Clinical Study Protocol

Drug Substance	AZD3355
Study Code	D9120C00019
Edition Number	01

A randomized, double-blind, placebo controlled, multi-centre phase IIb dose finding study to assess the effect on GERD symptoms, safety and tolerability during four weeks treatment with AZD3355 in doses 60 mg, 120 mg, 180 mg and 240 mg bid as add-on treatment to a PPI in patients with GERD that are partial responders 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

PROTOCOL SYNOPSIS

A randomized, double-blind, placebo controlled, multi-centre phase IIb dose finding study to assess the effect on GERD symptoms, safety and tolerability during four weeks treatment with AZD3355 in doses 60 mg, 120 mg, 180 mg and 240 mg bid as add-on treatment to a PPI in patients with GERD that are partial responders to PPI treatment

International Co-ordinating Investigator

Study centre(s) and number of patients planned

The study will be carried out in approximately 650 patients from approximately 130 sites in Canada, France, Germany, Hungary, Latvia, Romania and USA.

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

Objectives

The primary objective is to evaluate the effect on GERD symptoms of four doses of AZD3355 (60 mg, 120 mg, 180 mg and 240 mg bid) compared to placebo, as add-on treatment to a PPI by using the responder definition; ie, at least 3 more days of not more than mild GERD symptoms on average per week during the whole treatment period compared to baseline, based on the Overall symptoms domain in the Reflux Symptom Questionnaire electronic Diary (RESQ-eD).

The secondary objectives of the study are:

- To evaluate the effect on GERD symptoms of four doses of AZD3355 (60 mg, 120 mg, 180 mg and 240 mg bid) compared to placebo, as add-on treatment to a PPI by

using an alternative responder definition; ie, at least 5 days of not more than mild GERD symptoms on average per week during the whole treatment period based on the Overall symptoms domain in the RESQ-eD

- To evaluate the effect on GERD symptoms of four doses of AZD3355 (60 mg, 120 mg, 180 mg and 240 mg bid) compared to placebo, as add-on treatment to a PPI by analysing the change in the proportion of days of not more than mild GERD symptoms compared to baseline, based on the Overall symptoms domain and for each separate domain (Heartburn; Regurgitation; Hoarseness, cough and difficulty swallowing; Burping) in the RESQ-eD

- To evaluate the dose-response curve with respect to:

  – The responder definition

  – The change in the proportion of days of not more than mild GERD symptoms compared to baseline, based on the Overall symptoms domain in the RESQ-eD

- To assess basic measurement properties of the RESQ-eD

- To assess the pharmacokinetics of AZD3355 by population PK analyses with special regard to variability in the patient population

- To study the relationship between systemic exposure and response with special regard to the responder definition

- To study the prevalence of mucosal breaks in the target population through endoscopy at baseline during the study or historical reports

- To describe the endoscopic findings after the study treatment period, in patients with baseline endoscopy showing mucosal breaks.

Safety objectives:

To assess the safety and tolerability during 4 weeks treatment with four doses of AZD3355 as cadd-on treatment to a PPI, by evaluation of adverse events, laboratory variables, ECG, blood pressure, pulse, orthostatic tests and physical examination.

Exploratory objective:

– To collect and store DNA for future exploratory research into genes/genetic variation that may influence response (ie, distribution, safety, tolerability and efficacy) to AZD3355 and/or susceptibility to or prognosis of GERD and associated disease as under study.

Study design

This is a randomized, double blind, placebo-controlled, parallel group designed, multi-centre phase IIb dose finding study to assess the effect on GERD symptoms, safety and tolerability during 4 weeks of treatment with four doses of AZD3355 as add-on treatment to a PPI. The effect evaluation will be based on patient reported symptom data, using twice-daily recordings in an electronic diary device.

Target patient population

The target patient population are partial responders to PPI treatment who experience at least moderate intensity of GERD symptoms on at least 3 of the past 7 days despite optimized PPI treatment[1]. Patients will be males and females aged 18-70 years, inclusive, and must have a history of GERD symptoms for at least 6 months.

Investigational product, dosage and mode of administration

Oral administration of AZD3355 60 mg, 120 mg, 180 mg, 240 mg or placebo twice daily. The doses are given as modified release (MR 1 hour) capsules 30 mg, 60 mg, 120 mg or placebo.

Comparator, dosage and mode of administration

Placebo matching the investigational product.

Duration of treatment

The study consists of a screening phase of 8-26 days, a 4-weeks treatment phase and a follow-up phase of 2 weeks.

Outcome variable(s):

- Efficacy variables
  - The binary variable (yes/no) indicating a patient fulfilling the responder definition: at least 3 more days of not more than mild GERD symptoms on average per week during the whole treatment period compared to baseline based on the Overall symptoms domain in the RESQ-eD
  - The binary variable (yes/no) indicating a patient fulfilling the alternative responder definition: at least 5 days of not more than mild GERD symptoms on

[1] An optimized PPI treatment is a treatment which according to the investigator judgment can not be further improved by changing brand or dosing of the PPI. Patients should be continuously treated during the last 4 weeks before enrolment with daily optimized unchanged PPI therapy with dose according to the country label, or regional where applicable, for any GERD indication. Patients with endoscopy verified reflux esophagitis (Los Angeles classification grade A-D) within the last 8 weeks must have completed the prescribed 8 weeks of treatment with a PPI.

average per week during the whole treatment period based on the Overall symptoms domain in the RESQ-eD

– The change in the proportion of days of not more than mild GERD symptoms compared to baseline, based on the Overall symptom domain in the RESQ-eD and for each separate domain

- Pharmacokinetic variables:

  – Plasma concentration versus time

  – Total plasma clearance

- Safety variables:

  – Adverse events, laboratory values, vital signs (supine and standing blood pressure and pulse), physical examination and dECG.

Statistical methods

The primary objective, to evaluate the effect of four doses of AZD3355 compared to placebo using the responder definition, will be evaluated by pairwise comparisons. Each dose level will be compared to placebo using logistic regression with dose as a factor variable and proportion of days of not more than mild symptoms during baseline as a covariate. The tests will be one-sided with a significance level of 10%. The multiplicity issue will be handled by using a step down procedure where AZD3355 is tested sequentially starting with the highest dose.

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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)
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AMA	Anti-Mitochondrial antibodies
ANA	Anti-nuclear antibodies
anti-EBV-IgM	Epstein-Barr Virus antibodies immunoglobulin M
anti-CMV-IgM	Cytomegalo Virus antibodies immunoglobulin M
anti-HAV-IgM	Hepatitis A Virus antibodies immunoglobulin M
anti-HBc-IgM	Hepatitis B Virus core antigen immunoglobulin M
anti-HCV	Hepatitis C Virus antibodies
ASA	Acetylsalicylic acid
AST	Aspartate aminotransferase
AUC	Total area under the plasma concentration vs. time curve
BIL	Bilirubin
BMI	Body Mass Index
BP	Blood pressure
BUN	Blood urea nitrogen
C_{max}	Observed maximum plasma concentration
CDT	Carbohydrate deficient transferrin
CK	Creatine kinase
CMV	Cytomegalo Virus
CNS	Central nervous system
COC	Combined oral contraceptive
COX-2	Cyclo-oxygenase-2
CRF	Case Report Form (electronic/paper)
CRP	C-reactive protein
CSA	Clinical Study Agreement
CSP	Clinical Study Protocol

Abbreviation or special term	Explanation
CSR	Clinical Study Report
CT	Computer tomography
DAE	Discontinuation of Investigational Product due to Adverse Event
dECG	Digital electrocardiogram
DBP	Diastolic blood pressure
DNA	Deoxyribonucleic acid
DSMB	Data safety monitoring board
DUS	Disease under study
EBV VCA IgM	Epstein-Barr Virus Viral Capsid Antigen immunoglobulin M
EBNA IgG	Epstein-Barr Nuclear Antigen immunoglobulin G
EC	Ethics Committee, synonymous to Institutional Review Board (IRB) and Independent Ethics Committee (IEC)
ECG	Electrocardiogram
eCRF	Electronic case report form
e-diary	Electronic diary
ePRO	Electronic patient-reported outcome(s)
ES	Effect size
FAS	Full analysis set
FDA	Food and Drug Administration
$GABA_B$	Gamma-Amino-Butyric-Acid B receptor
GCP	Good Clinical Practice
GERD	Gastroesophageal Reflux Disease
GI	Gastrointestinal
GMP	Good Manufacturing Practice
Hb	Haemoglobin
HBsAg	Hepatitis B Virus surface antigen
H.pylori	*Helicobacter pylori*
HR	Heart rate
HRT	Hormone replacement therapy
IATA	International air transport association
IB	Investigator's Brochure
IBS	Irritable Bowel Syndrome

Abbreviation or special term	Explanation
ICC	Intraclass correlation coefficients
ICH	International Conference on Harmonisation
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
INR	International Normalized Ratio
International Co-ordinating investigator	If a study is conducted in several countries the International Co-ordinating Investigator is the Investigator co-ordinating the investigators and/or activities internationally
IP	Investigational product
IR	Immediate release
ITT	Intention to treat
IUD/IUS	Intra uterine device/system
iv	Intravenous
LES	Lower esophageal sphincter
LLOQ	Lower Limit of Quantification
LSLV	Last Subject Last Visit
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
MIC	Minimal important change
MID	Minimal important difference
min	Minutes
MR	Modified release
NSAID	Non-steroid anti-inflammatory drugs
OAE	Other Significant Adverse Event (see definition in Section 11.2.1)
optimized PPI treatment	An optimized PPI treatment is a treatment which according to the investigator judgment can not be further improved by changing brand or dosing of the PPI. Patients should be continuously treated during the last 4 weeks before enrolment with daily optimized unchanged PPI therapy with dose according to the country label, or regional where applicable, for any GERD indication. Patients with endoscopy verified reflux esophagitis (Los Angeles classification grade A-D) within the last 8 weeks must have completed the prescribed 8 weeks of treatment with a PPI
OTC	Over the counter
OTE	Overall Treatment Evaluation

Abbreviation or special term	Explanation
PGx	Pharmacogenetic research
PI	Principal Investigator
PK	Pharmacokinetic
PPI	Proton pump inhibitor
PR	ECG interval measured from the beginning of the P wave to the beginning of R wave, or as PQ to the beginning of Q wave, when present
PRO	Patient-reported outcome(s)
QRS	ECG interval measured from the beginning of the Q wave (or the R wave if Q is missing) to the J point
QT	ECG interval measured from the onset of the Q wave (or the R wave if Q is missing) to the T wave offset
QTcB	Heart rate corrected QT interval using the Bazett formula
QTcF	Heart rate corrected QT interval using the Fridericia formula
RBC	Red blood cell
RDQ	Reflux Disease Questionnaire
RE	Reflux esophagitis (Los Angeles classification grade A-D)
RESQ-eD	Reflux Symptom Questionnaire electronic Diary
RESQ-7	Reflux Symptom Questionnaire 7 day recall
RR	The time between corresponding points on 2 consecutive R waves on ECG
SAE	Serious adverse event (see definition in Section 6.4.2)
SAP	Statistical analysis plan
SBP	Systolic blood pressure
SMA	Smooth muscle antibodies
Target population	Patients with GERD who have a partial response to PPI treatment, as characterized by persistent GERD symptoms
TIBC	Total iron-binding capacity
TLESR	Transient lower esophageal sphincter relaxation
t_{max}	Time to reach maximum total plasma concentration following drug administration
ULN	Upper limit of normal
WBC	White blood cell
WBDC	Web Based Data Capture

1. INTRODUCTION

1.1 Background

Gastroesophageal Reflux Disease (GERD) is common, with an estimated prevalence of 10-20% in the Western world (Dent et al 2005). Heartburn and regurgitation are predominant symptoms of GERD, however other symptoms are also associated with the disease (Vakil et al 2006). It is becoming commonly accepted that the diagnosis and management of GERD can be based on patients' report of symptoms (Jones et al 2007). This has been formally addressed in a new definition and classification of GERD developed by an International Consensus Group, first presented at the World Congress of Gastroenterology in Montreal 2005 (Vakil et al 2006). Specifically, the definition concludes that GERD diagnosis is best based on a "patient-centered definition of troublesome symptoms".

Acid suppressive therapy with proton pump inhibitors (PPIs) is an effective treatment of GERD. However, approximately 20-30% of patients continue to experience GERD symptoms while on PPI treatment (Fass 2007a). Although PPIs are very effective in reducing acid, remaining acid reflux and other factors such as weakly acidic reflux, weakly alkaline reflux, and esophageal hypersensitivity, can produce symptoms in patients on PPI therapy who continue to reflux stomach contents into the esophagus. This may explain why some patients, despite all efforts by the treating physicians to optimize the PPI treatment eg, by changing dosing and brands, continue to experience GERD symptoms. There is a clear unmet medical need among the patients who continue to experience GERD symptoms while on PPI therapy, especially since GERD symptoms are associated with a negative impact on patients' lives (Wiklund at al 2003, Jones et al 2007). The most commonly reported impacts found in the literature are problems with sleep as a result of nighttime symptoms and food and drink problems, but also emotional problems and reduced vitality are commonly reported by patients with GERD.

AstraZeneca is developing AZD3355 as add-on treatment to PPIs to relieve GERD symptoms in patients with GERD who have a partial response to PPI treatment, as characterized by persistent GERD symptoms. This definition of the target patient population requires patients to be on optimized PPI treatment and excludes patients who do not respond at all to PPIs. The primary treatment goal for AZD3355 is to provide 24-hour symptom relief in this target patient population.

The major barrier for protecting the esophagus from constant exposure of stomach contents is the lower esophageal sphincter (LES). Transient LES relaxation (TLESR) triggered by gastric distension is a physiological phenomenon that allows for the evacuation of gas from the stomach. However, studies combining manometry and pH-metry have shown that TLESRs account for approximately 80% of all reflux episodes. TLESRs occur in both healthy volunteers and patients with GERD, however they are more often associated with reflux events in patients with GERD (Dent et al 1988, Holloway et al 1990). Thus, a physiological approach to reduce reflux episodes in patients with GERD would be to reduce the number of TLESRs. Stimulation of Gamma-Amino-Butyric-Acid B ($GABA_B$) receptors located

peripherally in the stomach and the esophagus (where they reduce mechanosensitivity) and centrally in the brain stem has demonstrated an inhibitory effect on the TLESR reflex pathway (Blackshaw 2001). Baclofen, which is a $GABA_B$ receptor agonist approved for the treatment of neurological disorders, has been shown to inhibit TLESRs and reflux episodes (Zhang et al 2002). However, central nervous system (CNS) side effects limit the usefulness of baclofen in the treatment of GERD.

The novel reflux inhibitor AZD3355 is a selective $GABA_B$ receptor agonist being developed for the treatment of GERD. AZD3355 has limited access to $GABA_B$ receptors in the CNS, due to an uptake by GABA transporters and sequestration in central neurons and glial cells. Therefore, AZD3355 acts mainly on peripheral $GABA_B$ receptors and has low potential for CNS side effects. A peripherally acting $GABA_B$ receptor agonist targeting TLESRs is thus a novel approach to improve the treatment of GERD.

AZD3355 65 mg twice daily (3 doses), given as add-on treatment to a PPI in patients with GERD symptoms despite PPI therapy, reduced the post-prandial number of TLESRs by 25%, increased the LES pressure by 28% and decreased the number of reflux episodes by approximately 47% compared to placebo (D9120C00020). In another study in patients who experienced GERD symptoms despite PPI treatment, AZD3355 65 mg twice daily was given for 4 weeks as add-on treatment to a PPI (D9120C00011). The results demonstrated a significant improvement of the patient reported GERD symptoms of burning feeling behind the breastbone (heartburn) and unpleasant movement of materials upward from the stomach (regurgitation) as compared to placebo. Although the purpose of the PRO validation study (D9120C00027) was not to evaluate efficacy, a post-hoc analysis was performed using the same responder definition as in the D9120C00011, and though there was a higher proportion of responders in the AZD3355 65 mg bid group as compared to placebo it was not statistically significant at a 5% significance level. The therapeutic dose has not yet been established however, this will be the purpose of this dose finding study.

Toxicological evaluation have not raised any safety concerns. AZD3355 has been well-tolerated in the clinical studies. The most common adverse events reported in patients taking AZD3355 have been diarrhoea, paraesthesia and pruritus.

Both proof-of-principle and proof-of-concept studies have been delivered for the AZD3355 programme. The outcome of this study will support the selection of the therapeutic dose(s) of AZD3355 for the confirmatory studies in the clinical development programme.

More information on the compound and the non-clinical and clinical studies can be found in the Investigator's Brochure (IB).

1.2 Research hypothesis

In the dog model, there was a dose response to the inhibition of TLESRs by AZD3355. In studies evaluating the effect of AZD3355 on GERD symptoms in patients, only one dose, AZD3355 65 mg twice daily (bid), has been tested thus far. The hypothesis is that there is a

dose response relationship for the effect of AZD3355 on GERD symptoms, which could be established using a Patient-Reported Outcome (PRO) diary.

