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**Amended Clinical Study Protocol No.3**

Drug Substance           AZD6140  
Study Code                D5130C00034  
Edition Number          4.0  
Date

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

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Sponsor: AstraZeneca LP, 1800 Concord Pike, Wilmington, DE 19850

AstraZeneca Research and Development  
site representative

Date

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

<b>Amendment No.</b>	<b>Date of Amendment</b>	<b>Local Amendment No.</b>	<b>Date of local Amendment</b>
<u>1.0</u>		<u>3.0</u>	
<u>2.0</u>			
<b>Administrative change No.</b>	<b>Date of Administrative Change</b>	<b>Local Administrative change No.</b>	<b>Date of local Administrative Change</b>

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

<b>Role in the study</b>	<b>Name</b>	<b>Address and Telephone number</b>
Study Delivery Team Leader		AstraZeneca LP 1800 Concord Pike FOC-SW1-745 Wilmington, DE 19850 Phone: 302-886-3483 Fax: 302-886-1099 Mobile: 302-463-6090
Study Delivery Team Physician		AstraZeneca LP 1800 Concord Pike FOC-SW1-934 Wilmington, DE 19850 Phone: 302-885-9887 Fax: 302-886-1512 Mobile: 302-420-9512
SAE Reports		Fax: 302-886-1099

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

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

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#### **Investigator**

#### **Study centre(s), type and number of patients planned**

Approximately 24 patients (male or female) including 12 mild asthmatic patients aged 35 to 75 years (inclusive) and 12 mild to moderate chronic obstructive pulmonary disease(COPD) patients aged 55 to 75 years (inclusive) will be randomized at a single center to assure the completion of 8 patients in each patient group.

#### **Study period**

Estimated date of first patient enrolled

Estimated date of last patient completed

#### **Phase of development**

Clinical Pharmacology (I)

#### **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 mild asthma patients and mild to moderate COPD patients;
- Compare respiratory parameters between the mild asthma patients, mild to moderate COPD patients and healthy volunteers from study D5130C00028;

- Compare the pharmacokinetics (PK) of AZD6140 and AR-C124910XX in mild asthma patients, mild to moderate COPD patients and healthy volunteers from study D5130C00028;
- Evaluate the tolerability of AZD6140 in mild asthma and mild to moderate COPD patients.

### **Study design**

This will be a randomized, double blind, placebo-controlled, PK/PD, single center study to assess the effect of AZD6140 on respiratory parameters in mild asthma patients aged 35 to 75 years (inclusive) and mild to moderate COPD patients aged 55 to 75 years (inclusive).

### **Investigational product, dosage and mode of administration**

Each patient 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 micronised 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 micronised tablets) 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 patient 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 patient 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  $AUC_{\tau}$ , 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<sub>1</sub>, FVC, PEF)

Pulse oximetry

Respiratory Rate

Modified Borg Scale

Bidirectional Dyspnea Index

### - Safety

Safety and tolerability will be assessed by collection of adverse events, 12-lead ECG (electrocardiogram), telemetry (continuous ECG evaluations) 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 including side effects will be performed.

All patients randomized into the study will be required to donate blood for pharmacogenetic analysis. Blood sample for this analysis will be taken only after the patient has given informed consent for the study. 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 using descriptive statistics. These will also be supported with graphical displays in order to ease making comparisons.

The plasma concentrations and pharmacokinetic parameters of AZD6140 and AR-C124910XX will be descriptively summarized. Furthermore, graphical plots of the respiratory parameter and concentration of AZD6140 will also be explored.

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 each of the 2 cohorts using descriptive statistics.

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

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**APPENDICES**

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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 aminotransferase
ANOVA	Analysis of variance
Assessment	An observation made on a variable involving a subjective judgment
ASA	Acetylsalicylic acid
AST	Aspartate aminotransferase
AUC <sub>t</sub>	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 <sub>max</sub>	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
HIPPA	Health Insurance Portability and Accountability Act

<b>Abbreviation or special term</b>	<b>Explanation</b>
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
MDI	Meter Dose Inhaler
Measurement	An observation made on a variable using a measurement device
Mg	Milligram
ML	Milliliter
OAE	Other significant adverse event (See <a href="#">Appendix B</a> )
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
PEF	Peak expiratory flow
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 <sub>2</sub>	Oxygen saturation
SAP	Statistical analyses plan
TBD	To be determined
THC	Tetrahydrocannabinol
t <sub>½</sub>	Terminal phase half-life
t <sub>max</sub>	Time to reach maximum (peak) concentration

<b>Abbreviation or special term</b>	<b>Explanation</b>
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 <sub>E</sub>	Minute ventilation
VO <sub>2</sub>	Oxygen uptake
V <sub>T</sub>	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<sub>1</sub> and P2Y<sub>12</sub>, 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<sub>12</sub>-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<sub>12</sub>-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<sub>12</sub>-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.

The data from the Phase I studies completed to date 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<sub>max</sub>) were reached within 1.5 to 3 hours after dose intake, and the mean terminal half-life (t<sub>1/2</sub>) of AZD6140 ranged from 6 to 13 hours. The metabolite area under the plasma concentration-time curve (AUC) and C<sub>max</sub> 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 (SAEs) were observed in the Phase I studies completed to date. The most common adverse events reported to date in the Phase I studies include: headaches, dizziness and vasovagal syncope. The causal relationship of these adverse events to AZD6140 is uncertain.

In Disperse 2, a 12 week study in patients with non-ST elevation acute coronary syndrome, Holter ECG monitor evaluation was conducted in patients for up to 7 days post event (average recording of about 4 days). There were Holter data available for 305 patients on the 90 mg dose of AZD6140, 284 patients on the 180 mg dose of AZD6140, 297 on the 75 mg dose of clopidogrel. At post hoc evaluation of this data demonstrated an increased incidence of the occurrence of pauses, dropped beats, and bradycardia on Holter monitoring in the AZD6140 groups compared to clopidogrel. These Holter observations were not generally associated with symptoms. The frequency of pauses over 2.5 seconds was 17 for the 90 mg of AZD6140, 28 for the 180 mg dose of AZD6140 and 13 for the 75 mg dose of clopidogrel. For dropped beats (>180% RR interval) there were 88 for the 90 mg dose of AZD6140, 89 for the 180 mg dose of AZD6140 and 73 for the 75 mg dose of clopidogrel. The occurrence of bradycardia (4 beats<45 bpm) was 103 for the 90 mg dose of AZD6140, 106 for the 180 mg dose of AZD6140, and 96 for the 75 mg dose of clopidogrel.

## 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 who were of a younger age range. One pre-clinical study in rats demonstrated an increase in respiratory rate at concentrations of AZD6140 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 patients with mild asthma and mild to moderate COPD are being included to assess the sensitivity of patients with respiratory disease. A similar phase I study is ongoing in healthy elderly volunteers to assess for changes in respiratory assessments following administration of AZD6140, and will complete the clinical phase prior to the start of the proposed study with mild asthma and mild to moderate COPD patients. The pharmacodynamic effect of AZD6140 on respiratory parameters will be assessed by cardiopulmonary exercise test, respiratory rate, minute ventilation, spirometry (FVC, FEV<sub>1</sub>), modified Borg Scale<sup>1</sup>, and Bidirectional Dyspnea Index<sup>2</sup>. 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 including side effects such as dyspnea will 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 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 mild asthma patients and mild to moderate COPD patients;
- Compare respiratory parameters between mild asthma patients, mild to moderate COPD patients and healthy volunteers from study D5130C00028;
- Compare PK of AZD6140 and AR-C124910XX in mild asthma patients, mild to moderate COPD patients and healthy volunteers from study D5130C00028.
- Examine the safety and tolerability of AZD6140 in mild asthma patients and mild to moderate COPD patients.

## **3. STUDY PLAN AND PROCEDURES**

### **3.1 Overall study design**

This will be a randomized, double blind, 2 cohort, placebo-controlled, PK/PD, single center study to assess the effect of AZD6140 on respiratory parameters in mild asthma patients aged 35 to 75 years (inclusive) and mild to moderate COPD patients aged 55 to 75 years (inclusive). Up to 12 men or women will be randomized in each cohort to ensure at least 8 patients complete the trial within each cohort.

