AstraZeneca

Amended Clinical Study Protocol	
Drug Substance	AZD1656
Study Code	D1020C00018
Edition Number	1
Date	

A Randomised, open, two-way Cross-over, Phase I Study to Evaluate the Response to Glucagon versus the spontaneous counter-regulatory response in T2DM Patients treated with AZD1656 and Metformin during hypoglycemia

Sponsor: AstraZeneca AB, 151 85 Södertälje, Sweden

AstraZeneca Research and Development site representative

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

Amendment No.	Date of Amendment	Local Amendment No.	Date of local Amendment
1			

Administrative change No.	Date of Administrative Change	Local Administrative change No.	Date of local Administrative Change
1			

PROTOCOL SYNOPSIS

A Randomised, open, two-way Cross-over, Phase I Study to Evaluate the Response to Glucagon versus the spontaneous counter-regulatory response in T2DM Patients treated with AZD1656 and Metformin during hypoglycemia

Principal Investigator

Dr. Marcus Hompesch

Study centre(s) and number of subjects planned

The study will be conducted at one single centre and 8 patients with type 2 diabetes (T2DM) will be randomised.

Study period		**Phase of development**
Estimated date of first subject enrolled	Q1 09	I
Estimated date of last subject completed	Q2 09	

Objectives

The primary objective of the study is to compare the recovery from hypoglycaemia in a fasting state induced by a single oral dose of AZD1656 in T2DM patients on Metformin treatment as induced by treatment with intramuscular glucagon versus spontaneous counter regulatory (CR) response. The recovery from hypoglycaemia is primarily assessed by the difference in plasma glucose levels between glucagon treatment and the control situation. As secondary pharmacodynamic variables time to recovery is measured.

Secondary objectives are:

- To describe safety and tolerability of AZD1656 in T2DM patients
- To evaluate pharmacokinetic (PK) properties of AZD1656
- To assess the response in C-peptide and insulin during hypoglycemia in T2DM patients

Exploratory objectives are:

- To assess the CR response (norepinephrine, epinephrine, glucagon, cortisol and growth hormone) during hypoglycemia in T2DM patients treated with Metformin
- To collect and store deoxyribonucleic acid (DNA) for future exploratory research into genes that may influence drug response ie, distribution, safety, tolerability and efficacy of AZD1656 treatment

Study design

The total study period is approximately 4-8 weeks and includes four visits to the clinic.

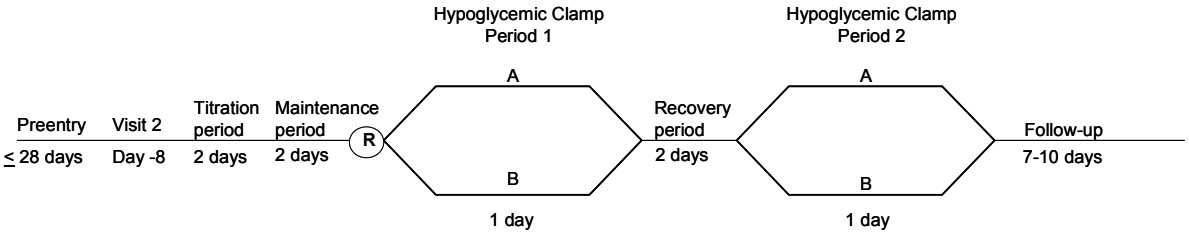
A pre-entry visit (Visit 1) will take place within 28 days before randomisation. If the subjects are eligible to enter the study they will come to the clinic 8 days (Day –8, Visit 2) before Visit 3 to switch to the Metformin provided by the centre. The Metformin treatment will be maintained until the end of the residential period, Visit 3. A follow-up visit (Visit 4) will take place 7-10 days after Visit 3.

Each subject will start their Visit 3 by entering a titration phase of two days, starting at 40 mg twice daily (bid) for one day and increase to 80 mg bid, if tolerated, the second day. Thereafter the tolerated dose, 40 or 80 mg bid, will be maintained for two more days.

The subjects will then (Day 5) be randomised to one of two treatment arms in a cross-over manner, where the full daily oral dose of AZD1656 will be given in the morning under fasting condition to induce hypoglycaemia. The single dose will be followed by either a subsequent injection of glucagon or spontaneous recovery from hypoglycaemia by the CR response depending on treatment arm.

After a recovery phase of two days (Day 6 and 7) where AZD1656 will be maintained at the same dose bid as Day 3-4, subjects will be subjected to a new period of controlled hypoglycaemia, see Figure S1.

Figure S1 Study design



A = Glucagon injection, 1 mg i.m.
B = Control, i.e. Spontaneous CR response

Plasma glucose, counter regulatory hormones and pharmacodynamic (PD) markers will be followed for 6 h post dose.

Target subject population

Men and non-fertile women with T2DM treated with Metformin will participate in the study. Ten subjects are planned to be randomised in order to get eight evaluable subjects.

Investigational product, dosage and mode of administration

AZD1656 will be administered twice daily (40 mg or 80 mg depending on tolerance), along with food, as an oral suspension on all days except Day 5 and 8, when the total daily dose (80 mg or 160 mg) will be administered as a single dose in the morning after an overnight fast. The AZD1656 formulation is oral suspension 25 mg/mL.

All subjects will keep their regular Metformin treatment (once daily [od] or bid), but switch to tablets provided by the site from Visit 2 through Visit 3.

On Day 5 and 8 subjects will or will not receive an intra muscular injection of 1 mg glucagon at 3 h post dose in a crossover manner.

Duration of treatment

Each subject will receive AZD1656 treatment for 8 days.

Outcome variable(s):

- Safety

  Adverse events, blood pressure, pulse, plasma glucose, safety laboratory variables and ECG

- Pharmacokinetics (PK)

  AUC0-24, C_{max} and $_{tmax}$ will be calculated for AZD1656 and its metabolite, AZ12555623

- Pharmacodynamics (PD)

  Plasma glucose level 20 minutes post release of the hypoglycaemic clamp, time to recovery from hypoglycaemia as well as plasma concentration values of glucose, insulin, C-peptide and the counter regulatory hormones norepinephrine, epinephrine, glucagon, cortisol and growth hormone

Statistical methods

The study data concerning safety variables, demographic variables and pharmacokinetic variables will be evaluated using descriptive statistics.

For the PD efficacy variables (glucose after 20 min and time to reach 3.5 and 5 mmol/L) confidence intervals (95%) will be presented. A mixed model ANOVA with fixed effects for sequence, period and treatment and a random effect for subject within sequence will be used..

All PD-variables will be presented with individual values and descriptive statistics by treatment (with and without glucagon).

