Clinical Study Protocol

Drug Substance	AZD3355
Study Code	D9120C00032
Edition Number	1
Date	

A double-blind, placebo controlled, randomised, phase IIA pharmacodynamic 4-way cross-over study to estimate the dose response relationship of AZD3355 on the number of reflux episodes assessed by impedance/pH in patients with GERD and a partial response to PPI treatment

Sponsor:
AstraZeneca AB
151 85 Södertälje
Sweden

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

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

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

AstraZeneca

PROTOCOL SYNOPSIS

A double-blind, placebo controlled, randomised, phase IIA pharmacodynamic 4-way cross-over study to estimate the dose response relationship of AZD3355 on the number of reflux episodes assessed by impedance/pH in patients with GERD and a partial response to PPI treatment

Principal Investigator

Study centre(s) and number of patients planned

The study will be conducted at one (1) study centre. The study will include 27 randomised patients to achieve the necessary number of evaluable patients on each of the doses.

Study period	Phase of development
Estimated date of first patient enrolled	Phase I
Estimated date of last patient completed	

Objectives

Primary objective

To estimate the dose-response relationship of AZD3355 on the total number of reflux episodes during 24 hours.

Secondary objectives

To estimate the effect over 24 hours as total values, and in upright and supine position of 4 different doses of AZD3355 compared to placebo measured as reduction in:

- total number of reflux episodes
- the number of acid-, weakly acidic- and weakly alkaline reflux episodes

- the height (mean proximal extent), content (liquid, gas or a mixture) of the refluxate and esophageal pH in the pH interval 4-6.5
- the time with esophageal pH<4
- the time with intragastric pH<4

To study the relationship between total number of reflux episodes, acid-, weakly acidic- and weakly alkaline reflux and GERD symptoms during 24 hours.

To study the pharmacokinetics of AZD3355 after 30, 90, 120 and 240 mg MR 1h capsules.

To study the relationship between exposure (area under curve (AUC)0-12h , AUC12-24h, AUC0-24h) and reflux episodes after the first and second doses of AZD3355.

Safety objectives

To assess the safety and tolerability of 4 different doses of AZD3355 as add-on treatment to a PPI, by evaluation of adverse events (AEs), laboratory variables, digital ECG (dECG) and physical examination.

To assess the effect of AZD3355 on orthostatic blood pressure and pulse, as well as sitting blood pressure and pulse over 24 hours.

Exploratory objectives

To collect and store DNA for future exploratory research into genes/genetic variation that may influence response (ie, PK profile, safety, tolerability and efficacy) to AZD3355 and/or susceptibility to GERD.

Study design

This is a randomised, double-blind, placebo controlled, 4-way crossover study to estimate the dose response relationship of AZD3355 on the number of reflux episodes assessed by esophageal impedance/pH monitoring in patients with GERD with a partial response to PPI treatment, as characterized by persistent GERD symptoms. Each patient will receive placebo and 3 of the 4 doses of AZD3355 in a randomised order.

Target patient population

Patients with GERD who have a partial response to PPI treatment, as characterized by persistent GERD symptoms.

Investigational product, dosage and mode of administration

AZD3355 30 mg, 90 mg, 120 mg or 240 mg MR1h capsule formulation taken orally twice daily.

Comparator, dosage and mode of administration

Placebo capsule to be taken orally twice daily.

Duration of treatment

The study starts with a pre-entry visit up to 21 day before treatment. This is followed by 4 treatment periods with a 7-28 days washout period between each. Each treatment period is one day, one dose in the morning and one dose in the evening. After the last treatment period the study ends with a follow-up visit 5-10 days later.

Outcome variable(s):

Primary Variable

Total number of reflux episodes (total, upright, supine).

Secondary Variables

Number and percentage of registration time of acid (pH<4), weakly acidic (4≤pH<6.5) and weakly alkaline (pH≥6.5) reflux episodes (total, upright, supine).

Number and percentage of liquid, gas and mixed gas/liquid reflux episodes (total, upright, supine).

Number and proportion of reflux events (liquid, gas or mixture) with proximal extent.

The percentage of time with intragastric pH<4 during the 24-hour period at 10 cm below LES (total, upright, supine).

GERD symptoms registration during 24 hours.

Mean bolus clearance time (sec).

Safety variables

Adverse events, laboratory variables, digital ECG (dECG), vital signs, physical examination, orthostatic testing, sitting blood pressure and pulse over 24 hours.

Pharmacokinetic variables

AUC_{0-12h}, AUC_{12-24h} , AUC_{0-24h}, C_{max} and t_{max}.

Exploratory variables

Genes/genetic variation that may influence response (ie, PK profile, safety, tolerability and efficacy) to AZD3355 and/or susceptibility to GERD.

Statistical methods

The estimation of the dose-response curve will primarily be made by estimating the parameters of a fixed effect Emax model. In addition, the same model will be estimated with

data from study D9120C00020 included, with a mixed effect Emax model with and without data from D9120C00020 included. The possibility of using additional model will also be explored.

The effect of 4 different doses of AZD3355 measured as reduction in the total number of reflux episodes during 24 hours, compared to placebo will be assessed by estimating the inhibition (%) in the primary variable after treatment with AZD3355 30 mg, 90 mg, 120 mg and 240 mg compared to placebo.

The analysis will be based on an mixed effect model with treatment, period and sequence as fixed effects and patient as a random effect. The same analysis will be made for the other variables addressed in the secondary objective. No correction for multiplicity will be made.

The primary and secondary analyses will be made on the efficacy analysis set, defined as all patients with data not affected by major protocol deviations and violations, relevant for the analysis of a specific variable.

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

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

Abbreviation or special term	Explanation
AE	Adverse event (see definition in Section 6.4.1)
ALT	Alanine aminotransferase
anti-HCV	hepatitis C antibody
AST	Aspartate aminotransferase
AUC	Area Under Curve
bid	"*bis in die"* twice daily
C_{max}	Maximum concentration in plasma
COC	Combined Oral Contraceptive
CRF	Case Report Form
DAE	Discontinuation of Investigational Product due to Adverse Event
DNA	Deoxyribonucleic acid
dECG	Digitally recorded Electrocardiogram
$GABA_B$	Gamma-Amino-Butyric-Acid B
GCP	Good Clinical Practice
GERD	Gastro Esophageal Reflux Disease
HBsAg	Hepatitis B surface Antigen
HIV	Human Immunodeficiency Virus
HP	Helicobacter Pylori
IP	Investigational Product
IR	Immediate release
IRB	Institutional Review Board
LES	Lower Esophageal Sphincter
LESP	Lower Esophageal Sphincter Pressure
LLOQ	Lower Limit of Quantification
MR	Modified Release
OAE	Other Significant Adverse Event (see definition in Section 11.2.1)
PD	Pharmacodynamics
PGx	Pharmacogenetic research

Abbreviation or special term	Explanation
PI	Principal Investigator
PK	Pharmacokinetics
PPI	Proton Pump Inhibitor
RESQ-7	Reflux Symptoms Questionnaire 7 days recall
RDQ	Reflux Disease Questionnaire
SAE	Serious adverse event (see definition in Section 6.4.2)
SAP	Symptom associated probability
SI	Symptom Index
t_{max}	Time to reach maximum plasma concentration
ULN	Upper Limit of Normal

1. INTRODUCTION

1.1 Background

Gastroesophageal Reflux Disease (GERD) is a common condition with the predominant symptoms of heartburn and/or regurgitation, and occurs at least weekly in 10-20% of the population in the Western world (Dent et al 2005). Acid suppressive therapy with proton pump inhibitors (PPI) has been very effective in the treatment of GERD. However, approximately 20 to 30% of patients with GERD only have a partial response to PPI treatment, as characterized by persistent GERD symptoms (Fass 2007, Jones et al 2007). There is an unmet medical need in the patients that continue to have symptoms while on PPI treatment, especially since available data indicate that these symptoms are associated with a negative impact on patients' lives (Wiklund et al 2003).

As part of AstraZeneca´s ongoing effort to develop treatments for patients with GERD, the current clinical program focuses on patients who are partial responders to PPI treatment with persistent GERD symptoms of an intensity and frequency that merit additional treatment. A logical approach to therapy in the group of patients with persistent GERD symptoms despite PPI treatment is to focus on known mechanisms for reflux episodes. Transient lower esophageal sphincter relaxations (TLESRs) have been demonstrated to be the predominant mechanism for reflux episodes (van Herwaarden et al 2002). Thus, reducing the number of TLESRs would offer a novel and physiological approach to GERD treatment.

AZD3355, a selective Gamma-Amino-Butyric-Acid B ($GABA_B$) receptor agonist, is a novel reflux inhibitor specifically being developed for the treatment of GERD (Lehmann et al 2009). The accumulated data suggest that the primary site of action for AZD3355 to inhibit TLESRs resides in the mechanosensitive vagal afferent nerve endings in the gastric wall.

In humans (study D9120C00020), AZD3355 as compared to placebo given as add-on treatment to PPI in patients with persistent GERD symptoms despite PPI therapy, reduced the number of TLESRs, increased the lower esophageal sphincter (LES) pressure and decreased the number of reflux episodes

AZD3355 is being developed as an add-on treatment to PPIs to provide symptom relief in patients with a partial response to PPI therapy, as characterized by persistent GERD symptoms. These patients may have symptoms due to acidic, weakly acidic or weakly alkaline refluxate entering the esophagus. It is anticipated that by targeting the LES and reducing TLESRs, increasing LES pressure and decreasing reflux episodes, this will decrease acid, weakly acidic and weakly alkaline reflux, resulting in a reduction of GERD symptoms.

To date the pharmacokinetic-pharmacodynamics (PK-PD) relationship has been studied only for single dose 0.8mg/kg in healthy volunteers and the corresponding dose of 65mg twice daily (bid) in patients. The present study therefore aims to explore the dose relationship for a span of doses, using reflux episodes measured by impedance/pH as the primary endpoint.

AstraZeneca also intends to perform genetic research in the AZD3355 clinical development programme to explore how genetic variations that may affect the clinical parameters associated with AZD3355. Collection of deoxyribonucleic acid (DNA) samples from populations with well described clinical characteristics may aid in the identification of future drug targets and projects to validate already identified targets.

Further non-clinical and clinical information regarding AZD3355 can be found in the Investigator's Brochure.

1.2 Research hypothesis

A dose-response relationship and the safety of potential therapeutic doses of AZD3355 may be demonstrated by administration of oral doses, both lower and higher than those previously evaluated in human efficacy studies.

Genetic variations may affect the clinical parameters associated with AZD3355 and may aid in the identification of future drug targets and projects to validate already identified targets.

1.3 Rationale for conducting this study

In a study in healthy volunteers (D9120C00001) an oral single dose of 0.8 mg/kg AZD3355 solution and placebo was given followed by a sleeve manometry combined with pH metry for 4 hours. A significant effect was demonstrated as compared to that of placebo, with a reduction of the number of TLESRs by 36% (95% CI 18% - 49%), an increase in the lower esophageal sphincter (LES) pressure as well as a reduction of acid reflux episodes.

In study D9120C00020 in patients with persistent GERD symptoms despite PPI treatment, oral bid dosing of 65 mg AZD3355 (drug in capsule) and placebo was given as 3 doses (bid on day 1 and 1 dose in the morning of day 2) while impedance/pH registration was measured during 24 h, followed by a 4 h combined impedance/pH and manometric measurement on day 2. A significant effect compared to that of placebo was demonstrated, with an average reduction of TLESRs of 25% (geometric mean ratio 0.75 (95% CI 0.60, 0.93)). AZD3355 increased the LES pressure by 28% (geometrical mean ratio 1.28 (95% CI:1.05,1.57)). In the AZD3355 treatment group the mean value for the total number of reflux episodes was reduced by 10 (95%CI:-15, -4.8), which corresponds to a relative reduction of approximately 47%. During 24 hours post first dose the mean value for the total number of reflux episodes was reduced by 39% (geometric mean ratio 0.61 (95% CI:0.52, 0.71)) in the AZD3355 treatment group.

These studies explored only one dose level (0.8 mg/kg corresponds to a dose of 65 mg) however a dose response relationship in humans has not been determined. The present study therefore aim to explore the dose-response relationship and safety of both lower and higher doses of AZD3355 than tested in the previous efficacy studies (see above) in patients with GERD with a partial response to PPI treatment.

Further, blood will be collected for pharmacogenetic research to explore how genetic variations may affect the clinical parameters associated with AZD3355. Future research may

suggest other genes or gene categories as candidates for influencing not only response to AZD3355 but also susceptibility to Adverse Events for which AZD3355 may be evaluated. Thus, this genetic research may involve study of as yet un-named genes or gene categories.

1.4 Benefit/risk and ethical assessment

Seventeen human phase I studies with AZD3355 have been completed and reported. In these studies, 489 healthy volunteers have been exposed to AZD3355. To date the maximal oral doses given to healthy volunteers are; 1800 mg as a single dose; 800 mg bid for 5 days; and 150 mg bid for 7 days. The most commonly reported adverse events (AE) over the range of doses described in healthy volunteers were paraesthesia (24.4%), headache (10.8%), feeling hot (8.3%), diarrhoea (7.9%), flatulence (6%) and dizziness (5.1%).

A total of 382 patients have been treated with AZD3355 in 3 clinical phase II studies, with repeated oral doses, 65 mg bid as add on to a PPI for up to 4 weeks (122 patients in study D9120C00011, and 235 patients in study D9120C00027) and as 3 doses (65 mg bid on day one, and a single dose of 65 mg on day 2) in 25 patients in study D9120C00020. The most commonly reported adverse events (AEs) in the 4 week patient studies were gastrointestinal symptoms (mainly diarrhoea (8.7%) and nausea (3.6%)) followed by AEs related to the nervous system (mainly paraesthesia (5%), fatigue (2.5%) and headache (2.2%) and skin disorders (pruritus, 3.9%). In the shorter patient study (3 doses over two days) the most common AEs were headache, paraesthesia and abdominal distension.

To date, 3 SAEs on active treatment with AZD3355 have been reported. One report of sinus arrest (sinus pause of 8.2 seconds in combination with presyncope) in a healthy volunteer on AZD3355 600 mg which was considered causally related to the study drug by the investigator. One report of hypertension in a patient and one report of operative haemorrhage in a patient admitted for elective surgery (Both patients were given AZD3355 65mg bid and the investigators considered the SAE´s not to be related to the study drug).

Potential or identified risks as of today

- A Thorough QT/QTc study (D9120C00012) was performed that failed to exclude an effect of AZD3355 on the prolongation of the QTc interval based on QTcF. In this study, the QTcF upper limit of the two-sided 90% CI exceeded 10 msec on Day 1, albeit by a small margin (no 90% CI values reaching 11 msec) at three time points.

- Orthostatic tests performed in healthy volunteers given single doses between 65 and 1800 mg and either as single or repeated doses 65 to 800 mg has provoked positive test with or with out dizziness. Apart from provoked dizziness, there are some reports of postural dizziness and one event of presyncope with an accompanied sinus arrest (sinus pause of 8.2 seconds) in phase I.