On Study Day 1 of Period I, each patient 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 micronised 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 micronised tablet) in the PM (12 hours after the first dose); thereafter, 180 mg AZD6140 (2 x 90 mg micronised 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 patients will crossover to receive the alternate treatment following at least a 7 outpatient 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.

### 3.1.1 Screening/Visit 1

In order to establish eligibility to participate in the study, potential patients 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).

Patients 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 patient eligibility to participate in the study. The results of the assessments must be confirmed before the patient is randomized and receives study medication on Day 1 of Period I.

All patients will be required to begin an overnight fast on the evening of Study Day –1. On Study Day 1 of Period I, each patient 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 micronised 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 micronised tablet) in the PM (12 hours after the first dose); thereafter, 180 mg AZD6140 (2 x 90 mg micronised 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.

A blood sample for genetic analysis will be taken from patients 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 patient 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 patient's condition. Other diagnostic studies, such as arterial blood gas (ABG), 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 patient's dyspnea should resolve, or return to patient's baseline respiratory parameters, before patient proceeds on to Period II (see Section [3.3.5.1](#)).

Patients 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, patients 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 at least 7 outpatient 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).

Patients 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 patient eligibility to participate in the study.

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

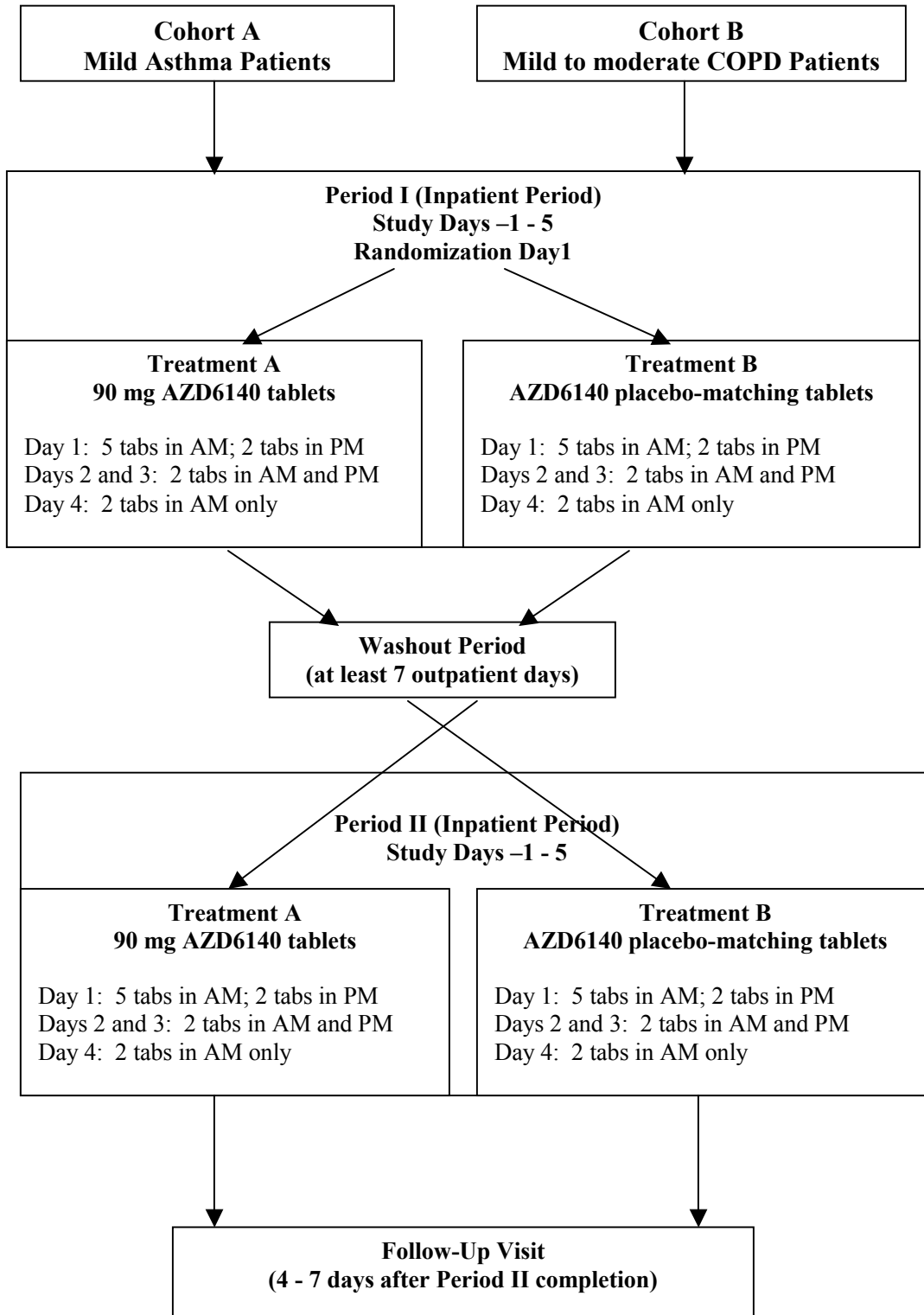
Patients will be discharged from the study center on Study Day 5, after the completion of all protocol-driven procedures and assessments and at the discretion of the investigator. At

discharge, patients 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**

Patients 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 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 <sup>a</sup> Screening	Visit 2 Period I	Visit 3 <sup>b</sup> Period II	Visit 4 <sup>c</sup> Follow-up
Informed consent	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	X	
Serum pregnancy test (females only)	X	X	X	X
Oral temperature	X	X	X	X
BP and heart rate	X	X	X	X
Height and weight	X			X <sup>d</sup>
Complete physical examination	X			X
Brief physical examination		X	X	
12-lead ECG <sup>e</sup>	X	X	X	X
Telemetry <sup>f</sup>		X	X	
Genetic blood sample <sup>g</sup>		X		
Inpatient stay		X	X	
Randomization <sup>g</sup>		X		
AZD6140 or placebo administration		X	X	
AZD6140/AR-C124910XX PK blood samples		X	X	
Modified Borg Scale/Bidirectional Dyspnea Index	X <sup>h</sup>	X	X	
Minute ventilation (including respiratory rate)	X <sup>h</sup>	X	X	
Spirometry (FEV <sub>1</sub> , FVC, PEF)	X <sup>h</sup>	X	X	
Cardiopulmonary exercise test	X <sup>h</sup>	X	X	
Hand-held peak flow meter <sup>i</sup>	X	X <sup>g</sup>	X <sup>g</sup>	
Pulse oximetry		X	X	
Concomitant medication monitoring	X	X	X	X
Adverse event monitoring <sup>j</sup>	X	X	X	X

- a. Within 21 days of Day 1, Period I.
- b. There will be at least a 7 outpatient day washout period between Period I and Period II.
- c. Within 4 to 7 days of Period II (Day 5).
- d. Weight only.
- e. A 12 lead ECG will be performed at screening and should not show any clinically significant abnormalities including significant AV block ( 2<sup>nd</sup> or 3<sup>rd</sup> degree).
- f. Telemetry will be performed from 30 minutes prior to the first administration of study drug (Day 1) until 24 hours after the last administration of study drug (Day5).
- g. Prior to study drug administration Period 1.
- h. Patients 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, patients will be asked to demonstrate that they can utilize the equipment for the test and will be familiarized with the procedure.
- i. Hand-held peak flow meter may be used during nocturnal occurrences of dyspnea, if the spirometry technician is unavailable. A baseline value will be obtained on Day 1 with spirometry testing (Pre-AM dose).
- j. SAE's will be collected from the time of the informed consent is obtained through the Follow-Up visit. Non SAE's will be collected from the time of the 1<sup>st</sup> 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) <sup>a</sup>																						
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	24
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																					
Serum pregnancy test (females only)	X																					
Urine drug screen	X																					
12-lead ECG <sup>b</sup>	X														X				X			X
Telemetry <sup>c</sup>		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Oral temperature	X																					X
BP and heart rate	X	X			X				X	X		X	X		X		X					X
Randomization (AZD6140 or placebo) <sup>d</sup>			X																			
Pharmacogenetic sample <sup>d</sup>		X																				
AZD6140/placebo administration			X					X	X		X	X		X	X							
AZD6140/AR-C124910XX PK sample <sup>e</sup>		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

Period I (Visit 2) and Period II (Visit 3) <sup>a</sup>																						
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	24
Spirometry (FEV <sub>1</sub> , FVC, PEF)	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
Hand-held peak flow meter <sup>f</sup>		X																				
Cardiopulmonary exercise test <sup>g</sup>																			X			
Concomitant medication monitoring		X-----X																				
Adverse event monitoring		X-----X																				
Discharge from study center																						X <sup>h</sup>

- a. Separated by at least a 7 outpatient day washout period.
- b. An ECG will be performed pre and post cycle exercise test on Study Day 4. ECG results obtained on Study Days 4 and 5 will not be collected on the pCRF. Any clinically significant changes will be recorded as an AE.
- c. Continuous ECGs (telemetry will be performed from 15 minutes pre-dose until 24 hours after the last dose (Day 5 ) per study period).
- d. Prior to dose administration on Study Day 1 of Period I only.
- e. If a PK sample and dose administration are scheduled at the same time, the PK sample should be obtained just prior to dose administration are scheduled at the same time.
- f. Hand-held peak flow meter may be used during nocturnal occurrences of dyspnea, if spirometry technician unavailable. A baseline value will be obtained predose on Study Day 1 with spirometry testing.
- g. Cardiopulmonary exercise will be performed from 5-7 hours post dose.
- h. After completion of all study related procedures.