1.3 Rationale for conducting this study

Proton pump inhibitors suppress acid and have a proven effect on treating GERD symptoms and healing reflux esophagitis (Kahrilas et al 2000, Richter et al 2001). However, a proportion of patients with GERD need more than acid suppression to achieve adequate symptom relief. For those patients with GERD who continue to have symptoms on PPI therapy, the cause of their symptoms may be from continued reflux of stomach contents directly irritating the lining of the esophagus or a hypersensitivity to the refluxate. This indicates that reflux inhibition would be a suitable treatment option for these patients. Patients with a partial response to PPI therapy who continue to have at least moderate symptoms of GERD 3 days a week or more, despite optimization of their current PPI therapy, are the target population for AZD3355. Patients with no response at all to PPIs are not part of the target population.

The pharmacodynamic effects of AZD3355 has translated into clinical efficacy in one study (D9120C00011), ie improvement of GERD symptoms in the target population, however the dose tested (65 mg bid) has not yielded consistent results (D9120C00027). AZD3355 therefore seems to have the potential to address an unmet medical need and improve the limited current treatment options in patients with a partial response to PPI treatment. However, the therapeutic dose of AZD3355 has yet to be established.

The results from this study will support the selection of the-therapeutic dose(s) of AZD3355 to be used in the confirmatory phase III studies in patients with GERD with a partial response to PPI treatment. The efficacy of AZD3355 will be evaluated using a PRO diary, the Reflux Symptom Questionnaire electronic Diary (RESQ-eD). A PRO validation study (D9120C00027) has confirmed that the RESQ-eD is valid, reliable and responsive to change, and therefore fit for purpose in the target patient population and the AZD3355 clinical programme. To build on the previous documentation of the RESQ-eD being fit for purpose in the target patient population, basic measurement properties of the RESQ-eD will also be assessed in this study.

The selection of the therapeutic dose(s) of AZD3355 will be based on the efficacy and the assessment of safety and tolerability during 4 weeks treatment with four doses of AZD3355 as add-on treatment to a PPI. In order to provide information on the choice of dosing regimen for future clinical development, the pharmacokinetics of AZD3355 by population PK analyses, with special regard to variability in the patient population, will be assessed. In addition, the relationship between systemic exposure and treatment response will be studied.

The majority of patients with reflux esophagitis (Los Angeles classification grade A-D) treated with a PPI heal their mucosal breaks and have symptom resolution. Therefore, it is expected that most of the patients enrolled in the study will have non-erosive disease. However, since the prevalence of erosive disease in patients with a partial response to PPI therapy with at least moderate symptoms is unknown, this will be assessed based on baseline

endoscopy or historical endoscopy reports. Esophageal mucosal status after the study treatment period will also be described in patients with mucosal breaks at baseline endoscopy.

Blood samples for DNA extraction will be collected in connection to this study in order to enable future genetic research if approved by appropriate Regulatory Authority/Ethics Committee. The genetic research is optional for study sites as well as for individual patients. Further description of the genetic research is given in Appendix D.

1.4 Benefit/risk and ethical assessment

It is estimated that 20-30% of patients with GERD continue to experience GERD symptoms while on PPI treatment (Fass 2007a). This finding reinforces the need for improved management options in this patient population. Therefore, research focusing on compounds with a different mechanism of action for the treatment of GERD, that complements acid suppressive therapy with PPIs, is of great clinical importance. The potential benefit of treatment with AZD3355 is the relief of persistent GERD symptoms in partial responders to PPI treatment. In addition, a determination of what would be considered a meaningful change as a response to treatment would also improve the understanding and management of these patients.

AZD3355 is a selective $GABA_B$ receptor agonist that acts predominately in the peripheral nervous system by targeting the LES. In therapeutic doses, the risk for CNS side effects is expected to be small, which is supported by the finding that the incidence of centrally related effects of the nervous system with AZD3355 has been low and comparable to placebo in studies both in healthy volunteers and in patients studied to date. The peripheral activity of AZD3355 offers a potential benefit over existing centrally acting agents.

There is low toxicity of AZD3355 in animals, with no specific organ toxic effects seen in animal studies up to 12 months of treatment. The only evident changes seen were a decrease in body weight and food consumption and a dose dependent diuretic effect.

The patient studies D9120C00011 and D9120C00020 showed that AZD3355 has an effect on both the underlying cause of GERD and the resultant symptoms, indicating that AZD3355 may effectively treat patients with persistent symptoms of GERD while on a PPI but the therapeutic dose is yet unclear.

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.

In 3 clinical phase II studies, a total of 382 patients have been treated with 65 mg bid immediate release (IR) formulation as add on to a PPI, as 3 doses over 2 days (25 patients, D9120C00020) and for up to 4 weeks in two studies (122 patients, D9120C00011 and 235 patients, D9120C00027).

The most commonly reported AEs after use of AZD3355 in healthy volunteers, all doses tested, were paraesthesia (24.4%), headache (10.8%), feeling hot (8.3%), diarrhoea (7.9%), flatulence (6%) and dizziness (5.1%). The most commonly reported AEs on AZD3355 65 mg bid in the 4 week patient studies (D9120C00011 and D9120C00027) 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%). Laboratory evaluation in humans has not shown any clinically important changes. However, mild liver enzyme (ALT, AST) increases, which returned to normal after stopping treatment, were observed in some healthy subjects after AZD3355 400 and 800 mg bid for 5 days (D9120C00030)

Symptoms of paraesthesia, defined as an abnormal cutaneous sensation in the absence of an external stimulus (eg tingling, pins and needles, prickling, skin crawling, formication, burning sensation and numbness) reported after the administration of AZD3355 have primarily been short lasting, ie often starts and ends within the first hour after dosing, of predominantly mild intensity and frequently located in the hands and feet. The reported paraesthesia have not been associated with any abnormal neurological findings on physical examination. Through evaluation of the data generated to date from clinical studies of AZD3355, it has been determined from a medical and safety perspective, that the transient and reversible paraesthesia do not appear to pose a risk to subjects enrolled in studies with AZD3355.

A moderate increase in urinary volume and decrease in urine osmolality have been noted during the first two hours after drug intake in healthy volunteers. However, no effect on the 24-hour urine volume was seen. In none of the clinical studies in healthy subjects and patients completed so far have there been any clinically relevant laboratory findings in urine or in serum related to renal function (ie, glucose, electrolytes, creatinine).

In phase I studies it was noted that there is an initial slight increase in supine pulse and a more marked pulse increase at standing after administration of AZD3355 compared to placebo, with reports of postural dizziness during orthostatic testing. A head-up tilt test study (D9120C00021) performed in healthy volunteers to investigate a possible influence of AZD3355 (500 mg oral IR single dose) on the baroreceptor reflex showed a shorter head-up endurance time when taking AZD3355. The findings did not indicate an effect on the baroreflex but rather is compatible with a vasovagal reaction or a vasodilatory effect that may be associated with AZD3355. Patients with a supine systolic blood pressure (BP) below 110 mm Hg, history of severe orthostatic reactions or syncope will not be included in clinical studies until potential hemodynamic effects of AZD3355 on patients at therapeutic dose levels have been further evaluated. In addition, patients in this study will be actively questioned whether they experience pre-syncope or dizziness at any time during the treatment period.

A thorough QT study (D9120C00012) was performed, however, this study failed to exclude an effect of AZD3355 on the prolongation of the QTc interval based on QTcF. Until the potential effect on the QT interval has been definitively assessed patients with history of a heart diagnosis (including ischemic heart disease, congestive heart failure, cardiac arrhythmias, congenital long QT syndrome), or signs or symptoms of any heart disease, or

persons with clinically significant ECG abnormalities as determined by the investigator, or QTcF >450 ms, history of electrolyte imbalances (specifically hypokalemia and hypomagnesemia) and concomitant drugs that may prolong the QT interval will be excluded from clinical studies with AZD3355.

Non-clinical evaluation indicates no genotoxicity, low fetal risk of exposure to AZD3355 during embryofetal development and low probability for drug interaction with hormonal contraceptives. However, no data exists on embryofetal development in humans. Women of childbearing potential can participate in the study provided one of the highly effective contraceptive methods described in the protocol is used (see Section 5.1.1).

The available toxicological and clinical documentation supports the proposed study design including treatment with the maximum daily dose of AZD3355 240 mg bid during 4 weeks. Based on the experience presented in the IB and the results from the previous human studies and the restrictions for participating together with close monitoring, the risk associated with participation in this study is assessed 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.

An overall risk benefit assessment of AZD3355 and details on the safety findings are presented in the IB for AZD3355 (Edition 13).

2. STUDY OBJECTIVES

2.1 Primary objective

The primary objective is to evaluate the effect on GERD symptoms of four doses of AZD3355 (60 mg, 120 mg, 180 mg and 240 mg bid) compared to placebo, as add-on treatment to a PPI by using the responder definition; ie, at least 3 more days of not more than mild GERD symptoms on average per week during the whole treatment period compared to baseline, based on the Overall symptoms domain in the RESQ-eD.

2.2 Secondary objectives

The secondary objectives of the study are:

- To evaluate the effect on GERD symptoms of four doses of AZD3355 (60 mg, 120 mg, 180 mg and 240 mg bid) compared to placebo, as add-on treatment to a PPI by using an alternative responder definition; ie, at least 5 days of not more than mild GERD symptoms on average per week during the whole treatment period based on the Overall symptoms domain in the RESQ-eD

- To evaluate the effect on GERD symptoms of four doses of AZD3355 (60 mg, 120 mg, 180 mg and 240 mg bid) compared to placebo, as add-on treatment to a PPI by analysing the change in the proportion of days of not more than mild GERD symptoms compared to baseline, based on the Overall symptoms domain and for

each separate domain (Heartburn; Regurgitation; Hoarseness, cough and difficulty swallowing; Burping) in the RESQ-eD

- To evaluate the dose-response curve with respect to:
  - The responder definition
  - The change in the proportion of days of not more than mild GERD symptoms compared to baseline, based on the Overall symptoms domain in the RESQ-eD
- To assess basic measurement properties of the RESQ-eD
- To assess the pharmacokinetics of AZD3355 by population PK analyses with special regard to variability in the patient population
- To study the relationship between systemic exposure and response with special regard to the responder definition
- To study the prevalence of mucosal breaks in the target population through endoscopy at baseline during the study or historical reports
- To describe the endoscopic findings after the study treatment period, in patients with baseline endoscopy showing mucosal breaks.

2.3 Safety objective

To assess the safety and tolerability during 4 weeks treatment with four doses of AZD3355 as add-on treatment to a PPI, by evaluation of adverse events, laboratory variables, ECG, blood pressure, pulse, orthostatic tests and physical examination.

2.4 Exploratory objectives

To collect and store DNA for future exploratory research into genes/genetic variation that may influence response (ie, distribution, safety, tolerability and efficacy) to AZD3355 and/or susceptibility to or prognosis of GERD and associated disease as under study.

3. STUDY PLAN AND PROCEDURES

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

3.1 Overall study design and flow chart

This is a randomized, double blind, placebo-controlled, parallel group designed, multi-centre phase IIb dose finding study to assess the effect on GERD symptoms, safety and tolerability during 4 weeks of treatment with four doses of AZD3355 as add-on treatment to a PPI. The target patient population in this study are partial responders to PPI treatment who experience

at least moderate intensity of GERD symptoms on at least 3 of the past 7 days despite optimised PPI treatment and must have a history of GERD symptoms for at least 6 months.

The effect evaluation will be based on patient reported symptom data, using twice-daily recordings in a hand-held electronic diary (e-diary) device using the RESQ-eD, (see Section 6.5).

The study consists of a screening phase, a treatment phase and a follow-up phase. The patients will be randomized to one of four doses of AZD3355 (60 mg, 120 mg, 180 mg or 240 mg bid) or matching placebo as add-on treatment to their optimized PPI treatment during 4 weeks, see Figure 1.

Approximately 650 patients from Canada, France, Germany, Hungary, Latvia, Romania and USA will be randomized in the study and approximately 130 sites will participate. The aim is to have an equal number of randomized patients in the five treatment arms.

Visit 1 (Enrolment visit)

At the enrolment visit 8-26 days before randomization after written informed consent is given, patients will receive brief training in how to use an e-diary device for completion of PRO assessments and will complete the screening questionnaire Reflux Symptom Questionnaire 7 day recall (RESQ-7), using the e-diary device, prior to any further assessment.

Each patient who fulfils the RESQ-7 eligibility criteria, which automatically is determined from an algorithm in the e-diary device, will continue with visit 1 screening procedures. The patients will then undergo a physical examination including weight and height, dECG, orthostatic test and a full laboratory screen. Women of childbearing potential will undergo a urine pregnancy test and will be asked to verify that they are using one of the accepted methods of contraception to be allowed to participate in the study.

Information regarding medical and surgical history and PPI usage will be collected. The patients will then be reevaluated for all inclusion and exclusion criteria. If not eligible for the study a minimum of required modules must be entered in the eCRF, see Section 5.9.

All patients who have provisionally met the eligibility criteria for the baseline symptom recording will receive additional training on how to use the e-diary device and will start recording in the e-diary the evening of visit 1. Baseline symptom recording in RESQ-eD at least 8 days twice daily is required to confirm eligibility and to ensure an adequate baseline for evaluation of efficacy variables (ie 7 evening and 7 morning reports). For patients where an endoscopy is required (see Section 6.2.1), the baseline symptom recordings must continue for at least 8 days after the day of the endoscopy.

During the screening phase, the patients will be asked to record GERD symptoms in the e-diary upon waking up in the morning and each evening right before bedtime (see Section 6.5.4). The patients will also be informed that they will be asked to record the use of PPI and antacids in the e-diary, see Section 6.5.3.

Visit 2 (Randomization visit)

At visit 2, an e-diary device check (eg, battery status, data transfer) and e-diary compliance review will be done. The RESQ-eD registrations during the last 7 days in the screening phase will be used to evaluate whether the patient meets the criteria for randomization (an algorithm which is programmed in the e-diary device, will determine eligibility automatically). If not eligible for the study a minimum of required modules must be entered in the eCRF, see Section 5.9.

In order to obtain baseline information before study treatment has started active questioning (see Section 6.4.3.2) will be done prior to the first dose of investigational product (IP) to determine if the patients report any episodes of the following: syncope, felt faint, lightheadness or dizziness.

Prior to the first dose of IP, a dECG will be recorded followed by an orthostatic test and full laboratory screen. Women of childbearing potential will undergo a second urine pregnancy test before dosing.

Patients who fulfil all the randomization criteria will be randomized and continue in the study and IP will be dispensed to cover the dosing required for 2 weeks until visit 4.

The first dose of IP will be taken at the site. It is most important that the date and exact time of the first dose of IP is recorded in the eCRF. A dECG followed by an orthostatic test will be repeated 2 hours (±15 min) post-dose (at expected t_{max}). A sample for DNA collection will be taken provided that consent is given.

Visit 3

One week (±2 days) after randomization a visit 3 will be scheduled. The patient will come to the clinic after intake of IP (morning dose) for a dECG recording followed by an orthostatic test, limited laboratory screen, drug compliance, e-diary device check, e-diary compliance review and AE recording followed by active questioning (see Section 6.4.3.2). The patients are instructed to record the exact time of dose intake in the evening before the next study visit.

Visit 4

Two weeks (±2 days) after randomization a visit 4 will be scheduled. At home, the patient will complete the morning e-diary, take their PPI as usual and eat breakfast without taking the IP. Thereafter, the patient will come to the clinic in the morning where the IP will be taken in accordance with the PK sampling procedure.

The visit specific ePRO assessment Overall Treatment Evaluation (OTE) will be completed at the clinic. E-diary device check and e-diary compliance review will be done. A pre-dose dECG recording followed by an orthostatic test, full laboratory screen, PK sampling and AE recording followed by active questioning (see Section 6.4.3.2) will be done.

After intake of IP, additional PK samples will be taken at 2 hours (±30 min) and 4 hours (±30 min) post-dose, see Section 6.6.1.

Drug accountability will be recorded and IP will be dispensed to cover the dosing required for 2 weeks until visit 6.

Visit 5

Three weeks (±2 days) after randomization a visit 5 will be scheduled. The patient will come to the clinic after intake of IP (morning dose) for a dECG recording followed by an orthostatic test, limited laboratory screen, drug compliance, e-diary device check, e-diary compliance review and AE recording followed by active questioning (see Section 6.4.3.2). The patients are instructed to record the exact time of dose intake in the evening before the next study visit.

Visit 6 (End of treatment visit)

Four weeks (±2 days) after randomization a visit 6 will be scheduled. At home, the patient will complete the morning e-diary, take their PPI as usual and eat breakfast without taking the IP. Thereafter, the patient will come to the clinic in the morning where the IP will be taken in accordance with the PK sampling procedure. The visit specific ePRO assessment (OTE) will be completed at the clinic. E-diary device check and e-diary compliance review will be done and the e-diary device will be collected as the last recording is done in the morning of this visit.

