permitted during the study.

2.1 ACE Inhibitors

Benazepril, captopril, cilazapril, enalapril, fosinopril, lisinopril, moexipril, perindopril, quinapril, ramipril, trandolapril.

2.2 Angiotensin II Inhibitors

Candesartan, eprosartan, irbesartan, losartan, telmisartan, valsartan

2.3 Thiazide Diuretics

Hydrochlorothiazide, indapamide, chlorothiazide

2.4 Thyroid Hormone Replacement

Synthroid, Levoxyl

(1)This list is provided as a guideline. The study sponsor should be contacted with any questions regarding concomitant medication.

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Clinical Study Protocol: Appendix D	
Drug Substance	AZD6140
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Appendix D
WHO Risk Categories

1. WHO RISK CATEGORIES

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

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

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Clinical Study Protocol: Appendix E	
Drug Substance	AZD6140
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Appendix E
Modified Borg Scale

Modified Borg Scale

Score		Sensation of breathlessness/dyspnea
0	=	Nothing At All
0.5	=	Very, very slight (just noticeable)
1	=	Very Slight
2	=	Slight
3	=	Moderate
4	=	Somewhat Severe
5	=	Severe
6	=	
7	=	Very severe
8	=	
9	=	Very, very severe (almost maximal)
10	=	Maximal

The subject should be given the following instructions:

"This is a scale for rating breathlessness. The number "0" represents no breathlessness. The number "10" represents the strongest or greatest breathlessness you have ever experienced. Prior to completing the minute ventilation test and spirometry tests, you will be asked to point to a number with your finger, which represents your perceived level of breathlessness at that time. Use the written description to the right of the number to help guide your selection. I will say the number out loud in order to confirm your choice. If you have an even stronger or greater intensity of breathlessness than you have ever previously experienced, you should then point to the word "maximal" if the severity is greater than 10."

The subject should be given the following additional instructions during the cycle exercise test:

"Each minute during the exercise test you will be asked to complete this assessment. During the cycle exercise you may have an even stronger or greater intensity of breathlessness than you have ever previously experienced. You should then point to the word "maximal", if the severity is greater than 10. You can tell us this number after the mouthpiece has been removed."

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Clinical Study Protocol: Appendix F	
Drug Substance	AZD6140
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Appendix Edition Number	1.0
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Appendix F
Bidirectional Dyspnea Index

Bidirectional Dyspnea Index

Score		Change in Breathlessness/Dyspnea
-5	=	Very marked improvement
-4	=	Marked improvement
-3	=	Moderate improvement
-2	=	Slight improvement
-1	=	Very slight improvement
0	=	No change
+1	=	Very slight worsening
+2	=	Slight worsening
+3	=	Moderate worsening
+4	=	Marked worsening
+5	=	Very marked worsening

The subject should be given the following instructions:

"This is a scale for rating changes in breathlessness. The number -5 represents a very marked improvement. The number +5 represents a very marked worsening. Prior to each set of three spirometry tests, you will be asked to point to a number with your finger, which represents your perceived level of breathlessness compared to how you felt prior to taking your most recent dose of medication. Use the written description to the right of the number to help guide your selection. I will say the number out loud in order to confirm your choice."