**Table 3 Procedures for Spontaneous Dyspnea Episode**

Period I (Visit 2) and Period II (Visit 3)	
Procedure <sup>a</sup>	Sequence
Modified Borg Scale/Bidirectional Dyspnea Index	1
Pulse oximetry	2
Minute ventilation including respiratory rate	3
Spirometry (FEV <sub>1</sub> , FVC, PEF) <sup>b</sup>	4
AZD6140/AR-C124910XX PK sample	5

- a. The first time a patient reports experiencing dyspnea, and for subsequent episodes thereafter, these procedures are to be completed sequentially. Additionally, at the investigator's discretion, other diagnostic tests such as an ABG, echocardiography, chest x-ray and auscultation over the chest and trachea may be performed to evaluate dyspnea
- i. b. Hand-held peak flow meter may be used during nocturnal occurrences of dyspnea, if spirometry technician unavailable.

### 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 mild asthma and mild to moderate COPD patients with age range similar to that of the expected treatment population. 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 receiving AZD6140 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, self-limited, and resolved with continued dosing. There were no notable clinical findings (including bronchospasm) in patients experiencing dyspnea who had diagnostic evaluation of their symptoms. One patient reported symptoms of moderate intensity and discontinued the study. Of approximately 400 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 A<sub>3</sub> 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 tolerability data on the effect of AZD6140 in mild asthma and mild to moderate COPD patients.

This will be a double-blind, randomized, 2 cohort, placebo-controlled, 2-period crossover, pharmacokinetic/pharmacodynamic study. Mild asthma and mild to moderate COPD patients are included so that qualification of the differences in physiologic response and subjective experience of dyspnea can be extrapolated to the expected treatment population. The age range encompasses that of the Phase II study where dyspnea was first observed.

The primary variable will be respiratory rate and minute ventilation, based on the hypothesis of affecting adenosine receptors and preclinical observations. Cardiopulmonary cycle exercise testing is included because provocation of the system may elicit measurable changes in physiological parameters that are not otherwise observed. A sub-maximal protocol will be used for the exercise testing, and patients may terminate the test prior to experiencing dyspnea if they experience symptoms, which limit further exercising. Exercise testing also provides an opportunity to simulate the conditions and study the respiratory responses that could contribute to the sensation of 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. In a healthy elderly volunteer study presently completing in the clinic this dose was well tolerated with no reports of dyspnea to date. This study will conclude the clinical phase prior to the start of the respiratory study in mid asthma and mild to moderate COPD patients.

Genetic polymorphisms may affect patients' responses to drugs. Genetic analysis on genes that may be involved in development of dyspnea associated with AZD6140 treatment will improve the understanding of the potential mechanism of this effect of AZD6140. Due to the limited number of patients in this study and low incidence of dyspnea associated with AZD6140 treatment, all patients participating in the study will be required to donate blood for genetic analysis.

A retrospective genetic analysis to explore the genetic variables in genes that are important to absorption and disposition of, and response to AZD6140 including dyspnea will be carried out. Genetic sampling will be mandatory to all patients enrolled. 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 patients who were considered for enrollment but never enrolled eg. patient screening log- according to local procedures. This information is necessary to establish that the patient population was selected without bias.

#### 3.3.2 Inclusion criteria

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

1. Give written informed consent and agree to comply with all requirements of the study (this includes giving consent to donate blood for genetic testing);
2. Generally healthy male or female, who have mild asthma or mild to moderate COPD according to the following criteria:

Cohort	Volunteer Type	Age Range	Respiratory criteria
A	Mild Asthmatic	35 –75 years (inclusive)	Clinical diagnosis > 1 year; FEV <sub>1</sub> ≥ 70% predicted; no exacerbation in past 8 weeks; reversibility demonstrated within the past year (≥ 12% or 200 mL)
B	Mild to Moderate COPD	55 – 75 years (inclusive)	Clinical diagnosis > 1 year; FEV <sub>1</sub> ≥50% predicted; no exacerbation in past 8 weeks; FEV <sub>1</sub> /FVC ratio < 75%; history of tobacco smoking > 10 pack year

3. Females must be of non-childbearing potential defined as surgically sterile (hysterectomized, tubal ligation, or bilateral oophorectomy) or post-menopausal (cessation of regular menses for 12 months);
4. Weight at least 50 kg and have a BMI between 18 to 32 kg/m<sup>2</sup> inclusive. (BMI will be calculated as weight in kg/height in m<sup>2</sup> and will not be reported on the case report form.);
5. Have normal physical examination, laboratory values and vital signs; stable chronic medical conditions unless the investigator considers an abnormality to not be clinically significant or a symptom of mild asthma or mild to moderate COPD;
6. Patients are able to communicate with the investigator, and to understand and comply with all study requirements.

#### 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 leukotriene antagonists within 7 days of Day 1 of Period I;
3. Use of prescription medication for a chronic medical condition within 2 months of Study Day 1 of Period I ([Appendix C](#) identifies several accepted/unaccepted class of drugs); Use of long-acting inhaled corticosteroid medications for asthmatics is permitted in the study; Use of Atrovent inhaler for maintenance therapy of COPD is permitted;
4. Use of prescription medication for an acute medical condition within 4 weeks of Study Day 1 of Period I with the exception of maintenance inhalers;
5. Use of aspirin or any other drug known to increase the propensity for bleeding within 7 days of Study Day 1 of Period I;
6. Use of NSAIDs (ibuprofen) within 3 days prior of Study Day 1 of Period I;
7. 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 Study Day 1 of Period I;
8. 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;
9. A personal history of any first, second or third degree heart block;
10. Screening ECG should not show any clinically significant abnormalities including significant AV block (2<sup>nd</sup> or 3<sup>rd</sup> degree heart block).
11. Any previous episodes of unexplained syncope;
12. 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;
13. History suggestive of peptic ulcer disease or bleeding diatheses;
14. History of diabetes, claudication, CHF (congestive heart failure) or myocardial infarction in the past year;
15. History of anxiety disorder;

16. History of intubation/mechanical ventilation due to respiratory disease or bronchospasms;
17. Platelet count  $<100,000/\text{mm}^3$  at screening;
18. A significant history of alcohol or substance abuse within the past year;
19. Surgery or significant trauma within 3 months prior to Study Day 1 of Period I;
20. Unable to discontinue tobacco use during the inpatient stay of Periods I and II. At the investigator's discretion, use of nicotine supplement may be used for current smokers during the inpatient period;
21. Positive test results for HIV, (human immunodeficiency virus) HBsAg, (hepatitis B surface antigen) or hepatitis C antibody (anti-HCV);
22. Positive urine drug screen unless resulting from declared and confirmed medication;
23. Receipt of an investigational drug within 60 days prior to Study Day 1 of Period I;
24. Previous participation in an AstraZeneca AZD6140 study with the exception of a screening visit or those who now meet the new inclusion criteria based on this amendment.
25. Consumption of Seville oranges (e.g. orange marmalade) or grapefruit-containing products, alcohol, medicines or nutritional supplements within 1 week before Study Day 1 of Period I;
26. Consumption of caffeine-containing or methylxanthine products within 48 hours prior to Study Day 1 of each study period;
27. Blood donation within 90 days before Study Day 1 of Period I;
28. Clinical judgment by the investigator that the patient should not participate in the study;
29. A suspected/manifested infection according to WHO risk categories 2, 3 and 4 (Refer to [Appendix D](#));
30. Involvement in the planning and conduct of the study (applies to both AstraZeneca staff or staff at the investigational site).