A physical examination including weight will be performed. A dECG recording followed by an orthostatic test, full laboratory screen, PK sampling and AE recording followed by active questioning (see Section 6.4.3.2) will be done.

After intake of IP, additional PK samples will be taken at 2 hours (±30 min) and 4 hours (±30 min) post-dose, see Section 6.6.1. An orthostatic test will be repeated 2 hours (±15 min) post-dose (at expected t_{max}). N.B. no dECG will be recorded prior to the orthostatic test post-dose at visit 6.

Drug accountability will be performed after the last morning dose has been administered. A follow-up endoscopy will be performed within 5 days for those patients who had evidence of mucosal breaks at baseline endoscopy.

Visit 7 (End of study visit)

Two weeks (±2 days) after visit 6, a visit 7 will be scheduled for physical examination, a dECG recording followed by an orthostatic test and a full laboratory screen. Women of childbearing potential will undergo a urine pregnancy test. Any ongoing AEs will be followed up by the investigator as long as medically indicated.

Important safety information regarding visit 2-7

- Following the spontaneous AE collection, there will be active questioning to determine whether patients experienced any episode of the following: syncope,

felt faint, lightheadedness or dizziness, see Section 6.4.3.2. The active questioning at visit 2 will be asked prior to first dose of IP.

- For instructions of handling of symptoms occurring during orthostatic tests, see Section 6.4.3.4.
- For handling of patients who spontaneously report symptoms of paraesthesia, see Section 6.4.3.1.
- Patients who have ALT or AST > 3 x ULN must be closely monitored and followed-up until they return to baseline according to a pre-specified handling plan, see Section 6.4.5.2.

Information on population PK sampling at visit 4 and 6

PK samples will be taken at visit 4 (after 2 weeks treatment) and at visit 6 (after 4 weeks treatment); pre-dose in the morning and then two additional samples, at 2 hours (±30 min) and at 4 hours (±30 min) post-dose, see Section 6.6.1.

It is very important, for the population PK analysis that:

- the patients are instructed to record the exact time of dose intake in the evening before the study visit and this information will be entered in the eCRF
- the exact time of dose intake in the morning is recorded and transferred to the eCRF by the site personnel
- the exact time of blood sampling is entered into the lab requisition form at all occasions and transferred to the eCRF by the site personnel.

Early end of treatment visit

If a patient for any reason is withdrawn early from the IP, an Early end of treatment visit should be performed without delay (see Section 5.8.1).

Unscheduled visit

Extra visits may be necessary for various reasons. These visits will be recorded as unscheduled visits and appropriate section(s) in the eCRF will be completed.

The study assessments are described in the Study plan, Table 1.

Figure 1 Study Flow Chart

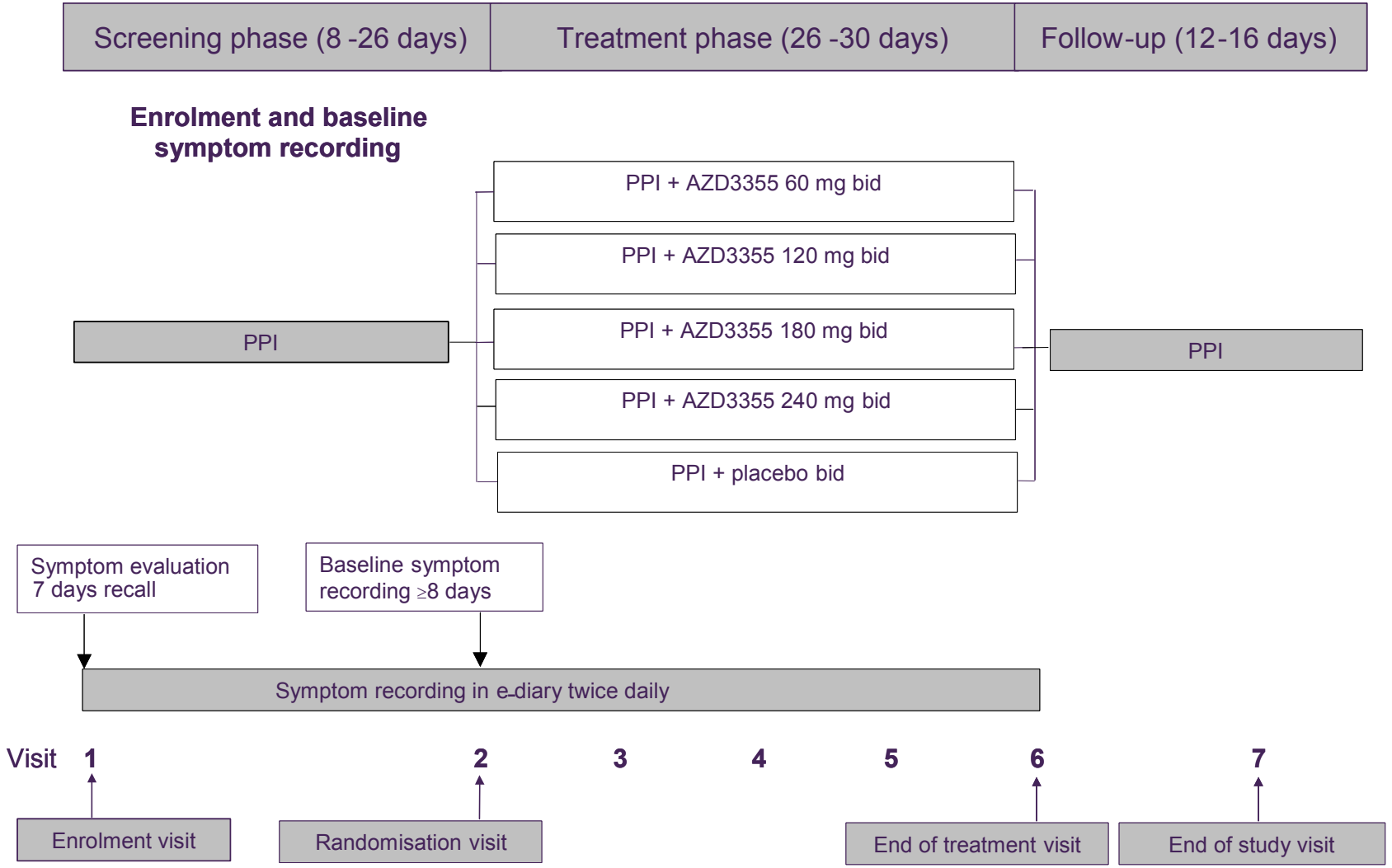

Table 1 Study Plan

Visit number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	
Visit Description	**Enrolment visit**	**Randomization visit**				**End of treatment visit**	**End of study visit**	**Early end of treatment visit**
Visit Window (No. Weeks ± No. Days)			1 week ± 2 days after first dose	2 weeks ± 2 days after first dose	3 weeks ± 2 days after first dose	4 weeks ± 2 days after first dose	2 weeks ± 2 days after visit 6	
Informed consent	X							
Inclusion/exclusion criteria	X	X						
Assignment of e-diary device	X							
ePRO training	X[a]	X[a]						
ePRO assessment at site	X			X		X		X
Demography	X							
Physical examination	X					X	X	X
dECG	X	X[c]	X	X[g]	X	X[g]	X	X
Orthostatic test[b]	X	X[c]	X	X[g]	X	X[c]	X	X
Weight, height	X					X[d]		X[d]
Urine pregnancy test	X[e]	X[e]					X[e]	X[e]

Visit number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	
Visit Description	**Enrolment visit**	**Randomization visit**				**End of treatment visit**	**End of study visit**	**Early end of treatment visit**
Visit Window (No. Weeks ± No. Days)			1 week ± 2 days after first dose	2 weeks ± 2 days after first dose	3 weeks ± 2 days after first dose	4 weeks ± 2 days after first dose	2 weeks ± 2 days after visit 6	
Medical/ surgical history	X							
Concomitant medication	X	X	X	X	X	X	X	X
Endoscopy	X[f]					X[f]		X[f]
Full laboratory screen	X	X[g]		X[g]		X[g]	X	X
Limited laboratory screen			X		X			
Check of e-diary device, review of e-diary compliance		X	X	X	X	X		X
Active questioning for specific symptoms (see Section 6.4.3.2)		X[h]	X	X	X	X	X	X
Randomization		X						

Visit number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	
Visit Description	**Enrolment visit**	**Randomization visit**				**End of treatment visit**	**End of study visit**	**Early end of treatment visit**
Visit Window (No. Weeks ± No. Days)			1 week ± 2 days after first dose	2 weeks ± 2 days after first dose	3 weeks ± 2 days after first dose	4 weeks ± 2 days after first dose	2 weeks ± 2 days after visit 6	
Dispensation of investigational product		X		X				
Intake of IP at site		X		X		X		
Twice daily e-diary recordings	X[i]	----------	----------	----------	----------	X[i]		
Collection of e-diary device						X		X
Genetic blood sampling		X[j]						
PK sampling				X[k]		X[k]		
Adverse Event[l]	X	X	X	X	X	X	X	X

a Brief training before initial completion of RESQ-7, extended training before patient brings home an e-diary device for the screening phase. Refresher training as needed at visit 2.
b Supine BP and pulse will be measured after 10 min of rest. 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.
c Pre-and post dose measurements.
d Only weight should be measured.

e Women of childbearing potential. If applicable, pre-dose at visit 2.
f If applicable. See Section 6.2.1.
g Pre-dose.
h Prior to first intake of IP, in order to obtain baseline information before study treatment has started.
i Starting right before bedtime the evening of visit 1 and ending in the morning the day of visit 6.
j Optional. Informed consent for genetic research required.
k Pre-dose in the morning, 2 hours (+/-30 min) and 4 hours (+/-30 min) post-dose.
l Serious AEs will be collected from signed informed consent. Non-serious AEs will be collected from the first administration of IP. Specific questions regarding the following: syncope, felt faint, lightheadedness and dizziness will be asked at visit 2-7.

3.2 Rationale for study design, doses and control groups

The purpose of this dose finding study is to provide information on efficacy and safety to support the selection of the therapeutic dose(s) of AZD3355. AstraZeneca is developing AZD3355 as add-on treatment to a PPI to relieve GERD symptoms in patients with GERD who have a partial response to PPI treatment, as characterized by persistent GERD symptoms of at least moderate intensity for three days per week or more. More specifically, the target patient population will be on optimized PPI treatment; patients who have not responded at all to a PPI will be excluded. Non-responders may have other causes for their symptoms other than reflux of stomach contents into the esophagus.

Only one dose of AZD3355 (65 mg bid) has been tested for efficacy, therefore the dose response relationship is unknown. However it is hypothesized that a higher dose will improve efficacy while maintaining safety, which will be tested in this study. The estimation of the dose response curve and evaluation of safety will therefore be performed on higher doses than previously tested in patients. A modified release (MR) formulations aiming at reducing the frequency of paraesthesia has been selected for the Phase IIb programme. Since earlier studies were performed with an IR formulation of 65 mg bid, the efficacy and safety of MR 1 hour formulation needs to be evaluated with a similar MR dose (60 mg bid). Based on all of these considerations, the doses chosen for this study are 60 mg, 120 mg, 180 mg and 240 mg MR 1 hour bid, which are in the predicted therapeutic range and within a range that is covered by the metabolite profile in toxicological animal studies.

To determine efficacy, PRO measures will be utilized. PROs are commonly used in various therapeutic areas and their use is supported by guidance documents from both the Food and Drug Administration (FDA 2006) and the European Medicines Agency (EMEA) (EMEA Committee for medicinal products for human use (CHMP) 2005).

Patient-reported data has been suggested to more reliably reflect the response to treatment of GERD than assessment of symptoms by the clinician (McColl et al 2005). Patient-reported symptoms are best evaluated based on daily recordings (Fass 2007b). Electronic capture of PRO (ePRO) data ensures that diary entries are not made in advance or retrospectively (Stone et al 2002) and allows for continuous review of diary compliance.

The specific diary that will be used to evaluate the efficacy of AZD3355 is the Reflux Disease Questionnaire, (RESQ-eD). Symptoms that are important and relevant, as determined by qualitative patient interviews with patients in the target population, have been added to an existing diary, the RDQ, to form the modified diary, the RESQ-eD. A PRO validation study (D9120C00027) has confirmed that the RESQ-eD is valid, reliable and responsive to change, and therefore fit for purpose in the target patient population. Based upon the same study, a responder definition and an alternative responder definition were established. The outcome of the validation study resulted in the RESQ-eD consisting of 13 items that combine into an Overall symptoms domain and 4 separate domains (Heartburn; Regurgitation; Hoarseness, cough, difficulty swallowing; and Burping). At least 8 days of twice daily recordings of GERD symptoms in the screening phase are required to confirm eligibility and to ensure an adequate baseline for evaluation of the primary variable.

A randomized, double-blind, parallel-group, multi-centre, placebo-controlled study design is standard in dose finding studies and is considered the best choice to achieve the safety and efficacy objectives of the study. A placebo treatment group in the trial as control is justified for two reasons; there is currently no reflux inhibitor approved as add-on treatment to PPIs on the market and thus it is not feasible to use an active comparator. Also, it is important to control for the effect of on-going therapy with a PPI that will be continued in both AZD3355 treatment and placebo groups.

In a recently published methodological workshop with reference to clinical trial design in adult reflux disease (Dent et al 2008), a 4-week treatment period is considered to be sufficient to observe a treatment response in patients with typical reflux syndrome and symptoms on at least 3 days per week at inclusion. This treatment period will also be sufficient to evaluate short-term safety and tolerability, while making a comparison to previous 4-week patient studies with AZD3355.

Since there is always a risk involved in testing new compounds in patients, safety precautions are taken by applying strict exclusion criteria and pre-specified discontinuation criteria. An internal safety committee will perform medical surveillance of AEs, ECG, orthostatic test and laboratory variables on blinded treatment data continuously throughout the study. In addition, an independent Data Safety Monitoring Board (DSMB) will review treatment data during the course of the study (see Section 12.4).

Evaluation for hemodynamic effects will be done through frequent monitoring of dECGs and orthostatic tests. To further appreciate and understand the incidence of symptoms that may be associated with hemodynamic effects, active questioning will be solicited from all patients, see Section 6.4.3.1. Any patient experiencing a severe hemodynamic episode will be subject to discontinuation of the IP, see Section 5.8. Strict exclusion criteria for heart disease and ECG abnormalities will be applied. Patients with QTcF > 450 ms should not be randomized and patients with QTcF ≥500 ms during treatment should discontinue intake of IP.

Liver tests will be obtained weekly during the treatment period in this study with criteria for more frequent laboratory monitoring and for discontinuation of the IP applied in the event of significantly increased liver enzymes in any patient, see Section 6.4.5.2.

General medical history information will be collected prior to enrolment in the study, with specific questions to identify previous episodes of paraesthesia. Patients with current neurological disorders will be excluded. All patients reporting paraesthesia during the study will be subjected to additional questioning about their symptoms and will undergo an extra physical examination by the investigator. Patients experiencing episodes of paraesthesia for 7 consecutive days will discontinue the IP and be referred to a board certified neurologist, see Section 6.4.3.1.

In order to describe endoscopic findings in this target population, a baseline endoscopy will be performed in all patients unless the patient has had an endoscopy within 2 years without mucosal breaks in the most recent examination. In the other patients, only historical endoscopy report data will be collected. In addition, those patients with mucosal breaks at the

baseline endoscopy will have a follow-up endoscopy to provide descriptive data on the esophageal mucosal status after the treatment.

Future Research Considerations

Blood samples will be collected in this study to enable future genetic research if approved by appropriate Regulatory Authority/Ethics Committee. The genetic research is optional for study sites as well as for individual patients. DNA samples are only collected from patients providing informed consent for this specific procedure. If a patient declines to participate in the genetic research, there will be no penalty or loss of benefit. The patient will not be excluded from the main study. The samples are collected 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. Further description of the genetic research is given in Appendix D.

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 should fulfil the following criteria:

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

2. Male or female. Females of childbearing potential must be using a highly effective contraceptive method for at least the previous 3 months, see Section 5.1.1

3. Age 18-70 years, inclusive

4. Body Mass Index (BMI) 18.5 – 35.0 kg/m^2, inclusive

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

6. Continuously treated during the last 4 weeks before enrolment with daily optimized unchanged PPI therapy with doses according to the country label, or regional where applicable, for any GERD indication. Patients with endoscopy verified reflux esophagitis (Los Angeles classification grade A-D) within the last 8 weeks must have completed the prescribed 8 weeks treatment with a PPI. An optimized PPI treatment is a treatment which according to the investigator judgment can not be further improved by changing brand or dosing of the PPI.