- Paraesthesia is an identified adverse drug reaction to AZD3355 both in healthy volunteers and patients. The reported symptoms were mainly mild to moderate in intensity, reversible, short lasting and usually started and ended within one hour after dosing. Paraesthesia has not been associated with any abnormal neurological findings of sensory or motor function on physical examination.

- No clinically relevant findings have been seen in clinical chemistry or haematology laboratory screen or urine analyses in any of the studies completed so far, except for mild liver enzyme increases after repeated dosing of higher doses than proposed in this study (400 mg bid).

The benefit to the subjects participating in this study is limited to the thorough medical investigation they will be given (physical examination, ECG, laboratory tests etc) before entering the study. The patients will be followed during the study period and at the follow-up visit. The safety of the patients has been considered when deciding the inclusion- and exclusion criteria, the proposed restrictions and the criteria for discontinuation. No adverse effects on fertility or embryofetal development have been seen in non-clinical studies. However, since no data exist on embryofetal development in humans, women of childbearing potential must use an adequate highly effective contraceptive method to be eligible for studies with AZD3355.

The methods for assessing reflux episodes and pH may be uncomfortable to the subject and involves 24 hours testing. Mild adverse events related to 24 hour catheter impedance/pH monitoring may be observed, eg. less than 5% of subjects may have nasal irritation and very rarely, patients may develop epistaxis.

In conclusion, in the present study the maximum daily dose of AZD3355 will be 240 mg bid for one day. Based on the non-clinical and clinical data presented in the IB, together with the restrictions and the close monitoring in this study, the discomfort and risks associated with an oral administration of AZD3355 modified release (MR) 1h formulation in doses up to 240 mg are judged as low. This risk may be justified by the benefit of developing a potential new effective treatment for patients with GERD who have a partial response to PPI treatment with persistent GERD symptoms.

The IB for AZD3355 contains the information supporting the overall benefit/risk assessment of the investigational product and is available as a reference.

2. STUDY OBJECTIVES

2.1 Primary objective

- To estimate the dose-response relationship of AZD3355 on the total number of reflux episodes during 24 hours.

2.2 Secondary objectives

- To estimate the effect over 24 hours as total values, and in upright and supine position of 4 different doses of AZD3355 compared to placebo measured as reduction in:
  - total number of reflux episodes
  - the number of acid-, weakly acidic- and weakly alkaline reflux episodes
  - the height (mean proximal extent), content (liquid, gas or a mixture) of the refluxate and esophageal pH in the pH interval 4-6.5
  - the time with esophageal pH<4
  - the time with intragastric pH<4
- To study the relationship between total number of reflux episodes, acid-, weakly acidic- and weakly alkaline reflux and GERD symptoms during 24 hours.
- To study the pharmacokinetics of AZD3355 after 30, 90, 120 and 240 mg MR 1h capsules.
- To study the relationship between exposure (area under curve (AUC)0-12h , AUC12-24h, AUC0-24h) and reflux episodes after the first and second doses of AZD3355.

2.3 Safety objectives

- To assess the safety and tolerability of 4 different doses of AZD3355 as add-on treatment to a PPI, by evaluation of adverse events (AEs), laboratory variables, digital ECG (dECG) and physical examination.
- To assess the effect of AZD3355 on orthostatic blood pressure and pulse, as well as sitting blood pressure and pulse over 24 hours.

2.4 Exploratory objectives

- To collect and store DNA for future exploratory research into genes/genetic variation that may influence response (ie, PK profile, safety, tolerability and efficacy) to AZD3355 and/or susceptibility to GERD.

3. STUDY PLAN AND PROCEDURES

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

3.1 Overall study design and flow chart

The study will include 27 randomised patients to achieve the necessary number of evaluable patients on each of the doses. See Section 12.3.

This is a randomised, double-blind, placebo controlled, 4-way crossover study to estimate the dose response relationship of AZD3355 on the number of reflux episodes assessed by esophageal impedance/pH monitoring in patients with GERD with a partial response to PPI treatment, as characterized by persistent GERD symptoms. Each patient will receive placebo and 3 of the 4 doses of AZD3355 in a randomised order.

- AZD3355 30 mg MR1h capsule formulation bid
- AZD3355 90 mg MR1h capsule formulation bid
- AZD3355 120 mg MR1h capsule formulation bid
- AZD3355 240 mg MR1h capsule formulation bid
- Placebo capsule formulation bid

Figure 1 Study Design

Each patient will receive placebo and 3 of the 4 doses of AZD3355 in a randomised order.

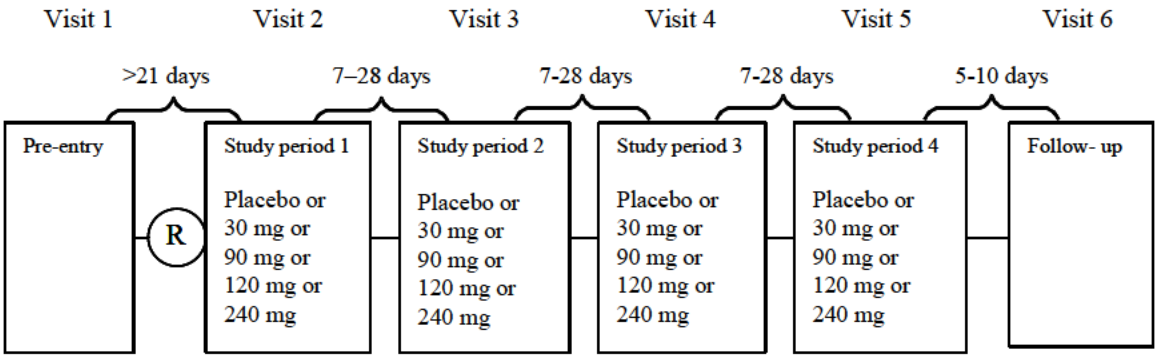

Table 1 Study Plan

Assessment	Visit 1	Visit 2 – Visit 5		Visit 6
	Pre-entry visit	Study Period Visits		Follow-up visit
		Day 1	Day 2	
Signed Informed Consent Form (ICF)	X			
Signed ICF for genetic research (if applicable)	X			
Demography	X			
RESQ-7	X			
Inclusion/exclusion criteria	X			
Medical/surgical history	X			
Physical examination	X			X
Weight and height (BMI calculated)	X			
dECG[a]	X	X		X
BP and pulse[b] (supine after 10 min rest)	X	X	X	X
Orthostatic BP and pulse[c]		X		
Drug screen[d]	X			
Hepatitis B, C, HIV	X			
Full lab screen	X			X
Pregnancy test[e]	X	X		X
Reduced lab screen[f],		X	X	
Randomisation[g]		X		
PGx[h]		X		
HP serology[i]	X			
Impedance/pH		X	X	
Manometry for localisation of LES[j]	X			
Study drug administration		X		
Concomitant Medications	X	X	X	X
PK-sampling[k]		X	X	
AE recording[l]		X	X	X
SAE recording[m]	X	X	X	X

[a] Resting 12-lead dECG after lying down for at least 10 minutes.
[b] See Table 2 for details.
[c] Should be done predose and 2 hours post dose on day 1 of each study period.
[d] The drug abuse screen will be repeated randomly once more during the study period.
[e] Urine pregnancy test in women of childbearing potential.

[f] Samples taken before and 12 hours after dose.
[g] Only at Visit 2.
[h] PGx will be collected after randomisation only if patient has signed optional Pharmacogenetic informed consent (see Appendix D).
[i] Unless HP eradication has been performed within the last 6 months prior to enrolment.
[j] Not necessary if done within 3 years of inclusion and at the same laboratory as this study is performed.
[k] For detailed PK sampling schedule see Table 2 .
[l] AE recording will start after administration of the first dose at visit 2.
[m] SAE recording will start after signing of Informed Consent.

Visit 1 (Pre-entry visit)

Within 21 days prior to first study day patients will be scheduled for the pre-entry visit. Patient with a history of GERD symptoms for 6 months (need not to be consecutive) and a partial response to PPI treatment, as characterized by persistent GERD symptoms will be screened regarding the inclusion/exclusion criteria. Partial response to PPI treatment implies that the patients have experienced partial symptom response to the PPI treatment and the patients must have been on optimised[1] continuous PPI treatment with approved doses for any GERD indication during the last 4 weeks prior to the enrolment visit. Patients with endoscopy verified reflux esophagitis, verified with endoscopy, within the last 8 weeks must have completed the prescribed 8 weeks treatment with a PPI. The patients will continue with the same PPI treatment during the study and avoid changing the dose or stopping treatment of their PPI during the study.

Informed consent will be signed and patients will complete the screening questionnaire (the Reflux Symptoms Questionnaire, 7 days recall (RESQ-7) prior to other examinations at the clinic.

Enrolled patients will undergo a physical examination, blood sampling for a full laboratory screen, Hepatitis B and C, Human Immunodeficiency Virus (HIV) and urine sampling for pregnancy test (if applicable) and drugs of abuse screen. Weight, height, dECG, supine and sitting blood pressure and pulse will also be recorded. A Helicobacter Pylori (HP) serological test will be performed unless eradication has been done within the last 6 months.

A standard water perfused stepwise withdrawal manometry is performed to establish the position of the LES (if not previously known). The distance from the nares should be noted in order to place the impedance/pH catheter at the same position at all visits (V2-V5).

Information about medical and surgical history will be collected, together with PPI usage and other concomitant medications.

Serious Adverse Events (SAEs) will be recorded from Visit 1 throughout the study.

[1] An optimised PPI treatment is a treatment which according to the investigator judgement can not be further improved by changing brand or dosing of the PPI and is within the label for any GERD indication (eg, no bid dosing allowed).

If the patient is not eligible for the study a minimum of required data must be entered in the CRF, see Section 5.10.

For all study related procedures see the Study Plan in Table 1.

Visit 2 – Visit 5 (Study periods)

During the 4 study periods each patient will receive 3 of the 4 different doses of AZD3355 and placebo in a randomised order. Each study period includes administrations of study drug on day 1, one hour prior to breakfast and one hour prior to dinner.

The patient should be fasting overnight before each study period (no food after 22:00 and no fluid after 24:00).

Day 1: Sitting BP and pulse is measured, then the patient should be resting in supine position before the dECG and an orthostatic test is performed with supine and standing BP and pulse (see Section 6.4.8.1).

Before dosing a reduced safety lab screen and pregnancy test (if applicable) is collected. A genetic blood sample is collected at the same time provided that the patient has signed the optional pharmacogenetic research (PGx) informed consent. (see Table 2).

The catheter with sensors for impedance, esophageal and intragastric pH are then inserted into the esophagus.

In order to get baseline information before study treatment has started, the active questioning for specific symptoms related to pre-syncope dizziness will take place (see Section 6.4.3.2) prior to first intake of investigational product (IP) at visit 2-5.

The patient is then randomised. After intake of the ordinary dose of PPI, the first dose of AZD3355 or placebo is given and the 24-hour recording is started. An additional dECG will be recorded 2 hours post dose. After the dECG, an orthostatic test is performed with supine and standing BP and pulse (see Section 6.4.8.1) and after which the PK blood samples are drawn. During the first 24 hours impedance/pH measurement, GERD symptoms if experienced, meal intake and periods in supine position will be recorded by the patient using the impedance/pH recording devise and a paper diary (time is recorded by the devise and the activity is described in the paper diary).

Pharmacokinetic (PK) samples as well as sitting BP and pulse should be taken from pre-dose until 24 hours after the first dose (for detailed time of PK samples, BP and pulse see Table 2). During the early morning PK sampling the patient should be encouraged to stay in supine position until their normal waking time

The patient will eat a standardized meal approximately one hour after each dose of AZD3355 and at the same time points during the different study periods. The meals (food and liquid) will be standardized primarily regarding fat content and pH. The patient should be encouraged to complete the meal. If not possible, the same amount should be eaten at each meal during

each study period. During the stay in the clinic the patients should be encouraged to move around to mimic normal activities as much as possible.

Day 2: In the morning of day two, the last PK sample and a reduced safety lab screen will be collected. Sitting BP and pulse is measured before the patients leaves the clinic.

The active questioning for specific symptoms will take place again (see Section 6.4.3.2) before the patient leaves in the mornings after the impedance is completed, but before the catheter is removed.

No antacids are allowed 24 hours before and during the treatment period (due to interference with pH measurement). The 4 study periods will be separated by a washout period of 7-28 days.

Adverse events (AEs) will be recorded after drug administration at Visit 2 throughout the study.

Table 2 Study period procedures

Time post dose (h:min)	Clock Time[a] (h:min)	PK blood sample[b] (No)	Blood pressure and pulse monitoring[c]	Activity
Day 1				
Pre-dose	07:00	1	Sitting, supine and standing after 1 min	BP, pulse, dECG, orthostatic test, reduced laboratory screen, PGx[e] urine sample for drug abuse screen[d] Placement of impedance/pH catheter
0:00	08:00			Active question specific symptoms Drug/placebo administration Start of impedance/pH recording
0:30	08:30	2		
1:00	09:00			Breakfast
1:30	09:30	3		
2:00	10:00	4	Supine + standing after 1 min	dECG, orthostatic test
2:30	10:30	5		
4:00	12:00	6	Sitting	
4:30	12:30			Lunch
8:00	16:00	7	Sitting	Snack
12:00	20:00	8	Sitting	Drug/placebo administration
12:30	20:30	9		
13:00	21:00			Evening meal
13:30	21:30	10		
14:00	22:00	11	Sitting	
14:30	22:30	12		
Day 2				
16:00	00:00	13		
21:00	05:00	14		
24:00	08:00	15	Sitting	BP and pulse, end of 24-hour impedance/pH recording, active question specific symptoms, removal of catheter, reduced lab screen, breakfast (optional)

[a] Approximate time. Exact time of dose and subsequent PK samples must be recorded.
[b] PK samples must be taken as close to the actual time as possible with exact time noted.
[c] BP and pulse (sitting and supine) must be taken after 10 minutes of rest.

[d] A drug abuse screen will be repeated randomly once more during the study period.
[e] PGx will be collected after randomisation only if patient is participating in the optional PGx part of the study (see Appendix D).

Follow-up visit

Patients will return to the study site within 5-10 days after completion of Study Period 4 for the follow-up visit. This will include a dECG, sitting BP and pulse, physical examination, a full laboratory screen, pregnancy test (if applicable) and follow-up of any AE or serious adverse event (SAE).

Assessments will be performed as detailed in the Study Plan (see Table 1).

3.2 Rationale for study design, doses and control groups

The doses were selected in order to evaluate the effect of AZD3355 in a dose range from 30 mg to 240 mg bid, in order to evaluate doses both higher and lower than the 65 mg bid dose that was used in study D9120C00020. The 240 mg bid dose was chosen in order to evaluate the effect at the highest possible therapeutic dose. In order to estimate the efficacy and compare and assess safety information for AZD3355 each patient will receive placebo in addition to 3 of the 4 doses of AZD3355.