#### **3.3.4 Restrictions**

Patients will be required to adhere to the following restrictions:

1. No alcohol consumption will be allowed from 1 week prior to Study 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 Study 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 Study Day 1 of Period I, and through completion of the Follow-up Visit;
4. Patients will be required to refrain from strenuous physical exercise within 48 hours of Study 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. Patients 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 Study 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 or any other drug known to increase the propensity for bleeding within 7 days prior to Study Day 1 of Period I, and through completion of the Follow-up Visit unless otherwise agreed to by the investigator and sponsor;
7. Refrain from taking NSAIDs (ibuprofen) within 3 days prior to Study Day 1 of Period I, and through completion of the Follow-up Visit unless otherwise agreed to by the investigator and sponsor;
8. Scheduled surgery, including dental surgery, at anytime following the screening visit, and through completion of the Follow-up Visit.
9. No use of tobacco or other nicotine-containing products from screening and throughout the Follow-up Visit. At the investigator's discretion, use of a nicotine supplement may be used for current smokers during the study period.

### **3.3.5 Discontinuation of patients from treatment or assessment**

#### **3.3.5.1 Criteria for discontinuation**

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

- Voluntary discontinuation by the patient, who 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 patient's baseline within 2 weeks.
- Severe non-compliance to protocol as judged by the investigator and/or AstraZeneca.

- Incorrect enrollment (ie, the patient does not meet the required inclusion/exclusion criteria) or randomization (ie, the patient is not allocated study drug as described in the protocol) of the patient.
- Patient lost to follow-up
- A suspected/manifested infection according to WHO risk categories 2, 3, and 4 (Refer to [Appendix D](#)).
- Non-exercising respiratory rate >25 per minute, heart rate >120 per minute or SaO<sub>2</sub> <85%; or FEV1 < 1 Liter; or Peak Expiratory flow < 80L/min; or negative change in Modified Borg Scale /Bidirectional Dyspnea scale >3 points on 2 successive assessments (exclude exercising). All these criteria should be at investigator's judgment in conjunction with clinical findings.

### **3.3.5.2 Procedures for discontinuation**

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

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

### **3.3.5.3 Procedures for Discontinuation from Genetic aspects of the study**

Patients 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 patient:

- 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 patient 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 patients 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 micronised tablets	AstraZeneca R & D Charnwood, UK	307	AZD6140, Mannitol, Dibasic calcium phosphate, Povidone K30 (PVP K30), Croscarmellose sodium, Magnesium stearate. <u>Film coat:</u> 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 <u>Film coat:</u> Hydroxypropyl methylcellulose 2910, titanium dioxide, lactose monohydrate, polyethylene glycol 3000,



**Table 4 Identity of Investigational Product**

<b>Investigational product</b>	<b>Dosage form and strength</b>	<b>Manufacturer</b>	<b>Formulation Number</b>	<b>Ingredients</b>
				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**
- **study code**
- **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 patient 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 micronised 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 micronised tablet) in the PM (12 hours after the first dose); thereafter, 180 mg AZD6140 (2 x 90 mg micronised 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 patient is sitting in an upright or in a semi-recumbent position. Patients 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 patients will receive the AM study medication following an overnight (10 hour) fast and will continue to fast for 3-4 hours post dose. On Study Day 4 of each treatment, the patients will be allowed to drink water beginning 2 hours after study drug administration and patients should be encouraged to drink water in order to maintain an adequate hydration state for the cycle exercise test.

The PM dose on Study Days 1, 2 and 3 will be given either 1 hour before or 2 hours 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 patients to treatment groups

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

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

Cohort	Volunteer Type	Randomization Number
A	Mild Asthma	201, 202, 203, 204, etc.
B	Mild to moderate COPD	301, 302, 303, 304, etc.

Patients will be randomized strictly sequentially within their Cohort as patients are eligible for randomization on Day 1 of Period I before study drug administration. Written informed consent

will be obtained before enrollment and the volunteers identified with an enrollment number starting with E0001001. Using the AZ Global Randomization system (Grand) by AstraZeneca LP, patient treatment will be allocated as generated by the system. Patients fulfilling the eligibility criteria will be assigned patient numbers starting with number 201, 202, 203, 204, etc.

Patients will be randomized strictly sequentially as patients are eligible for randomization on Day 1 of Period I before study drug administration. If a patient should be incorrectly randomized, randomization should continue with no attempt to correct the error. If a patient discontinues from the study, the randomization number will not be re-used and the patient will not be allowed to re-enter the study. Patients who withdraw after having received study medication will not be replaced.

### **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 patients 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 patient 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 patient have been made and documented.

### **3.4.5 Concomitant medication**

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

1. prescribed medication from 4 weeks prior to the 1<sup>st</sup> 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; 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. ([Appendix C](#) identifies several accepted/unaccepted class of drugs). Maintenance inhalers such as long-acting corticosteroid inhalers and Atrovent are permitted.

2. OTC preparations that include herbal remedies and vitamin preparations from 1 week prior to the 1<sup>st</sup> dose of study drug and through completion of the assessments and procedures scheduled during the Follow-up Visit.
3. aspirin or any other drug known to increase the propensity for bleeding are specifically prohibited within 7 days prior to Study Day 1 of Period I and through completion of the assessments and procedures scheduled during the Follow-up Visit.
4. NSAIDs (ibuprofen) within 3 days prior to Study Day 1 of Period I and through completion of the assessments and procedures scheduled during the Follow-up Visit.
5. Tiotropium within 7 days of Study Day 1 of Period I and through completion of the assessments and procedures scheduled on Study Day 5 of Period II.
6. Long-acting  $\beta$ -agonists within 72 hours of Study Day 1 and through completion of the assessments and procedures scheduled on Study day 5 of each Period;
7. Short-acting  $\beta$ -agonists (ie, albuterol) within 8 hours of Study Day 1 until completion of the assessments and procedures scheduled on Study Day 5 of each Period. These may be allowed on an 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.
8. Leukotriene antagonists within 7 days of Study Day 1 of Period I and through completion of the assessments and procedures on Study Day 5 of Period II;
9. OTC bronchodilators (ie, Primatine Mist) within 7 days of Study Day 1 of Period I and through completion of the assessments and procedures on Study Day 5 of Period II.

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

#### **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
- Spirometry - A hand-held peak flow meter may be used during nocturnal occurrences of dyspnea, if spirometry technician unavailable.
- Blood pressure/heart rate
- ECG
- Telemetry
- 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 patient 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
- Serum  $\beta$ -HCG (females only)
- 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  $^{\circ}\text{C}$
- Adverse event and concomitant medication monitoring.

#### **4.1.2 Post-study medical examination**

Patients 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
- Serum  $\beta$ -HCG (females only)
- 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 ( $^{\circ}\text{C}$ )
- 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. If an indwelling catheter is used, it should be kept patent with isotonic saline, The isotonic saline must be withdrawn (1 mL) prior to sample collection. 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 samples* in Periods I and II:

Study number: D5130C00034

Patient 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
	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	24	17

j. \* 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: D5130C00034

Patient number:

Period I or II/Study Day:

Collection time<sup>a</sup>:

Analyte: AZD6140/AR-C124910XX

Matrix: PLASMA

k. <sup>a</sup> Defined as time after intake of last dose.

#### 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 patient 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**

**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 patient'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 patient's source document files. (Refer to [Appendix E.](#))

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

“Every 2 minutes 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 patient 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, patients 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 patient reports should be recorded on the pCRF.

#### **4.3.3 Pulse oximetry**

Pulse oximetry (SpO<sub>2</sub>) should be assessed at the times specified in [Table 1 through Table 3](#). SpO<sub>2</sub> should be recorded on the pCRF.

#### **4.3.4 Respiratory rate**

Respiratory rate should be assessed after the patient has been quietly at rest for at least 5 minutes. Respiratory rate should be assessed by counting the number of breaths for a full minute by direct observation or palpation of the patient’s chest wall. If a measurement of minute ventilation with Hans Rudolph Pneumotach system is scheduled, the respiratory rate may be taken as part of this test for a period of one minute. 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. Quality of breaths will not be measured during the cycle exercise test.