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

8. Able to read and write in the local language and use the e-diary device

9. **To be eligible for the screening phase** the patients must have reported in the RESQ-7 using 7 days recall of symptoms, a minimum of 3 days and a rating of at least moderate intensity on at least one of the following items; a burning feeling behind the breastbone or unpleasant movement of material upwards from the stomach. (An algorithm which is programmed in the e-diary device, will determine eligibility automatically).

10. **To be eligible for randomization** the patients must have recorded in the RESQ-eD on the last 7 days before randomization, a minimum of 3 days with a symptom intensity of at least moderate on either item, or any combination of both items (eg 1 day on one item and 2 days on the other); a burning feeling behind the breastbone or unpleasant movement of material upwards from the stomach. (An algorithm, which is programmed in the e-diary device, based on the e-diary recordings, will determine whether the patient fulfils the criteria).

11. An upper gastrointestinal endoscopy is required before randomization provided that:

    - no endoscopy has been performed within the past 24 months
    - the most recent endoscopy within 24 months demonstrated mucosal breaks in the esophagus
    - no documented endoscopy report from the most recent endoscopy within 24 months is available to the investigator.

    If the most recent available documented report from an upper endoscopy within the past 24 months confirms the absence of mucosal breaks then the patient does not need to undergo an endoscopy during this study.

Inclusion criteria for the optional genetic research are described in Appendix D.

4.2 Exclusion criteria

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

1. Patients that have not experienced any GERD symptom improvement at all during PPI treatment

2. PPI treatment with doses not according to the country label, or regional where applicable, for any GERD indication. Note: bid dosing is not allowed

3. Working night-shift during the period of the study

4. 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

5. Current neurological disorders including nerve compression syndromes. Patients with well controlled migraine and other headache disorders can be included

6. History of clinically significant orthostatic reaction or syncope. Clinically significant orthostatic reaction at visit 1 or pre-dose at visit 2

7. Supine systolic blood pressure below 110 mm Hg at visit 1 and pre-dose visit 2

8. History of a heart disease (including ischemic heart disease, congestive heart failure, cardiac arrhythmias, congenital long QT syndrome), or current signs or symptoms of any heart disease, or patients with clinically significant ECG abnormalities or QTcF >450 ms as determined by the investigator (see section 6.4.7)

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

10. History of clinically significant electrolyte imbalances

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

12. Need for concomitant medication with:

- Drugs or any compound that may interfere with the pharmacodynamic effect of investigational product (eg, Baclofen, pure GABA, supplements containing GABA)

- Drugs that by their mode of actions may alter gastrointestinal (GI) symptoms, with exception of the PPI used in the study and antacids, (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/day
- Bisphosphonates
- Antineoplastic drugs

- Drugs that may prolong the QT interval. Common examples of such drugs are listed in Appendix E. This list should not be considered comprehensive therefore investigators need to use their judgement when reviewing the medication list from individual patients and restrict patients who must stay on drugs that may increase the QT interval
- Drugs that have a narrow therapeutic window (eg, warfarin, digoxin, phenytoin, carbamazepine)

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

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

15. Pregnant or breastfeeding females

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

17. Blood donation within 8 weeks prior to the first dose of the investigational product

18. Involvement in the planning and/or conduct of the study (applies to all AstraZeneca/ AstraZeneca representative/study site personnel)

19. Previous enrolment or randomization in the present study or in another study with AZD3355

20. Participation in another clinical study, with administration of any investigational product during the last 2 months prior to enrolment

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

    - S-creatinine >1.2 x ULN

- S-AST or ALT > 2 x ULN
- S-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.

Exclusion criteria for the optional genetic research are described in Appendix D.

Procedures for withdrawal of incorrectly enrolled patients, see Section 5.3.

5. STUDY CONDUCT

5.1 Restrictions during the study

5.1.1 Precautions to minimize risk of pregnancy

Women of childbearing potential must have started to use a highly effective contraceptive method, at least 3 months before enrolment and continue to use the same method strictly as prescribed throughout the study.

Acceptable highly effective contraceptive methods:

- 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 (eg Evra™ patch)
- Intravaginal device with a combination of progestin and estrogen (eg NuvaRing™)
- Progestin-releasing IUS (eg Mirena™)
- Progestin-releasing implants (eg Implanon™ , Norplant™)
- Medroxyprogesterone for depot injection (eg 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. Randomized women of childbearing potential should be made aware of the availability of emergency "post-coital" contraception if there is an indication of 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.

5.1.2 Other restrictions

Patients should not plan surgeries during the study and any other period that in the opinion of the investigator would interfere with their participation and collection of study data.

As safety laboratory monitoring will be done throughout the study and blood loss might impact laboratory results, patients must abstain from blood donation while participating in the study.

5.2 Patient enrolment and randomization and initiation of investigational product

The Principal Investigator will be responsible for the following: Once a patient has signed the written informed consent, they will be assigned with a unique identifying number (enrolment number, beginning with "E"). This enrolment number will be used as the identification number throughout the study and once allocated it will not be re-used. The enrolment number will be a combination of the country number, site number and the sequential patient at that site. Signed written informed consent must be obtained from potential patients before any study specific procedures are performed. Patient eligibility will be established at visit 1 at the start of the screening phase (see Sections 4.1 and 4.2) and at visit 2 (before treatment randomization).

If a patient withdraws from participation in the study, the patient will not be allowed to re-enter the study and the patient's enrolment or randomization code cannot be re-used.

5.2.1 Procedures for randomization

Patients who meet all of the inclusion criteria and none of the exclusion criteria at visit 2 will be randomized by blinded randomization to receive either AZD3355 (60 mg, 120 mg, 180 mg or 240 mg bid) or placebo, as add-on treatment to a PPI. The randomization scheme will be generated by AstraZeneca R&D Mölndal using the global randomization system (GRand). Randomization codes will be assigned strictly in sequential order at each site as patients become eligible for randomization and each patient will be assigned a unique randomization code.

The following personnel will have access to the randomization scheme:

- The AstraZeneca personnel generating the randomization scheme
- The AstraZeneca personnel carrying out the labelling and packaging of IP
- The personnel analysing the PK samples
- The independent DSMB.

No other person will have knowledge about the scheme. Hence, the investigators will have no knowledge beforehand of the treatment the patient will receive.

If a patient is provided wrong treatment by mistake which is not the treatment assigned by the randomization scheme the patient should continue with the treatment provided. AstraZeneca or its representative (ICON) should be notified as soon as the error is discovered.

5.3 Procedures for handling patients incorrectly enrolled or randomized or initiated on investigational product

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

Patients who are incorrectly enrolled but are not yet randomized or initiated on treatment should be withdrawn from the study.

In the event that a patient has been randomized and has started intake of IP in error despite best efforts to ensure that the inclusion and exclusion criteria were adhered to by the investigator, with respect to continuing intake of IP, medical judgement should apply on a case-by-case basis in regards to continuation of IP.

The likely benefits and risks to the patient should be assessed and a discussion should occur between the study delivery team physician and the investigator regarding whether to continue or discontinue the patient from treatment. In cases where it could not be excluded that exposure to the IP might compromise individual safety of the specific patient the intake of IP must be stopped. In situations where an agreement cannot be reached, the patient should have their intake of IP stopped. If decided to stop the intake of IP the procedures described in Section 5.8.1 must be followed.

The study delivery team physician is to ensure all such decisions are appropriately documented.

5.4 Blinding and procedures for unblinding the study

5.4.1 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.

5.4.2 Methods for unblinding the study

Individual treatment codes, indicating the treatment randomization for each randomized patient, will be available to the investigator(s) or pharmacists at the study centre.

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

AstraZeneca retains the right to break the code for Serious Adverse Events (SAE)s that are unexpected and are suspected to be causally related to an investigational product and that potentially require expedited reporting to regulatory authorities. Care will be taken to ensure the study team within AstraZeneca/AstraZeneca representative (ICON) other than those responsible for SUSAR reporting will remain blinded.

An independent DSMB will have access to the randomization list to, if necessary, be able to review unblinded study data, see Section 12.4.

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.

5.5 Treatments

5.5.1 Identity of investigational product(s)

Investigational product	Dosage form and strength	Manufacturer
AZD3355, MR 1h	Capsules 30 mg	AstraZeneca AB
	Capsules 60 mg	AstraZeneca AB
	Capsules 120 mg	AstraZeneca AB
Placebo for AZD3355	Capsules	AstraZeneca AB

The capsules contain no lactose or gelatine.

5.5.2 Doses and treatment regimens

At visit 2 the patients will be randomized to receive one of five treatments, as add-on to their optimized PPI treatment, for 4 weeks:

- AZD3355 60 mg bid
- AZD3355 120 mg bid
- AZD3355 180 mg bid
- AZD3355 240 mg bid
- Placebo bid

At both visit 2 and visit 4 the patients will receive one labelled box with two bottles, each labelled and filled with 36 capsules AZD3355 or placebo. To obtain the correct dose the patients should take one capsule from each bottle twice daily.

The five different dose groups will be packed as follows:

- 60 mg: both bottles will contain 30 mg capsules
- 120 mg: both bottles will contain 60 mg capsules
- 180 mg: one bottle will contain 60 mg capsules and one bottle will contain 120 mg capsules
- 240 mg: both bottles will contain 120 mg capsules

– Placebo: both bottles will contain placebo capsules.

The capsules should be swallowed whole together with a glass of water twice daily, 30 minutes before breakfast and in the evening 30 minutes before the main meal and are not to be chewed, crushed or divided. At visit 2, 4 and 6, the morning dose should instead be taken at the clinic (ie, the morning doses at visit 2, 4 and 6 are allowed to be taken **after** breakfast).

5.5.3 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. Label text will be translated into local language.

The investigational product, AZD3355 capsules, will be packed and labelled by IPS, Mölndal or its designee according to current EU GMP guidelines. A booklet label will be used for the boxes and the bottles. Labels for several countries are included in the booklet together with an index page. Variables such as expiry date, P Lot ID and subject number are printed on the front cover. The study personnel should fill in enrolment code (E-code), visit number and the investigator's name on the front page if applicable. To be able to read the country specific label the resealable front cover has to be opened.

The box booklet label has a tear-off part that will be inserted into the source document worksheets, in the study records for each respective patient.

The investigational product may be labelled with the following information, depending on local requirements:

- Name, address and telephone number of the sponsor
- Pharmaceutical dosage form, quantity of dosage units and name and all possible strengths (30 mg or 60 mg or 120 mg or placebo) of the IP
- Route of administration
- Study code
- Order number
- Randomization code and visit number (where relevant)
- The name of the investigator
- Directions for use
- "For clinical trial use only"
- Storage conditions

- Period of use (expiry date)
- "Keep out of reach of children"

5.5.4 Storage

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

5.6 Concomitant and post-study treatment(s)

The patient should not change their PPI treatment during the study and should also avoid changes of other medications. Antacids are allowed to be used by the patient during the study if needed when experiencing GERD symptoms.

Women of childbearing potential must have started to use one of the highly effective contraceptive methods listed in Section 5.1.1 at least 3 months before enrolment and continue to use the same method strictly as prescribed throughout the study.

Patients should not receive eradication therapy for *Helicobacter pylori (H.pylori)* during the study.

The following concomitant medications should not be used during the study:

- Drugs or any compound that may interfere with the pharmacodynamic effect of the investigational product (eg, Baclofen, pure GABA or supplements containing GABA)
- Drugs that by their mode of actions may alter gastrointestinal (GI) symptoms:
  - PPI (except the PPI that was optimized prior to study enrolment and will be taken during the study, neither the dose or brand of PPI should be changed during the study)
  - H_2 receptor antagonists
  - Sucralfate
  - Alginates
  - Tegaserod
  - Domperidone
  - Metoclopramide
  - Erythromycin

  - Drugs with significant anticholinergic effect (eg anticholinergics used in GI 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
  - NSAIDs or COX-2 inhibitors (more than 2 days/week)
  - ASA >162 mg/day
  - Bisphosphonates
  - Antineoplastic drugs
- Drugs that may prolong the QT interval. Common examples of such drugs are listed in Appendix E. This list should not be considered comprehensive therefore investigators need to use their judgement when reviewing the medication list from individual patients and restrict patients who must stay on drugs that may increase the QT interval.
- Drugs that have a narrow therapeutic window (eg warfarin, digoxin, phenytoin, carbamazepine).

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 eCRF.

After the completion of the study, patients should be treated according to standard clinical practice, at the discretion of the investigator.

5.7 Treatment compliance

The administration of all medication (including investigational products) should be recorded in the appropriate sections of the eCRF. Treatment compliance will be assessed by reviewing drug accountability records and will be recorded in the study source documents. The date and time of intake of the first and last dose of the IP will be recorded in the eCRF and checked by the monitor at the monitoring visits.

5.7.1 Accountability

It is essential that all medication is accounted for by the investigator or institution, and that any discrepancies are explained and documented.

The study treatment(s) must be used only as directed in the CSP. The investigator must maintain accurate records accounting for the receipt of the IP and for the disposition of the material. This accounting of treatment consists of a dispensing record including the identification of the person to whom the drug is dispensed (enrolment and randomization

code), the quantity and the date of dispensing, and any unused drug returned to the investigator. This record is in addition to any drug accountability recorded in the electronic data capture system.

Patients must return all unused medication and empty containers to the investigator. The number of capsules returned must be checked against the number dispensed to determine patient compliance.

The investigator will retain the returned medication until AstraZeneca authorized personnel collect it, along with any study treatments not dispensed. At the termination of the study or at the request of the sponsor, the investigator must return any unused supplies to AstraZeneca (or its designee). This return will be documented by using an IP Return Invoice (or an equivalent form) supplied by AstraZeneca representative (ICON). Certificates of delivery and return should be signed.

The investigator is responsible for ensuring that the patient has returned all unused IP.

5.8 Discontinuation of investigational product

Patients may be discontinued from IP in the following situations:

- Patient decision. The patient is at any time free to discontinue treatment, without prejudice to further treatment
- AE
- Severe non-compliance to study protocol
- Incorrectly randomized patient, see Section 5.3
- Development of any of the following study specific criteria for individual patients, which **must** lead to discontinuation of IP:
  - Patient experiencing symptoms of paraesthesia daily for at least 7 consecutive days, see Section 6.4.3.1
  - Patient experiencing a syncopal episode (loss of consciousness) from any cause
  - Patient describing an AE 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 at home when getting up)
  - Patient with a corrected QT interval (QTcF) ≥500 ms
  - Patient with ALT or AST > 5 x ULN, see Section 6.4.5.2

  - Patient with ALT or AST >3 x ULN in combination with BIL >2 x ULN, see Section 6.4.5.2

  - Patient with ALT or AST >3 x ULN and clinical signs or symptoms indicating liver dysfunction, see Section 6.4.5.2

- AstraZeneca may decide to stop a dose group in the study based on a recommendation from the DSMB. The following criteria, included in the DSMB charter, serve as a guide for the DSMB to stop a dose group. However, a more restrictive approach may be applied based on the DSMB's and AstraZeneca's evaluation during the study:

  - two or more unique events of syncope in different patients in the same dose group that cannot definitively be attributed to another cause (eg, syncope while having blood drawn would be attributable to the blood draw, not the investigational product)

  - corrected QT interval (QTcF) ≥ 500 ms in 20% of the planned total number of patients to be enrolled in the same dose group.

  - dizziness causing discontinuation from the IP in 10% of the planned total number of patients to be enrolled in the same dose group.

If a dose group is stopped, any treatment group(s) on a higher dose will also be stopped.

If a decision has been taken to stop a dose group the investigator will receive information about which patients (randomization codes) should be discontinued from treatment. The investigator at each site must immediately inform the patient to stop intake of the IP and to schedule an end of treatment visit within 1 week and a subsequent end of study visit. The investigator must also ensure that no new patients will be randomized to the stopped dose group. Patients on lower doses of IP and placebo will continue in the study unless the site is informed otherwise.

In all patients discontinued from the IP, data collection and procedures should continue as described in Section 5.8.1. If the patient does not agree to attend an end of treatment visit, a modified follow up should be arranged (eg, telephone contact to follow-up with respect to any ongoing AEs or a reminder to return the e-diary device and unused IP), if agreed to by the patient and in compliance with local data privacy laws/practices. Relevant information should be recorded in the unscheduled visit section in the eCRF.

5.8.1 Procedures for discontinuation of a patient from investigational product

A patient that decides to discontinue the IP will always be asked about the reason(s) and the presence of any AEs. If possible, they will be seen and assessed by an investigator(s). AEs will be followed up (see Sections 6.4.3 and 6.4.4), e-diary device and IP should be returned by the patient.

For early discontinuation visits, assessments according to end-of-treatment visit (visit 6) should be done including completion of the visit 6 ePRO assessments (OTE). In addition, a urine pregnancy test for women of childbearing potential, should be performed. All patients should be strongly encouraged to attend the end of study visit (visit 7). AEs and/or SAEs, including QTcF prolongations and clinically significant abnormal laboratory values, must be followed up. The e-diary device and IP must be returned by the patient, drug accountability, and an e-diary device check must be done.