The study population was chosen to resemble the study population in D9120C00020 which allowed mild symptoms for three days. Therefore, the study population will not be exactly comparable with the study population in the planned dose finding study (D9120C00019) where only patients with persistent symptoms of moderate severity will be included. Although symptoms are not the primary endpoint to this study, the information on the effect of AZD3355 on reflux episodes is still valuable in understanding if there is a dose relationship in patients who have reflux irrespective of the symptom burden.

The double-blind, cross over design was chosen in order to avoid bias and reduce variability and thereby sample size. Placebo was included as a comparator to determine the difference between PPI alone and the addition of AZD3355. The sample size was based on previous experience in a similar setting.

The effect on reflux as measured by 24h combined impedance/pH registration was chosen as primary endpoint since it is believed that AZD3355 reduces the total number of reflux episodes, whether the reflux is acid, weakly acidic or weakly alkaline. Symptoms are thought to be produced by the refluxate in the esophagus therefore, decreasing the number of reflux episodes should impact on the symptom burden of patients who have GERD and are partial responders to PPI treatment. Further, intragastric pH will be monitored to evaluate if AZD3355 has an effect on gastric acid secretion, as has been suggested in some animal studies, and can reduce the time with pH<4 in the dose range used in the study.

Genetic samples will be collected for future exploratory research into genes/genetic variation that may influence response (ie, PK profile, safety, tolerability and efficacy) to AZD3355 and/or agents used in combination and/or as comparators and/or susceptibility to GERD.

4. PATIENT SELECTION CRITERIA

Investigator(s) should keep a record, the patient screening log, of patients who entered pre-study screening.

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

4.1 Inclusion criteria

For inclusion in the study patients must fulfil the following criteria.

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

2. Male or female patients aged 18-70 years. Females of childbearing potential should use highly effective contraceptive methods. For allowed contraception see Section 5.2.

3. Body Mass Index between 18.5-35 kg/m^2 calculated from height and weight given in demographic data.

4. Have at least a 6 months history of GERD symptoms (need not to be consecutive).

5. Have reported symptoms in the RESQ-7 (7 days recall) a minimum of 3 days and a rating of at least mild intensity on at least one of the following items; a burning feeling behind the breastbone or unpleasant movement of material upwards from the stomach.

6. Clinically normal physical findings, dECG and laboratory values at the time of pre-entry visit, as judged by the Investigator.

7. Continuously treated during the last 4 weeks before enrolment with daily optimised[2] unchanged PPI therapy with doses according to the US label, for any GERD indication. Patients with endoscopy verified reflux esophagitis within the last 8 weeks must have completed the prescribed 8 weeks treatment with a PPI.

8. Have a PPI prescription with refills that cover the whole study period or instructions by a physician to use an OTC PPI in accordance with the labelling of their prescription counter-part.

[2] An optimised PPI treatment is a treatment which according to the investigator judgement can not be further improved by changing brand or dosing of the PPI and is within the label for any GERD indication (eg, no bid dosing allowed).

Inclusion criteria for the optional participation in the pharmacogenetics part of the study are described in Appendix D.

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

4.2 Exclusion criteria

Patients must not enter the study if any of the following exclusion criteria are fulfilled

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

2. Previous enrolment or randomisation in the present study, previous exposure to AZD3355.

3. Participation in another clinical study with an investigational product during the last 8 weeks.

4. Patients that have not experienced any GERD symptoms improvements at all during PPI treatment.

5. Prior surgery of the upper gastrointestinal (GI) tract (open, endoscopic and laparoscopic surgery on the esophagus, the stomach and the duodenum with the exception of oversewing or endoscopic treatment of bleeding ulcer).

6. History of a heart disease (including but not limited to ischemic heart disease, congestive heart failure, cardiac arrhythmias, congenital long QT syndrome), or current signs or symptoms of any heart disease, or persons with clinically significant dECG abnormalities as determined by the investigator, or QTcF ≥450 ms.

7. Unstable or clinically significant cardiovascular, respiratory, hepatic, renal, metabolic, psychiatric, other clinical disorders, or gastrointestinal and esophageal disorders besides GERD. Clinically significant is defined as disorders that could compromise patients' safety or interfere with the evaluation of the study as judged by the investigator. Patients with uncomplicated, well-controlled hypertension (SBP≤ 160 and DBP≤ 90) and patients with uncomplicated, well-controlled Diabetes Mellitus, as judged by the investigator, can be included.

8. Current neurological disorders including nerve compression syndromes/rhizophathy, history of paraesthesia, or history of clinically significant orthostatic reaction or syncope. Supine systolic blood pressure below 110 mmHg at enrolment or randomisation. (Patients with well controlled migraine and other headache disorders can be included.)

9. History of or current malignant disease. (Radically treated basal cell cancer is allowed.)

10. History of clinically significant electrolyte imbalances.

11. History of severe allergic or hypersensitivity reactions (such as Stevens Johnson syndrome, anaphylactic shock, angioedema-urticaria).

12. History of drug addiction, drug abuse (including cannabinoids) or alcohol abuse or other circumstances which in the investigators judgment may compromise the patient's ability to comply with the study requirements.

13. Pregnant or breast feeding females.

14. Need for concomitant medication with:

- Drugs that may interfere with the pharmacodynamic effect of Investigational Product (eg, Baclofen, pure GABA, supplements containing GABA).
- Drugs that by their mode of action may alter gastrointestinal symptoms, with exception of the PPI used in the study, (eg, H_2 receptor antagonists, sucralfate, alginates, tegaserod, domperidone, metoclopramide, erythromycin), drugs with significant anticholinergic effect (eg, anticholinergics used in gastro-intestinal disorders; anticholinergics used for Parkinson's disease; anticholinergics used for urine bladder disorders; tricyclic antidepressants).
- Drugs that may cause mucosal damage in the GI tract:
  - Non-steroid anti-inflammatory drugs (NSAIDs), or cyclo-oxygenase-2 (COX-2) inhibitors, more than 2 days/week
  - Acetylsalicylic acid (ASA) >162 mg
  - Bisphosphonates
  - Antineoplastic drugs
- Drugs that may prolong the QT interval, see Appendix G.
- Drugs that have a narrow therapeutic window (eg, warfarin, digoxin, phenytoin, carbamazepine).

15. The following laboratory exclusion criteria based on laboratory samples from visit 1 assessed at visit 2:

    - S-creatinine >1.2 x ULN
    - AST or ALT >2 x ULN
    - Bilirubin >1.5 x ULN
    - S-potassium below the lower reference range
    - S-magnesium below the lower reference range
    - Other clinically significant electrolyte imbalances as judged by the investigator

16. Plasma donation within one month of screening or blood donation or any blood loss equivalent to the amount of a blood donation during the 3 months prior to screening.

17. Any other condition which in the opinion of the investigator would render the patient unsuitable for inclusion in the study.

Exclusion criteria for the optional participation in the pharmacogenetics part of the study are described in Appendix D.

Procedures for withdrawal of incorrectly enrolled patients see Section 5.4.

5. STUDY CONDUCT

5.1 Restrictions during the study

During the whole study (V1-V6) patients will be required to:

- Abstain from blood and plasma donation at any time during the study and up to 3 months after completion of the study.
- Abstain from starting any new physical training activities or increase the intensity of their usual physical training one week prior to and during the study.
- Use of drugs with enzyme inducing properties such as St John's Wort within 3 weeks prior to the administration of investigational product.
- Abstain from taking drugs of abuse, or ingest foods containing poppy seeds 72 hours prior to the pre-entry visit until completion of the study.

- Abstain from taking any medication (including herbal medicines and high-dose or "mega" vitamins). Paracetamol/acetaminophen (1 g) may be administered for minor symptoms such as headache. However a maximum total dose of 2 g should not be exceeded during any 24-hour period.

- Patients should not receive eradication therapy for Helicobacter Pylori during the study.

- Abstain from consumption of energy drink containing taurine or glucuronolactone from the pre-entry visit until the follow up visit examples.

Within 7 days of study periods (V2-V5) patients will be required to:

- Refrain from consumption of Seville oranges and grapefruit containing products until completion of the follow-up visit.

Within 72 hours of study periods (V2-V5) patients will be required to:

- Abstain from intake of alcohol or acidic liquids like fruit juices and soft drinks eg, colas during each study period.

Within 24 hours of study periods (V2-V5) patients will be required to:

- Fast overnight (no food after 22:00 and no fluid after 24.00).

- Abstain from antacid intake during each study period.

During study periods (V2-V5) patients will be required to:

- Abstain from nicotine use (smoking, snuff, nicotine or chewing gum). Nicotine patches are allowed.

5.2 Precautions to minimize risk of pregnancy

Women of childbearing potential:

Women of childbearing potential must have started to use one of the highly effective contraceptive methods (as listed below), defined as one that results in a failure rate of less than 1% per year when used consistently and correctly, at least 3 months before enrolment and continue to use the same method strictly as prescribed throughout the study:

- Bilateral tubal ligation/occlusion

- IUD with copper-banded coils

- Combined oral contraceptives with fixed doses of progestin and estrogen during each treatment cycle

- Transdermal system with a combination of progestin and estrogen (Evra™ patch)

- Intravaginal device with a combination of progestin and estrogen (NuvaRing™)
- Progestin-releasing IUS (Mirena™)
- Progestin-releasing implants (Implanon™, Norplant™)
- Medroxyprogesterone for depot injection (Depo-Provera™)
- Cerazette™ (desogestrel) - the only accepted progestin-only pill

The informed consent information explicitly states that women of childbearing potential must comply with the user prescription for the contraceptive methods to be allowed to participate in the study.

Vomiting within 3 hours of taking oral contraception does pose a risk equivalent to a missed pill and patients should follow the guidelines for a missed pill. Randomised women of childbearing potential should be made aware of the availability of emergency "post-coital" contraception if there is an indication that their contraceptive method may have failed (eg, missing IUD treads).

Not acceptable contraception methods:

- Triphasic combined oral contraceptives with variable doses of progestin and/or estrogen during each treatment cycle
- All progestin only pills, except Cerazette™
- All barrier methods, if intended to be used alone
- Non copper containing IUDs
- Fertility awareness methods
- Sexual abstinence
- Vasectomised sexual partner
- Coitus interruptus

Women considered to be of no childbearing potential criteria:

- Post-menopausal females (either of);
  - Females >50 and have been amenorrheic for 12 months or more following cessation of all exogenous hormonal treatments or have not used exogenous hormonal treatment.

  - Females >57 regardless of whether they are on Hormonal Replacement Therapy (HRT).

- Permanent sterilisation by hysterectomy, bilateral oophorectomy or bilateral salpingectomy.

5.3 Patient enrolment and randomisation and initiation of investigational product

The Principal Investigator will:

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

2. Assign potential patient a unique enrolment number, beginning with E#.

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

4. Assign eligible patient unique randomisation code (patient number).

If a patient withdraws from participation in the study, then his/her enrolment/randomisation code cannot be reused.

5.3.1 Procedures for randomisation

A randomisation scheme will be produced by AstraZeneca R&D Mölndal using the global randomisation system (GRand).

Informed consent will be obtained before enrolment and the patients identified with an enrolment number starting with E0001001. Patients fulfilling the eligibility criteria will at Visit 2 be assigned individual randomisation codes (patient numbers) in a strict sequential order, as patients are eligible for randomisation starting with number 101.

Study drug will be given in a double-blind, cross-over manner with all patients during each study period to receive either AZD3355 or placebo in addition to their usual PPI dosage. The patients will be assigned to 4 of the 5 treatments by being randomised to one of the following 10 treatment sequences EABC, ABCE, BCED, CEDB, EDBA, DABE, ABEC, BECD, ECAD and CADE where A=30 mg, B=90 mg, C=120 mg, D=240 mg and E=placebo. The randomisation will be performed in blocks of consecutive patient numbers.

5.4 Procedures for handling patients incorrectly enrolled or randomised

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

Where patients that do not meet the selection criteria are randomised in error or incorrectly started on treatment, or where patients subsequently fail to meet the study criteria post

initiation, a discussion should occur between the AstraZeneca Study Delivery Team Physician and the Investigator regarding whether to continue or discontinue the patient from treatment.

The AstraZeneca Study Delivery Team Physician is to ensure all such decisions are appropriately documented. In situations where an agreement cannot be reached, the patient should have their study therapy stopped.

5.5 Blinding and procedures for unblinding the study

5.5.1 Methods for ensuring blinding

All packaging and labelling will be done in such way as to ensure blinding. The capsules for AZD3355 and placebo will appear identical. All patients, study personnel, AstraZeneca personnel, as well as the central reader of impedance/pH data will be blinded to the treatment given during the 4 treatment periods.

5.5.2 Methods for unblinding the study

Individual treatment codes, indicating the treatment randomisation for each randomised patient, will be available to the investigator or pharmacists at the study centre.

The drug kit for each visit will be labelled with a double panel label, with a tear-off section containing a black scratch portion hiding the treatment code.

The treatment code should not be broken except in medical emergencies when the appropriate management of the patient requires knowledge of the treatment randomisation. The investigator documents and reports the action to AstraZeneca, without revealing the treatment given to patient to the AstraZeneca staff.

AstraZeneca retains the right to break the code for SAEs that are unexpected and are 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.

At the end of the study the treatment codes will be collected and accounted for by the study monitor and then be destructed.

5.6 Treatments

5.6.1 Identity of investigational product(s)

The study medication will be supplied to the investigator by AstraZeneca. The study medication will be supplied as capsules for oral use as follows:

Investigational product	Dosage form and strength	Manufacturer	Formulation number
AZD3355, MR 1hr	Capsules 30 mg	AstraZenenca AB	H 2095-01-02 / D0900168
	Capsules 60 mg	AstraZenenca AB	H 2099-01-01 / 2034387
	Capsules 120 mg	AstraZenenca AB	H 2096-01-02 / 2034395
Placebo	Capsules	AstraZenenca AB	H 2102-01-01 / 2034403

AstraZeneca will provide the study site with blinded clinical material packaged into patient specific bottles. Each bottle will contain various combinations of either 30 mg, 60 mg, 120 mg of AZD3355 capsules or placebo capsules to match the treatment arms of 30 mg, 90 mg, 120 mg, 240 mg or placebo. There will be no difference in appearance between the capsules. The study mediation will be packaged into tamper-evident high-density polyethylene (HDPE) bottles and contain 2 capsules/bottle.

The capsules contain no lactose or gelatine.

5.6.2 Doses and treatment regimens

Blinded study medication will be taken orally, twice daily. One dose will consist of 2 capsules. Patients will be instructed to take the 2 capsules from the AM bottle in the morning and the 2 capsules from the PM bottle in the evening, see Table 2 for details.