#### **4.3.5 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. Tidal volume ( $V_T$ ), and breathing frequency ( $f$ ) will be determined with Hans Rudolph Pneumotach Research System. Calibrations will be completed before each test period.

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

#### **4.3.6 Spirometry**

Patients 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. A minimum of three maneuvers will be completed at each timepoint. Two(2) of the measurements must be closely correlated (within 10%). The highest values for forced vital capacity (FVC), forced expiratory volume in one second (FEV<sub>1</sub>) and peak expiratory flow (PEF) selected from the two measurements will be recorded on the pCRF. Patients 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<sup>3</sup>.

FVC, FEV<sub>1</sub>, FEV<sub>1</sub>/FVC ratio and PEF should be recorded on the pCRF.

#### **4.3.7 Cycle exercise testing**

Tidal volume (V<sub>T</sub>) and oxygen saturation (SpO<sub>2</sub>) by pulse oximetry 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. 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). Minute ventilation, tidal volume and respiratory rate will be monitored during the cycle exercise testing using Hans Rudolph Pneumotach system. 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.

##### **4.3.7.2 Patient preparation**

Patients should not eat for at least 2 hours before any exercise challenge. Patients 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 patient, 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 patient will be informed that he/she must signal any unexpected difficulty, ie, the patient 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 patient wishes to terminate the test. Good communication with the patient throughout the whole procedure will increase the patient's confidence and helps to ensure a good effort.

Seat height should be adjusted so that the patient's legs are almost completely extended when the pedals are at the lowest point and the cycling rhythm practiced. When the patient 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 patient.

#### **4.3.7.3 Symptom-limited incremental cycle exercise test**

Before exercise, while seated comfortably on the cycle ergometer, the patient will be fitted with the Hans Rudolph mask and will breathe for 3 minutes.  $V_T$  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 workload 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 patient will perform 2 minute of loadless pedaling prior to increasing the work rate. The patient will begin to pedal at 50-70 rpm and will be encouraged to maintain this pedaling frequency throughout the exercise challenge. The patient is encouraged to continue exercise for as long as possible (i.e., to symptoms that limit exercise or maximal exercise).

At the end of each 2 minute interval, the work rate will be increased in increments of 25 watts.

The patient will exercise until:

- Until 80% of maximum predicted heart rate achieved; OR
- 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); OR

*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. During recovery, the patient 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.*

ECG monitoring will continue during the recovery period. Heart rate will be recorded from ECG at various intervals (i.e., 1, 2, 3, 5 minutes and as needed up to 10 minutes) during recovery.

Upon completion of the cycle exercise test and at the end of the recovery period, the following assessments need to be completed Modified Borg Scale, Bidirectional Dyspnea Index, Minute Ventilation including respiratory rate, pulse oximetry and spirometry with inspiratory loop.

At any time, a patient may discontinue the test if he/she feels symptoms that are uncomfortable and limit the volunteer's ability to continue exercising. Additionally, 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;
- 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 patient 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:*** Patients will fast 3-4 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, patients will be served lunch. Patients 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:*** Patients 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. Patients 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 patient has not performed these activities recently.

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

**Time of exercise:** No visual cues regarding the time of exercise should be provided to the volunteer. The patient'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 patient 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".

**Comfort with equipment:** Patients 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 positioning and the equipment prior to starting the exercise.

**Familiarity with test:** Before starting exercise, a member of the study team should make sure that the patient is completely familiar with the type of exercise that is to be performed; for the incremental test, patients 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.

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

The cycle exercise test will be captured on the pCRF in these categories: baseline-loadless pedalling, exercise and recovery phase. Each category will include (as appropriate): work load (watts) at the end of each 2 minute interval, HR (BPM), systolic BP (mmHg), diastolic BP (mmHg), RR (BPM),  $V_t$  (Liters), 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 patient'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 patient 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 patient 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 patient 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 (ie, 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:

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**Periods I and II**

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<b>Clinical chemistry</b>	<b>Hematology</b>	<b>Urinalysis</b>
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 <sup>a</sup>
Alanine aminotransferase	Differential leukocytes including:	
Sodium	Lymphocytes	
Potassium	Basophils	
Magnesium	Monocytes	
Phosphate	Neutrophils	
Blood urea nitrogen	Eosinophils	
Chloride		
Uric acid		
Gamma glutamyl transferase		
Creatine kinase (CK)		

l.

m. <sup>a</sup>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  $\geq 3 \times$  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, phencyclidine (PCP), and barbiturates.

If a patient 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 patient 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 patients at screening only. If a test result is positive, the patient will not be allowed to proceed in the study.

Note: Although the results of the HIV and hepatitis screens must be documented in the patient'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 patient will not be permitted to proceed in the study.

#### **4.4.8 Serum pregnancy test**

All women will have a serum  $\beta$ -HCG test at the times specified in [Table 1](#) and [Table 2](#). If at any point a pregnancy test result is positive, the patient will not be allowed to proceed in the study.

Serum pregnancy tests may be run on the same blood collected from the serum chemistry analysis. Refer to [Section 8.4](#) for instructions regarding the reporting and follow-up of pregnancies. No study medication may be given to a woman who has not had negative results on the initial serum pregnancy test as well as the subsequent serum pregnancy tests. The results of the pregnancy tests will be recorded on the CRF as "positive" or "negative".

#### **4.4.9 Electrocardiographic measurements**

For timing of individual measurements refer to study plans [Table 1](#) and [Table 2](#).

##### **4.4.9.1 Resting 12-lead ECG**

Twelve-lead ECGs will be obtained after the patient has been lying down for 5 minutes in each case. The following ECG results will not be collected on the pCRFs unless a clinically significant result is obtained: Study Day 4 pre dose, pre and post cycle exercise test. If a clinically significant result occurs, the result will be recorded as an adverse event.

#### **4.4.9.2 Telemetry**

Continuous ECG monitoring will be performed from 15 minutes prior to the first administration of study drug (Day 1) until 24 hours after the last administration of study drug (Day 5) per study period. If there are multiple pauses on the telemetry of 2.5 seconds or greater in ventricular beats caused by sinoatrial arrest or AV block the subject may be discontinued from the trial. If possible, AstraZeneca should be consulted before discontinuing a subject.

#### **4.4.10 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 patient's clinical condition. These assessments should be entered as unscheduled assessments in the appropriate sections of the pCRF.

##### **4.4.10.1 Blood pressure and heart rate**

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

For timing of individual measurements refer to [Table 1](#) and [Table 2](#). The following blood pressure and heart rate measurements will not be captured on the pCRFs unless a clinically significant result is obtained. Study Day 1: 3 hour post AM dose; study Days 2, 3 and 4: predose (AM) and 3 hours post AM dose. If a clinically significant result is obtained at this time it will be recorded as an adverse event.

##### **4.4.10.2 Respiratory rate**

One minute respiratory rate will be assessed after the patient has been sitting for 5 minutes. Refer to [Table 1 through Table 3](#) for the timing of individual measurements.

##### **4.4.10.3 Oral temperature**

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

#### **4.4.11 Height and weight**

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

#### **4.4.12 Physical examination**

##### **4.4.12.1 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.

#### **4.4.12.2 Brief Physical Examination**

The brief physical examination will include an assessment of the following: general appearance, 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**

Patients 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 (patient name, initials or date of birth) will be placed on the tube or accompanying documentation. A record of the date of the patient 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. Processing, labelling and shipping instructions are provided in [Appendix G](#).

Genetic blood samples will be sent to the following address:

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 is important to maintain patient 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 patient number. Only the investigator will be able to link the blood sample to the individual patient. The sample and data will not be labelled with a personal identifier. The link between the patient 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 patients.

#### **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 including side effects such as dyspnea. 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 patient in this study is as follows:

**Table 6 Volume of blood to be drawn from each patient**

Assessment		Sample volume (mL)	n of samples	Total volume (mL)
AZD6140 PK samples		2 mL	34	68 mL
Blood Sample for genotyping		9 mL	1	9 mL
β-HCG (females only)		Drawn from chemistry	4	
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
<b>Total</b>				<b>207 ML<sup>a</sup></b>

a. Total blood volume may vary if patient experiences a spontaneous dyspnea episode, which requires a 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 is 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 patient 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 Study Delivery 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 patient from study treatment, will be classified as OAEs. Examples of these are marked haematological and other laboratory abnormalities, and certain events that lead to intervention (other than those already classified as serious), dose reduction or significant additional treatment. For each OAE, a narrative may be written and included in the Clinical Study Report.