If the patient does not agree to this option, a modified follow-up should be arranged, see Section 5.8. Relevant information should be recorded in the unscheduled visit section in the eCRF.

Patients withdrawn from the IP due to reporting daily episodes of symptoms of paraesthesia for at least 7 consecutive days must be referred to a board certified neurologist for examination and must be followed up by the investigator after discontinuation of IP at the end of study visit, see Section 6.4.3.1.

All patients who discontinue the IP due to elevated liver associated tests must be followed until their liver tests have returned to baseline or until a firm explanation (diagnosis) for the elevated liver tests has been established, see Section 6.4.5.2.

If a patient is withdrawn from the study, see Section 5.9.

5.9 Withdrawal from study

Patients are at any time free to withdraw from the study (IP and assessments), without prejudice to further treatment (withdrawal of consent). Such patients will always be asked about the reason(s) and the presence of AEs. If possible, they will be seen and assessed by an investigator. AEs will be followed up (see Sections 6.4.3 and 6.4.4), e-diary device must be returned by the patient and checked (data transfer) and IP returned with drug accountability completed.

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

- Visit
- Demography
- Eligibility criteria
- If SAE criteria was fulfilled, the AE and SAE modules must be completed
- Study termination
- Electronic signature.

6. COLLECTION OF STUDY VARIABLES

6.1 Recording of data

The Web Based Data Capture (WBDC) system, RAVE, will be used for data collection and query handling. The investigator will ensure that data are recorded in the eCRFs as specified in the study protocol and in accordance with the instructions provided. See Section 10.

The investigator ensures the accuracy, completeness and timeliness of the data recorded and provides answers to data queries according to the Clinical Study Agreement (CSA). The investigator will sign the completed eCRFs and a copy will be archived at the study site.

The patients will use e-diary devices for collection of the PRO data at visits and in the morning and evening, see Sections 6.5.2 and 10. Trained study personnel at the study site will be responsible for entering the patient identifier data and visit data in the e-diary device, see Sections 6.5.2 and 10.

The dECGs are recorded using ECG equipment provided by a central ECG vendor, eResearch Technology (ERT). The ECG machines will record dECGs for transmission to the central vendor over the site's analogue phone line using the ECG machine's modem, or via the ERT web upload system. The ECG machine will also print off 2 copies of the ECG by default, one copy of which can be provided to ERT for digitization and analysis if necessary (see section 6.4.7).

Laboratory data will be delivered by a central laboratory (Quintiles) and will be loaded into the study database.

6.2 Data collection and enrolment

The following data will be collected during the study:

- Obtaining written informed consent prior to start of any study specific procedures
- Demographic data
- Medical and surgical history and medication
- GI history, Irritable Bowel Syndrome (IBS) symptoms, PPI use, history of paraesthesia
- A complete physical examination
- Height, weight for calculation of BMI
- 12-lead dECG recordings
- Orthostatic test

- Endoscopy results
- AEs
- Active questioning regarding specific symptoms (see section 6.4.3.2)
- Urine pregnancy tests
- Blood samples for clinical chemistry and haematology, urine samples, PK samples and genetic samples (optional)
- ePRO data (see section 6.5).

6.2.1 Upper gastrointestinal endoscopy

An upper gastrointestinal endoscopy is required within 14 days after the enrolment visit provided that:

- no endoscopy has been performed within the past 24 months
- the most recent endoscopy within 24 months demonstrated mucosal breaks in the esophagus
- no documented endoscopy report from the most recent endoscopy within 24 months is available to the investigator.

If the most recent available documented report from an upper endoscopy within the past 24 months confirms the absence of mucosal breaks then the patient does not need to undergo an endoscopy during this study. Data from these reports will be recorded in the eCRF by the investigator.

The study endoscopy may be performed by a physician other than the Principal Investigator. Information on the endoscopic status including esophagus, stomach and duodenum will be recorded in the eCRF. The Los Angeles (LA) classification of reflux (erosive) esophagitis is to be applied (Lundell L et al 1999).

The LA classification:

Grade A	One (or more) mucosal break(s) no longer than 5 mm that does not extend between the tops of two mucosal folds
Grade B	One (or more) mucosal break(s) more than 5 mm that does not extend between the tops of two mucosal folds
Grade C	One (or more) mucosal break(s) that is continuous between the tops of two or more mucosal folds but which involves less than 75% of the circumference

Grade D One (or more) mucosal break(s) that involves at least 75% of the circumference.

Baseline symptom recording in RESQ-eD must continue for at least 8 days after the day of the endoscopy, so as not to interfere with the baseline symptoms recordings. The endoscopy performed as a study requirement should not result in a change of PPI therapy, otherwise the patient will not be eligible for randomization.

Patients with mucosal breaks at the baseline endoscopy will have a repeat endoscopy within 5 days after the end of treatment. In case of early discontinuation of such patients it is at the discretion of the investigator to decide whether a repeat endoscopy is appropriate based on the duration of study treatment and the reason for early discontinuation.

6.3 Efficacy

6.3.1 Efficacy variable

The efficacy variables in the study will be based on the twice daily recordings of patient-reported symptoms using the RESQ-eD, which are described in Section 6.5.

6.4 Safety

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

6.4.1 Definition of adverse events

An AE 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 SAE is an AE occurring during any study phase (ie, run-in, treatment, 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 CSP.

6.4.3 Recording of adverse events

Time period for collection of adverse events

Non serious AEs will be collected from the first time of administration of IP throughout the treatment period and including the follow-up period. SAEs will be recorded from the time of signed informed consent throughout the treatment period and including the follow-up period.

Follow-up of unresolved adverse events

Any AEs that are unresolved at patient's last AE assessment in the study are followed up by the investigator for as long as medically indicated, but without further recording in the eCRF. 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 recorded in the eCRF for each AE:

- AE (verbatim)
- the date when the AE started and stopped
- maximum intensity
- whether the AE is serious or not
- investigator causality rating against the IP (yes or no)
- action taken with regard to IP
- 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 hospitalization (as applicable)
- date of discharge (as applicable)
- probable cause of death (as applicable)
- date of death (as applicable)
- autopsy performed (as applicable)
- causality assessment in relation to study procedure(s)
- causality assessment in relation to other medication
- description of AE.

Intensity rating is defined as:

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

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria in Section 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 AE, 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 CSP.

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 eCRF.

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.

See Section 5.8 regarding discontinuation criteria.

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 (CSR). Deterioration as compared to baseline in protocol-mandated laboratory values, vital signs, ECGs 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 IP.

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 eCRF. Furthermore, a complete physical examination including neurological examination with evaluation of cranial nerve functions must be completed and documented in the eCRF 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 5.8.1), and be referred to a board certified neurologist for evaluation. A copy of the report from the neurologist must be provided to the AstraZeneca representative (ICON).

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, lightheadedness or dizziness. In order to get baseline information before study treatment has started, the question will also be asked prior to first intake of IP at visit 2. All reported

episodes will be recorded in a specific module in the eCRF. 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 eCRF.

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
- an acid taste in the 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 the mouth
- stomach contents (liquid or food) moving upwards to the throat or mouth
- heartburn.

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 eCRF.

If any SAE occurs in the course of the study, then investigators or other site personnel must inform appropriate AstraZeneca representative (ICON) 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 (ICON) 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 (ICON) 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.

Once the investigators or other site personnel indicate an AE is serious in the WBDC system, an automated email alert is sent to the designated AstraZeneca representative (ICON).

If the WBDC system is not available, then the investigator or other study site personnel reports a SAE to the appropriate AstraZeneca representative (ICON) by telephone or fax. The AstraZeneca representative (ICON) will advise the investigator/study site personnel how to proceed.

The reference document for definition of expectedness/listedness is the IB for the AstraZeneca drug AZD3355.

6.4.5 Laboratory safety assessment

Blood and urine samples for determination of clinical chemistry, haematology and urine analysis, will be taken at the times indicated in the Study Plan, see Table 1.

6.4.5.1 Regular laboratory safety monitoring

A full laboratory screen with blood and urine samples for determination of clinical chemistry, haematology and urine analysis parameters will be taken at visit 1 (including *H. pylori* serology), visit 2, visit 4, visit 6 and visit 7. If an early discontinuation visit is done, a full laboratory screen (including a urine pregnancy test for women of childbearing potential) should be performed.

If there is an unscheduled visit and depending on the reason for this visit, it is the judgement of the investigator to decide if laboratory samples need to be taken to ensure safety monitoring. A limited laboratory screen will be taken at visit 3 and visit 5 to include the following analysis: AST, ALT, ALP, total BIL and CK. Patients with elevated liver transaminases > 3 x ULN will be closely monitored, see Section 6.4.5.2.

A urine pregnancy test for women of childbearing potential will be conducted at visit 1, 2, 7 and at early discontinuation visits.

The laboratory samples will be analysed at a central laboratory (Quintiles), except for the urine tests. These tests will be done by dipstick at the study site and the results will be entered into the appropriate modules in the eCRF. See Section 7.1 for the total volume of blood that will be drawn from the patients throughout the conduct of the study.

Table 2 Laboratory safety variables, full laboratory screen

Clinical chemistry	**Haematology**
S-Aspartate aminotransferase (AST)	B-Haemoglobin (Hb)
S-Alanine aminotransferase (ALT)	B-Red blood cell (RBC) count
S-Alkaline phosphatase (ALP)	B-Erythrocyte mean cell volume (MCV)
S-Total bilirubin (BIL)	B-Mean corpuscular haemoglobin concentration (MCHC)
S-Creatinine	B-White blood cell (WBC) count
S-Blood urea nitrogen (BUN)/urea	B-neutrophils count
S-Albumin	B-eosinophils count
S-Sodium	B-basophils count
S-Potassium	B-lymphocyte count
S-Magnesium	B-monocyte count
S-Calcium	B-Platelet count
S-Creatinine kinase (CK)	
S-C-reactive protein (CRP)	**Urinalysis (dipstick)**
S-Non-fasting serum glucose	U-Protein
H. pylori antibody immunoglobulin G (IgG) test[a]	U-Glucose
	U-Haemoglobin
	Urine pregnancy test[b] (dipstick)

[a] Conducted at visit 1 only.
[b] Conducted at visit 1, visit 2, visit 7 and early discontinuation visits only for women of childbearing potential.

Laboratory values outside the reference ranges suspected to be of any clinically significance could be re-checked during an unscheduled visit. Randomized patients who develop clinically significant abnormal laboratory values must be followed until normalization or for as long as the investigator considers necessary.

6.4.5.2 Handling of patients with elevated liver transaminases

The investigator will be alerted from the central laboratory regarding patients developing ALT or AST > 3 x ULN during the study ie, all values above > 3 x ULN with no upper limit will be alerted. How to handle these patients is described in detail in this section.

All patients, regardless of whether they stop or continue the intake of IP, must be closely monitored with repeated laboratory liver tests every third day or more frequently if judged necessary by the investigator until the liver tests begin to improve. Thereafter liver tests will be performed at an interval decided to be appropriate by the investigator. All patients must be followed until the liver tests have returned to baseline or until a firm explanation (diagnosis) to the elevated liver transaminases has been established.

The specific laboratory kit, (Liver kit 1) to be used for confirmation and monitoring includes ALT, AST, ALP, BIL, conjugated BIL, INR, albumin, CK, Hb, WBC, neutrophils, eosinophils, basophils, lymphocytes, monocytes, sodium, potassium and creatinine.

- **Patients who can continue the intake of IP**

  - Patients with ALT or AST > 3 x ULN but ≤ 5 x ULN and no clinical signs or symptoms indicating liver dysfunction can, at the discretion of the investigator, continue the intake of IP with close monitoring.

The patients must be brought back to the clinic for an unscheduled visit without any delay, but not later than 72 hrs after the test results have been received, for specific evaluation of the underlying cause for the ALT or AST elevation and confirmatory laboratory testing.

A medical history focused on risk factors for liver injury (alcohol consumption, exposure to toxic agents, infections, medications and drug use including herbal remedies etc) should be obtained, evaluation of recent symptoms (AEs) and physical examination should be done and all relevant information should be captured in appropriate eCRF modules.

Confirmatory laboratory testing should be done and more frequent monitoring of liver tests should be initiated.

- **Patients who stop intake of IP**

Patients with the following findings should immediately be contacted and instructed to stop intake of IP:

  - ALT or AST > 5 x ULN

  - ALT or AST >3 x ULN in combination with BIL >2 x ULN

  - ALT or AST >3 x ULN with clinical signs or symptoms indicating liver dysfunction (such as nausea, fatigue, right upper quadrant pain or tenderness, fever, rash or eosinophilia).

The patients must be brought back to the clinic for an unscheduled visit without any delay, but not later than 72 hrs after the test results have been received, for specific evaluation of the underlying cause of the ALT or AST elevation and confirmatory laboratory testing.

A medical history focused on risk factors for liver injury (alcohol consumption, exposure to toxic agents, infections, medications and drug use including herbal remedies etc) should be obtained, evaluation of recent symptoms (AEs) and physical examination should be done and all relevant information should be captured in appropriate eCRF modules.

Confirmatory laboratory testing should be done and more frequent monitoring of liver tests should be initiated.

In addition, the following blood samples (Liver kit 2) for differential diagnosis purposes must be taken in all patients who stop intake of IP:

- Alcohol misuse: carbohydrate deficient transferring (CDT), S-ethanol
- Viral hepatitis: anti-HAV-IgM, HBsAg, anti-HBc-IgM, anti-HCV, HCV RNA, EBV VCA IgM + EBNA IgG, anti-CMV-IgM
- Autoimmune hepatitis: ANA, AMA, SMA, IgG, IgM, IgA
- Hereditary disorders: S-Iron and TIBC, S-Ferritin, ceruloplasmin, alfa1-antitrypsin.

Imaging techniques and additional examinations can be done if there is a clinical indication as judged by the investigator (eg, ultrasound, CT, liver biopsy). The results of all testing should be entered in the appropriate eCRF modules. It is important that every effort is made to find an explanation for the elevated liver enzymes.

6.4.6 Physical examination

A complete physical examination will be performed at visit 1, 6, 7 and at early discontinuation visits and include an assessment of the following: general appearance, skin, head and neck including throat, lymph nodes and thyroid, abdomen, musculoskeletal/extremities, lungs, cardiovascular, respiratory and neurological systems including cranial nerves.

A complete physical examination as described above must be done in all patients reporting symptoms of paraesthesia, see Section 6.4.3.1.

6.4.7 ECG

The dECGs will be recorded at all visits including early discontinuation visits before orthostatic test and blood sampling. At visit 2, when the dECG will be performed both pre-dose and at estimated t_{max} (2 hours ±15 min post-dose), the ECG electrodes should remain on the patient to ensure that the same position is used for both dECG recordings. After the patient has been supine for at least 10 min, three standard 12-lead dECG recordings (triplicate) will be performed within a 5-minute period while the patient remains supine.

The central vendor (ERT) will provide the equipment (ELI-150) for recording and evaluation of dECGs. The ECG equipment will allow for both digital transfer of the data for central evaluation and for paper print-outs (paper speed 25mm/sec) for the investigator's judgement.

The investigators judgement will be used for enrolment /randomization by classifying the ECG as "normal" or "abnormal", whether any abnormality is clinically significant or not.

All dECGs will be electronically submitted by the investigator to ERT. Once received at ERT the patient's demography and visit information on the ECGs will be checked for inconsistencies and possible errors. Queries will be sent to the sites if necessary to clarify the data before the ECG can be analyzed. If no queries are needed, or once all queries on the ECG are resolved, ECG's will be analyzed using the 3 beats on lead II methodology, performed by a cardiac safety specialist who will select beats and place calipers to produce the interval duration measurements. The ECG then passes to a cardiologist for review. The cardiologist will check for correct beat selection and caliper placements, and also reviews the morphology and note any findings. The turn around time for the complete analysis is 72 hours starting from when the ECG is received at ERT. This time is paused however whilst there are any outstanding queries, and will start again once these are resolved.

ECG reports will be provided to the sites (preferably by e-mail although fax is possible) once the analysis is complete. The reports will contain the mean interval duration measurements, the ERT cardiologist's findings, as well as an evaluation of the ECG (normal, abnormal, unable to evaluate). The standard interval duration measurements produced from a good quality ECG are PR, RR, QRS, and QT. The report will also contain the derived HR, QTcB and QTcF values. It is the investigator's judgement whether the findings/results on the ERT report are clinically relevant or not and whether the findings will result in the discontinuation of the patient from the study based on the inclusion/exclusion or discontinuation criteria.

6.4.8 Vital signs

6.4.8.1 Weight and height

Height will be measured (centimetres) at visit 1 and weight (kilograms) at visit 1, 6 and at early discontinuation visits. Measurements should be taken without shoes and preferably on the same scale used for all measurements. BMI will be calculated from the height and weight.