Patients will be randomised at Visit 2 to 1 of 5 treatment groups, 30 mg AZD3355, 90 mg AZD3355, 120 mg AZD3355, 240 mg AZD3355 or placebo for 1 day of that treatment. Treatment will be given as follows:

Both AM and PM bottles will contain 2 capsules of drug, either AZD3355 30 mg, 60 mg, 120 mg or matching placebo or a combination (see below).

- Treatment A: One 30 mg capsule + One placebo capsule
- Treatment B: One 30 mg capsule + One 60 mg capsule
- Treatment C: One 120 mg capsule + One placebo capsule
- Treatment D: One 120 mg capsule + One 120 mg capsule
- Treatment E: One placebo capsule + One placebo capsule.

This is a 4-way crossover study so each patient will receive placebo and 3 of the 4 doses of AZD3355 in a randomised order. At visits 3, 4 and 5 patients will crossover to one of the other treatment groups according to the randomisation scheme from Visit 2.

5.6.3 Labelling

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

All bottles will be clearly labelled with a single panel label with the study number, study drug name, randomisation code, visit number and storage conditions. Two bottles will be placed in a carton to create a visit kit. The visit kits will be clearly labeled with a detachable tear-off label that will show the study number, the randomisation code, visit number, and a space for the E-Code.

5.6.4 Storage

All study drugs should be kept in a secure place under appropriate storage conditions. The investigational product label on the bottle specifies the appropriate storage.

All study drugs will be stored in original containers in a lockable storage facility until dispensed to the volunteers.

Each patients study drug kit will need to be stored between the different study periods in a secure place. Special attention must be given to dispensation at the different study visit 2-5 in order to avoid errors or mix-ups in given treatment between patients or study periods.

5.7 Concomitant and post-study treatment(s)

Included patients should have been treated during the last 4 weeks before enrolment with daily optimised unchanged PPI therapy with doses according to the US label, for any GERD indication. Patients with endoscopy verified reflux esophagitis within the last 8 weeks must have completed the prescribed 8 weeks treatment with a PPI. An optimised PPI treatment is a treatment, which according to the investigator judgment cannot be further improved by changing brand or dosing of the PPI.

Patients will continue with their regular, optimised PPI treatment throughout the study. Change of PPI dose or brand is not allowed until the follow-up visit. The patient should have a PPI prescription with refills that cover the whole study period or instructions by a physician to use an OTC PPI in accordance with the labelling of their prescription counter-part.

Unless otherwise indicated, the patient will continue their regular PPI treatment after the study completion.

Drugs that may interfere with the pharmacodynamic effect of the investigational product, that have anticholinergic effects or that influence gastrointestinal symptoms are not allowed from one week prior to study day one until follow-up. Antacids should not be used within 24 hours or during the investigational periods

Medications that may prolong the QT interval (see appendix G) or that have a narrow therapeutic window (see Section 4.2) are not allowed during the study.

For restrictions in contraceptive medication see Section 5.1.

Other medication, which is considered necessary for the patient's safety and well being, may be given at the discretion of the investigator and recorded in the appropriate sections of the Case Report Form (CRF).

5.8 Treatment compliance

The administration of all medication (including investigational products) should be recorded in the appropriate sections of the CRF.

A patient is defined as treatment compliant for a study period if he/she has taken the study medication in accordance with the protocol.

5.8.1 Accountability

The study drug provided for this study will be used only as directed in the study protocol.

It is the investigator's responsibility to establish a system for handling study treatments, including investigational products to ensure that deliveries are correctly received and recorded by a responsible person (eg, a pharmacist).

The study personnel will account for all study drugs dispensed to and returned from the patient.

The AZ monitor will account for all received study drugs received at the site, unused study drugs and for appropriate destruction. Any discrepancies must be accounted for. Certificates of delivery and return should be signed.

5.9 Discontinuation of investigational product

Patients may be discontinued from investigational product (IP) in the following situations:

- Patient decision. The patient is at any time free to discontinue treatment, without prejudice to further treatment
- Adverse Event
- Severe non-compliance to study protocol
- Not fulfilling study specific inclusion/exclusion criteria
- Need for disallowed medication
- Inability to perform impedance/pH measurements or manometric evaluation

- Development of any study specific criteria:

  - Patient experiencing symptoms of paraesthesia daily for at least 7 consecutive days, see Section 6.4.3.1

  - Patient experiencing syncopal episode from any cause or if a patient describes an adverse event of pre-syncope or dizziness as severe. Orthostatic dizziness provoked during testing would not automatically discontinue a patient unless this continued without provocation from orthostatic testing (eg, when getting up from bed).

  - Patient with a corrected QTc (QTcF) ≥500 ms

  - Patients requiring a change in dose or to stop treatment of their prescribed PPI

  - Patients who develops abnormalities in laboratory values:

    - S-creatinine >1.2 x ULN
    - AST or ALT >2 x ULN
    - Bilirubin >1.5 x ULN
    - S-potassium below the lower reference range
    - S-magnesium below the lower reference range
    - other clinically significant electrolyte imbalances as judged by the investigator

Patients who discontinue from investigational product may not continue in the study. See Section 5.9.1 for procedures for discontinuation.

5.9.1 Procedures for discontinuation of a patient from investigational product and from the study

A patient that decides to discontinue investigational product will always be asked about the reason(s) and the presence of any adverse events. If possible, they will be seen and assessed by an investigator(s) and perform a follow-up visit (Visit 6). Adverse events will be followed up (see Sections 6.4.3 and 6.4.4) and the patient should return diary cards and study drug.

If a patient is withdrawn from study, see Section 5.10.

5.10 Withdrawal from study

Patients are at any time free to withdraw from the study (investigational product and assessments), without any prejudice to further treatment (withdrawal of consent). Such patients will always be asked about the reason(s) and the presence of any adverse events. If

possible they will be seen and assessed by an Investigator. Adverse events will be followed up (see Sections 6.4.3 and 6.4.4) and the patient should return diary cards and study drug.

For enrolled but not randomised patients, the following modules in the CRF must be entered at a minimum:

- Demography
- Eligibility criteria, incl. RESQ-7
- AE
- If SAE criteria was fulfilled, the AE and SAE modules must be completed
- Study termination
- Investigators signature

6. COLLECTION OF STUDY VARIABLES

6.1 Recording of data

A paper Case Report Form will be used for data collection and query handling. The investigator will ensure that data are recorded on the CRF as specified in the study protocol and in accordance with the instructions provided.

The investigator ensures the accuracy, completeness, and timeliness of the data recorded and of the provision of answers to data queries according to the Clinical Study Agreement. The investigator will sign the completed CRFs. A copy of the completed paper CRFs will be archived at the study site.

The diary card completed by the patient during the 24-hours impedance/pH measurements will not be entered into the CRF. The diary will be analysed together with the data from the data logger by the central reader of impedance/pH data, see Section 6.3.1.1.

6.2 Data collection and enrolment

6.2.1 Screening assessments

Each patient will complete the following assessments during the screening.

- Obtaining written informed consent prior to any study specific procedures
- RESQ-7 (see Section 6.5.1)
- Demography data - date of birth, sex, race

- Medical and surgical history, including medication and PPI use
- GI history, IBS symptoms and history of paraesthesia, syncope or orthostatic reaction
- Complete physical examination (see Section 6.4.6)
- Vital signs (Supine resting SBP and DBP, pulse)
- Height (cm) and weight (kg) (for BMI calculation)
- 12-lead dECG
- Blood and urine samples for standard clinical chemistry, haematology assessments, hepatitis C antibody (HIV antibody, Hepatitis B surface Antigen (HBsAg), hepatitis C antibody (anti-HCV) and PK samples, and drugs of abuse screen (see Section 6.4.5). If applicable, a urine pregnancy test and/or HP serology. Genetic sampling is optional.

6.3 Efficacy

6.3.1 Efficacy variable

6.3.1.1 Impedance/pH measurements

Esophageal impedance/pH monitoring will be performed using a Sleuth® Multi-channel Intraluminal Impedance Ambulatory system (Sandhill Scientific, Inc; Highlands Ranch, CO). The system includes a portable data logger with impedance/pH amplifiers and a catheter, 2.13 mm outer diameter, with two antimony pH sensors that will be placed 5 cm above the LES and 10 cm below the LES as well as eight impedance electrodes at 2, 4, 6, 8, 10,14, 16 and 18 cm above the tip of the electrode. Each pair of electrodes measures impedance over a 2 cm segment at 3, 5, 7, 9, 15 and 17 cm above the LES. Before and after the recordings, the pH electrodes will be calibrated using standard buffers of pH 7 and pH 1. The catheters will be passed transnasally under topical anaesthesia and positioned in relation to the LES as located by a manometric recording. After 24-hours continuous recording, the impedance/pH catheters will be removed.

For acquisition of pH data, the data logger measures and stores the potential differences between recording and reference sensors in the esophagus. The impedance measurements allow detection of gastroesophageal reflux based on changes in resistance (Ohms) to alternating electrical current flow <6 microamperes at a frequency of 1-2 kHz between a series of ring sensors on the probe, when liquid and/or gas bolus moves between them. The six impedance and pH signals are recorded at 50 Hz on a 128 MB Compact Flash card for further computer analysis. The impedance/pH data will be downloaded on a personal computer and then stored as the original acquisition file in Bioview™ Autoscan™ format and will also be converted to an deidentified ACII file format on a CD, which will be sent to the central reader for analysis. All data and CDs will be sent back to the study team at AstraZeneca.

During the 24 hours of ambulatory impedance/pH measurement, the patient will be using the data logger and a diary card to record meal intake and periods in supine position, as well as GERD symptoms. Time is recorded on the devise and the corresponding event is described in the paper diary on which the patient will be recording the type of GERD symptoms. The diary card can be seen in Appendix H. A copy of the de-identified diary card will also be sent to the central reader for uploading and confirmation of event marker recordings.

Data will be analysed visually and with the support of a dedicated software (Bioview Analysis®, Version 5.5.4, Sandhill Scientific, Inc.) Analysis will include identification and characterization of individual reflux events, measurements of clearance times (pH and bolus), calculation of symptom and change in position in association to reflux episodes, measurement of esophageal exposure to reflux of various composition and pH as well as measurements of gastric pH.

The data will be analysed by one central reader blinded to treatment allocation, Dr John Pandolfino at Northwestern University Medical School, Division of Gastroenterology, Chicago, USA. The purpose of utilizing a central reader is to have a consistent assessment and analysis of measurements for all study impedance/pH data.

The central reader will determine the validity of the pH recordings based on the following criteria established for evaluability: A minimum of 20 hours of impedance/pH recording must be obtained in order to have evaluable data. Data points that will be excluded are: pH<0 or >9, flat recording artifact associated with loss of signal or drift artifact. The recording must not have technical failures of the pH recording and must not have 1 continuous hour or more with pH data outside the reference range. If the recording is less than 20 hours due to these technical issues the data is not evaluable.

6.4 Safety

The Principal Investigator is responsible for ensuring that all staff involved in the study is familiar with the content of this section.

6.4.1 Definition of adverse events

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.

The term AE is used to include both serious and non-serious AEs.

6.4.2 Definitions of serious adverse event

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

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

For further guidance on the definition of a SAE, see Appendix B to the Clinical Study Protocol.

6.4.3 Recording of adverse events

Time period for collection of adverse events

Adverse Events will be collected from administration of the first dose at visit 2 throughout the treatment period and including the follow-up period, visit 6.

SAEs will be recorded from the time of informed consent.

Follow-up of unresolved adverse events

Any AEs that are unresolved at the patient's last AE assessment or other assessment/visit as appropriate in the study are followed up by the investigator for as long as medically indicated, but without further recording in the CRF. AstraZeneca retains the right to request additional information for any patient with ongoing AE(s)/SAE(s) at the end of the study, if judged necessary.

Variables

The following variables will be collected for each AE;

- AE (verbatim)
- the date and time when the AE started and stopped
- maximum intensity
- whether the AE is serious or not

- investigator causality rating against the Investigational Product (yes or no)
- action taken with regard to investigational product
- AE caused patient's withdrawal from study (yes or no)
- outcome.

In addition, the following variables will be collected for SAEs:

- Date AE met criteria for serious AE
- Date Investigator became aware of serious AE
- AE is serious due to
- Date of hospitalisation
- Date of discharge
- Probable cause of death
- Date of death
- Autopsy performed
- Causality assessment in relation to Study procedure(s)
- Causality assessment in relation to Other medication
- Description of AE

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria in Section 6.4.2. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not 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.

Causality collection

The Investigator will assess causal relationship between Investigational Product and each Adverse Event, and answer "yes" or "no" to the question "Do you consider that there is a reasonable possibility that the event may have been caused by the investigational product?"

For SAEs causal relationship will also be assessed for other medication and study procedures. Note that for SAEs that could be associated with any study procedure the causal relationship is implied as "yes".

A guide to the interpretation of the causality question is found in Appendix B to the Clinical Study Protocol.

Adverse Events based on signs and symptoms

All AEs spontaneously reported by the patient or reported in response to the open question from the study personnel: "Have you had any health problems since the previous visit/you were last asked?", or revealed by observation will be collected and recorded in the CRF. When collecting AEs, the recording of diagnoses is preferred (when possible) to recording a list of signs and symptoms. However, if a diagnosis is known and there are other signs or symptoms that are not generally part of the diagnosis, the diagnosis and each sign or symptom will be recorded separately.

Adverse Events based on examinations and tests

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

If deterioration in a laboratory value/vital sign is associated with clinical signs and symptoms, the sign or symptom will be reported as an AE and the associated laboratory result/vital sign will be considered as additional information. Wherever possible the reporting investigator uses the clinical, rather than the laboratory term (eg, anaemia versus low haemoglobin value). In the absence of clinical signs or symptoms, clinically relevant deteriorations in non-mandated parameters should be reported as AE(s).

Deterioration of a laboratory value, which is unequivocally due to disease progression, should not be reported as an AE/SAE.

Any new or aggravated clinically relevant abnormal medical finding at a physical examination as compared with the baseline assessment will be reported as an AE.

6.4.3.1 Spontaneously report of symptoms of paraesthesia

If the patient spontaneously reports cutaneous sensations such as tingling, pins and needles, prickling, skin crawling, formication, burning sensation and numbness, a more thorough questioning will be done, asking for frequency, start of symptoms in relation to drug intake, duration of symptoms and level of discomfort. The responses should be recorded in the paraesthesia module in the CRF. Furthermore, a complete physical examination including neurological examination with evaluation of cranial nerve functions must be completed and documented in the CRF by the investigator.

If the patient reports episodes of symptoms of paraesthesia for 7 consecutive days the patient will permanently stop intake of IP (see Section 5.9.1), and be referred to a board certified neurologist for evaluation. A copy of the report from the neurologist must be provided to AstraZeneca.

6.4.3.2 Active questioning of specific symptoms

Following the spontaneous AE collection, there will be active questioning to determine whether patients experienced any episode of the following: syncope, felt faint, light-headedness or dizziness. In order to get baseline information before study treatment has started, the question will also be asked prior to first intake of investigational product (IP) at visit 2-5. It will also be asked on day 2 prior to removing the pH/impedance catheter before the patient leaves in the mornings after the impedance is completed. All reported episodes will be recorded in a specific module in the CRF. Only reported episodes that fulfil any of the SAE criteria (see Section 6.4.2) or are the reason for discontinuation from the study will be recorded in the AE module in the CRF.