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

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

Abbreviation or special term	Explanation
AE	Adverse Event (see definition in Section 7.3.1)
ALT	Alaninaminotransferas
AST	Aspartataminotransferas
AUC	Area under plasma concentration time curve from zero to infinity
AUC_{0-t}	The area under the plasma concentration versus time curve from time zero to the last quantifiable concentration (C_{last})
AZ	AstraZeneca
AZDD	AstraZeneca Drug Dictionary
Bid	Twice daily
BP	Blood Pressure
CK	Creatinkinase
CL/F	Apparent oral clearance
C_{max}	Maximum plasma drug concentration
CNS	Central Nervous System
CR	Counter Regulatory
CRF	Case Report Form (electronic/paper)
CRO	Clinical Research Organisation
CSA	Clinical Study Agreement
CSP	Clinical Study Protocol
CSR	Clinical Study Report
CYP450	Cytochrome P450, liver enzyme
CV	Coefficient of variation
DAE	Discontinuation due to Adverse Event
DCCT	Diabetes Control and Complications Trial
DNA	Deoxyribonucleic acid
DPP-4	Dipeptidyl peptidase-4
EC	Ethics Committee, synonymous to Institutional Review Board (IRB) and Independent Ethics Committee (IEC)
ECG	Electrocardiogram

Abbreviation or special term	Explanation
eCRF	Electronic Case Report Form
FPG	Fasting Plasma Glucose
FSH	Follicle Stimulating Hormone
GAD	Glutamic Acid Decarboxylate
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
GH	Growth Hormone
GK	Glucokinase
GKA	Glucokinase Activation
GLP-1	Glucagon-Like-Peptide-1
GMP	Good Manufacturing Practice
GRand	Global Randomisation system
HbA1c	Glycated haemoglobin A1c
HIV	Human Immunodeficiency Virus
IB	Investigational Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IP	Investigational Product
ISF	Investigator Study File
JSAD	Japanese Single Ascending Dose
LIMS	Laboratory Information Management System
LLOQ	Lower Limit of Quantification
MAD	Multiple Ascending Dose
MedDRA	Medical Dictionary for Regulatory Activities
MDRD	Modification of diet in renal disease
OAE	Other Significant Adverse Event (ie, , adverse events of particular clinical importance, other than SAEs and those AEs leading to discontinuation of the healthy volunteer from study treatment; see definition in Section 12.1.1
Od	Once daily
OTC	Over The Counter
pCRF	Paper Case Report Form
PD	Pharmacodynamic

Abbreviation or special term	Explanation
PI	Principal Investigator
PK	Pharmacokinetic
RA	Regulatory Authorities
SAD	Single Ascending Dose
SAE	Serious Adverse Event (see definition in Section 7.3.2).
SD	Standard Deviation
SDT	Study Delivery Team
SDV	Source Data Verification
SMF	Study Master File
SU	sulfonylureas
T2DM	Type 2 diabetes mellitus
$t_{1/2}$	Elimination half-life
t_{max}	Time to reach peak or maximum concentration or maximum response following drug administration
ULN	Upper Limit of Normal

1. IMPORTANT MEDICAL PROCEDURES TO BE FOLLOWED BY THE INVESTIGATOR

1.1 Medical emergencies and AstraZeneca contacts

The principal investigator(s) (PI) is responsible for ensuring that procedures and expertise are available to handle medical emergencies during the study. **A medical emergency usually constitutes an Serious Adverse Event (SAE) and is to be reported as such, see Section 7.3.4**.

In the case of a medical emergency the investigator may contact the Physician responsible for the protocol at the AstraZeneca (AZ) Mölndal.

Name	Role in the study	Address & telephone number

1.2 Overdose

At present, a dose of AZD1656 in excess of that planned according to the protocol, is to be considered an overdose.

There is no experience regarding an overdose of AZD1656. In case of known or suspected overdose symptomatic treatment as well as monitoring of vital functions should be performed according to routine clinical practice. Plasma glucose should always be monitored and low levels of glucose should be treated with oral or intravenous glucose to counteract hypoglycaemia as prolonged periods of hypoglycaemia may have detrimental effect on central nervous system (CNS), peripheral nerves and other tissues.

In order to collect more information concerning excessive doses of AZD1656, a drug intake fulfilling the overdose definition needs to be reported to AZ as an overdose, regardless of clinical consequences.

- An overdose with associated Adverse Events (AEs) is recorded as the AE diagnosis/symptoms on the relevant AE modules in the Case Report Form (CRF) and on the Overdose CRF module
- An overdose without associated symptoms is only reported on the Overdose CRF module

Overdoses with non-AZ products should be handled according to the label.

1.3 Pregnancy

Women of childbearing potential are not allowed to be included in this study and male patients must refrain from fathering a child, including sperm donation, during the study and three months following the last dose. If the investigator receives information that a pregnancy has occurred during the study (ie, a pregnancy in the partner of a male patient) despite these restrictions, the AZ representative should be informed.

2. INTRODUCTION

The aim of this study is to assess the effect of exogenous glucagon on the glycaemic recovery from pronounced hypoglycaemia in patients with type 2 diabetes mellitus (T2DM) treated with AZD1656 on top of Metformin. Patients with T2DM will be randomised to alternate exogenous glucagon or spontaneous recovery in an open, two-way cross-over design using a glycaemic clamp with stepwise lowering of plasma glucose levels.

2.1 Background

Currently, there are five different classes of oral antidiabetic drugs available on the market sulfonylureas (SU), biguanides (metformin), alpha glucosidase inhibitors, thiazolidinediones and just recently glucagon-like peptide-1 (GLP-1) analogues and GLP-1 providers (dipeptidyl peptidase-4 [DPP-4] inhibitors) have been launched. Despite different mechanisms of action with potent glucose lowering effects, current agents do not provide optimal treatment for patients with T2DM. Each of the existing therapies is associated with adverse effects on T2DM itself (ie, weight gain and β-cell dysfunction) and/or has adverse effects that limit the use. There is therefore a rationale to develop oral products with new mechanisms of action and more favourable benefit/risk profiles.

Glucokinase (GK) is present in liver and pancreatic β-cells and catalyses the conversion of glucose to glucose 6-phosphate. GK can be regarded as a glucose sensor and is rate limiting for glucose uptake and utilisation in pancreatic β-cells, where it plays a major role in regulating insulin secretion. GK is also present in liver parenchymal cells (hepatocytes), where it regulates hepatic glucose utilisation. Defects in these two processes significantly contribute to the development of hyperglycaemia in T2DM. AZD1656 is a new potent glucose kinase activator (GKA). Due to its dual-compartment mode of action, AZD1656 has the potential to provide superior glycaemic control relative to existing oral agents. There are as yet no products targeting activation of GK on the market.

AZD1656 has in pre-clinical studies been shown to be a potent activator of rat and human GK in vitro. Preliminary data from the ongoing single ascending dose (SAD) study in healthy males (D1020C00001), Japanese single ascending dose (JSAD) study in healthy males (D1020C00003) and the multiple ascending dose (MAD) study in patients with T2DM (D1020C00002) have shown that AZD1656 is well tolerated and a dose-dependent glucose lowering effect has been seen in healthy volunteers during euglycemic clamp.

Type 1 and 2 diabetic patients with severe hypoglycemia are treated with glucose injections or intra muscular injections with the pancreas hormone glucagon. The latter treatment is crucial for out of hospital events and is often used by bystanders (relatives) as the first treatment when the patient is unconscious. Glucagon acts quickly on the liver and converts stored glycogen into glucose which subsequently is released into the bloodstream. A GKA may interfere with this process due to its effect on liver by increasing available glucose 6-phosphate and thereby the substrate for glycogen production.