6.4.8.2 Pulse and blood pressure

Supine and standing BP and pulse rate (orthostatic test) will be measured (mm Hg and beats/min) using standard equipment in all patients at each visit and at early discontinuation visits.

6.4.8.3 Instructions for orthostatic test

The patient will rest on a bed for 10 min and supine 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. The orthostatic test will be

performed immediately following the dECG recording and prior to any blood sampling (N.B.no dECG will be performed prior to orthostatic test post-dose at visit 6).

6.5 Patient reported outcomes (PRO)

Since there was not a PRO instrument for assessment of GERD symptoms documented to be fit for purpose in the target patient population, a new diary, the RESQ-eD, was developed by modifying an existing PRO instrument, the RDQ.

The validity, reliability and responsiveness of the RDQ has 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 e-diary, the RESQ-eD, was determined to be fit for purpose in the target population for this programme, in a subsequent measurement property validation study (D9120C00027).

6.5.1 Reflux Symptom Questionnaire e-diary and 7 day recall

The RESQ-7, using 7-day recall, will be used to determine eligibility for inclusion into the screening phase. Patients will be asked to complete the RESQ-7 questionnaire in an electronic format at visit 1.

The RESQ-eD will be used to evaluate efficacy and to determine eligibility for randomization. To measure patient-reported symptoms during the screening and the treatment phase, patients will be asked to complete the RESQ-eD twice daily, upon waking up in the morning and just prior to bedtime in the evening, starting in the evening on the day of visit 1 and ending in the morning on the day of visit 6.

In the RESQ-7, the frequency of symptoms over the past 7 days are 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). In the RESQ-eD, using twice daily recordings, recall periods of “since waking today” and “during the nighttime” is used. The RESQ-eD uses the same response format for intensity as the RESQ-7.

The RESQ-eD and 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 the 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 first 6 symptom items were from the original RDQ (Shaw et al 2001). The results from cognitive interviews and focus groups with patients with GERD who had symptoms despite PPI treatment (Report from Mapi Values), supplemented by clinical experts and literature review, lead to the addition of 7 symptom items to the RDQ in study D9120C00027, to become the RESQ-eD.

The validation study (D9120C00027) results show that the RESQ-eD is valid, reliable and responsive ie, is fit for purpose in the target patient population. In addition, based on the results from the study, the items in the RESQ-eD are combined into an Overall symptoms domain and 4 separate domains, see Section 11.3.1.

The wording of the RESQ-7 used at screening and the RESQ-eD can be found in Appendices F and G respectively.

As in study D9120C00027, the RESQ-7 and the RESQ-eD will be used together with a labelled torso, see Appendix H, as suggested by direct feedback in cognitive interviews (Report from UBC, Report from Mapi Values).

6.5.2 Overall Treatment Evaluation

The OTE questionnaire has been developed from similar questionnaires used to determine the minimal important difference (MID) or minimal important change (MIC) in various PRO instruments (Juniper et al 1994, Jaeschke et al 1989).

The OTE, in which patients assess to what extent their symptoms have changed and rate the importance of this change since the start of treatment with IP, will be used to classify patients with various magnitude and importance of symptom change. This allows for the confirmation of the RESQ-eD's ability to detect change (responsiveness) and subsequently to confirm the responder definition, based upon clinically meaningful change.

The OTE will also be used to identify patients in a stable condition for the test-retest reliability analysis of the RESQ-eD.

Patients will be asked to complete a study specific version of the OTE in an electronic form at visit 4, visit 6, and in the case of early discontinuation.

The wording of the OTE to be used in this study can be found in Appendix I.

Scoring and classifications are described in Section 11.3.2.

6.5.3 Recordings of use of proton pump inhibitor and antacid

Patients will be asked to report their use of PPI in the e-diary once daily just prior to bedtime in the evening before reporting the use of antacids.

They will be asked following question:

“Have you taken your PPI today?”

Response categories: “No” or “Yes”.

Patients will also be asked to report their use of antacids in the e-diary twice daily, upon waking up in the morning and just prior to bedtime in the evening, starting in the evening on the day of visit 1 and ending in the morning the day of visit 6.

They will be asked the following questions in the evening:

“Have you taken any antacids since waking today?”

Response categories: “Yes” or “No”.

Only if yes: “How many doses of antacid did you take since waking today?”

In the morning, the questions will have the following wording:

“Have you taken any antacids during the nighttime?”

Response categories: “Yes” or “No”.

Only if yes: “How many doses of antacid did you take during the nighttime?

6.5.4 Administration of PRO questionnaires

The PRO instruments will be self-administered and the patients will be asked to complete the PRO questionnaires and questions in electronic format using hand-held e-diary devices. Patients will use the same e-diary device at visits and during the twice daily registrations to complete the RESQ-eD and questions on the use of PPI and antacids.

All patients will receive training in how to use the e-diary device before completing the RESQ-7 questionnaire at visit 1, and extended training on how to use the e-diary devices and transmit data before entering the screening phase of the study. The site staff will document the training of each patient.

At study visits, the PRO questionnaires will be completed as mentioned in Table 1 and in Sections 6.5.1 and 6.5.2, and will automatically appear on the screen of the e-diary, when the site staff selects the appropriate visit in the device.

Patients will be instructed to start recording in their e-diary in the evening on the day of visit 1, with the last recording in the morning on the day of visit 6.

During the training the importance of the twice-daily e-diary recordings upon waking up in the morning (reporting night-time symptoms) and just prior to bedtime in the evening (reporting day-time symptoms) will be emphazised.

Patients will be instructed that a torso picture, which will be provided in the inside of the e-diary case, is available to show the anatomical location of the GERD symptoms that are referenced in the RESQ-7 and RESQ-eD questions. Additionally, for each patients' reference the site staff should record the PPI name and dose as well as the names of the antacids the patients most frequently uses on the back side of the torso picture to help when answering the PPI and antacid use questions.

The RESQ-eD questions and questions on antacid use for twice daily e-diary recordings will automatically appear on the screen of the e-diary device, when the patient activates the bedtime and morning diaries, respectively. A question regarding PPI use for once daily recording will appear on the screen, prior to the antacid recording, when activating the bedtime diary. The bedtime diary will be available daily from 8:00 pm until 2:00 am the next day, for completion by the patient. The morning diary will be available daily from 05:00 am until 11:00 am, for completion by the patient. The patient will be asked to set a flexible alarm for the morning and bedtime diaries, respectively.

Standard procedures for minimizing bias and enhancing ePRO compliance will be followed throughout the study.

The patients will be instructed to complete all questionnaires and questions independently, at study visits as well as when completing the e-diary questions, so that the responses reflect the patient's own perception. During training, patients should be told that there are no right or wrong answers to any diary questions and that they should choose the responses that best describes them. Site staff should be neutral in their response to any questions from the patient and not help the patient to choose an answer or interpret the question to the patient. However, the site staff should encourage the patient to answer each question as well as he/she can.

Each site will have a designated quiet space for patients to use when completing the PRO instruments at the study visits. Dedicated study staff at each site will be responsible for ensuring that the ePRO process will be followed according to the specific instructions from

the clinical study team and the ePRO provider. A detailed manual, which covers the description of the ePRO solution and practical issues that may arise, will be provided to the study staff. Training of staff is described in Section 9.2.

The site staff will transmit the data from the e-diary device via a wireless or analogue modem, depending on feasibility, to a centralized database, as soon as possible after completion of the PRO instruments at the study visit. Similarly, the patient will transmit their e-diary data via a wireless or analogue modem to a centralised database, as soon as possible after completion of an e-diary assessment. To carry out the data transmission, the patient needs to place the e-diary device on the modem for automatic transmission of the data. Once transmitted, the data will be accessible in a secure fashion on a web site that is available to the specific study site. In addition, the data will be accessible to the study monitor and the AstraZeneca/AstraZeneca representative (ICON) clinical study team. If a data transmission cannot be completed from the patient's home, it may be done from work or another suitable location. If none of the previous means can be used, the data transmission can be done at the regularly scheduled study visits.

The study staff will be required to monitor that the patients have completed the appropriate PRO instruments at visits 1, 4 and 6.

Further, the study staff will be required to monitor the patients' compliance with the twice daily recordings in the e-diary on a daily basis using the web site. If the e-diary compliance is deemed not optimal, ie, if the web reports indicate that bedtime or morning diaries have not been completed or transmitted as expected, the study staff will contact the patient via telephone to assess if the issue is technical or if the patient needs additional instructions or support.

In addition, a check of the e-diary device (battery status and data transfer) and a review of e-diary compliance together with the patient will be done at visits 2, 3, 4, 5 and 6. At visit 6 the e-diary device will be collected.

6.6 Pharmacokinetics

6.6.1 Collection of samples

Venous blood samples (approximately 2 mL) for the determination of concentrations of AZD3355 in plasma will be taken at the times presented in the Study plan, Table 1.

PK samples will be collected for all patients regardless of treatment allocation to keep the study blind.

At visit 4 and 6 a blood sample will be taken just before dose intake, when the patient arrives to the clinic in the morning. In addition, two more samples will be taken at 2 hours (±30 min) and at 4 hours (±30 min) post-dose at these visits. There will be no food or water restrictions prior to the PK sampling.

For the population PK sampling it is very important to collect the exact time of sampling and dose intake (both in the morning and the previous evening). Samples outside these recommended time-frames are still useful provided that the exact times are recorded, see Section 3.1.

All samples will be analyzed 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 the 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 PRA International-Bioanalytical Laboratory B.V., The Netherlands, on behalf of Clinical Pharmacology & DMPK, AstraZeneca, Mölndal, Sweden, using liquid chromatography and mass spectrometric detection. The lower limit of quantification (LLOQ) of AZD3355 in plasma is 0.030 µmol/L.

6.7 Pharmacogenetics

6.7.1 Collection of pharmacogenetic samples

For information on collection of pharmacogenetic samples see Appendix D.

For blood volume, see Section 7.1.

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 3 Volume of blood to be drawn from each patient

Assessment		**Sample volume (mL)**	**No. of samples**	**Total volume (mL)**
Safety	Clinical chemistry	5	7	35
	Haematology	4	7	28
Pharmacokinetic		2	6	12
Pharmacogenetics		10	1	10
Total				85

However, if additional tests are required for the patient's safety (eg unscheduled visits for elevated liver tests, retests etc) the volume of blood might be increased.

7.2 Handling, storage and destruction of biological samples

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

7.2.1 Pharmacokinetic samples

Samples will be disposed of after the CSR has been finalised, unless retained for future analyses, see below.

Key samples for metabolite analysis will be sent to Clinical Pharmacology & DMPK, AstraZeneca R&D, Mölndal, and retained for a maximum of 2 years following the finalisation of the CSR. The results from the investigation will not be reported in the CSR but separately in a bioanalytical report. The key samples will be disposed after 2 years storage or earlier if it is decided that the metabolite analysis will not be conducted.

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 IATA 6.2 Guidance Document, see Appendix C.

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 at each centre 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 the genetic sampling is optional in the study this will not withdraw the patient from the study.

The Principal Investigator:

- Ensures patients' withdrawal of informed consent to the use of donated samples is notified immediately to AstraZeneca representative (ICON)
- 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 representative (ICON) are informed about the sample disposal.

AstraZeneca representative (ICON) 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.

For further information about genotype results from the optional genetic research, see Appendix D.

8.3 Ethics and regulatory review

An Ethics Committee 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 Ethics Committee, and to the study site staff.

The opinion of the Ethics Committee should be given in writing. The investigator should submit the written approval to AstraZeneca representative (ICON) before enrolment of any patient into the study.

The Ethics Committee 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 Ethics Committee 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 provide Regulatory Authorities, Ethics Committees and Principal Investigators with safety updates/reports according to local requirements. For studies in countries implementing the EU Clinical Trials Directive, informing Ethics Committees and Regulatory Authorities will be performed by AstraZeneca.

Each Principal Investigator is responsible for providing the Ethics Committees/IRB with reports of any serious and unexpected adverse drug reactions from any other study conducted with the IP. 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 Ethics Committee.

The genetic research component of this study is optional. Patients must provide a separate Informed Consent Form for genetic sample and analysis.

8.5 Changes to the protocol and informed consent form

Study procedures will not be changed without the mutual agreement of the International co-ordinating Investigator, National Co-ordinating Investigator, and the Principal 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 Ethics Committee 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 Ethics Committee, see Section 8.3.

If a protocol amendment requires a change to a centre's Informed Consent Form, AstraZeneca and the centre's Ethics Committee 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 Ethics Committee.

8.6 Audits and inspections

Authorised representatives of AstraZeneca, a regulatory authority, or an Ethics Committee 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 representative (ICON) immediately if contacted by a regulatory agency about an inspection at the centre.

9. STUDY MANAGEMENT BY ASTRAZENECA REPRESENTATIVE (ICON)

9.1 Pre-study activities

Before the first patient is entered into the study, it is necessary for an AstraZeneca representative (ICON) 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 representative (ICON). This will be documented in a CSA between AstraZeneca representative (ICON) and the investigator.

9.2 Training of study site personnel

Before the first patient is entered into the study, an AstraZeneca representative (ICON) will review and discuss the requirements of the CSP and related documents with the investigational staff and also train them in any study specific procedures and the WBDC system 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 site staff will also be trained to use the electronic devices, training devices, ePRO web sites (Study Works), perform transmission, monitor e-diary compliance, handle queries and will be authorised to train patients in using the electronic devices and perform transmission prior to their participation in the study. The site staff will receive a detailed user manual for the use of the electronic device and the ePRO web sites. Retraining will be provided if needed. The investigators are not required to complete the full level training, if they will not train patients, nor assign nor use the electronic devices. Investigators will however, be trained to log into the web report system (Study Works) and follow the procedures for navigating to the patient data for their site. A training certificate will be issued to site staff participating in training at investigator meeting. Training of site staff outside the investigator meeting will be documented at the site level.

The site staff will be trained to use the dECG equipment and a detailed user manual will be included with the dECG equipment when delivered at the site.

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 (ICON) 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 eCRFs, that biological samples are handled in accordance with the Laboratory Manual and that IP accountability checks are being performed.
- Perform source data verification (a comparison of the data in the eCRFs 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 (ICON) 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 CSA.

9.4 Study agreements

The Principal Investigator at each/the centre should comply with all the terms, conditions, and obligations of the CSA for this study. In the event of any inconsistency between this CSP and the CSA, the terms of CSP 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 CSA shall prevail.

Agreements between AstraZeneca representative (ICON) 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 CSA.

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 Q4 2009 and to end by Q2 2010.

The study may be terminated at individual centres 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.

10. DATA MANAGEMENT BY ASTRAZENECA REPRESENTATIVE (ICON)

Data management will be performed by AstraZeneca representative (ICON). Data will be entered in the WBDC system at the study site. Trained study personnel will be responsible for entering data on the observations, tests and assessments specified in the protocol into the WBDC system and according to the eCRF Instructions. The eCRF instructions will also provide the study site with data entry instructions. Data queries will be raised for inconsistent, impossible or missing data. Data entered in the WBDC system will be immediately saved to a central database and will be available in an audit trail. When data have been entered, reviewed, edited and Source Data Verification (SDV) performed, the Principal Investigator will be notified to sign the eCRF electronically as per the agreed project process and data will be locked to prevent further editing.

Data collected from the completed eCRFs, laboratory data and through third party sources will be received electronically by an AstraZeneca representative (ICON) and reconciled against study data. The process will be documented in the Data Management Plan and the validation performed under the direction of the responsible Data Manager, according to the Data Validation Manual. Quality control procedures will be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly.

AEs and medical history/surgical history will be classified according to the terminology of the latest version of the Medical Dictionary for Regulatory Activities (MedDRA). Medications will be classified according to the AstraZeneca Drug Dictionary. All coding will be done performed by an AstraZeneca representative (ICON). Queries can be raised by the coding expert at any time during the study period and the query tool in RAVE will be used. When all data have been coded, validated, signed and locked, clean file will be declared. Any treatment revealing data may thereafter be added and the final database will be locked.

The central ECG vendor (ERT) will be used to collect dECG data and sites will transmit the dECG data to ERT’s central database using equipment provided by the vendor. ERT will identify, examine and analyse the data and will send the ECG reports to site. See Section 6.4.7.

Laboratory data will be delivered to an AstraZeneca representative (ICON) by the central laboratory (Quintiles). The laboratory data will be loaded into the study database.

The ePRO system is a specialised data capture appliance designed for entry of data in a way that is attributable, secure and accurate in compliance with FDA's Final Rule: Electronic Records; Electronic Signature (21 CFR part 11). The ePRO system will be provided by a central vendor (PHT Corporation). The ePRO data will be transmitted either via analog or wireless modems to a central database.

After the ePRO data have been received at the central database, it can be reviewed via a secure access to a web-server. All data will be date and time stamped and all changes will be tracked in the systems audit trail. Each user needs to have system training before any access will be given out. All actions on data are to be accomplished using the system controls compliant with 21 CFR part 11 that are built in to the system application used to collect, clean, review and archive clinical study data.