#### **4.7.1.2 Recording of adverse events**

The patients 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 patient. The AE open standardized questioning should be done discretely in order to prevent the patients from influencing each other.

Any AEs observed or reported by a patient 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 patient 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 patient’s medical notes (permission from the patient will be sought as part of the consent process). Such verification is an essential element of quality control, as it allows the rectification of transcription errors and omissions.

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 patients 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 AstraZeneca 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 patient 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 patients' 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 center'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 April 2005 and to be completed by May 2006.

## **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 patient before clean file status is declared.

### **5.7.2 Pharmacogenetic data**

In the case of genotypic data, only the date the patient gave consent to participation in the genetic component of the study and the date the blood sample was taken from the patient 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 patients, 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 patient's DNA will not be used for any purpose other than those described in the study protocol.

Individual patients 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 patient'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.

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 2 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, and metabolite to parent  $C_{max}$  and AUC ratios

Day 4:  $C_{max}$ ,  $t_{max}$ ,  $AUC_{\tau}$ , 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  $AUC_{\tau}$  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  $AUC_{\tau}$  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 mild asthma and mild to moderate COPD elderly patients.

## **6.4.2 Description of analysis sets**

### **6.4.2.1 Pharmacodynamic analysis set**

All patients who have evaluable respiratory measurements in both periods of the study without any protocol deviations 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 patients who have evaluable pharmacokinetic data without any protocol deviations 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 patients 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 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. AZD6140 and AR-C124910XX plasma concentrations will be summarized and, reported as descriptive statistics and listed by individual at each scheduled time point.

### **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,  $V_{T,f}$ , FVC, FEV<sub>1</sub>, PEF 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 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 patient 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 patients 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 patient 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 patient is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study. Patients must also be notified that they are free to discontinue from the study at any time. The patient should be given the opportunity to ask questions and allowed time to consider the information provided.

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

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

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

The genetic component of this study is optional and the patient may participate in other components of the study without participating in the genetic component. To participate in the genetic component of the study the patient 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 patient and the original filed at the study centre. The principal investigator(s) is responsible for ensuring that consent is given freely and that the patient 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 Patient data protection**

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

The Master Informed Consent Form will explain that study data will be stored in a computer database, maintaining confidentiality in accordance with national data legislation. All data

computer processed by AstraZeneca will be identified by 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 patients' medical history.

Reference to participation in the genetic research component of the study should not be recorded into the patients' 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**

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

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

## **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.





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**Clinical Study Protocol: Appendix B**

Drug Substance AZD6140

Study Code D5130C00034

Appendix Edition Number 1.0

Appendix Date

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**Appendix B**  
**Additional Safety Information**

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## **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
Study Code	D5130C00034
Appendix Edition Number	1.0
Appendix Date	

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**Restricted Medications**

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## **1. MEDICATIONS NOT ALLOWED DURING THE STUDY<sup>(1)</sup>**

### **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<sup>(1)</sup>**

*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 stable 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

<sup>(1)</sup>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
Study Code	D5130C00034
Appendix Edition Number	1.0
Appendix Date	

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**Appendix D**  
**WHO Risk Categories**

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## 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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**Amended Clinical Study Protocol: Appendix E**

Drug Substance	AZD6140
Study Code	D5130C00034
Appendix Edition Number	2.0
Appendix Date	

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**Appendix E**  
**Modified Borg Scale**

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## 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:

“Every two minutes 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

Study Code                              D5130C00034

Appendix Edition Number      1.0

Appendix Date

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**Appendix F**  
**Bidirectional Dyspnea Index**

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## 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.”



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**Clinical Study Protocol Appendix G**

Drug Substance	AZD6140
Study Code	D5130C00034
Appendix Edition Number	1.0
Appendix Date	

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**Appendix G**  
**Instructions for Collection, Storage and Collection of Blood Samples for**  
**Genetic Analysis**

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## 1. BLOOD SAMPLE COLLECTION

Ideally, blood should be collected into *9/10ml polypropylene tubes* containing the *anticoagulant EDTA*. Recommended tubes are detailed in the table below. **Please check tubes are suitable before ordering them.** After collection, blood tubes must be gently *inverted* several times to ensure thorough mixing of EDTA with the sample to prevent clotting.

**Table 1 Recommended blood collection tubes for genotyping sample**

Polypropylene Collection Tube	Part Number	Comments
	1066 USA 1066.001 UK	SARSTEDT Monovette® EDTA KE-9mL
	367525 USA/UK	Becton-Dickinson Vacutainer™ K2E-10mL
	455036 USA/UK	Greiner Bio-one Vacuette® K3E EDTA K3-9mL

- ***Glass tubes MUST NOT be used*** as they may break during transport and freeze-thaw cycles.
- ***Heparin MUST NOT be used as an anticoagulant*** as it may interfere with downstream genotyping methodology.

### 1.1 Labelling of the Collection Tubes

The collection tubes must be labelled with the following information:

Unique sample ID (compliant with protocol)

Study ID (& Study Centre ID, if available)

Date of sample collection

DNA Processing Laboratories have encountered scanning incompatibility due to inclusion of hidden digits in barcode labels. If barcode labels are to be used, a sample will be requested at a later date. All labels must be freezer-proof.

## 2. STORAGE AT THE STUDY CENTER AND TRANSPORT

After collection, blood samples must be stored appropriately at the site of collection and transported to the Central Handling Facility, or Designated DNA Processing Laboratory, *as soon as possible*. The table below shows guidelines for sample storage and transport:

**Table 2 Recommended storage conditions for blood samples immediately after collection**

Option	Storage Temperature at Study Center	Maximum Duration	Transport Temperature	Delivery Time
1	+ 4°C (fridge)	24 hours	0 - 4°C (ice bricks)	24 hours
2	+ 4°C (fridge)	24 hours	Less than -20°C (dry ice)	24-72 hours
3	-20°C (freezer) or -70°C	Up to 1 month	Less than -20°C (dry ice)	24-72 hours

- If blood samples are to be stored at -20°C or less, non-frost free freezer must be used to prevent repeated freeze-thaw of blood which may reduce yield & quality of the DNA obtained
- Samples must NOT be thawed and then re-frozen at any point

The Central Handling Facility, or Designated DNA Processing Laboratory, must be notified of the shipment of any samples prior to dispatch. Ideally, the dispatch note must be sent by either fax or email and must contain the following information:

Study ID, number of samples & list of sample ID's

Courier name, airway bill number & date of shipment

Shipment condition (wet ice or dry ice)

Contact name & address

Considerations should be made to ensure that the samples are delivered during working hours and within 24-72 hours of dispatch.

### 3. RECOMMENDED PACKAGING INSTRUCTIONS

For safety reasons all blood samples must be contained. Samples should be individually placed in a clip-lock bag labelled with the sample ID and sealed. Samples may then be batched and again sealed within a second clip-lock bag labelled with the study ID. For ease of further packaging and protection from damage, samples should then be placed within another plastic bag labelled with the study ID and study centre ID. A bio-safety label should also be applied.

#### 3.1 Sample Shipment.

IATA (International Air Transport Association) approved polystyrene transport boxes must be used.

*For samples transported on wet ice:*

The box should contain frozen ice blocks and protective packaging (polystyrene flocking), to allow for a minimum of 24 hours transport.

*For samples transported on dry ice:*

The box should contain dry-ice pellets (if pellets are not available then blocks may be used if protective packaging such as polystyrene flocking is included) to allow for a minimum of 72 hours transport.