Preliminary data from the global SAD (D1020C00001) and MAD (D1020C00002) studies have demonstrated that AZD1656 is rapidly absorbed and maximum concentration (C_{max}) is reached within 1 hour. The compound is rapidly eliminated with a half-life ($t_{1\2}$) of approximately 3-4 hours. AZD1656 is metabolised to the active metabolite AZD12555623. The mean exposure to the metabolite is approximately 20% compared to AZD1656. The C_{max} of the metabolite is reached at the same time as for the parent compound. The initial AZD12555623 decline is parallel to AZD1656 decline but a slower terminal elimination phase is evident. In rats, the exposure to the active metabolite AZD12555623 is approximately 2 fold higher compared to the exposure to AZD1656.

AZ intends to perform genetic research in the AZD1656 clinical development programme to explore how genetic variations may affect the clinical parameters associated with AZD1656.

2.2 Research hypothesis

The primary research hypothesis is that severe hypoglycemia in T2DM patients treated with AZD1656 is counteracted more effectively with intra muscular injections with the pancreas hormone glucagon as compared to spontaneous recovery in plasma glucose induced by counter regulatory response (norepinephrine, epinephrine, glucagon, cortisol and growth hormone).

2.3 Rationale for conducting this study

This study is performed to investigate the response to exogenous glucagon in T2DM patients treated with AZD1656. Such knowledge is valuable early in the clinical program since intramuscular glucagon is an important emergency treatment of severe hypoglycaemia. Enrolled subjects will be T2DM patients treated with Metformin, an important future target population.

2.4 Benefit/risk and ethical assessment

The subjects will be hospitalised during the study in order to carefully monitor glucose levels, achieve maximal tolerated dose of AZD1656 given twice-daily (bid) and avoiding spontaneous hypoglycaemia.

The dose will be titrated over two days up to the maximal tolerated dose, where it will be maintained for another two days. Giving the maximally tolerated bid dose of AZD1656 as a single dose after an overnight fast will induce hypoglycaemia in both treatment arms (Day 5 and Day 8). Insulin may be needed as a supplement in order to achieve stable glucose levels during these study days.

A glycaemic clamp with a continuously titrated glucose and/or insulin infusion (via an automated glucose clamp device, Biostator®) will be used to allow a controlled step-wise lowering of plasma glucose, and the plasma glucose level will not be allowed to be lower than 2.7 mmol/L

The recovery of plasma glucose will be measured with and without intramuscular glucagon. The Biostator® will also be used to guarantee a safe recovery of the plasma glucose levels.

In the ongoing SAD study (D1020C00001), single doses up to 180 mg AZD1656 have been given to healthy volunteers during euglycemic clamp and in the ongoing MAD study (D1020C00002) multiple oral doses of up to 80 mg AZD1656 have been given bid to patients with T2DM. These ongoing studies have not raised any safety concern.

All of the significant adverse findings seen in the toxicology studies are considered to be secondary to hypoglycaemia and/or relatively minor, reversible or can be monitored in clinical studies.

Careful monitoring regarding symptoms or laboratory abnormalities indicative of any adverse effects, including muscle symptoms, creatinekinase (CK) and aspartataminotransferas (AST) will be observed during the entire study.

Blood samples for deoxyribonucleic acid (DNA) extraction will be taken (optional) and DNA will be saved for potential future research into genes that may influence response to AZD1656.

There are no direct benefits for the subjects participating in this study.

3. STUDY OBJECTIVES

3.1 Primary objective

The primary objective of the study is to compare the recovery from hypoglycaemia in a fasting state induced by a single oral dose of AZD1656 in T2DM patients on Metformin treatment as induced by treatment with intramuscular glucagon versus spontaneous counter regulatory (CR) response. The recovery from hypoglycaemia is primarily assessed by the difference in plasma glucose levels between glucagon treatment and the control situation. As secondary pharmacodynamic variables time to recovery is measured.

3.2 Secondary objectives

- To describe safety and tolerability of AZD1656 in T2DM patients
- To evaluate pharmacokinetic (PK) properties of AZD1656
- To assess the response in glucose, C-peptide and insulin during hypoglycemia in T2DM patients

3.3 Exploratory objective

- To assess the CR response (norepinephrine, epinephrine, glucagon, cortisol and growth hormone) during hypoglycemia in T2DM patients treated with Metformin

- To collect and store DNA for future exploratory research into genes that may influence drug response ie, distribution, safety, tolerability and efficacy of AZD1656 treatment

4. STUDY PLAN AND PROCEDURES

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

4.1 Overall study design and flow chart

In total, 10 T2DM patients treated with Metformin, aged ≥18 to ≤75 years, will participate in the study to ensure 8 evaluable patients, hereafter called subjects.

The total study period is approximately 4-8 weeks and includes four visits to the clinic, see Figure 1.

Visit 3, treatment/residential period

Each subject will enter a titration phase of two days, with 7-point plasma glucose monitoring, starting at 40 mg bid for one day and increased to 80 mg bid, if tolerated, the second day. Thereafter the tolerated dose, 40 or 80 mg bid, will be maintained for two more days.

The subjects will then (Day 5) be randomised to one of two treatment arms in a cross-over manner, where the full daily oral dose of AZD1656 will be given in the morning under fasting condition to induce hypoglycaemia. The treatment sequence will be given in the randomisation list. The single dose will be followed by either a subsequent injection of glucagon or spontaneous recovery from hypoglycaemia by the CR response depending on treatment arm.

After a recovery phase of two days (Day 6 and 7) where AZD1656 will be maintained at the same dose bid as Day 3-4, subjects will be subjected to a new period of controlled hypoglycaemia followed by alternate treatment as to the previous hypoglycaemic study period, see Figure 1.

On Day 5 and 8, after an overnight fast, the full daily dose from Day 3-4 (80 mg or 160 mg) will be given as a single oral dose (time 0 min). Blood samples for glucose and hormone analyses will be drawn from a catheter in a cubital vein. Another vein will be used for infusion of insulin and/or glucose, controlled by the Biostator®, in order to reach glycaemic plateaus of 5 mmol/L (0 to 60 min), 4 mmol/L (60 to 90 min), and 3.2 mmol/L (90 to 150 min). Plasma glucose will then be clamped to a target nadir of 2.7 mmol/L and released at 180 min, ie, insulin infusion will be stopped, see Figure 3.

The fasting state will be maintained 270 min after dosing. Plasma glucose will be followed continuously. When plasma glucose has been clamped aiming at 2.7 mmol/L for 30 minutes (ie, 180 min after dose), the clamp will be released and either 1 mg glucagon will be

administered intra muscular or a spontaneous recovery in glucose levels will be awaited. After the release of the hypoglycaemic clamp, the Biostator® will continue to follow glucose on a minute by minute basis at least until two consecutive plasma glucose values are obtained at or above 5.0 mmol/L without concomitant glucose infusion. The Biostator's glucose analyzer will be re-calibrated in regular intervals by means of a YSI STAT2300 glucose analyzer throughout the clamp procedure, at least every 10-15 minutes.

Ninety minutes past clamp release (4.5 h post dose), the subjects will be served a meal with food ad libitum. Plasma glucose, counter regulatory hormones and pharmacodynamic (PD) markers will be followed for 3 hours after the clamp release (6 h post dose).