6.4.3.3 Disease under study

The following symptoms of disease under study are not to be reported as AEs during treatment with IP unless they fulfil the criteria for SAEs or lead to early discontinuation of the IP. These are considered as symptoms of disease under study and are measured outcome variables of interest in the study:

- a burning feeling behind the breastbone
- pain behind the breastbone
- a burning feeling in the centre of the upper stomach
- a pain in the centre of the upper stomach
- heartburn
- an acid taste in the mouth
- a bitter taste in the mouth
- unpleasant movement of material upwards from the stomach
- stomach contents (liquid or food) moving upwards to the throat or mouth

6.4.3.4 Recording of adverse events in connection with orthostatic test

A change in BP or pulse during an orthostatic test without accompanying symptoms should not be recorded as an AE. Other signs and symptoms occurring during the orthostatic test should be reported as AEs. Dizziness occurring in upright position during an orthostatic test should be reported as postural dizziness.

6.4.4 Reporting of serious adverse events

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

If any SAE occurs in the course of the study, then investigators or other site personnel inform appropriate AstraZeneca representatives within one day ie, immediately but **no later than the end of the next business day** of when he or she becomes aware of it.

The designated AstraZeneca representative works with the investigator to ensure that all the necessary information is provided to the AstraZeneca Patient Safety data entry site **within one calendar day** of initial receipt for fatal and life threatening events **and within five calendar days** of initial receipt for all other SAEs.

For fatal or life-threatening adverse events where important or relevant information is missing, active follow-up is undertaken immediately. Investigators or other site personnel inform AstraZeneca representatives of any follow-up information on a previously reported SAE within one calendar day ie, immediately but **no later than the end of the next business day** of when he or she becomes aware of it.

Investigators and other site personnel send relevant CRF modules by fax or e-mail to the designated AstraZeneca representative.

6.4.5 Laboratory safety assessment

Blood and urine samples for determination of clinical chemistry, haematology, and urinalysis will be taken at the times indicated in the Study Plan (see Table 1) and Study period procedures (see Table 2).

All the laboratory safety samples will be analysed at the local laboratory, except for the urine test, which will be done by dipstick at the study site. All results from both blood and urine tests should be entered into the appropriate parts of the CRF. See Table 5 for the total volume of blood that will be drawn from the patient throughout the study.

Laboratory values outside of the reference ranges suspected to be of any clinical significance could be re-checked. Randomised patients who develop clinically significant abnormal laboratory values must be followed until normalisation or for as long as the Investigator deems necessary.

See also Section 6.4.3 paragraph Adverse Events based on examinations and tests.

The following laboratory variables will be measured:

6.4.5.1 Full laboratory screen

Blood samples (~12 ml) and urine samples for a full laboratory screen will be collected at the pre-entry visit within 21 days prior to the first study period and at the follow-up visit

5-10 days after the last study period. The full laboratory screen will include the variables in Table 3 below.

Table 3 Full laboratory screen

Test	Unit	Comment
Clinical Chemistry		
S- Aspartate aminotransfease (ASAT)	µkat/L (U/L)	
S- Alanine aminotransferase (ALAT)	µkat/L (U/L)	
S- Alkanine phosphatase (ALP)	µkat/L (U/L)	
S- Total bilirubin	µmol/L	
S- Albumin (Alb)	g/L	
S- Creatine Kinase (CK)	µkat/L (U/L)	
S- Creatinine (Creat)	µmol/L	
S- Urea	mmol/L	
S- Sodium (Na)	mmol/L	
S- Potassium (K)	mmol/L	
S- Magnesium (Mg)	mmol/L	
S- Calcium (Ca)	mmol/L	
P- Glucose	mmol/L	Fasting
S- C-Reactive Protein (CRP)	µmol/L	
Clinical Haematology		
B- Haemoglobin (Hb)	g/L	
B- Erythrocyte Particle Concentration	10^{12}/L	
B- Erythrocyte Mean cell Volume (Ery-MCV)	L/L (%)	
B- Mean Cell Haemoglobin Concentration (MCHC)	10^{9}/L	
B- Leukocyte Particle Concentration (LPC)	10^{9}/L	
B- Platelet Particle Concentration (PPC)	10^{9}/L	
Urinalysis (dip-stick)		
U- Protein		
U-Glucose		
U-Haemoglobin		
U-Pregnancy test		If applicable

For blood volume see Section 7.1.

6.4.5.2 Reduced laboratory screen

Blood samples (~8 ml) and urine samples for a reduced laboratory screen will be collected before first dose at each study period and 12 hours after the last dose. The reduced laboratory screen will include the variables in Table 4 below.

Table 4 Reduced laboratory screen

Test	Unit	Comment
Clinical Chemistry		
S- Aspartate aminotransfease (ASAT)	µkat/L (U/L)	
S- Alanine aminotransferase (ALAT)	µkat/L (U/L)	
S- Alkanine phosphatase (ALP)	µkat/L (U/L)	
S- Total bilirubin	µmol/L	
S- Creatine Kinase (CK)	µkat/L (U/L)	
S- Creatinine (Creat)	µmol/L	
S- Sodium (Na)	mmol/L	
S- Potassium (K)	mmol/L	
S- Calcium (Ca)	mmol/L	
P- Glucose	mmol/L	Fasting
Clinical Haematology		
B- Haemoglobin (Hb)	g/L	
B- Erythrocyte Particle Concentration	10^{12}/L	
B- Leukocyte Particle Concentration (LPC)	10^{9}/L	
B- Platelet Particle Concentration (PPC)	10^{9}/L	
Urinalysis (dip-stick)		
U- Protein		
U-Glucose		
U-Haemoglobin		

For blood volume see Section 7.1.

Drugs of abuse

Urine will be tested for drugs of abuse at screening (Visit1) and repeated randomly once more during the study. The following substances will be tested: benzodiazepines, cocaine, and/or metabolites, amphetamines, tetrahydrocannabinol (THC), opiates, methamphetamines (including ecstasy), phencyclidine (PCP) and barbiturates. The result of the drug screen will be recorded in the patients medical records and recorded in the CRF, but will not be included

in the analysed data. If a patient test positive for drugs of abuse they will be excluded from entering or continuing in the study.

HIV and hepatitis screens

Testing for the HIV antibody, HBsAg, and anti-HCV (~8 ml) is to be performed on all patients at screening (Visit 1) only. If a test result is positive, the patient will not be allowed to proceed in the study. The results of the HIV and hepatitis screens will be documented in the patient medical records and recorded in the CRF but will not be included in the analysed data.

6.4.6 Physical examination

A physical examination will be performed at visit 1 and visit 6 (pre-entry and follow-up) and include an assessment of the following:

- General appearance
- Skin
- Head and neck (including ears, eyes and throat)
- Lymph nodes
- Thyroid
- Abdomen
- Musculo-skeletal (including extremities)
- Cardiovascular, respiratory and neurological systems
- Neurological systems (including cranial nerves and reflexes)

6.4.7 dECG

A 12-lead dECG will be taken at visit 1 (screening), visit 2-5 (both pre-dose and 2 hours after the first dose), visit 6 (follow-up) and any other time the investigator deems necessary for safety during the dosing period. The 12 lead dECGs will be obtained after the patient has been lying down for at least 10 minutes.

The dECGs will be reviewed by the investigator or a qualified cardiologist who will judge the overall interpretation as normal or abnormal. If abnormal, it will be decided as to whether or not the abnormality is clinically significant or not. The evaluation will be recorded in the appropriate sections of the CRF.

All dECGs will be transferred to a central reader for evaluation for general evaluation of ECG trends.

6.4.8 Vital signs

Vital signs (resting BP, pulse, orthostatic test) will be collected at the time points specified in the Study Plan (see Table 1 and Table 2).

Vital sign assessments can also be made at the discretion of the investigator in order to follow the patient's clinical condition. These assessments should not be part of the study.

Both supine and sitting BP, a s well as pulse (mmHg and beats/min) will be measured after 10 minutes of rest preferably using the same semi-automatic blood pressure recording device with an appropriate cuff size in all patients at all study days.

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

6.4.8.1 Orthostatic test

The patient will rest in supine position for 10 min and BP and pulse will be measured. The patient will then be instructed to stand up with arms hanging relaxed down at their sides and BP and pulse will be measured after 1 min in the upright position.

An orthostatic test will be performed before and after first dose at the time of $C_{max.}$ The test will be performed only after the dECG has been recorded (see Table 2).

6.5 Patient reported outcomes (PRO)

During the 24 hours of ambulatory impedance/pH measurement, the patient will use a diary card to record meal intake and periods in supine position as well as symptoms. See Section 6.3.1.1.

6.5.1 The Reflux Symptoms Questionnaire (RESQ-7 days recall)

Since there was no validated GERD instrument for the patients in the target patient population, a new instrument was developed by modifying an existing PRO instrument, the Reflux Disease Questionnaire (RDQ). The validity, reliability and responsiveness of the RDQ have previously been documented for use in patients with GERD (Shaw et al 2001). Based upon a patient interview study with patients with GERD who had symptoms despite PPI treatment, additional items were added to the RDQ. The modified version is hereafter referred to as the RESQ-7. The RESQ-7, was determined to be fit for purpose in the target population for this programme, in a subsequent measurement property validation study (D9120C00027).

In the RESQ-7, the frequency of symptoms over the past 7 days is rated by the patients using a 6-graded Likert scale (Did not have; 1 day; 2 days; 3-4 days; 5-6 days; Daily). Similarly, the intensity is rated by the patients using a six-graded Likert scale (Did not have; Very mild; Mild; Moderate; Moderately severe; Severe).

The RESQ-7 address the frequency and intensity of the following symptoms:

- a burning feeling behind the breastbone
- pain behind the breastbone
- a burning feeling in the centre of the upper stomach
- a pain in the centre of the upper stomach
- an acid taste in your mouth
- unpleasant movement of material upwards from the stomach
- burping (gas coming from the stomach through the mouth)
- hoarseness
- cough
- difficulty swallowing
- a bitter taste in your mouth
- stomach contents (liquid or food) moving upwards to your throat or mouth
- heartburn

The wording of the RESQ-7 used at screening can be found in Appendix G.

The RESQ-7 will be used together with a labelled torso, see Appendix H, as suggested by direct feedback in cognitive interviews (Report from Mapi Values).

To be eligible for the study the patient must have reported in the RESQ-7, a minimum of 3 days and a rating of at least mild intensity on at least one of the following items; a burning feeling behind the breastbone, unpleasant movement of material upwards from the stomach.

The individual RESQ-7 responses will be recorded in the CRF.

6.5.2 Administration of PRO questionnaires

The RESQ-7, with a 7-days recall period, will be used to determine eligibility for inclusion into the screening phase. The patients will be instructed to complete the RESQ-7 at the Pre-entry visit (visit 1). The study personnel should point out the importance of answering all the questions in the RESQ-7 but should not help the patient to choose an answer. The study personnel must be neutral in their response to any questions from the patient. Each site will have a designated quiet space for patients to use when completing the PRO instruments at the

study visit. The patient should be given adequate time to complete all items, ie, no time limits for completing the questions should be given.

Patients will be asked to complete the RESQ-7 questionnaire in a paper format, after signing informed consent. The study staff will be required to monitor that the patients have completed the RESQ-7 at visit 1. After completion of the RESQ-7, the study personnel will review the questionnaire for determining the eligibility of the patient for the study.

Patients will be given a torso picture together with the RESQ-7. The torso is available to show the anatomical location of the GERD symptoms that are referenced in the RESQ-7 questions.

6.6 Pharmacokinetics

6.6.1 Collection of samples

Venous blood samples (approximately 2 mL) for determination of concentrations of AZD3355 in plasma will be taken at the times presented in the Table 2.

All samples will be analysed within a timeframe for which the stability of AZD3355 in the samples has been validated and shown to be acceptable.

Samples will be collected, labelled stored and shipped as detailed in a separate Laboratory Manual.

For blood volume see Section 7.1.

6.6.2 Determination of drug concentration

Samples for determination of drug concentration in plasma will be analysed by

using liquid chromatography and mass spectrometric detection. The lower limit of quantification (LLOQ) of AZD3355 in plasma is 0.030 µmol/L.

Samples from patients on placebo treatment may be analysed if judged appropriate, by the investigator(s) and/or pharmacokineticist, eg, confirmation that these patients have not been given AZD3355.

Full details of the analytical method used will be detailed in a separate bioanalytical report.

6.7 Pharmacodynamics

See Section 6.3.1.

6.8 Pharmacogenetics

6.8.1 Collection of pharmacogenetic samples

The blood sample for genetic research will be obtained from the patients at Visit 2. Although genotype is a stable parameter, early sample collection is preferred to avoid introducing bias through excluding patients who may withdraw due to an adverse event (AE), such patients would be important to include in any genetic analysis. If for any reason the sample is not drawn at Visit 2, it may be taken at any visit until the last study visit. Only one sample should be collected per patient for genetics during the study. Samples will be collected, labelled, stored and shipped as detailed in the Laboratory Manual.

For information on collection of pharmacogenetic samples, see Appendix D.

For blood volume see Section 7.1.

6.9 Health economics (Not Applicable)

7. BIOLOGICAL SAMPLING PROCEDURES

7.1 Volume of blood

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

Table 5 Volume of blood to be drawn from each patient

Assessment		Sample volume (mL)	No. of samples	Total volume (mL)
Safety	Full laboratory screen, HP	12	2	24
	Reduced laboratory screen	8	8	64
	Hepatitis B, C, HIV	8	1	8
Pharmacokinetic		2	60	120
Pharmacogenetics[a]		10	1	10
Total				226

[a] Only if the patient is participating in the optional PGx part of the study, see Appendix D.

By discretion of the investigator additional blood samples may be taken for patient safety reasons outside the scope of this protocol.

7.2 Handling, storage and destruction of biological samples

The samples will be used up or disposed of after analyses or retained for further use as described here.

7.2.1 Pharmacokinetic and/or pharmacodynamic samples

Samples will be disposed of after the clinical study report has been finalised.

7.2.2 Pharmacogenetic samples

The process adopted for the coding and storage of samples for genetic analysis are important to maintain patient confidentiality. Samples will be stored for a maximum of 25 years, from the date of the Last Patient's Last visit, after which they will be destroyed. DNA is a finite resource that may be used up during analyses. Samples will be store and used until no further analyses are possible or the maximum storage time has been reached.