The investigators (or study personnel designated by the investigator) are responsible for any modification of data on study patients. No changes to the patient's symptom recording should be made. Investigators, study monitors, (and any auditors or FDA inspectors) will have access to the data and electronic records via connection to the web-server from the site. Clinical investigators shall retain the recordings during the study in the sense that they can access them and they uniquely and specifically control the contents of the data in the records.

Online study reports will be accessible to the study team during the study. The source files will be returned to AstraZeneca R&D, Mölndal.

For information regarding genetic data, see Appendix D.

11. EVALUATION AND CALCULATION OF VARIABLES BY ASTRAZENECA

11.1 Calculation or derivation of efficacy variable(s)

Efficacy variables will be based on patient reported outcomes. Therefore, see section 11.3 for derivation of efficacy variables.

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 and Discontinuation of IP due to Adverse Events (DAE)s. Based on the expert's judgement, significant AEs of particular clinical importance may, after consultation with the Global Patient Safety Physician, be considered OAEs and reported as such in the CSR. A similar review of laboratory/vital signs/ECG 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

11.3.1 Reflux Symptom Questionnaire e-diary

The RESQ-eD consists of 13 items that combine into an Overall symptoms domain and 4 separate domains:

- Heartburn
  - burning feeling behind the breastbone
  - pain behind the breastbone
  - heartburn
  - burning feeling in centre of the upper stomach
  - pain in centre of the upper stomach
- Regurgitation
  - acid taste in the mouth
  - bitter taste in the mouth
  - unpleasant movement of material upwards from the stomach
  - stomach contents (liquid or food) moving upwards to your throat and mouth
- Hoarseness, cough and difficulty swallowing
  - hoarseness
  - cough
  - difficulty swallowing
- Burping
  - Burping.

The e-diary ratings of the 6-graded Likert scale are transformed to numerical values, from “Did not have” = 0 to “Severe” = 5.

When imputing missing values, morning registrations will be regarded as one sequence of observations and evening registrations as another sequence of observations. Missing values in the sequence of morning or evening registrations respectively will be imputed according to the following rules. If a single observation is missing, the missing value will be replaced by the larger of the two surrounding values. If two or more consecutive observations are missing the values will remain missing.

Values of intensity for a day (approximately a 24 h period) will then be constructed using the imputed sequences of evening and morning registrations of item intensities. A day will consist of an evening and the following morning registration. The daily item intensity will be defined as the maximum of the two registrations. If either the morning or the evening registration is missing, the corresponding daily value will be set to missing in the statistical analyses. In the algorithm determining whether a patient is eligible for randomization missing values are not imputed, and the daily value will be the maximum value of the evening and morning registration even if one of them is missing.

The domain intensity on a given day will be derived as the mean daily intensities of the items that form the domain.

The proportion of days of not more than mild symptoms with respect to a domain during a specified time period will be calculated as: the number of days when none of the items in the domain has a daily intensity that exceeds “Mild” (numerical value 2) divided by the number of expected values in the specified time period. That is, a day with missing value will be regarded as a day with more than mild symptoms.

Baseline will be defined as the seven days prior to visit 2 (Randomization visit). Hence, the last e-diary registration that contributes to the baseline period will be the morning registration on the day of visit 2.

A patient’s treatment period will be defined as the days from the day of first dose up to and including the day of last dose. Hence, the first e-diary registration contributing to the treatment period will be the evening registration on the day of the first dose, and the last e-diary registration contributing to the treatment period will be the morning registration on the day of the last dose. If the date of the first dose has not been entered in the database, the date of the first dose will be imputed to the date of visit 2 (Randomization visit). If the date of the last dose has not been entered in the database, the date of the last dose will be imputed to the date of visit 6 (End of treatment visit).

The change from baseline to the treatment period in proportion of days of not more than mild symptoms with respect to a domain will be calculated as the proportion of days of not more than mild symptoms with respect to the domain during the patient’s treatment period minus the proportion of days of not more than mild symptoms with respect to the domain during the patient’s baseline period.

Primary outcome variable:

A responder is defined as a patient who experiences at least three more days of not more than mild symptoms on average per week during treatment compared to baseline, based on the Overall symptoms domain in the RESQ-eD. This means that the increase from baseline to the treatment period in the proportion of days of not more than mild symptoms will exceed or equal 3/7 for a responder. A patient that is not classified as a responder according to this criteria will be classified as a non-responder. The primary outcome variable is the binary variable (yes/no) indicating a patient who meets the responder definition.

Secondary outcome variables:

An alternative definition of a responder is a patient who experiences at least five days of not more than mild symptoms on average per week during treatment, based on the Overall symptoms domain in the RESQ-eD. This means that the proportion of days of not more than mild symptoms during the treatment period will exceed or equal 5/7 for a responder. A patient that is not classified as a responder according to these criteria will be classified as a non-responder. A secondary outcome variable is the binary variable (yes/no) indicating a patient who meets the alternative responder definition.

The change from baseline to the treatment period in proportion of days of not more than mild symptoms, as defined above, with respect to the Overall symptoms domain, the Heartburn domain, the Regurgitation domain, the Hoarseness, cough, difficulty swallowing domain and the Burping domain will be analysed as secondary outcome variables.

For the assessment of basic measurement properties of the RESQ-eD the following variables will be derived:

- Item intensity over baseline will be derived as the mean of the daily item intensities during the seven days that form the baseline period.

- Domain intensity over baseline will be derived as the mean of the daily domain intensities during the seven days that form the baseline period. If more than two daily domain intensity values are missing during the seven day period for a patient, the baseline intensity will be set to missing.

- Domain intensity over the seven days prior to visit 4 will be derived as the mean of the daily domain intensities during the seven days. The last e-diary registration that contributes to the seven day period will be the morning registration on the day of visit 4. If more than two daily domain intensity values are missing during the seven day period for a patient, the intensity for the seven days prior to visit 4 will be set to missing.

- Domain intensity over the treatment period will be derived as the mean of the daily domain intensities during the treatment period. If the patient has less than 7 days on treatment or if more than 30% of the daily domain intensity values

during treatment are missing then the domain intensity over the treatment period will be set to missing.

11.3.2 Overall Treatment Evaluation

The 15-graded OTE responses will be broken down into categories as follows:

Table 4 OTE responses and collapsed categories

OTE responses, Improvement/deterioration	Collapsed categories
A very great deal better	Large improvement
A great deal better	
A good deal better	Moderate improvement
Moderately better	
Somewhat better	Small improvement
A little better	
Almost the same, hardly better at all	Unchanged
About the same	
Almost the same, hardly worse at all	
A little worse	Deteriorated
Somewhat worse	
Moderately worse	
A good deal worse	
A great deal worse	
A very great deal worse	

11.4 Calculation or derivation of pharmacokinetic variables

The PK analyses will be performed at AstraZeneca R&D. Population mean and variability parameters will be estimated for oral total plasma clearance (CL/F), which determines total drug exposure, AUC, and for other pharmacokinetic parameters as applicable.

12. STATISTICAL METHODS AND SAMPLE SIZE DETERMINATION BY ASTRAZENECA

12.1 Description of analysis sets

12.1.1 Efficacy analysis set

All randomized patients, who receive at least one dose of IP, will be included in the Full Analysis Set (FAS). The FAS data will be analysed as randomized ie, in as-randomized analyses patients are analysed based on their randomized treatment. Randomized treatment is defined as the treatment associated with the randomization code given to the patient, identified via the randomization schedule.

12.1.2 Safety analysis set

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

12.2 Methods of statistical analyses

12.2.1 Statistical evaluation – general aspects

The FAS will be used in the efficacy analyses described in this section. Analyses on RESQ-eD data will be based on imputed data. Unless otherwise stated, daily (approximately a 24 h period) values will be used. All patient reported outcome analyses will be calculated by using pooled data from all languages and cultures, if nothing else is stated under each paragraph. A Statistical analysis plan (SAP) will be prepared before unblinding data.

In the event of a dose group being terminated due to safety stopping criteria, the dose group would be excluded from the primary efficacy analyses comparing AZD3355 to placebo but included in safety presentations, PK analyses and the estimations of dose-response curves.

The statistical analysis will be performed by Statistics and Informatics, AstraZeneca R&D Mölndal, using mainly SAS® version 8.2. If available and necessary SAS® version 9.0 will be used.

12.2.2 Primary objective

The primary objective, to evaluate the effect of four doses of AZD3355 compared to placebo using the responder definition, will be evaluated by pairwise comparisons. Each dose level will be compared to placebo using logistic regression with dose as a factor variable. Based on experience from previous studies (D9120C00011 and D9120C00027), the symptom frequency during baseline is expected to be associated with the response variable. Therefore, the proportion of days of not more than mild symptoms during baseline will be included as a covariate. The tests will be one-sided with a significance level of 10%. The multiplicity issue will be handled by using a step down procedure where AZD3355 is tested sequentially

starting with the highest dose. If the null hypothesis of no difference between AZD3355 240 mg and placebo is not rejected no further dose level will be tested. If the null hypothesis is rejected, AZD3355 240 mg is demonstrated to have an effect and AZD3355 180 mg will be tested compared to placebo. If the null hypothesis of no difference between AZD3355 180 mg and placebo is not rejected, no further dose level is tested. If the null hypothesis is rejected, AZD3355 180 mg is demonstrated to have effect and AZD3355 120 mg will be tested versus placebo.

Estimates and two-sided 95% confidence intervals of the odds ratios of each dose level compared to placebo will be calculated.

An additional explorative logistic regression with additional covariates will be fitted to the data. Details of this analysis will be presented in the SAP.

In addition, the proportion of responders per treatment will be presented.

Since the number of patients at each centre is expected to be few, the country-treatment interaction will be evaluated instead of centre-treatment interaction. A Breslow-Day's test (two-sided with a significance level of 10%) will be used to evaluate the country-treatment interactions for the primary variable.

12.2.3 Secondary objectives

12.2.3.1 Alternative responder definition

As in the primary objective, the pairwise comparisons to placebo will be evaluated, but this time using the alternative responder definition. The secondary analysis will not be adjusted for multiplicity, hence the interpretation of these results should be made with caution of the multiplicity issue.

Estimates and two-sided 95% confidence intervals of the odds ratios of each dose level compared to placebo will be calculated.

12.2.3.2 Change in the proportion of days of not more than mild symptoms compared to baseline

The change from baseline to the treatment period in the proportion of days of not more than mild symptoms, both on the Overall symptoms domain in the RESQ-eD as well as for each domain separately will be analysed assuming data follows approximately a normal distribution. If the latter criteria is not fulfilled an appropriate transformation of the variable will be performed. Each dose level will be compared to placebo using an ANCOVA, where the baseline is included as a covariate. The results of the ANCOVA will be presented as estimates and two-sided 95% confidence intervals.

12.2.3.3 Dose response curves

Two versions of the dose response curve will be estimated, one based on the primary variable and one based on the change in the proportion of days of not more than mild symptoms

compared to baseline on the Overall symptoms domain in the RESQ-eD. The dose response curve for the primary variable will be estimated by a logistic regression model where dose is included as a continuous variable. The dose response curve for the change in proportion of days of not more than mild symptoms will be estimated using a 4-parameter E-max model. The main focus will be on the E-max model, and the logistic regression will be regarded as a supportive model. A more detailed description of the estimation of the dose response curves will be described in the SAP.

12.2.3.4 Efficacy over time

The proportion of patients with not more than mild symptoms by day will be presented graphically.

12.2.3.5 Internal consistency

The internal consistency of RESQ-eD domains will be evaluated by using Cronbach's alpha (Cronbach 1951). Cronbach's alpha will be estimated as

$$\alpha_c = \frac{m}{m-1}\left(1 - \frac{\sum Var(x_i)}{Var(\sum x_i)}\right)$$

where m is the number of items in the domain, $Var(x_i)$ is the estimated variance of the i^{th} item and the summation is over all items within a domain. Ninety-five % confidence intervals for the Cronbach's alpha coefficients will be presented. An alpha coefficient ≥ 0.70 will be interpreted as sufficient (Fayers et al 2007) to suggest that the items within a domain measure the same construct.

Cronbach's alpha will be calculated for each separate domain with more than one item, using the variable "item intensity over baseline".

If possible, Cronbach's alpha will be calculated with a subpopulation defined by the same languages version of the RESQ-eD. This will be done only for the subpopulation which is large enough to provide a reliable result.

Assuming the coefficient is 0.8 for a domain with 5 items, with 74 patients - or for a domain with 3 items, with 89 patients - the length of a 95% confidence interval for Cronbach's alpha is approximately ± 0.07. The calculations are based on formulas given in Bonett 2002.

12.2.3.6 Test-retest reliability

Test-retest reliability of the RESQ-eD will be investigated using Intraclass Correlation Coefficients (ICC), using patients in a stable condition between the randomization visit (visit 2) and the visit after 2 weeks treatment with IP (visit 4). A stable patient will be defined as a patient who scored "About the same", "Almost the same, hardly worse at all" or "Almost the same, hardly better at all" on the OTE after 2 weeks treatment.

Test-retest reliability will be calculated using the variables "domain intensity over baseline" and "domain intensity over the seven days prior to visit 4", for each separate domain.

The test-retest reliability intraclass correlation coefficient (R) will be estimated as:

$$\hat{R} = \frac{BMS - WMS}{BMS + (k_0 - 1)WMS}$$

where BMS and WMS are the mean square values between-patients and within-patients respectively, and k_0 denotes the number of replicates per patient (two in this case).

A 95% confidence interval for R will be estimated as

$$R \leq \frac{\frac{BMS}{WMS} - F_{N-1,K-N,\alpha/2}}{\frac{BMS}{WMS} + (2-1)F_{N-1,K-N,\alpha/2}}, \quad R \geq \frac{\frac{BMS}{WMS} - F_{N-1,K-N,1-\alpha/2}}{\frac{BMS}{WMS} + (2-1)F_{N-1,K-N,1-\alpha/2}}$$

where N = number of patients, K = number of measurements and $\alpha = 0.05$.

The within-patient and between-patient mean square will be calculated by two-way analysis of variance (ANOVA) with the factors of patient and visit.

Patients with a missing value for either domain intensity over baseline or domain intensity over the seven days prior to visit 4 will be excluded from the test-retest calculations.

Intraclass correlation coefficients < 0.4 will be considered to represent poor reliability, values > 0.75 excellent reliability, and values in between fair to good reliability (Fleiss 1986).

12.2.3.7 The ability to detect change (responsiveness)

The ability to detect change (responsiveness) will be analyzed by calculating the effect size (ES), defined as the mean change over time divided by the standard deviation at baseline.

The ES will be estimated as ES = $\frac{\bar{y}}{sd_{x_1}}$, where $y_i = x_{i2} - x_{i1}$ and x_{i1} = domain intensity over baseline for patient i, x_{i2} = domain intensity over the treatment period for patient i, and sd_{x1} = standard deviation of the domain intensity over baseline.

The ES will be presented graphically, by collapsed improvement/deterioration OTE-categories (Table 4).

Data from patients with available data for both terms in the change calculation ($x_{i1} - x_{i2}$) will be used when calculating the SD at baseline.

12.2.3.8 Responder definition

The proposed values, which form the responder definitions (both the responder definition and the alternative responder definition originally derived in study D9120C00027), based upon clinically meaningful change, will be evaluated by graphical depiction of the intra-patient change in proportion of days of not more than mild symptoms from baseline to the entire treatment period, versus the cumulative proportion of patients achieving that change for each of the collapsed OTE categories unchanged, small improvement, moderate improvement and large improvement, as assessed at the visit after 4 weeks treatment (visit 6) or at early discontinuation.

12.2.3.9 Pharmacokinetic evaluation

A population PK evaluation will be made to fit a pharmacokinetic model to plasma concentration versus time data, and tables and graphs will describe PK analyses. The influence of patient covariates such as gender, age, body weight and creatinine clearance on the pharmacokinetic parameters will be explored. The AUC of plasma concentration will be correlated to proportion of days of not more than mild symptoms per dose level, to be able to analyse the relationship between the systemic exposure and the response.

12.2.3.10 The prevalence of mucosal breaks

The prevalence of mucosal breaks obtained through upper endoscopy or historical reports and endoscopy findings after the treatment period will be described descriptively and reported in tables.

12.2.4 Safety objectives

Tables and graphs will describe the safety objective descriptively. In addition, the relationship between dose and AE will be evaluated by a logistic regression, using merged AE data from both spontaneous registrations and from active inquiry.

Laboratory data will be analyzed using descriptive statistics. Truncated laboratory values (values below the limit of quantification) will be set to LLOQ in all analyses.

12.2.5 Interim analyses

No interim analysis of efficacy data will be performed. However, the DSMB will have the possibility to unblind treatment and evaluate safety data. See Section 5.4.2 and Section 12.4.