In order to avoid prolonged hypoglycaemia, a reversed clamp will be applied at 240 minutes post dosing with AZD1656. A glucose infusion will be used as needed to achieve a minimum plasma glucose level of 3.2 mmol/L at 240-270 min, 4.0 mmol/L at 270-300 min and ≥5.0 mmol/L after 300 min post dosing with AZD1656. The Biostator® will be disconnected at 360 min post dosing with AZD1656 provided that two consecutive plasma glucose values of at least 5.0 mmol/L are measured without ongoing glucose infusion. Plasma glucose values will be monitored also during the recovery period, Day 6-7, and in the morning on the day of discharge (Day 9), see Table 4.

A pre-entry visit will take place within 28 days before randomisation, Visit 1. If the subjects are eligible to enter the study they will come to the clinic 8 days (Day –8, Visit 2) before Visit 3 to switch to the Metformin provided by the centre and to check their fasting glucose level. The Metformin treatment will be maintained until the end of the residential period, Visit 3. A follow-up visit (Visit 4) will take place 7-10 days after Visit 3.

4.1.1 Titration procedure

The dose of AZD1656 will be individually titrated stepwise provided that the glucose values of the subject are within acceptable limits. The decision to increase the dose on study Day 2 (dose titration) and the decision to continue on the individual tolerated dose on study Day 3-4 will be based on the morning fasting glucose on the actual day and the post-prandial glucose values from the previous day (Figure 2). After Day 2 no further dose increases are allowed, but down-titration to 40 mg bid may be needed. Study specific discontinuation criteria applies for hypoglycaemia or pronounced hyperglycaemia as described in Section 5.4.3.

4.1.2 Rescue treatment – Low plasma glucose outside titration period

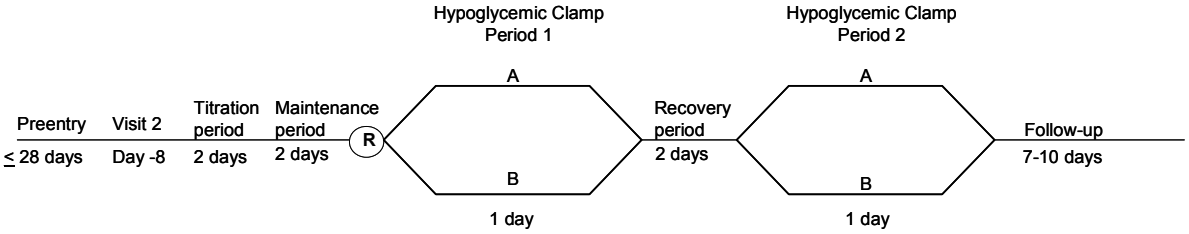
The following section applies during the residential period of the study (Visit 3), excluding 0-6 hours post dosing with AZD1656 on study Day 5 and 8.

If the subject experiences hypoglycaemic symptoms, a plasma glucose measurement should be obtained (before any treatment) and the subject should be treated according to local practice. For subjects with hypoglycaemia, defined as P Glucose <2.5 mmol/L (45 mg/dL), approximately 25 g glucose (juice) orally should be given as rescue treatment. Intravenous glucose could also be considered according to the judgement of the investigator.

If recurrent hypoglycaemia (defined as P-Glucose <2.5 mmol/L [45 mg/dL]), or hypoglycaemia that is difficult to cure, the subject should be discontinued from the study, Section 5.4.3.

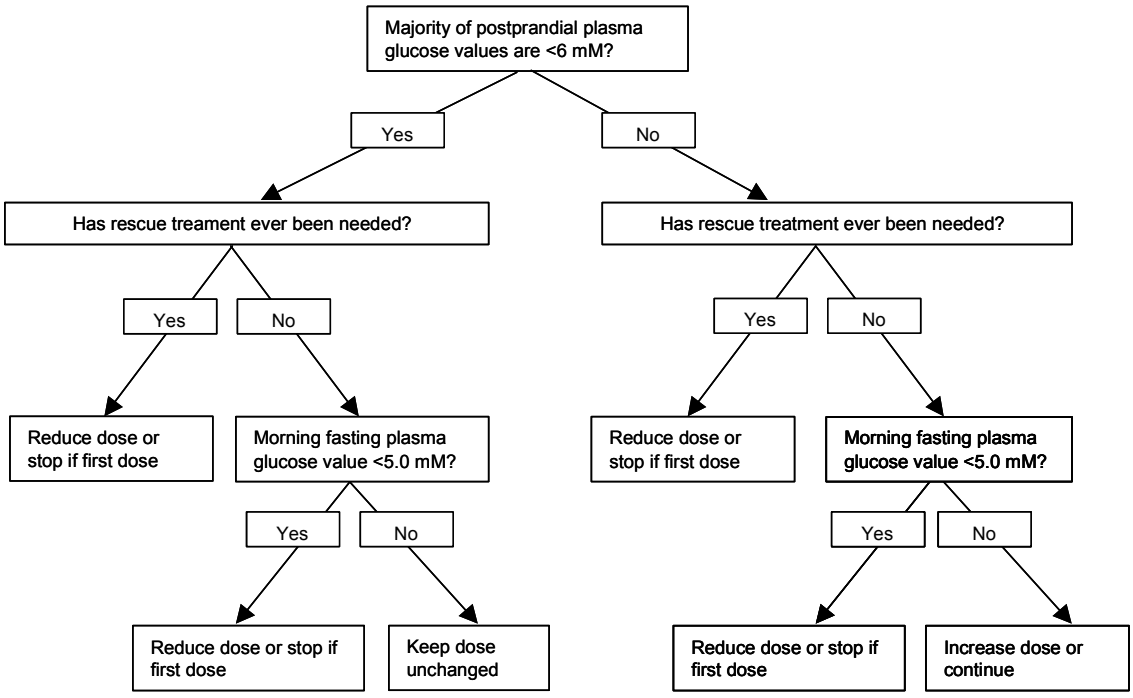
If rescue treatment is needed during titration of investigational product (IP), the dose of AZD1656 should be reduced, see Figure 2. If rescue treatment is needed already after the initial dose the subject should be discontinued from the study.

Figure 1 Study design



A = Glucagon injection, 1 mg i.m.
B = Control, i.e. Spontaneous CR response

Figure 2 Dose titration and glucose value



Note: the titration period is limited to Day 1 and Day 2 for each subject. On Day 3 the dose will be kept or reduced if needed.