**Each package must be sealed in a cardboard box labelled with the courier airway bill.**

### 4. STORAGE AT THE CENTRAL HANDLING FACILITY OR DESIGNATED DNA PROCESSING LABORATORY

#### 4.1 Central Handling Facility (short term storage)

Blood/EDTA samples can be stored temporarily at the Central Handling Facility and subsequently transported to the Designated Processing Laboratory following the guidelines below:

Storage Temperature at Central Handling Facility	Maximum Duration	Transport Temperature	Delivery Time
-20°C (freezer) or -70°C	6 months	Less than -20°C (dry ice)	24-72 hours

- If Blood Samples Are To Be Stored At -20°C Or Less, Non-Frost Free Freezers Must Be Used To Prevent Repeated Freeze-Thaw Of Blood Which May Reduce Yield & Quality Of The DNA Obtained



- Samples Must Not Be Thawed And Then Re-Frozen At Any Point

#### **4.2 Designated DNA Processing Laboratory (final destination)**

On arrival at the Designated DNA Processing Laboratory, blood/EDTA samples should be stored at - 20°C or -70°C (freezer) until they are processed. **Ideally, samples should be processed within 6 months of collection.**



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**Clinical Study Protocol Amendment**

Amendment Number	3.0
Drug Substance	AZD6140
Study Code	D5130C00034
Date	

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

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This submission /document contains trade secrets and confidential commercial information, disclosure of which is prohibited without providing advance notice to AstraZeneca and opportunity to object.

**Sponsor:**

AstraZeneca Pharmaceuticals, LP, PO Box 15437, Wilmington, DE 19850-5437 USA

**Centers affected by the Amendment:****The protocol for the study is to be amended as follows:**

The protocol is being amended to change the inclusion criteria for patients with COPD to ease recruitment issues. This amendment will also allow patients who have previously screened but who did not meet the inclusion criteria of mild COPD.

**Section of protocol affected:**

Title, Cover Page

**Previous Text:**

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

**Revised Text:**

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

**Section of protocol affected:**

Protocol Synopsis, Title, Page 3

**Previous text:**

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

**Revised text:**

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

**Section of protocol affected:**

Study Centers, type and number of patients planned, Page 3

**Previous text:**

Approximately 24 patients (male or female) including 12 mild asthmatic patients aged 35 to 75 years (inclusive) and 12 mild chronic obstructive pulmonary disease (COPD) patients aged 55 to 75 years (inclusive) will be randomized at a single center to assure the completion of 8 patients in each patient group.

**Revised text:**

Approximately 24 patients (male or female) including 12 mild asthmatic patients aged 35 to 75 years (inclusive) and 12 **mild to moderate chronic obstructive pulmonary disease (COPD)** patients aged 55 to 75 years (inclusive) will be randomized at a single center to assure the completion of 8 patients in each patient group.

**Section of protocol affected:**

Secondary objectives, bullet 2, Page 3

**Previous text:**

Evaluate the pharmacokinetic/pharmacodynamic (PK/PD)relationship between AZD6140/AR-C124910XX concentrations and respiratory parameters in mild asthma patients and mild COPD patients;

**Revised text:**

Evaluate the pharmacokinetic/pharmacodynamic (PK/PD)relationship between AZD6140/AR-C124910XX concentrations and respiratory parameters in mild asthma patients **and mild or moderate COPD patients;**

**Section of protocol affected**

Secondary objectives, bullet 3, page 3

**Previous text:**

Compare respiratory parameters between the mild asthma patients, mild COPD patients and healthy volunteers from study D5130C00028; Compare respiratory parameters between the mild asthma patients, mild COPD patients and healthy volunteers from study D5130C00028

**Revised text:**

Compare respiratory parameters between the mild asthma patients, **mild to moderate COPD** patients and healthy volunteers from study D5130C00028

Study Period Dates, Page 3

**Previous text:**

**Table 1**

**Study period**

Estimated date of first patient enrolled

**Phase of development**

**Revised text:**

**Table 2**

**Study period**

Estimated date of first patient enrolled

Estimated date of last patient completed

**Phase of development**

Clinical Pharmacology (I)

**Section of protocol affected:**

Secondary objectives, bullet 1, Page 4

**Previous text:**

Compare the pharmacokinetics (PK) of AZD6140 and AR-C124910XX in mild asthma patients, mild COPD patients and healthy volunteers from study D5130C00028;

**Revised text:**

Compare the pharmacokinetics (PK) of AZD6140 and AR-C124910XX in mild asthma patients, **mild to moderate COPD** patients and healthy volunteers from study D5130C00028

**Section of protocol affected:**

Study design, Page 4

**Previous text:**

This will be a randomized, double blind, placebo-controlled, PK/PD, single center study to access the effect of AZD6140 on respiratory parameters in mild asthma patients aged 35 to 75 years (inclusive) and mild COPD patients aged 55 to 75 years (inclusive).

**Revised text:**

This will be a randomized, double blind, placebo-controlled, PK/PD, single center study to access the effect of AZD6140 on respiratory parameters in mild asthma patients aged 35 to 75 years (inclusive) and **mild to moderate** COPD patients aged 55 to 75 years (inclusive).

**Section of protocol affected:**

Rationale, Page16

**Previous text:**

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 who were of a younger age range. One pre-clinical study in rats demonstrated an increase in respiratory rate at concentrations of AZD6140 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 patients with mild asthma and mild COPD are being included to assess the sensitivity of patients with respiratory disease. A similar phase I study is ongoing in healthy elderly volunteers to assess for changes in respiratory assessments following administration of AZD6140, and will complete the clinical phase prior to the start of the proposed study with mild asthma and mild COPD patients. The pharmacodynamic effect of AZD6140 on respiratory parameters will be assessed by cardiopulmonary exercise test, respiratory rate, minute

ventilation, spirometry (FVC, FEV<sub>1</sub>), modified Borg Scale<sup>1</sup>, and Bidirectional Dyspnea Index<sup>2</sup>. 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.

**Revised text:**

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 who were of a younger age range. One pre-clinical study in rats demonstrated an increase in respiratory rate at concentrations of AZD6140 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 patients with mild asthma and **mild to moderate** COPD are being included to assess the sensitivity of patients with respiratory disease. A similar phase I study is ongoing in healthy elderly volunteers to assess for changes in respiratory assessments following administration of AZD6140, and will complete the clinical phase prior to the start of the proposed study with mild asthma and **mild to moderate** COPD patients. The pharmacodynamic effect of AZD6140 on respiratory parameters will be assessed by cardiopulmonary exercise test, respiratory rate, minute ventilation, spirometry (FVC, FEV<sub>1</sub>), modified Borg Scale<sup>1</sup>, and Bidirectional Dyspnea Index<sup>2</sup>. 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.

**Section of protocol affected:**

Secondary objective, bullet 2, Page 17

**Previous text:**

Evaluate the pharmacokinetic/pharmacodynamic (PK/PD) relationship between AZD6140/AR-C124910XX concentrations and respiratory parameters in mild asthma patients and mild COPD patients;

**Revised text:**

Evaluate the pharmacokinetic/pharmacodynamic (PK/PD) relationship between AZD6140/AR-C124910XX concentrations and respiratory parameters in mild asthma patients and **mild to moderate** COPD patients;

**Section of protocol affected:**

Secondary objective, bullet 4, Page 17

**Previous text:**

Compare PK of AZD6140 and AR-C124910XX in mild asthma patients, mild COPD patients and healthy volunteers from study D5130C00028;

**Revised text:**

Compare PK of AZD6140 and AR-C124910XX in mild asthma patients, **mild to moderate** COPD patients and healthy volunteers from study D5130C00028;

**Section of protocol affected:**

Secondary objective, bullet 5, Page 17

**Previous text:**

Examine the safety and tolerability of AZD6140 in mild asthma patients and mild COPD patients.

**Revised text:**

Examine the safety and tolerability of AZD6140 in mild asthma patients and **mild to moderate** COPD patients.

**Section of protocol affected:**

Section 3.1, overall study design, Page 17

**Previous text:**

This will be a randomized, double blind, 2 cohort, placebo-controlled, PK/PD, single center study to assess the effect of AZD6140 on respiratory parameters in mild asthma patients aged 35 to 75 years (inclusive) and mild COPD patients aged 55 to 75 years (inclusive). Up to 12 men or women will be randomized in each cohort to ensure at least 8 patients complete the trial within each cohort.

**Revised text:**

This will be a randomized, double blind, 2 cohort, placebo-controlled, PK/PD, single center study to assess the effect of AZD6140 on respiratory parameters in mild asthma patients aged 35 to 75 years (inclusive) and **mild to moderate** COPD patients aged 55 to 75 years (inclusive). Up to 12 men or women will be randomized in each cohort to ensure at least 8 patients complete the trial within each cohort.