For all samples irrespectively 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 identified by the unique DNA number only. The DNA number is used to identify the sample and corresponding data at the AstraZeneca genetics laboratories, or at the designated contract laboratory. No personal details identifying the individual will be available to any person (AstraZeneca employee or contract laboratory staff working with the DNA:

The samples and data for genetic analysis in this study will be single coded. The link between the patients enrolment/randomisation code and the DNA number will be maintained and stored in a secure environment, with restricted access
t AstraZeneca. The link will be used to identify the relevant DNA samples for analysis, facilitate correlation of genotypic results with clinical data, allow regulatory audit and to trace samples for destruction in the case of withdrawal of consent when the patient has requested disposal/destruction of samples not yet analysed.

7.3 Labelling and shipment of biohazard samples

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

Any samples identified as Infectious Category A materials are not shipped and no further samples will be taken from the patient unless agreed with AstraZeneca and appropriate labelling, shipment and containment provisions are approved.

7.4 Chain of custody of biological samples

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

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

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

AstraZeneca keeps oversight of the entire life cycle through internal procedures, monitoring of study sites and auditing of external laboratory providers.

Samples retained for further use are registered in the AstraZeneca biobank system during the entire life cycle.

7.5 Withdrawal of informed consent for donated biological samples

If a patient withdraws consent to the use of donated biological samples, the samples will be disposed of/destroyed, and the action documented. If samples are already analysed, AstraZeneca is not obliged to destroy the results of this research.

As collection of the biological sample is an optional part of the study, then the patient may continue in the study after this consent is withdrawn.

The Principal Investigator:

- Ensures patients' withdrawal of informed consent to the use of donated samples is notified immediately to AstraZeneca.
- Ensures that biological samples from that patient, if stored at the study site, are immediately identified, disposed of /destroyed, and the action documented.
- Ensures the laboratory(ies) holding the samples is/are informed about the withdrawn consent immediately and that samples are disposed/destroyed, the action documented and the signed document returned to the study site.
- Ensures that the patient and AstraZeneca are informed about the sample disposal.

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

8. ETHICAL AND REGULATORY REQUIREMENTS

8.1 Ethical conduct of the study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/Good Clinical Practice, applicable regulatory requirements and the AstraZeneca policy on Bioethics and Human Biological Samples.

8.2 Patient data protection

The 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.

Patient data protection for the pharmacogenetic research part of the study is described in Appendix D.

8.3 Ethics and regulatory review

An Institutional Review Board (IRB) should approve the final study protocol, including the final version of the Informed Consent Form and any other written information and/or materials to be provided to the patients. The investigator will ensure the distribution of these documents to the applicable IRB, and to the study site staff.

The opinion of the IRB should be given in writing. The investigator should submit the written approval to AstraZeneca before enrolment of any patient into the study.

The IRB should approve all advertising used to recruit patients for the study.

AstraZeneca should approve any modifications to the Informed Consent Form that are needed to meet local requirements.

If required by local regulations, the protocol should be re-approved by the IRB annually.

Before enrolment of any patient into the study, the final study protocol, including the final version of the Informed Consent Form, is approved by the national regulatory authority or a notification to the national regulatory authority is done, according to local regulations.

AstraZeneca will handle the distribution of any of these documents to the national regulatory authorities.

AstraZeneca will provide Regulatory Authorities, IRBs and Principal Investigators with safety updates/reports according to local requirements.

Each Principal Investigator is 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 so that he/she can meet these reporting requirements.

8.4 Informed consent

The Principal Investigator(s) at each centre will:

- Ensure each patient is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study.

- Ensure each patient is notified that they are free to discontinue from the study at any time.
- Ensure that each patient is given the opportunity to ask questions and allowed time to consider the information provided.
- Ensure each patient provides signed and dated informed consent before conducting any procedure specifically for the study.
- Ensure the original, signed Informed Consent Form(s) is/are stored in the Investigator's Study File.
- Ensure a copy of the signed Informed Consent Form is given to the patient.
- Ensure that any incentives for patients who participate in the study as well as any provisions for patients harmed as a consequence of study participation are described in the informed consent form that is approved by an IRB.

8.5 Changes to the protocol and informed consent form

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

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

The amendment should be approved by each IRB and if applicable, also the national regulatory authority, before implementation. Local requirements should be followed for revised protocols.

AstraZeneca will distribute any subsequent amendments and new versions of the protocol to each Principal Investigator(s). For distribution to IRB see Section 8.3.

If a protocol amendment requires a change to a centre's Informed Consent Form, AstraZeneca and the centre's IRB should approve the revised Informed Consent Form before the revised form is used.

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

8.6 Audits and inspections

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

analysed, and accurately reported according to the protocol, Good Clinical Practice (GCP), guidelines of the International Conference on Harmonisation (ICH), and any applicable regulatory requirements. The investigator will contact AstraZeneca immediately if contacted by a regulatory agency about an inspection at the centre.

9. STUDY MANAGEMENT BY ASTRAZENECA

9.1 Pre-study activities

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

- Determine the adequacy of the facilities.
- Determine availability of appropriate patients for the study.
- Discuss with the investigator(s) (and other personnel involved with the study) their responsibilities with regard to protocol adherence, and the responsibilities of AstraZeneca or its representatives. This will be documented in a Clinical Study Agreement between AstraZeneca and the investigator.

9.2 Training of study site personnel

Before the first patient is entered into the study, an AstraZeneca representative will review and discuss the requirements of the Clinical Study Protocol and related documents with the investigational staff and also train them in any study specific procedures and the system(s) utilised.

The Principal Investigator will ensure that appropriate training relevant to the study is given to all of these staff, and that any new information relevant to the performance of this study is forwarded to the staff involved.

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

9.3 Monitoring of the study

During the study, an AstraZeneca representative will have regular contacts with the study site, including visits to:

- Provide information and support to the investigator(s).
- Confirm that facilities remain acceptable.
- Confirm that the investigational team is adhering to the protocol, that data are being accurately and timely recorded in the CRFs, that biological samples are handled in

accordance with the Laboratory Manual and that investigational product accountability checks are being performed.

- Perform source data verification (a comparison of the data in the CRFs with the patient's medical records at the hospital or practice, and other records relevant to the study) including verification of informed consent of participating patients. This will require direct access to all original records for each patient (eg, clinic charts).

- Ensure withdrawal of informed consent to the use of the patient's biological samples is reported and biological samples are identified and disposed of/destroyed accordingly, and the action is documented, and reported to the patient.

The AstraZeneca representative will be available between visits if the investigator(s) or other staff at the centre needs information and advice about the study conduct.

9.3.1 Source data

Source data locations are specified in the Clinical Study Agreement.

9.4 Study agreements

The Principal Investigator at each/the centre should comply with all the terms, conditions, and obligations of the Clinical Study Agreement for this study. In the event of any inconsistency between this Clinical Study Protocol and the Clinical Study Agreement, the terms of Clinical Study Protocol shall prevail with respect to the conduct of the study and the treatment of patients and in all other respects, not relating to study conduct or treatment of patients, the terms of the Clinical Study Agreement shall prevail.

Agreements between AstraZeneca and the Principal Investigator should be in place before any study-related procedures can take place, or patients are enrolled.

9.4.1 Archiving of study documents

The Investigator follows the principles outlined in the Clinical Study Agreement.

9.5 Study timetable and end of study

The end of the study is defined as "the last visit of the last patient undergoing the study".

The study is expected to start in

The study may be terminated at the centre if the study procedures are not being performed according to GCP, or if recruitment is slow. AstraZeneca may also terminate the entire study prematurely if concerns for safety arise within this study or in any other study with AZD3355.

The recruitment period will end when the targeted number of randomised patients has been reached. Individual investigators will be notified by AstraZeneca.

10. DATA MANAGEMENT BY COGNIZANT DMC

The CRF and the clinical database is created in accordance with Cognizant standard operating procedures using existing project standards. CRF instructions are provided to sites for recording data. The data are entered, verified and cleaned and data sets prepared according to Cognizant procedures. The data management staff is responsible for conducting and/or overseeing the following information.

Data Management Plan (DMP)

The study DMP will describe the methods used to collect, check and process clinical data in detail. It will also clarify the roles and responsibilities for the different functions and personnel involved in the data management process.

Dictionary coding

Medical coding is done using the most current version of MedDRA and AstraZeneca Drug Dictionary.

Management of external data

Data Management determines the format of the data to be received from external vendors and coordinates the flow of data to an external environment or clinical database (if applicable). Data Management will ensure that the data collection tool will be tested/validated as needed. External data reconciliation will be done with the clinical database as applicable.

Serious Adverse Event (SAE) Reconciliation

SAE reconciliation reports are produced and reconciled with Patient Safety database and/or the Investigational Site.

Quality Control Process

Data Management performs the quality control of the data in accordance with the Cognizant SOPs. Clean file occurs when all data have been declared clean.

Genotype Data

Genotype data generated in this study will be stored in the AstraZeneca genotyping LIMS database, or other appropriate secure system, separate from the database used for the main study.

Some or all of the clinical datasets from the main study may be merged with the genetic data in a suitable secure environment separate from the clinical database. The results from this genetic research will be reported separately from the clinical study report for the main study.

11. EVALUATION AND CALCULATION OF VARIABLES BY ASTRAZENECA

11.1 Calculation or derivation of efficacy variable(s)

11.1.1 Impedance/pH measurements

In this study the following parameters will be studied for the total period, as well as for periods with upright and supine position:

1. Total number of reflux episodes
2. Number and percentage of registration time of acid reflux episodes (pH<4)
3. Number and percentage of registration time of weakly acidic reflux episodes (4≤pH<6.5)
4. Number and percentage of registration time of weakly alkaline reflux episodes (pH≥6.5)
5. Number and percentage of liquid reflux episodes
6. Number and percentage of gas reflux episodes
7. Number and percentage of mixed gas/liquid reflux episodes
8. Number and proportion of reflux events (liquid, gas or mixture) with proximal extent
9. The percentage of time with intragastric pH<4 during the 24-hour period at 10 cm below LES
10. The relation between reflux episodes, acid- and weakly acid or weakly alkaline reflux and GERD symptoms during 24 hours (Symptom Index (SI), Symptom associated probability (SAP))
11. Mean bolus clearance time (sec)

11.1.2 Definition of variables

- An episode with acid reflux is defined as an episode with an intra-esophageal pH<4 lasting longer than 5 seconds. If pH is already below 4 a further fall in pH of at least one pH unit lasting longer than 5 seconds is required.
- Weakly acidic reflux is defined as a reflux episode with an intra-esophageal pH between 4 and 6.5 lasting longer than 5 seconds.

- Weakly alkaline reflux is defined as a reflux episode, lasting longer than 5 seconds, during which the intra-esophageal pH does not drop below 6.5.

- A liquid reflux episode is defined as an episode of reflux that manifested as a drop in the impedance (in Ohms) of more than 50% from its baseline, and propagates aborally at least two impedance measurement segments from the most distal channel.

- A gas reflux episode is defined as an episode of reflux that is associated with an rapid (3000 Ω/s) increase in the impedance level (>5000 Ω) from the baseline level, and propagates aborally at least two impedance measurement segment from the most distal channel in the absence of swallowing.

- A mixed gas/liquid reflux episode is defined as an episode of reflux in which there is a combination of liquid and gas criteria in the same time period.

- Proximal extent of the refluxate is defined as the number of reflux events reaching the most proximal impedance measuring site

- Acid clearance time is defined as the mean total duration of an acid reflux episode and will be calculated as the total time with pH<4 divided by the total number of acid reflux episodes.

- Bolus clearance time (sec) is defined as the time in seconds from the drop in impedance to below 50% of baseline until impedance has recovered to above 50% of baseline.

- Symptoms reported as GERD related by the patients on the diary card

11.1.3 Symptom analysis variables

During the 24 hours of ambulatory impedance/pH measurement, time for possible GERD symptoms, meal intake and periods in supine position will be recorded by the patient using the data logger. On the diary card the patient will also be recording the type of GERD symptoms.

Each time the patient records a symptom it is counted as a symptom episode. The type of the episode is determined by the specific type of symptom that occurred during the recording.

Symptom Index (SI)

For calculation of symptom index, symptoms will be identified on the impedance/pH tracing time line. Symptoms associated with a reflux event occurring within a 2 minute window before and 2 minute window after the symptom will be considered reflux positive. SI is calculated by the number of reflux positive symptoms divided by the number of symptoms (50% or more is considered to support a positive correlation between symptoms and reflux) (Wiener et al 1988).

Symptom associated probability (SAP)

For calculation of SAP, consecutive two minute periods of pH recording will be analysed for the presence of reflux and symptoms. A symptom episode will be considered as reflux positive if either of the two minute periods preceding or following the start of the symptom was reflux positive (pH<4 for ≥5 seconds). A contingency table will be constructed and Fisher's exact test will be used to calculate the p value according to Weusten et al. SAP was calculated as (1.0–P)x100% and a value greater than 95% is considered to support a positive correlation between symptoms and reflux. (Weusten et al 1994)

11.2 Calculation or derivation of safety variable(s)

11.2.1 Other significant adverse events (OAE)

During the evaluation of the AE data, an AstraZeneca medically qualified expert will review the list of AEs that were not reported as SAEs or Discontinuation of Investigational Product due to Adverse Event (DAE)s. Based on the expert's judgement, significant adverse events of particular clinical importance may, after consultation with the Global Patient Safety Physician, be considered OAEs and reported as such in the Clinical Study Report. A similar review of laboratory, vital signs and dECG data will be performed for identification of OAEs.

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

11.3 Calculation or derivation of patient reported outcome variables

To be eligible for randomisation the patients must have reported symptoms in the RESQ-7 (7 days recall) a minimum of 3 days and a rating of at least mild intensity on at least one of the following items;

- a burning feeling behind the breastbone
- unpleasant movement of material upwards form the stomach.

The patient diary used during impedance/pH measurements (see appendix H) will be analysed and evaluated in connection to the assessment of the results by the central reader.

The patients use of the data logger and recording on the diary card for meal intake and periods in supine position as well as symptoms will be used for determination of; relation between reflux episodes and symptoms during the 24-hours.

11.4 Calculation or derivation of pharmacokinetic variables

The pharmacokinetic analyses will be performed at AstraZeneca R&D, Mölndal, Sweden, by standard non-compartmental methods using WinNonlin Enterprise.

The actual sampling times, if different from protocol times, will be used in the final pharmacokinetic calculations. Plasma concentrations below LLOQ will be excluded except for the pre-dose samples after the first dose, which will be taken as zero. Furthermore, if there are more than one plasma concentration below LLOQ prior to C_{max}, then the last one before the first measurable plasma concentration will be calculated as LLOQ/2. The following PK parameters will be determined:

Table 6 Pharmacokinetic parameters to be determined in the study

Parameter	Description
AUC_{0-12h}	The area under the plasma concentration-time curve from time 0 to 12 hours after the first dose of AZD3355 calculated by the log/linear trapezoidal method
AUC_{12-24h}	The area under the plasma concentration-time curve from time 12 to 24 hours after the first dose (0-12 hours after the second dose) of AZD3355 calculated by the log/linear trapezoidal method
AUC_{0-24h}	The sum of AUC_{0-12h} and AUC_{12-24h} after the first and second doses of AZD3355
C_{max}	The observed maximum plasma concentration after each dose of AZD3355
t_{max}	The time to reach C_{max} after each dose of AZD3355

11.5 Calculation or derivation of pharmacodynamic variables

See also Section 6.3.1.1.