Figure 3 Overview step wise hypoglycaemic clamp

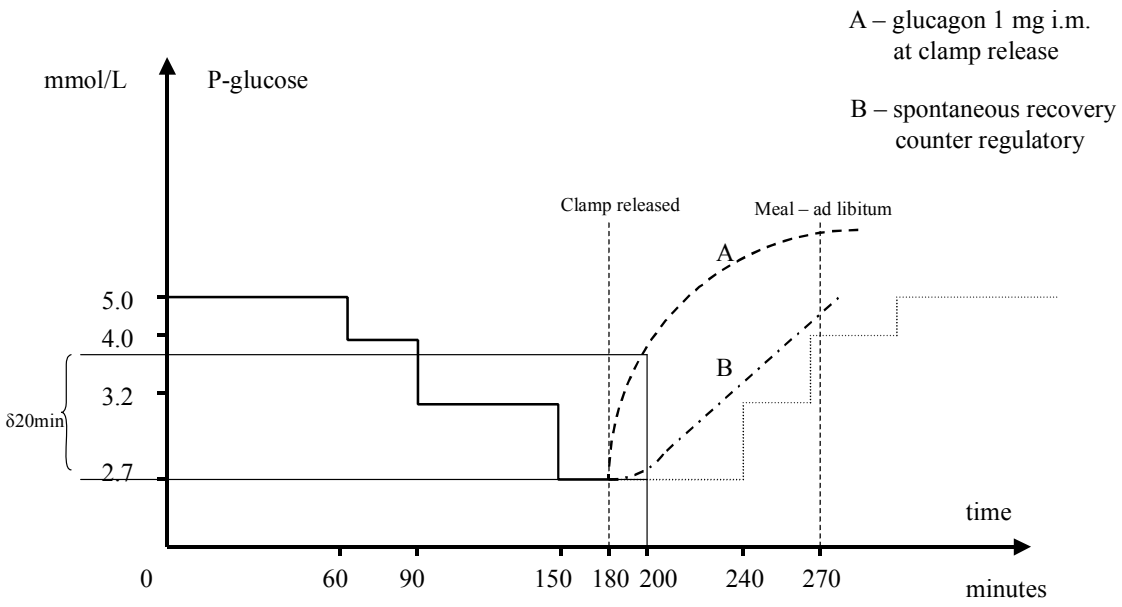


Table 1 Study plan

Event	Enrolment	Metfor-min treatment	Titration period	Maintenance period	Hypo-glycemic Clamp period 1	Recovery period	Hypo-glycemic Clamp period 2	Follow-up
Visit	**1**	**2**	**3**	**3**	**3**	**3**	**3**	**4**
Study Day	**≤28 days before rando-misation**	**Day -8**	**D1-D2**	**D3-D4**	**D 5**	**D 6-7**	**D 8**	**7-10 days after Visit 3**
Informed consent	X							
Inclusion/exclusion criteria	X				X			
Medical and surgical history including T2DM history	X							
Physical examination	X							X
Ophtamologic examination[a]	X							
Laboratory screening	X[b]		X[c]		X[c]	X[c]	X[c]	X
C-peptide, HbA1c & GAD antibodies	X							

Event	Enrolment	Metfor-min treatment	Titration period	Maintenance period	Hypo-glycemic Clamp period 1	Recovery period	Hypo-glycemic Clamp period 2	Follow-up
Visit	**1**	**2**	**3**	**3**	**3**	**3**	**3**	**4**
Study Day	**≤28 days before rando-misation**	**Day -8**	**D1-D2**	**D3-D4**	**D 5**	**D 6-7**	**D 8**	**7-10 days after Visit 3**
Body weight/height[d]	X							X
Drug screen test	X							
Change to clinic provided Metformin treatment		X						
Arrival at clinic			X					
Randomisation					X			
7-point glucose (bedside)		X[e]	X	X		X		
24 h glucose[f]					X		X	
ECG/BP/pulse	X		X[g]	X[g]	X[g]	X[g]	X[g]	X
Genetic blood sample (optional)					X			
Telemetry					X		X	
Administration of IP			X	X	X	X	X	
Standardised meals			X	X		X		
Hypoglycemic clamp					X		X	
PK/PD/CR blood sampling					X[h]		X[h]	
AE recording[i]	X	X	X	X	X	X	X	X

[a] Excluded for subjects who have done such examination within 3 months from study start.
[b] Also includes HIV, Hepatitis B and C test and for women FSH unless age >50 years and 2 years without menstruation periods.
[c] Laboratory screening will be done pre dose on Day 1, 5, 6, 8 and morning day 9 (24 hours after last dose).
[d] Height only measured at Visit 1.
[e] Only morning sample.
[f] Time points specified in Table 2.
[g] ECG, BP and pulse will be recorded pre dose on Day 1, 4 and 7 and pre dose and 12 h post dose on Day 5 and Day 8 (see Table 2).
[h] PK, PD and CR blood sampling will be done repeatedly during the 24-hour period after dose (see Table 2).
[i] Adverse Events will be collected from the time when informed consent is obtained until the end of the study (including the follow-up visit). Before the time of the first administration of investigational product only serious adverse events need to be collected.

Table 2 Time schedule during Day 5 and Day 8

Protocol Time	CR/PD	ECG/ BP/ Pulse	Safety lab screen	24 h Glucose (bedside)	PK	Other
Pre-dose	X	X	X	X		Start telemetry
0000						Adm of IP and start Clamp (Biostator)
0015				X	1	
0030				X	2	
0045				X		
0100	X			X	3	
0130	X			X	4	
0200	X			X	5	
0230	X			X		
0245	X					
0300	X			X		Clamp release and Glucagon injection
0315				X		
0320				X		
0330	X			X		
0345				X		
0400	X			X	6	Reversed clamp[a]
0430	X			**X**		Meal[b]
0500	X			X		Normalised P-glucose[c]
0600	X			X		Stop telemetry
1200		X			7	
2400				X	8	

a Minimal Plasma glucose value increased from 2.7 to 3.2 mmol/L.
b Minimal plasma glucose value increased from 3.2 to 4.0 mmol/L.
c Minimal plasma glucose value increased from 4.0 to ≥5.0 mmol/L.

Table 3 Time schedule during Day 1, 2, 3, 4, 6, 7 and 9[a]

Protocol Time	Glucose sampling (bedside)	ECG/ BP/ Pulse	Safety lab screen	Other
Pre-dose	X	X[b]	X[c]	
0000				Adm of IP followed by breakfast
0200	X			Snacks
0400	X			Lunch
0600	X			Snacks
0900	X			Adm of IP followed by dinner
1200	X			Evening meal
1400	X			At bedtime

[a] Day 9 only includes pre dose safety lab screen and one glucose sampling after breakfast, thereafter discharge from clinic.
[b] Only on Day 1, 4 and 7
[c] Only on Day 1, 6 and 9

4.2 Rationale for study design, doses and control groups

The study will be carried out in subjects with T2DM to allow for results applicable in the target population ie, subjects with T2DM failing on the first line of treatment, Metformin. Before proceeding into larger studies, the availability and security of effective treatment for severe hypoglycaemia are important to define. The CR response, safety aspects and recovery after hypoglycaemia are being assessed in healthy volunteers with unaffected insulin secretion (ie, highly responsive to GK stimulation). The current study will provide important complementary information on these aspects in T2DM patients as well as a comparison between spontaneous recovery from hypoglycaemia with recovery induced by administration of the pancreatic hormone glucagon.

Each subject with T2DM will receive AZD1656 at maximal tolerated dose on top of their normal dose of Metformin. In both hypoglycaemic clamp periods, giving the total daily dose of AZD 1656 and the normal morning dose of Metformin, will induce hypoglycaemia after an overnight fast. For safety reasons as well as to guarantee comparable degrees of hypoglycaemia in both study groups, a hypoglycaemic clamp method will be applied where glucose and insulin will be infused as required in response to a continuous plasma glucose measurement by an automated device, the Biostator®. Thereby, plasma glucose levels will be decreased in a step-wise controlled fashion and severe hypoglycaemia with plasma glucose 2.7 mmol/L will not be allowed.