**Section of protocol affected:**

Figure 1 Study Flow Chart, Page 21, Cohort B

**Previous text:**

Mild COPD Patients

**Revised text:**

**Mild to moderate COPD**

**Section of protocol affected:**

Section 3.2, Rationale for study design, doses and control groups, second paragraph, Page 25

**Previous text:**

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 mild asthma and mild COPD patients with age range similar to that of the expected treatment population. 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 receiving AZD6140 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, self-limited, and resolved with continued dosing. There were no notable clinical findings (including bronchospasm) in patients experiencing dyspnea who had diagnostic evaluation of their symptoms. One patient reported symptoms of moderate intensity and discontinued the study. Of approximately 400 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).

**Revised text:**

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 mild asthma and , COPD patients with age range similar to that of the expected treatment population. 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 receiving AZD6140 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, self-limited, and resolved with continued dosing. There were no notable clinical findings (including bronchospasm) in patients experiencing dyspnea who had diagnostic evaluation of their symptoms. One patient reported symptoms of moderate intensity and discontinued the study. Of approximately 400 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).



**Section of protocol affected:**

Rationale, Section 3.2, Rationale for study design, doses and control groups, paragraphs 1-4, Page 26

**Previous text:**

Therefore, this study will assess the effect of AZD6140 on respiratory parameters, and will also provide tolerability data on the effect of AZD6140 in mild asthma and mild COPD patients.

This will be a double-blind, randomized, 2 cohort, placebo-controlled, 2-period crossover, pharmacokinetic/pharmacodynamic study. Mild asthma and mild COPD patients are included so that qualification of the differences in physiologic response and subjective experience of dyspnea can be extrapolated to the expected treatment population. The age range encompasses that of the Phase II study where dyspnea was first observed.

The primary variable will be respiratory rate and minute ventilation, based on the hypothesis of affecting adenosine receptors and preclinical observations. Cardiopulmonary cycle exercise testing is included because provocation of the system may elicit measurable changes in physiological parameters that are not otherwise observed. A sub-maximal protocol will be used for the exercise testing, and patients may terminate the test prior to experiencing dyspnea if they experience symptoms, which limit further exercising. Exercise testing also provides an opportunity to simulate the conditions and study the respiratory responses that could contribute to the sensation of 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. In a healthy elderly volunteer study presently completing in the clinic this dose was well tolerated with no reports of dyspnea to date. This study will conclude the clinical phase prior to the start of the respiratory study in mid asthma and mild COPD patients.

**Revised text:**

Therefore, this study will assess the effect of AZD6140 on respiratory parameters, and will also provide tolerability data on the effect of AZD6140 in mild asthma and **mild to moderate** COPD patients.

This will be a double-blind, randomized, 2 cohort, placebo-controlled, 2-period crossover, pharmacokinetic/pharmacodynamic study. Mild asthma and **mild to moderate** COPD patients are included so that qualification of the differences in physiologic response and subjective experience of dyspnea can be extrapolated to the expected treatment population. The age range encompasses that of the Phase II study where dyspnea was first observed.

The primary variable will be respiratory rate and minute ventilation, based on the hypothesis of affecting adenosine receptors and preclinical observations. Cardiopulmonary cycle exercise

testing is included because provocation of the system may elicit measurable changes in physiological parameters that are not otherwise observed. A sub-maximal protocol will be used for the exercise testing, and patients may terminate the test prior to experiencing dyspnea if they experience symptoms, which limit further exercising. Exercise testing also provides an opportunity to simulate the conditions and study the respiratory responses that could contribute to the sensation of 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. In a healthy elderly volunteer study presently completing in the clinic this dose was well tolerated with no reports of dyspnea to date. This study will conclude the clinical phase prior to the start of the respiratory study in mid asthma and **mild to moderate** COPD patients.

**Section of protocol affected:**

Section 3.3.2, Page 27, Table 3

**Previous text:**

**Table 3**

<b>Cohort</b>	<b>Volunteer Type</b>	<b>Age Range</b>	<b>Respiratory criteria</b>
<b>A</b>	Mild Asthmatic	35 –75 years (inclusive)	Clinical diagnosis > 1 year; FEV <sub>1</sub> ≥ 70% predicted; no exacerbation in past 8 weeks; reversibility demonstrated within the past year (≥ 12% or 200 mL)
<b>B</b>	Mild COPD	55 – 75 years (inclusive)	Clinical diagnosis > 1 year; FEV <sub>1</sub> ≥ 60% predicted; no exacerbation in past 8 weeks; FEV <sub>1</sub> /FVC ratio < 75%; history of tobacco smoking > 10 pack year

## Revised Text

**Table 4**

<b>Cohort</b>	<b>Volunteer Type</b>	<b>Age Range</b>	<b>Respiratory criteria</b>
<b>A</b>	Mild Asthmatic	35 –75 years (inclusive)	Clinical diagnosis > 1 year; FEV <sub>1</sub> ≥ 70% predicted; no exacerbation in past 8 weeks; reversibility demonstrated within the past year (≥ 12% or 200 mL)
<b>B</b>	<b>Mild to Moderate COPD</b>	55 – 75 years (inclusive)	Clinical diagnosis > 1 year; FEV <sub>1</sub> ≥ <b>50%</b> predicted; no exacerbation in past 8 weeks; FEV <sub>1</sub> /FVC ratio < 75%; history of tobacco smoking > 10 pack year

### Section of protocol affected:

Inclusion criteria #5, Page 27

### Previous text:

Have normal physical examination, laboratory values and vital signs; stable chronic medical conditions unless the investigator considers an abnormality to not be clinically significant or a symptom of mild asthma or mild COPD;

### Revised text:

Have normal physical examination, laboratory values and vital signs; stable chronic medical conditions unless the investigator considers an abnormality to not be clinically significant or a symptom of mild asthma or **mild to moderate** COPD

### Section of protocol affected:

Exclusion Criteria # 24, page 29

### Previous text:

Previous participation in an AstraZeneca AZD6140 study;

### Revised Text

**Previous participation in an AstraZeneca AZD6140 study with the exception of a screening visit or those who now meet the new inclusion criteria based on this amendment;**

### Section of protocol affected:

Section 3.4.3 Method of assigning patients to treatment groups.

**Previous text:**

<b>Cohort</b>	<b>Volunteer Type</b>	<b>Randomization Number</b>
A	Mild Asthma	201, 202, 203, 204, etc.
B	Mild COPD	301, 302, 303, 304, etc.

**Revised text:**

<b>Cohort</b>	<b>Volunteer Type</b>	<b>Randomization Number</b>
A	Mild Asthma	201, 202, 203, 204, etc.
B	<b>Mild to moderate</b> COPD	301, 302, 303, 304, etc.

**Section of protocol affected:**

Section 6.4.2 ,Statistical evaluation, Page 59

**Previous text:**

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 mild asthma and mild COPD elderly patients.

**Revised text:**

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 mild asthma and **mild to moderate** COPD elderly patients

**Reason for Amendment:**

The reason for the amendment is to allow inclusion of patients with moderate COPD to enter the trial. This will allow the site to complete the recruitment of patients for this study.

**Persons who initiated the Amendment:**



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***Clinical Protocol Amendment No.3.0 Appendix A***

Drug Substance	AZD6140
Study Code	D5130C00034
Appendix Edition Number	3.0
Appendix Date	

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**Appendix A**  
**Signatures**

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## **ASTRAZENECA SIGNATURE(S)**

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

---

This Clinical Study Protocol and all Amendments to the CSP have been subjected to an internal AstraZeneca peer review

I agree to the terms of this study protocol/amendment.

**AstraZeneca Research and Development  
site representative**

\_\_\_\_\_  
Date  
(Day Month Year)

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## **ASTRAZENECA SIGNATURE(S)**

---

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

---

This Clinical Study Protocol and all Amendments to the CSP have been subjected to an internal AstraZeneca peer review

I agree to the terms of this study protocol/amendment.

**AstraZeneca Research and Development**  
site representative

Date  
(Day Month Year)

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## **SIGNATURE OF PRINCIPAL INVESTIGATOR**

---

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

---

This Clinical Study Protocol and all Amendments to the CSP have 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)

This document contains confidential information, which should not be copied, referred to, released or published without written approval from AstraZeneca. Investigators are cautioned that the information in this protocol may be subject to change and revision.