Relationship between AZD3355 exposure and effect on impedance measurements will be presented graphically. If appropriate, exposure-effect relationship will be further explored.

11.6 Calculation or derivation of pharmacogenetic variables (Not applicable)

11.7 Calculation or derivation of health economic variables (Not applicable)

12. STATISTICAL METHODS AND SAMPLE SIZE DETERMINATION BY ASTRAZENECA

12.1 Description of analysis sets

12.1.1 Efficacy analysis set

The evaluation of the efficacy of AZD3355 30 mg, 90 mg, 120 mg, 240 mg and placebo, will be based on all patients and data not affected by major protocol deviations and violations, relevant for the analysis of a specific variable.

The PK Analysis Set will be a subset of the Safety Analysis Set and will include only subjects with no major protocol deviations thought to significantly affect the PK (eg, subject vomited at or before 2 times median t_{max}; wrong dose administered; prohibited concomitant medication, etc.). A strategy for dealing with protocol deviations will be specified prior to clean file by the Study Delivery Team (SDT Leader, Physician, Pharmacokineticist and Statistician).

The evaluability of the data will be determined and documented prior to starting the statistical analysis (and before database lock).

12.1.2 Safety analysis set

All patients who received at least one dose of AZD3355 or placebo and for whom post-dose data are available will be included in the safety analysis set. Throughout the safety results sections, erroneously treated patients (eg, those randomised to treatment A but actually given treatment B) will be accounted for in the actual treatment group.

12.2 Methods of statistical analyses

The estimation of the dose-response curve will primarily be made by estimating the parameters of a fixed effect Emax model. In addition, the same model will be estimated with data from study D9120C00020 (using 65 mg bid) included with a mixed effect Emax model with and without data from D9120C00020 included. The possibility of using additional model will also be explored. The data will be logarithmically transformed prior to estimation of the dose-response curves. If zero values are obtained during a period then the logarithm is undefined and no transformation prior to analysis will be made. If the logarithmic transform is used the estimated mean curves and 2-sided 90% confidence interval (CI) limits will be transformed back to the original scale to give geometric mean curves and corresponding confidence intervals.

The effect of 4 different doses of AZD3355 measured as reduction in the total number of reflux episodes during 24 hours (total, upright and supine position), compared to placebo will be assessed by estimating the inhibition (%) in the primary variables after treatment with AZD3355 30 mg, 90 mg, 120 mg and 240 mg compared to placebo. Inhibition defined as 100*(1-R) where R is the ratio (AZD3355/placebo) of estimated geometric means. The estimated geometric means for each treatment (AZD3355 30 mg, 90 mg, 120 mg and 240 mg and placebo) will also be presented. For estimating the geometric means the data will be logarithmically transformed prior to statistical analysis. If zero values are obtained during a period then the logarithm is undefined and no transformation prior to analysis will be made. Then the primary objective will be assessed by the differences in arithmetic means.

The analysis will be based on an mixed effect model with treatment, period and sequence as fixed effects and patient as a random effect. If the logarithmic transform is used the estimated arithmetic means and their differences and 2-sided 90% confidence interval (CI) limits will be transformed back to the original scale to give geometric means, ratios and corresponding confidence intervals. The same analysis will be made for the other variables addressed in the secondary objective.

No correction for multiplicity will be made.

Further details of the statistical analysis will be given in the Statistical Analysis Plan.

12.3 Determination of sample size

In study D9120C00020 the between and within-subject standard deviation (STD) for the logarithm of total number of reflux episodes during 24 hour ambulatory impedance monitoring was estimated to 0.79 and 0.25, respectively, using 21 evaluable patients. The geometric mean number of total number of reflux episodes was 30.6 (95% CI 20.9, 44.7) for AZD3355. This corresponded to a distance between the upper and lower 95% CI for the estimated mean of the logarithm of the total number of reflux episodes during 24 hours of 0.77. This precision was regarded as satisfactory.

By simulation of data with this variability, 20 patients and the treatment sequence used here, the width of the 95% CI for the estimated mean curve of the Emax model and the mixed effect Emax model was estimated to 0.70 and 1.73, respectively. When the data from D9120C00020 was added to the simulated data, the width was estimated to 0.53 and 1.43, respectively. For a geometric mean of 30.6 these widths would correspond to 95% CI of (23.5, 39.9) and (15.0, 62.6), respectively.

With 20 randomised patients, all will be assigned to placebo, 14 will be assigned to AZD3355 30 mg, 16 will be assigned to AZD3355 90 mg, 16 will be assigned to AZD3355 120 mg and 14 will be assigned to AZD3355 240 mg. In order to achieving these numbers of evaluable patients on each of the doses the study will include 27 randomised patients.

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

13. IMPORTANT MEDICAL PROCEDURES TO BE FOLLOWED BY THE INVESTIGATOR

13.1 Medical emergencies and AstraZeneca contacts

The Principal Investigator is responsible for ensuring that procedures and expertise are available to handle medical emergencies during the study. **A medical emergency usually constitutes an SAE and is to be reported as such, see Section 6.4.4.**

The treatment code should not be broken except in medical emergencies when the appropriate management of the patient requires knowledge of the treatment code, see Section 5.5.2.

In the case of a medical emergency the investigator may contact the Study Delivery Team Leader. If the Study Delivery Team Leader is not available, contact the Study Delivery Team Physician.

Name	Role in the study	Address & telephone number

13.2 Overdose

A dose of AZD3355 in excess of that planned according to this protocol is to be considered an overdose. There are no data on overdosing. There is no known antidote. In case of known or suspected overdose, symptomatic treatment as well as close monitoring of vital functions should be carried out. This should be recorded as follows

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

If an overdose on an AstraZeneca study drug occurs in the course of the study, then investigators or other site personnel inform appropriate AstraZeneca representatives **within one day**, ie, immediately but no later than **the end of the next business day** of when he or she becomes aware of it.

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

For overdoses associated with SAE, standard reporting timelines apply, see Section 6.4.4. For other overdoses, reporting should be done within 30 days.

13.3 Pregnancy

All outcomes of pregnancy should be reported to AstraZeneca.

13.3.1 Maternal exposure

If a patient becomes pregnant during the course of the study investigational product should be discontinued immediately.

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. Congenital abnormalities/birth defects and spontaneous

miscarriages should be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth or congenital abnormality) should be followed up and documented even if the patient was discontinued from the study.

If any pregnancy occurs in the course of the study, then investigators or other site personnel inform appropriate AstraZeneca representatives **within one day** ie, immediately but no later than the **end of the next business day** of when he or she becomes aware of it.

The designated AstraZeneca representative works with the investigator to ensure that all relevant information is provided to the AstraZeneca Patient Safety data entry site within 1 or 5 days for SAEs, see Section 6.4.4 and within 30 days for all other pregnancies.

Since AZD3355 is a new drug, there is no information on its effects on foetus or newborn children. Consequently, women of childbearing potential can only be included if they are using a highly effective method of birth control (see recommendations in Section 5.2). Postmenopausal women (natural menopause with last menses >1 year ago and >50 years old) and women who are bilateral oophorectomised or hysterectomised are allowed to participate in the study.

The same timelines apply when outcome information is available.

13.3.2 Paternal exposure

There is no restriction on fathering children or donating sperm during the study.

14. LIST OF REFERENCES

Dent et al 2005
Dent J, El-Serag HB, Wallander M-A, Johansson S. Epidemiology of gastro-oesophageal reflux disease: a systematic review. Gut 2005;54:710-7.

Fass 2007
Fass R. Proton-Pump Inhibitor Therapy in Patients with Gastro-Oesophageal Reflux Disease Putative Mechanisms of Failure. Drugs 2007;67(11):1521-30.

Jones et al 2007
Jones R, Liker HR, Ducrotté P. Relationship between symptoms, subjective well-being and medication use in gastro-oesophageal reflux disease. Int J Clin Pract 2007;61(8):1301-7.

Lehmann et al 2009
Lehmann A, Antonsson M, Aurell Holmberg A, Blackshaw LA, Brändén L, Bräuner-Osborne H et al. AZD3355, a novel GABAB receptor agonist inhibits transient lower esophageal sphincter relaxation through a peripheral mode of action. J Pharmacol Exp Ther 2009; In Press. (Epub ahead of print) PMID 19648470.

Report from Mapi Values
Cognitive debriefing of the RDQ and XQS in subjects with symptoms of GERD despite PPI treatment. (Report AZ5668A v3.0 prepared by Mapi Values for AstraZeneca 2007.) Unpublished.

Shaw et al 2001
Shaw MJ, Talley NJ, Beebe TJ, Rockwood T, Carlsson R, Adlis S et al. Initial validation of a sdiagnostic questionnaire for gastroesophageal reflux disease. Am J Gastroenterol 2001;96:52-7.

van Herwaarden et al 2002
van Herwaarden MA, Samsom M, Rydholm H, Smout AJ. The effect of baclofen on gastrooesophageal reflux, lower oesophageal sphincter function and reflux symptoms in patients with reflux disease. Aliment Pharmacol Ther 2002;16:1655-62.

Weusten et al 1994
Weusten BL, Rolfs JMM, Akkermans LMA et al. The symptom association probability: An improved method for symptom analysis of 24-hour esophageal pH data. Gastroenterology 1994;107:1741-5.

Wiklund et al 2003
Wiklund I, Talley NJ. Update on health-related quality of life in patients with gastroesophageal reflux disease. Exp Rev Pharmacoeconomics Outcomes Res 2003;3(3):341-50.

Wiener et al 1988
Wiener GJ, Richter JE, Copper JB et al. The symptom index: A clinically important parameter of ambulatory 24-hour esophageal pH monitoring. Am J Gastroenterol 1988;83:358-61.

D9120C00001
A single blind, placebo controlled, randomized, single-centre phase I pharmacodynamic study to assess the effect of a single oral dose (0.8 mg/kg) of AZD3355 (AR-H061746XX) solution in comparison with baclofen (40 mg), measured as reduction in the number of transient lower esophageal sphincter relaxations in healthy male subjects. Clinical Pharmacology Study Report, AstraZeneca, .

D9120C00011
A randomized, double-blind, placebo controlled, multi-centre, phase IIA study to assess the effect on GERD symptoms, pharmacokinetics, safety and tolerability of four weeks treatment with AZD3355 65 mg bid as add-on treatment to a PPI in patients with an incomplete response to PPI treatment. Clinical Study Report, AstraZeneca, .

D9120C00012
A blinded, randomised, two-centre, placebo controlled phase I study in healthy volunteers to A) investigate the safety and tolerability after repeated administration of escalating doses of AZD3355 single blinded followed by B) and analysis of the effect of AZD3355 (highest dose) on cardiac depolarisation and repolarisation as measured by the QT/QTc interval in the ECG, with double blind, double dummy administration of moxifloxacin (400mg) as positive control. Clinical Pharmacology Study Report, AstraZeneca, .

D9120C00020
A double-blind, placebo controlled, randomized, two centre phase IIA pharmacodynamic cross-over study to assess the effect of AZD3355, 65 mg bid, on transient lower esophageal sphincter relaxations (TLESRs) in GERD patients with an incomplete response to PPI treatment. Clinical Pharmacology Study Report, AstraZeneca, .

D9120C00027
Validation of patient-reported outcome measures for the assessment of GERD symptoms and their subsequent impact on patients with a partial response to PPI treatment in a two part multi-center phase IIA study including a four week randomised, double-blind, placebo-controlled parallel-group treatment period with AZD3355, 65 mg bid as add-on treatment to a PPI. Clinical Study Report, AstraZeneca, .

Clinical Study Protocol	Appendix A
Drug Substance	AZD3355
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Protocol Dated	

Appendix A
Signatures

ASTRAZENECA SIGNATURE(S)

A double-blind, placebo controlled, randomized, phase IIA pharmacodynamic 4-way cross-over study to estimate the dose response relationship of AZD3355 on the number of reflux episodes assessed by impedance/pH in patients with GERD and a partial response to PPI treatment

---

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

I agree to the terms of this study protocol.

AstraZeneca Research and Development site representat

ASTRAZENECA SIGNATURE(S)

A double-blind, placebo controlled, randomized, phase IIA pharmacodynamic 4-way cross-over study to estimate the dose response relationship of AZD3355 on the number of reflux episodes assessed by impedance/pH in patients with GERD and a partial response to PPI treatment

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AstraZeneca Research and Development site representative

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AstraZeneca Research and Development site representativ

SIGNATURE OF PRINCIPAL INVESTIGATOR

A double-blind, placebo controlled, randomized, phase IIA pharmacodynamic 4-way cross-over study to estimate the dose response relationship of AZD3355 on the number of reflux episodes assessed by impedance/pH in patients with GERD and a partial response to PPI treatment

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

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

Centre No.:

Signature:

Clinical Study Protocol Appendix B

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Appendix B
Additional Safety Information

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

Life threatening

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

Hospitalisation

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

Important medical event or medical intervention

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

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

Examples of such events are:

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

A GUIDE TO INTERPRETING THE CAUSALITY QUESTION

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

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

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

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

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

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

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

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

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

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

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

- Is there a known mechanism?

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

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

LABELLING AND SHIPMENT OF BIOHAZARD SAMPLES

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

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

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

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

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

Exempt - all other materials with minimal risk of containing pathogens

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

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

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Appendix D
Pharmacogenetics Research

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation or special term	Explanation
CSR	Clinical Study Report
DNA	Deoxyribonucleic acid
GERD	Gastroesophageal Reflux Disease
LIMS	Laboratory information management system
PGx	Pharmacogenetics

1. BACKGROUND AND RATIONALE

Genetic variation within a population can contribute to inter-individual differences in drug response (where the term "response" is used broadly to include drug disposition, safety, efficacy and tolerability). Characterisation of such variation can help clarify the biology of drug action and elucidate processes that may influence the safety and tolerability of a drug or class of drugs (Roses 2004).

With the increase in information about genes and their variations (polymorphisms), there is an increasing understanding of how these may impact drug response (the field of "pharmacogenetics"). Genetic variation in drug metabolising enzymes has been extensively studied and has been shown to be of clinical significance for a number of drugs (Daly 2003).

For a number of these enzymes, particularly the cytochrome P450 isoenzymes, the genetic basis for altered activity is already well understood. This work is being expanded into other genes important to the absorption, distribution, metabolism and excretion (ADME) of drugs within the body, such as the drug transports proteins. Whilst there are many factors affecting the pharmacokinetic profile of a given drug, genetic variation can be an important determinant and genotyping may allow for more rational dosage and safety predictions in patients.

Variations in genes that encode the molecular target of a drug, and genes involved in the signalling pathways related to that target, are also candidates for influencing variability in therapeutic response. In comparison to the drug metabolising enzymes, these genes (and the variations within them) are generally less well characterised and understood at the present time; although some relevant examples are beginning to emerge (Evans and Relling 1999). In the future, however, it is likely that more information will become available on genes that are important in determining therapeutic efficacy.