The study subjects will be randomised to receive either an intramuscular dose of glucagon or to participate in a control arm where the recovery from hypoglycaemia is dependent on the CR response. Subjects will then crossover to the reverse treatment in the second hypoglycaemic period after a two days recovery period when the same dose of AZD1656 will be given as during Day 3 and 4 to ensure similar physiological conditions both treatment periods.

A follow-up visit will be conducted within 7 to 10 days after the last dose to assure that all study subjects have a stable glycaemic control and are otherwise healthy leaving the study.

This study is open, thus no blinding of treatment will be done. The primary variable will be assessed by investigation of laboratory variables and the secondary variables will be measurements of time to recovery from hypoglycaemia, which results are independent of blinding. Furthermore, in this crossover design the T2DM study subjects are their own controls.

The daily AZD1656 dose, 80 mg or 160 mg, is within the dose span studied in the early clinical development program for AZD1656.

To challenge the CR response, it is of utmost importance that the selected dose AZD1656 results in a plasma glucose nadir of 2.7 mmol/L, a glucose level that induces a CR response in hormones (D0280C00003). In order to increase the likelihood of reaching this nadir, all study subjects will start at a AZD1656 dose of 40 mg bid and will be titrated to 80 mg bid if tolerated as defined in the protocol. If administration of the highest tolerated total daily dose of AZD1656 as a single dose is insufficient for inducing the intended reduction of plasma glucose, insulin infusion will be used as needed. As a safety measurement, a glucose infusion will be continuously titrated to avoid prolonged and severe hypoglycaemia.

5. SUBJECT SELECTION CRITERIA

Subject population should be selected without bias.

Investigator(s) must keep a record of subjects who entered pre-trial screening but were never enrolled eg, subject screening log. Each subject must 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.

5.1 Inclusion criteria

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

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

2. Men or women of non-childbearing potential (postmenopausal, and/or have undergone hysterectomy and/or bilateral oophorectomy or salpingectomy/ tubal ligation) aged ≥18 to ≤75 years. Women will be defined as postmenopausal if last menstruation period was >1 year ago and serum follicle stimulating hormone (FSH)

is within the postmenopausal range, or if age >50 years and with last menstruation period >2 years ago

3. Body mass index between ≥19 and ≤40 kg/m^2

4. T2DM diagnosis confirmed by C-peptide >0.3 nmol/L and no glutamic acid decarboxylate (GAD) antibodies at enrolment (screening)

5. Treatment with Metformin alone with a total daily dose not less than 1000 mg. Stable glycaemic control indicated by unchanged treatment within 3 months prior to enrolment

6. HbA1c ≤9,5% at enrolment (HbA1c value according to international Diabetes Control and Complications Trial [DCCT] standard)

7. Subjects should have a fasting plasma glucose (FPG) in the range of 6,0 to 11,0 mmol/L) at screening

5.2 Exclusion criteria

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

1. Participation in another clinical study during the last 30 days prior to enrolment

2. History of ischemic heart disease, symptomatic heart failure, stroke, transitory ischemic attack or symptomatic peripheral vascular disease

3. Positive test for Hepatitis B surface antigen or antibodies to human immunodeficiency virus (HIV) or antibodies to Hepatitis C virus

4. AST or alanine aminotranferease (ALT) laboratory results >3xULN

5. History of psychiatric or somatic disease/condition (eg, gastrointestinal disease) that may interfere with the objectives of the study, as judged by the investigator

6. Signs of diabetic proliferative retinopathy or diabetic maculopathy, at screening or on an ophthalmological examination within 3 months from start of study

7. History or ongoing symptoms/signs of severe allergy/hypersensitivity as judged by the investigator

8. History of malignancy within the last 5 years, excluding successful treatment of basal or squamous cell skin carcinoma or in-situ carcinoma of the cervix

9. Impaired renal function in terms of Glomerular Filtration Rate <60 ml/min, based on Modification of diet in renal disease (MDRD)study calculation (Levey et al 2003)

10. Past or present alcohol or drug abuse within the last 5 years or excessive intake of alcohol of greater than 15 units/week or positive test in drugs of abuse screens

11. Systolic blood pressure (BP) >160 mmHg or diastolic BP >95 mmHg at screening

12. Any ongoing oral anti-diabetic treatment apart from Metformin within 8 weeks prior to randomisation

13. Changes in any current medication (initiation, dose change or cessation), within the last 4 weeks prior to randomisation. The criterion does not apply to medication prescribed for occasional use

14. Use of insulin, glitazones, warfarin, amiodarone within 3 months prior to enrolment (screening) and use of potent Cytochrome 450 (CYP450) inhibitors, eg, ketoconazole and macrolide antibiotics within 14 days before randomisation

15. Use of anabolic steroids and systemic treatment with glucosteroids within 3 months before enrolment (inhaled and local treatment allowed)

16. Blood loss in excess of 450 mL 3 months prior to enrolment

17. Intake of another investigational drug within 30 days (or at least five t1/2 of the drug) before the first administration of the IPs

18. Previous randomisation to treatment in the present study

19. Previous intake of the IP tested in this study, AZD1656

20. Current smokers who smoke more than 10 cigarettes per day (or equivalent use of tobacco products) or cannot give up smoking for duration of in-patient period

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

22. Subject has a sibling participating in the same study

23. Clinically significant neuropathy according to the investigator

24. Suspected or confirmed poor protocol or medication compliance as judged by the investigator

25. Any clinically significant abnormality identified on physical examination, laboratory tests or electrocardiogram (ECG), which in the judgment of the investigator would compromise the patients' safety or successful participation in the clinical study

The following is regarded as criteria for exclusion from the genetic component of the study:

1. Previous bone marrow transplant

2. Whole blood transfusion within 120 days of the date of genetic sample collection

5.3 Procedures for handling incorrectly included subjects

Subjects that do not meet the inclusion/exclusion criteria for a study should not, under any circumstances, be enrolled into the study– there can be no exceptions to this rule.

Where subjects that do not meet the study criteria are enrolled in error, incorrectly randomised, or where subjects subsequently fail to meet the criteria for the study post enrolment, the procedures included in the protocol for the discontinuation of such subjects must be followed. These procedures must be included in the protocol and must take into consideration ethical and safety factors and how these subjects will be treated in the analyses.

Once the error is identified a discussion must occur between the AZ SDT Physician and the Investigator regarding whether to continue or discontinue the subject from the study. The AZ SDT Physician is to ensure all such decisions are appropriately documented. In situations where an agreement cannot be reached, the subject should have their randomised therapy stopped and be discontinued from the study.