12.3 Determination of sample size

In the placebo-controlled study, D9120C00027, with a similar patient population as in this study, the proportion of patients fulfilling the responder definition was 24% in the placebo group. Assuming a true placebo response rate of 24% and with 130 patients in each treatment group, a one sided Chi-square test at a significance level of 10% for the difference in proportion between dose and placebo group, will discover an increase of 15 percent points (ie the expected effect of the dose is assumed to be at least 39%) with an approximate power of

90%. This means that an observed difference of at least 8% will be statistically significant on the 10% level. The sample size has been calculated using nQuery, version 4.0.

Based on these calculations, 130 randomized patients per treatment group are considered to be sufficient to address the primary objective.

12.4 Data monitoring committees

Continuous safety surveillance of the study will be conducted as follows:

- The AstraZeneca representative (ICON) will provide weekly updates from participating countries to evaluate AEs and discontinuations due to AEs.

- A joint safety committee consisting of physicians and safety specialists from the AstraZeneca representative (ICON) and AstraZeneca will meet every second week to review blinded treatment data including adverse events (AEs, SAEs, DAEs), safety laboratory variables and orthostatic testing.

- An independent DSMB consisting of two physicians and a statistician will review/analyse preliminary data from a safety perspective to evaluate overall and specific safety parameters. The DSMB will be external to and independent from AstraZeneca and the AstraZenca representative (ICON). The DSMB, will receive data regularly during the study and will have access to the randomization list. The DSMB alone will thus be able to do analyses on unblinded treatment data if necessary, see Section 5.4.1.

Safety reviews will be performed by the DSMB as defined in the charter. Included in the DSMB charter will be stopping criteria for either a single dose group or the whole study. The DSMB will continually survey DAEs and SAEs, and will review all of the safety data on at least two occasions during the study. The DSMB will remain available for extra meetings and analyses if potential safety concerns are identified by the joint safety monitoring committees of AstraZeneca and the AstraZeneca representative (ICON) or if the chairman of the DSMB deems it necessary for any reason.

Following each safety review, the DSMB will provide a recommendation regarding study continuation to the AstraZeneca representative (ICON). A decision body, which consists of the International coordinating investigator in the study, physicians and a statistician from AstraZeneca and a study physician at the AstraZeneca representative (ICON), will decide how to proceed with the study based on the DSMB recommendation.

13. IMPORTANT MEDICAL PROCEDURES TO BE FOLLOWED BY THE INVESTIGATOR

13.1 Medical emergencies and AstraZeneca representative (ICON) 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.**

In the case of a medical emergency the investigator may contact:

Name	Role in the study	Address & telephone number

13.2 Overdose

There is no data on overdosing. All intake of AZD3355 that exceeds the dosing described in the protocol is defined as an overdose.

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

13.3.1 Maternal exposure

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

Pregnancy itself is not regarded as an AE unless there is a suspicion that the IP 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 (ICON) **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 (ICON) 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.

The same timelines apply when outcome information is available.

The pregnancy module in the eCRF is used to report the pregnancy and the AstraZeneca representative (ICON) is responsible for providing reports of outcomes to the AstraZeneca Patient Safety data entry site and to inform the study team at AstraZeneca.

13.3.2 Paternal exposure

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

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Clinical Study Protocol: Appendix A

Drug Substance	AZD3355
Study Code	D9120C00019
Edition Number	01
Protocol Dated	

Appendix A
Signatures

ASTRAZENECA SIGNATURE(S)

A randomized, double-blind, placebo controlled, multi-centre phase IIb dose finding study to assess the effect on GERD symptoms, safety and tolerability during four weeks treatment with AZD3355 in doses 60 mg, 120 mg, 180 mg and 240 mg bid as add-on treatment to a PPI in patients with GERD that are partial responders 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 SIGNATURE(S)

A randomized, double-blind, placebo controlled, multi-centre phase IIb dose finding study to assess the effect on GERD symptoms, safety and tolerability during four weeks treatment with AZD3355 in doses 60 mg, 120 mg, 180 mg and 240 mg bid as add-on treatment to a PPI in patients with GERD that are partial responders to PPI treatment

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

I agree to the terms of this study protocol.

ASTRAZENECA SIGNATURE(S)

A randomized, double-blind, placebo controlled, multi-centre phase IIb dose finding study to assess the effect on GERD symptoms, safety and tolerability during four weeks treatment with AZD3355 in doses 60 mg, 120 mg, 180 mg and 240 mg bid as add-on treatment to a PPI in patients with GERD that are partial responders 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 REPRESENTATIVE SIGNATURE

A randomized, double-blind, placebo controlled, multi-centre phase IIb dose finding study to assess the effect on GERD symptoms, safety and tolerability during four weeks treatment with AZD3355 in doses 60 mg, 120 mg, 180 mg and 240 mg bid as add-on treatment to a PPI in patients with GERD that are partial responders 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.

SIGNATURE OF INTERNATIONAL CO-ORDINATING INVESTIGATOR

A randomized, double-blind, placebo controlled, multi-centre phase IIb dose finding study to assess the effect on GERD symptoms, safety and tolerability during four weeks treatment with AZD3355 in doses 60 mg, 120 mg, 180 mg and 240 mg bid as add-on treatment to a PPI in patients with GERD that are partial responders 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.

Clinical Study Protocol Appendix B

Drug Substance	AZD3355
Study Code	D9120C00019
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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

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

Important medical event or medical intervention

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

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

Examples of such events are:

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

A GUIDE TO INTERPRETING THE CAUSALITY QUESTION

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

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

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

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

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

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

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

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

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

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

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

- Is there a known mechanism?

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

Clinical Study Protocol Appendix C

Drug Substance	AZD3355
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Appendix C
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. Cat 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. Cat 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 Cat 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.

Clinical Study Protocol Appendix D	
Drug Substance	AZD3355
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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 transporter 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 analyse the samples.

Results from this genetic research will be reported separately from the CSR 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.

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.

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Clinical Study Protocol Appendix E

Drug Substance	AZD3355
Study Code	D9120C00019
Edition Number	01
Date	

Appendix E
Examples of drugs prohibited in study D9120C00019 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

Concomitant Medication	Class
Imipramine	Antidepressant
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 F	
Drug Substance	AZD3355
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Edition Number	01
Date	

Appendix F
RESQ-7 ePRO wording – US English

Reflux Symptom Questionnaire - 7 day recall (RESQ-7); wording (US English) for ePRO implementation

Wording
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.
Over the past 7 days, how often did you have a burning feeling behind your breastbone? Did not have 1 day 2 days 3-4 days 5-6 days Daily
Over the past 7 days, how often did you have pain behind your breastbone? Did not have 1 day 2 days 3-4 days 5-6 days Daily
Over the past 7 days, how often did you have a burning feeling in the center of the upper stomach? Did not have 1 day 2 days 3-4 days 5-6 days Daily
Over the past 7 days, how often did you have a pain in the center of the upper stomach? Did not have 1 day 2 days 3-4 days 5-6 days Daily

Wording
Over the past 7 days, how often did you have an acid taste in your mouth? Did not have 1 day 2 days 3-4 days 5-6 days Daily
Over the past 7 days, how often did you have unpleasant movement of material upwards from the stomach? Did not have 1 day 2 days 3-4 days 5-6 days Daily
Over the past 7 days, how often did you have burping (gas coming from the stomach through the mouth)? Did not have 1 day 2 days 3-4 days 5-6 days Daily
Over the past 7 days, how often did you have hoarseness? Did not have 1 day 2 days 3-4 days 5-6 days Daily
Over the past 7 days, how often did you have cough? Did not have 1 day 2 days 3-4 days 5-6 days Daily

Wording
Over the past 7 days, how often did you have difficulty swallowing? Did not have 1 day 2 days 3-4 days 5-6 days Daily
Over the past 7 days, how often did you have a bitter taste in your mouth? Did not have 1 day 2 days 3-4 days 5-6 days Daily
Over the past 7 days, how often did you have stomach contents (liquid or food) moving upwards to your throat or mouth? Did not have 1 day 2 days 3-4 days 5-6 days Daily
Over the past 7 days, how often did you have heartburn? Did not have 1 day 2 days 3-4 days 5-6 days Daily

continues on the following page

Wording
Over the past 7 days, how would you rate the intensity of a burning feeling behind your breastbone? Did not have Very mild Mild Moderate Moderately severe Severe
Over the past 7 days, how would you rate the intensity of pain behind your breastbone? Did not have Very mild Mild Moderate Moderately severe Severe
Over the past 7 days, how would you rate the intensity of a burning feeling in the center of the upper stomach? Did not have Very mild Mild Moderate Moderately severe Severe
Over the past 7 days, how would you rate the intensity of a pain in the center of the upper stomach? Did not have Very mild Mild Moderate Moderately severe Severe
Over the past 7 days, how would you rate the intensity of an acid taste in your mouth? Did not have Very mild Mild Moderate Moderately severe Severe

Wording
Over the past 7 days, how would you rate the intensity of unpleasant movement of material upwards from the stomach? Did not have Very mild Mild Moderate Moderately severe Severe
Over the past 7 days, how would you rate the intensity of burping (gas coming from the stomach through the mouth)? Did not have Very mild Mild Moderate Moderately severe Severe
Over the past 7 days, how would you rate the intensity of hoarseness? Did not have Very mild Mild Moderate Moderately severe Severe
Over the past 7 days, how would you rate the intensity of cough? Did not have Very mild Mild Moderate Moderately severe Severe
Over the past 7 days, how would you rate the intensity of difficulty swallowing? Did not have Very mild Mild Moderate Moderately severe Severe

Wording
Over the past 7 days, how would you rate the intensity of a bitter taste in your mouth? Did not have Very mild Mild Moderate Moderately severe Severe
Over the past 7 days, how would you rate the intensity of stomach contents (liquid or food) moving upwards to your throat or mouth? Did not have Very mild Mild Moderate Moderately severe Severe
Over the past 7 days, how would you rate the intensity of heartburn? Did not have Very mild Mild Moderate Moderately severe Severe

Clinical Study Protocol Appendix G	
Drug Substance	AZD3355
Study Code	D9120C00019
Edition Number	01
Date	

Appendix G
RESQ-eD – US English

Reflux Symptom Questionnaire – e-Diary (RESQ-eD); wording (US English) for ePRO implementation

Bedtime diary

Wording in bedtime diary
Please answer the following questions to help us better understand the symptoms you have been experiencing since waking today (from the time you wake up until the time you go to bed) because of your reflux disease. For each question, please choose the answer that is most appropriate to you.
Since waking today, how would you rate the intensity of a burning feeling behind your breastbone? Did not have Very mild Mild Moderate Moderately severe Severe
Since waking today, how would you rate the intensity of pain behind your breastbone? Did not have Very mild Mild Moderate Moderately severe Severe
Since waking today, how would you rate the intensity of a burning feeling in the center of the upper stomach? Did not have Very mild Mild Moderate Moderately severe Severe
Since waking today, how would you rate the intensity of a pain in the center of the upper stomach? Did not have Very mild Mild Moderate Moderately severe Severe

Wording in bedtime diary
Since waking today, how would you rate the intensity of an acid taste in your mouth? Did not have Very mild Mild Moderate Moderately severe Severe
Since waking today, how would you rate the intensity of unpleasant movement of material upwards from the stomach? Did not have Very mild Mild Moderate Moderately severe Severe
Since waking today, how would you rate the intensity of burping (gas coming from the stomach through the mouth)? Did not have Very mild Mild Moderate Moderately severe Severe
Since waking today, how would you rate the intensity of hoarseness? Did not have Very mild Mild Moderate Moderately severe Severe
Since waking today, how would you rate the intensity of cough? Did not have Very mild Mild Moderate Moderately severe Severe

Wording in bedtime diary
Since waking today, how would you rate the intensity of difficulty swallowing? Did not have Very mild Mild Moderate Moderately severe Severe
Since waking today, how would you rate the intensity of a bitter taste in your mouth? Did not have Very mild Mild Moderate Moderately severe Severe
Since waking today, how would you rate the intensity of stomach contents (liquid or food) moving upwards to your throat or mouth? Did not have Very mild Mild Moderate Moderately severe Severe
Since waking today, how would you rate the intensity of heartburn? Did not have Very mild Mild Moderate Moderately severe Severe

Morning diary

Wording in morning diary
Please answer the following questions to help us better understand the symptoms you have been experiencing during the nighttime (from the time you go to bed until the time you wake up) because of your reflux disease. For each question, please choose the answer that is most appropriate to you.
During the nighttime, how would you rate the intensity of a burning feeling behind your breastbone? Did not have Very mild Mild Moderate Moderately severe Severe
During the nighttime, how would you rate the intensity of pain behind your breastbone? Did not have Very mild Mild Moderate Moderately severe Severe
During the nighttime, how would you rate the intensity of a burning feeling in the center of the upper stomach? Did not have Very mild Mild Moderate Moderately severe Severe
During the nighttime, how would you rate the intensity of a pain in the center of the upper stomach? Did not have Very mild Mild Moderate Moderately severe Severe

Wording in morning diary
During the nighttime, how would you rate the intensity of an acid taste in your mouth? Did not have Very mild Mild Moderate Moderately severe Severe
During the nighttime, how would you rate the intensity of unpleasant movement of material upwards from the stomach? Did not have Very mild Mild Moderate Moderately severe Severe
During the nighttime, how would you rate the intensity of burping (gas coming from the stomach through the mouth)? Did not have Very mild Mild Moderate Moderately severe Severe
During the nighttime, how would you rate the intensity of hoarseness? Did not have Very mild Mild Moderate Moderately severe Severe
During the nighttime, how would you rate the intensity of cough? Did not have Very mild Mild Moderate Moderately severe Severe

Wording in morning diary
During the nighttime, how would you rate the intensity of difficulty swallowing? Did not have Very mild Mild Moderate Moderately severe Severe
During the nighttime, how would you rate the intensity of a bitter taste in your mouth? Did not have Very mild Mild Moderate Moderately severe Severe
During the nighttime, how would you rate the intensity of stomach contents (liquid or food) moving upwards to your throat or mouth? Did not have Very mild Mild Moderate Moderately severe Severe
During the nighttime, how would you rate the intensity of heartburn? Did not have Very mild Mild Moderate Moderately severe Severe

Clinical Study Protocol: Appendix H

Drug Substance	AZD3355
Study Code	D9120C00019
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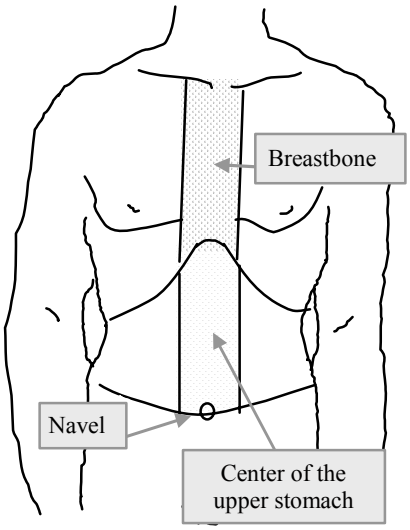
Appendix Date	

Appendix H

RESQ-7 RESQ-eD Torso picture - US English



AstraZeneca

Clinical Study Protocol Appendix I	
Drug Substance	AZD3355
Study Code	D9120C00019
Edition Number	01
Date	

Appendix I
Overall Treatment Evaluation (OTE) ePRO wording - US English

Overall Treatment Evaluation (OTE) – RI; wording (US English) for ePRO implementation

Wording when administered after a period of treatment with investigational product:		
We would like to find out if there are any changes in the way you have been feeling since treatment with the study medication started, i.e. the medication that you have been taking twice daily.		
Since treatment with the study medication started, has there been any change in your reflux symptoms (e.g. heartburn or regurgitation)? Better About the same Worse		
if "Better":		*if "Worse":*
How much better would you say your reflux symptoms (e.g. heartburn or regurgitation) have become since treatment with the study medication started? Almost the same, hardly better at all A little better Somewhat better Moderately better A good deal better A great deal better A very great deal better		How much worse would you say your reflux symptoms (e.g. heartburn or regurgitation) have become since treatment with the study medication started? Almost the same, hardly worse at all A little worse Somewhat worse Moderately worse A good deal worse A great deal worse A very great deal worse
How important is this improvement to you in carrying out your daily activities (e.g. work outside the home, housework, normal physical activities, sport, leisure activities, etc.)?		How important is this deterioration to you in carrying out your daily activities (e.g. work outside the home, housework, normal physical activities, sport, leisure activities, etc.)?

The OTE was developed from the Global Ratings of Change Questionnaire (GRCQ) with the permission of McMaster University.

Not important		Not important
Slightly important		Slightly important
Somewhat important		Somewhat important
Moderately important		Moderately important
Important		Important
Very important		Very important
Extremely important		Extremely important

The OTE was developed from the Global Ratings of Change Questionnaire (GRCQ) with the permission of McMaster University.