AstraZeneca intends to perform genetic research in the AZD3355 clinical development programme to explore how genetic variations may affect the clinical parameters associated with AZD3355. Collection of DNA samples from populations with well described clinical characteristics may lead to improvements in the design and interpretation of clinical trials and, possibly, to genetically guided treatment strategies.

Future research may suggest other genes or gene categories as candidates for influencing not only response to AZD3355 but also susceptibility to or prognosis of Gastroesophageal Reflux Disease (GERD) for which AZD3355 may be evaluated. Thus, this genetic research may involve study of additional un-named genes or gene categories, but only as related to disease susceptibility and drug action.

2. GENETIC RESEARCH OBJECTIVES

The objective of this research is to collect and store DNA for potential future research into genes which may influence pharmacokinetics, drug disposition, efficacy, safety and

tolerability of AZD3355 or influence the susceptibility to or prognosis of GERD and associated disease as under study.

3. GENETIC RESEARCH PLAN AND PROCEDURES

3.1 Selection of genetic research population

3.1.1 Study selection record

All randomised subjects will be asked to participate in this genetic research. Participation is voluntary and if a subject declines to participate there will be no penalty or loss of benefit. The subject will not be excluded from any aspect of the main study.

3.1.2 Inclusion criteria

For inclusion in this genetic research, subjects must fulfil all of the inclusion criteria described in the main body of the Clinical Study Protocol **and**:

- Provide informed consent for the genetic sampling and analyses.

3.1.3 Exclusion criteria

Exclusion from this genetic research may be for any of the exclusion criteria specified in the main study or any of the following:

- Previous allogeneic bone marrow transplant
- Non-leukocyte depleted whole blood transfusion in 120 days of genetic sample collection

3.1.4 Discontinuation of subjects from this genetic research

Specific reasons for discontinuing a subject from this genetic research are:

Withdrawal of consent for genetic research: Subjects may withdraw from this genetic research at any time, independent of any decision concerning participation in other aspects of the main study. Voluntary discontinuation will not prejudice further treatment. Procedures for discontinuation are outlined in Section 7.5 of the main Clinical Study Protocol.

3.2 Collection of samples for genetic research

The blood sample for genetic research will be obtained from the subjects at Visit 2 at randomisation. Although genotype is a stable parameter, early sample collection is preferred to avoid introducing bias through excluding patients who may withdraw due to an adverse event (AE), such patients would be important to include in any genetic analysis. If for any reason the sample is not drawn at Visit 2 it may be taken at any visit until the last study visit. Only one sample should be collected per subject for genetics during the study. Samples will be collected, labelled, stored and shipped as detailed in the Laboratory Manual.

For blood volume, see Section 7.1 of the Clinical Study Protocol.

3.3 Coding and storage of DNA samples

The processes adopted for the coding and storage of samples for genetic analysis are important to maintain subject confidentiality. Samples will be stored for a maximum of 25 years, from the date of last subject last visit, after which they will be destroyed. DNA is a finite resource that is used up during analyses. Samples will be stored and used until no further analyses are possible or the maximum storage time has been reached.

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

The samples and data for genetic analysis in this study will be coded. The link between the subject enrolment/randomisation code and the DNA number will be maintained and stored in a secure environment, with restricted access WITHIN the Clinical Genotyping Group Laboratory Information Management System (LIMS) at AstraZeneca. The link will be used to identify the relevant DNA samples for analysis, facilitate correlation of genotypic results with clinical data, allow regulatory audit and to trace samples for destruction in the case of withdrawal of consent.

4. ETHICAL AND REGULATORY REQUIREMENTS

The principles for ethical and regulatory requirements for the study, including this genetics research component, are outlined in Section 8 of the main Clinical Study Protocol.

4.1 Informed consent

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

4.2 Subject data protection

AstraZeneca will not provide individual genotype results to subjects, any insurance company, any employer, their family members, general physician or any other third party, unless required to do so by law.

Extra precautions are taken to preserve confidentiality and prevent genetic data being linked to the identity of the subject. In exceptional circumstances, however, certain individuals might see both the genetic data and the personal identifiers of a subject. For example, in the case of a medical emergency, an AstraZeneca Physician or an investigator might know a subject's identity and also have access to his or her genetic data. Also Regulatory authorities may require access to the relevant files, though the subject's medical information and the genetic files would remain physically separate.

5. DATA MANAGEMENT

Any genotype data generated in this study will be stored in the AstraZeneca genotyping LIMS database, or other appropriate secure system within AstraZeneca and/or third party contracted to work with AstraZeneca to analyze the samples.

The results from this genetic research may be reported in the CSR for the main study, or in a separate report as appropriate.

Some or all of the clinical datasets from the main study may be merged with the genetic data in a suitable secure environment separate from the clinical database.

6. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

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

7. LIST OF REFERENCES

Roses 2004
Roses AD. Pharmacogenetics and drug development: the path to safer and more effective drugs. Nat Rev Genet 2004;5(9):645-56.

Daly 2003
Daly AK. Pharmacogenetics of the major polymorphic metabolising enzymes. Fundamental & Clinical Pharmacology 2003;17:27-41.

Evans and Relling 1999
Evans WE, Relling MV. Pharmacogenomics: Translating functional genomics into rational therapeutics. Science 1999;286:487-91.

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Clinical Study Protocol Appendix E	
Drug Substance	AZD3355
Study Code	D9120C00032
Edition Number	1
Date	

Appendix E
The Reflux Symptoms Questionnaire, 7-days recall (RESQ-7)

Reflux Symptoms Questionnaire - RESQ-7

E-code: _____ Date:_____ Study code:_____

Please answer the following questions to help us better understand the symptoms you have been experiencing over the past 7 days because of your reflux disease. For each question, please choose the answer that is most appropriate to you. Please answer each question by ticking **one** box per row.

1. Thinking about your symptoms over the past 7 days, how often did you have the following?

		Did not have	1 day	2 days	3-4 days	5-6 days	Daily
a.	A burning feeling behind your breastbone	☐	☐	☐	☐	☐	☐
b.	Pain behind your breastbone	☐	☐	☐	☐	☐	☐
c.	A burning feeling in the centre of the upper stomach	☐	☐	☐	☐	☐	☐
d.	A pain in the centre of the upper stomach	☐	☐	☐	☐	☐	☐
e.	An acid taste in your mouth	☐	☐	☐	☐	☐	☐
f.	Unpleasant movement of material upwards from the stomach	☐	☐	☐	☐	☐	☐
g.	Burping (gas coming from the stomach through the mouth)	☐	☐	☐	☐	☐	☐
h.	Hoarseness	☐	☐	☐	☐	☐	☐
i.	Cough	☐	☐	☐	☐	☐	☐
j.	Difficulty swallowing	☐	☐	☐	☐	☐	☐
k.	A bitter taste in your mouth	☐	☐	☐	☐	☐	☐
l.	Stomach contents (liquid or food) moving upwards to your throat or mouth	☐	☐	☐	☐	☐	☐
m.	Heartburn	☐	☐	☐	☐	☐	☐

E-code: _____ Date:_____ Study code:_____

2. Thinking about your symptoms over the past 7 days, how would you rate the following?

		Did not have	Very mild	Mild	Moderate	Moderately severe	Severe
a.	A burning feeling behind your breastbone	☐	☐	☐	☐	☐	☐
b.	Pain behind your breastbone	☐	☐	☐	☐	☐	☐
c.	A burning feeling in the centre of the upper stomach	☐	☐	☐	☐	☐	☐
d.	A pain in the centre of the upper stomach	☐	☐	☐	☐	☐	☐
e.	An acid taste in your mouth	☐	☐	☐	☐	☐	☐
f.	Unpleasant movement of material upwards from the stomach	☐	☐	☐	☐	☐	☐
g.	Burping (gas coming from the stomach through the mouth)	☐	☐	☐	☐	☐	☐
h.	Hoarseness	☐	☐	☐	☐	☐	☐
i.	Cough	☐	☐	☐	☐	☐	☐
j.	Difficulty swallowing	☐	☐	☐	☐	☐	☐
k.	A bitter taste in your mouth	☐	☐	☐	☐	☐	☐
l.	Stomach contents (liquid or food) moving upwards to your throat or mouth	☐	☐	☐	☐	☐	☐
m.	Heartburn	☐	☐	☐	☐	☐	☐

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Clinical Study Protocol Appendix F	
Drug Substance	AZD3355
Study Code	D9120C00032
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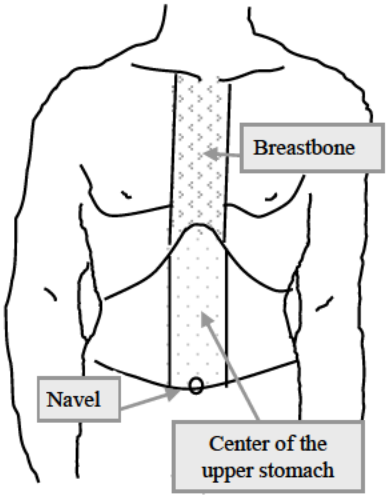
Appendix F
The Reflux Symptoms Questionnaire, 7-days recall (RESQ-7) Torso picture



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Clinical Study Protocol Appendix G

Drug Substance	AZD3355
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Appendix G
Examples of drugs prohibited in study D9120C00032 due to their potential to prolong the QT interval*

This list should not be considered comprehensive therefore investigators need to use their judgment when reviewing the medication list from individual patients and restrict patients who must stay on drugs that may increase the QT interval.

Concomitant Medication	**Class**
Disopyramide	Antiarrhythmic 1A
Procainamide	Antiarrhythmic 1A
Quinidine	Antiarrhythmic 1A
Mexiletine	Antiarrhythmic 1B
Propafenone	Antiarrhythmic 1C
Flecainide	Antiarrhythmic 1C
Amiodarone	Antiarrhythmic III
Dofetilide	Antiarrhythmic III
Ibutilide	Antiarrhythmic III
Sotalol	β blocking agent III
Bepridil	Ca channel blocker IV
Metoclopramide	Prokinetic
Dolasetron	Anti-emetic
Granisetron	Anti-emetic
Ondansetron	Anti-emetic
Droperidol	Anti-emetic
Levomethadyl	Opioid agonist
Chlorpromazine	Antipsychotic
Haloperidol	Antipsychotic
Pimozide	Antipsychotic
Thioridazine	Antipsychotic
Risperidone	Antipsychotic
Ziprasidone	Antipsychotic
Amitriptyline	Antidepressant
Nortriptyline	Antidepressant
Protriptyline	Antidepressant
Desipramine	Antidepressant
Imipramine	Antidepressant

Concomitant Medication	Class
Venlafaxine	Antidepressant
Fluxetine	Antidepressant
Chloroquin	Anti-malarial
Pentamidine	Anti-infective
Solifenacine	Anti-spasmodic
Vardenafil	Anti-spasmodic
Erythromycin	Antibiotic
Clarithromycin	Antibiotic
Azithromycin	Antibiotic
Telithromycin	Antibiotic
Gatifloxacin	Antibiotic
Gemifloxacin	Antibiotic
Levofloxacin	Antibiotic
Moxifloxacin	Antibiotic
Ofloxacin	Antibiotic
Sparfloxacin	Antibiotic
Fluconazole	Antimycotic
Ketoconazole	Antimycotic
Tacrolimus	Immunosuppressive
Alfuzosin	α-blocker

*Based on US Prescribing Information

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Clinical Study Protocol Appendix H	
Drug Substance	AZD3355
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Appendix H
Diary Card for 24-hours impedance pH measurement

24-Hour Ambulatory Impedance-pH Patient Diary Sheet - D9120C00032

Patient: ______________________________________ **Date:** __________________

ID #: ______________________________________ **Start time:** _____:_____am/pm

End Time: _____:_____am/pm

MEALS / SNACKS

Press once	BEGIN TIME	*Press once*	END TIME	NOTES
	_____:_____am/pm		_____:_____am/pm	
	_____:_____am/pm		_____:_____am/pm	
	_____:_____am/pm		_____:_____am/pm	
	_____:_____am/pm		_____:_____am/pm	
	_____:_____am/pm		_____:_____am/pm	
	_____:_____am/pm		_____:_____am/pm	
	_____:_____am/pm		_____:_____am/pm	

SLEEP / LYING DOWN

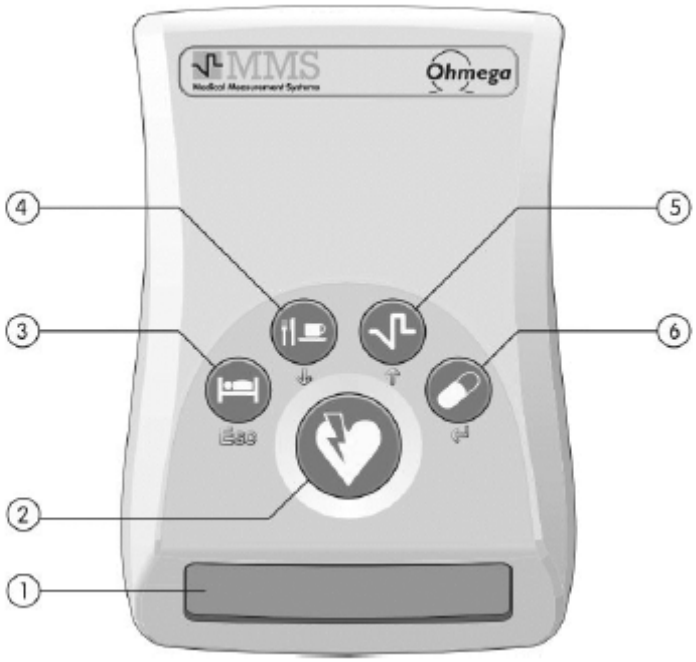
Press once	BEGIN TIME	*Press once*	END TIME	NOTES
	_____:_____am/pm		_____:_____am/pm	
	_____:_____am/pm		_____:_____am/pm	
	_____:_____am/pm		_____:_____am/pm	

SYMPTOMS

Press once	TIME	NOTES	*Press once*	TIME	NOTES
	_____:_____am/pm			_____:_____am/pm	
	_____:_____am/pm			_____:_____am/pm	
	_____:_____am/pm			_____:_____am/pm	
	_____:_____am/pm			_____:_____am/pm	
	_____:_____am/pm			_____:_____am/pm	
	_____:_____am/pm			_____:_____am/pm	
	_____:_____am/pm			_____:_____am/pm	
	_____:_____am/pm			_____:_____am/pm	
	_____:_____am/pm			_____:_____am/pm	
	_____:_____am/pm			_____:_____am/pm	

MEDICATIONS

	_____:_____am/pm		_____:_____am/pm		_____:_____am/pm		_____:_____am/pm


MMS
Medical Measurement Systems
Ohmega
4
5
3
6
2
1