5.4 Withdrawal of subjects

5.4.1 Criteria for discontinuation from the study

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

- Voluntary discontinuation by the subject who is at any time free to discontinue his/her participation in the study, without prejudice to further treatment
- Risk to subjects as judged by the investigator and /or AZ
- Severe non-compliance to protocol as judged by the investigator and/or AZ
- Incorrectly enrolled subjects
- Subject lost to follow-up
- Adverse Events
- Withdrawal of informed consent to the use of biological samples collected as an integral part (including the optional genetic sample) of the study, see Section 8.5

5.4.2 Procedures for discontinuation of a subject from the study

A subject that discontinues will always be asked about the reason(s) for discontinuation and the presence of any AE. If possible, they will be seen and assessed by an investigator(s). AEs will be followed up (See Sections 7.3.3 and 7.3.4).

5.4.3 Study specific criteria for discontinuation

Events of hypoglycaemia (defined as plasma glucose <2.5 mmol/L (45 mg/dL), at 2 occasions or 1 event of hypoglycaemia that is difficult to cure despite glucose administration (according to the judgement of the investigator) or prolonged hyperglycaemia (defined as 2 consecutive days with fasting morning plasma glucose >15 mmol/L (270 mg/dL) should lead to discontinuation of treatment and the event should be reported as AE.

6. STUDY CONDUCT

6.1 Restrictions during the study

Subjects will have to comply with the following restrictions:

1. Day 5 and 8: Fast (except for water) from 2200 (10 pm). Subject will be allowed to drink a moderate amount (<500 mL) of water from midnight until morning dose of IPs

2. Abstain from tobacco/nicotine-containing substances and refrain from alcohol within 12 hours prior to and during the clinic visit

3. Abstain from blood and plasma donation during the study and up to 3 months after completion of the study

4. The subjects should neither start any new physical training nor increase the intensity of their usual physical training during the study (from pre entry to follow up)

5. Male subjects should use appropriate contraceptive (double barrier method or vasectomy) from the 1st administration of the IP and refrain from sperm donation until at least 3 months after the last dose, since no data on male reproduction is available

6. Subjects should eat standard, BMI corrected, weight maintained diet during the residential period, except from day 5 and 8

6.2 Subject enrolment and randomization

The PI will:

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

2. Assign potential subject a unique enrolment number, beginning with "E0001001"

3. Determine subject eligibility. See Sections 5.1 and 5.2

4. Assign eligible subject unique randomization code (subject number), beginning with "101"

Randomisation codes will be assigned strictly sequentially as subjects are eligible for randomisation.

If a subject discontinues participation in the study, then his/her enrolment/randomisation code cannot be reused.

If subjects have discontinued their participation in the study then they cannot re-enter into the study.

6.2.1 Procedures for randomization

A randomisation scheme will be computer generated by AZ R&D Mölndal using the global randomisation system (GRand).

The subjects will be randomised to the two treatment sequences AB and BA in equal proportion. (A = 1 mg Glucagon i.m; B = control, ie, spontaneous CR response). The randomisation will be performed in blocks.

A total of 10 subjects will be randomised to the two treatment arms.

6.3 Blinding and procedures for unblinding the study (Not Applicable)

6.4 Treatments

6.4.1 Identity of investigational product(s)

Investigational product	Dosage form and strength	Manufacturer	Formulation number
AZD1656	Oral Suspension 25 mg/mL	AstraZeneca R&D	H 2001-01-01

Oral suspension of AZD1656 will be prepared by AstraZeneca R&D, Sweden. Before dosing the AZD1656 suspension will be diluted if needed to obtain the correct dose volume according to the handling instructions. Purified water will be used as dilution media. AZD1656 25mg/mL oral suspensions will be supplied in vials of 20 mL containing 10 mL oral suspension as bulk supply. One vial of AZD1656 oral suspension can be used to more than one (1) subject. Further information will be available in the handling instructions that will accompany the IP.

6.4.2 Doses and treatment regimens

The Metformin treatment will be provided by the site 8 days before subjects start the treatment with AZD1656 (Visit 3). All subjects will be given the same generic commercial Metformin immediate release formulation (major brand). Commercial Metformin in original packages will be purchased locally by the Clinical Research Organisation (CRO) and will be labelled by the study site personnel.

The Metformin will be administered once or twice daily with the first dose in the morning. If the subject normally takes Metformin more than twice daily, half the total daily dose will be administered bid, with the first dose in the morning and the second dose in the evening. If the subject normally takes Metformin once daily the total daily dose will be administered in the morning.

The Metformin dose should be taken after IP in the morning (before breakfast) and, if applicable, before dinner (9 hours later), after the second daily dose of IP.

At Visit 3, Day 1, all subjects will start their AZD1656 treatment with a titration period where they will receive oral solution of AZD1656 40 mg bid for one day on top of their Metformin treatment. Day 2 they will receive 80 mg bid, and, if tolerated, continue with that dose for another 2 days or, if not tolerated, maintain the dose of 40 mg bid for another 2 days. On Day 5 each subject will be randomised to the two treatment regimens AB or BA in random order. On Day 5 and Day 8 the AZD1656 dose, the total daily dose of 80 mg or 160 mg, will be given as a single dose in the morning after an overnight fast.

The suspension will be administered with the subjects in an upright position and the container will be rinsed with 2x30 mL of tap water to collect the remainings of the study drug in the container. The subjects will then drink an extra glass (150 mL) of tap water.

The A treatment includes an intra muscular injection of 1 mg glucagon at 3 hours post dose. Glucagon will be purchased locally in commercially available packages by the CRO, and will be labelled by the study personnel.

During Day 6-7 the subjects will receive the same dose of AZD1656 oral solution bid as on Day 3 and 4.

Insulin solution for intravenous use during the Hypoglycemic Clamp will be locally purchased by the CRO, Profil.

6.4.3 Additional study drug

Metformin, see above, see Section 6.4.2.

6.4.4 Labelling

The bulk supply of AZD 1656 will be labelled at Investigational Products, AstraZeneca R&D, Wilmington according to Good Manufacturing Practice (GMP). The IP will be labelled with a

2-panel label. One part of the label will be permanently affixed to the vial and the other part will be a peal-off part for insertion into the Drug Accountability document.

6.4.5 Storage

All study drugs must be kept in a secure place under appropriate storage conditions. The IP label on the bottle and the Investigator Brochure (IB) specifies the appropriate storage and shipment.

6.5 Concomitant and post-study treatment(s)

No oral anti-diabetic treatment apart from a stable dose of Metformin and study drug is allowed from 8 weeks before enrolment until the follow up visit.

No changes (initiation, dose change or cessation) in any current medication (including over the counter [OTC] products) are allowed from 4 weeks prior to randomisation until the follow up visit (except for drugs prescribed for occasional use, eg, paracetamol and nasal spray for nasal congestion).

Insulin, glitazones, warfarin and amiodarone are not allowed from 3 months before enrolment until the follow up visit.

Potent CYP450 inhibitors, eg, ketoconazole and macrolide antibiotics are not allowed from 14 days before randomisation until the follow up visit.

Anabolic steroids and systemic treatment with glucosteroids are not allowed from 3 month before enrolment (inhaled and local treatment allowed) until the follow up visit.

Other medication, which is considered necessary for the subject's safety and well-being, may be given at the discretion of the investigator.

If changes in current medication are necessary, the investigator must decide if the subject should remain in study or be dismissed from study. The subjects must be instructed that no new additional medication will be allowed without the prior consent of the investigator.

6.6 Treatment compliance

The administration of all medication (including IP) must be recorded in the appropriate sections of the CRF.

Compliance will be assured by supervised administration of the IP by the investigator or his/her delegate.

6.6.1 Accountability

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

The study personnel will account for all drugs dispensed and returned.

The study personnel will account for all unused drugs and for appropriate destruction. Certificates of delivery, destruction and return must be signed.

7. COLLECTION OF STUDY VARIABLES

7.1 Recording of data

The investigator will ensure that all data collected in the study are provided to AZ. He/she ensures the accuracy, completeness, legibility and timeliness of the data recorded in the appropriate sections of the paper CRF (pCRF) or electronic CRF (eCRF) and according to any instructions provided.

The PI will provide AZ with all data produced during the study from the scheduled study assessments. He/she ensures the accuracy, completeness, legibility, and timeliness of the data reported to AZ in the pCRF and in all required reports.

7.2 Screening and demography procedures

Each subject will undergo an enrolment (screening) medical examination within 28 days prior to Visit 3. This will consist of:

- Recording of demographic data - date of birth, sex, height, weight, race
- A standard medical/surgical history and a physical examination including general appearance, skin, lymph nodes, thyroid, musculoskeletal/extremities, cardiovascular system, respiratory system, abdomen and a neurological examination (reflexes only)
- T2DM history (year of diagnosis, any diabetic complications, current diabetes treatment)
- An ophtamological examination if not done within 3 months
- A blood sample for standard clinical chemistry and haematology assessments, HbA1c, C-peptide and GAD antibodies and a mid-stream urine sample for urinalysis, and drugs of abuse screen
- A resting blood pressure and pulse measurement
- Recording of a resting 12-lead ECG
- A blood sample for test of hepatitis B surface antigen, antibodies to HIV and antibodies to hepatitis C virus.
- Female subjects: Blood sample for analyses of follicle-stimulating hormone (FSH)
- Habits of nicotine and alcohol use

7.2.1 Follow-up procedures

A similar post-study examination (excluding the demographic data, height, drug test, medical and surgical history) will be performed within 7 to 10 days after last dosing of IP.

7.3 Safety

It is of the utmost importance that all staff involved in the study is familiar with the content of this section. The PI is responsible for ensuring this.

7.3.1 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.

7.3.2 Definitions of serious adverse event

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

- Results in death
- Is immediately life-threatening
- Requires in-patient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability or incapacity
- Is a congenital abnormality or birth defect
- Is an important medical event that may jeopardise the subject 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.

7.3.3 Recording of adverse events

AEs will be collected from the first administration of IP throughout the treatment period and including the follow-up period. AEs will be recorded during the study days at the investigational site and at the follow-up visit. Serious AEs will be recorded from the time when informed consent is obtained at the pre-entry visit until the follow-up visit.

Variables

The following variables will be recorded in the CRF for each AE: verbatim, date (time only during residential period) when the AE started and stopped, maximum intensity, whether the AE is serious or not, causality rating (Yes/No), action taken with regard to IP and outcome.

In addition, the following variables will be collected for all SAEs: Date AE met criteria for serious AE, Date Investigator aware of serious AE, AE is serious due to, Date of hospitalisation, Date of discharge, Probable cause of death, Date of death, Autopsy performed, Causality assessment in relation to Study procedure(s), Description of AE, Causality assessment in relation to Additional drug, Causality assessment in relation to Other medication.

The maximum intensity of each AE should be recorded and rated according to the following definitions:

- 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 7.3.2. 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.

The Investigator will assess causal relationship between IP and AEs, 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.

Causal relationship in cases where the disease under study has deteriorated due to lack of effect will be classified as no reasonable possibility.

For SAEs causal relationship will also be assessed for other medication and study procedure. 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 subject or reported in response to the open question from the study personnel: *“Have you had any health problems since the previous visit?”*, or revealed by observation will be collected and recorded in the

CRF. When collecting AEs, the recording of diagnoses is preferred (when possible) to recording a list of signs and symptoms. However, if a diagnosis is known and there are other signs or symptoms that are not generally part of the diagnosis, the diagnosis and each sign or symptom will be recorded separately.

Adverse Events based on examinations and tests

Deterioration as compared to baseline in protocol-mandated laboratory values, vital signs and other safety variables will 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 a deterioration in a laboratory value/vital sign is associated with clinical signs and symptoms, the sign/symptom will be reported as an AE and the associated laboratory result/vital sign will be considered as additional information. 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.

Clinically relevant deterioration in non-protocol-mandated measurements will be reported as AE(s).

Wherever possible the reporting investigator uses the clinical, rather than the laboratory term (eg, anaemia versus low haemoglobin value).

Follow-up of unresolved adverse events

Any AEs that are unresolved at the 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 CRF. AZ 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.

7.3.4 Reporting of serious adverse events

All SAEs have to be reported, whether or not considered causally related to the IP, or to the study procedure(s). All SAEs will be recorded in the CRF. SAEs will be recorded from the time of informed consent.

The investigator and/or Sponsor are responsible for informing the Ethics Committee (EC) and/or the Regulatory Authority (RA) of the SAE as per local requirements.

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

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 appropriate AZ representatives of any follow-up information on a previously reported SAE

immediately but no later than the end of the next **business day** of when he or she becomes aware of it.

The AZ representative will advise the Investigator/study site personnel how to proceed.

Investigators or other site personnel send relevant CRF modules by fax to the designated AZ representative.

If the Clinical Study SAE Report Form is used, then the Investigators or other site personnel fax on the same day the completed form and any other relevant supporting documentation (eg, ECG, laboratory results, autopsy report) and relevant CRF modules to the AZ representative.

The designated AZ representative works with the investigator to ensure that all the necessary information is provided to the appropriate AZ clinical drug safety data entry site within **one business day** for fatal and life threatening events and within **five calendar** days for other SAEs. If the report arrives late in the day, it can be sent the following morning. If the report arrives during a weekend or public holiday, the information is forwarded as early as possible on the first business day following the weekend or holiday. The clock start date is then the next business day.

The reference document for definition of expectness/listedness is the IB for the AZ drug.

7.3.5 Laboratory safety assessment

Blood and urine samples for determination of clinical chemistry, haematology and urinalysis parameters will be taken at the times given in the study plan. The date and time of collection will be recorded in the appropriate CRF section.

The following laboratory variables will be measured:

Laboratory variables:

Clinical chemistry	**Haematology**
S-Albumin	B-Haemoglobin
S-ALT	B-Leukocyte count
S-AST	B-platelet count
S-Alkaline phosphatase	B-Leukocyte differential count (absolute count)
S-Bilirubin, total	**Urinalysis**
S -Calcium, total	U-Hb
S -Creatinine	U-Protein
S –CRP	U-Glucose
S- γ-GT	

S-Potassium

S-Sodium

S-Creatine kinase

P-Glucose

P-Prothrombin complex (INR)[a]

[a] Only on Visit 1 and pre dose Day 9.

The blood samples for the clinical chemistry and haematology will be analysed using routine methods at the contracted Laboratory, Covance. Urine samples will be analysed at the CRO by urine dipsticks. Laboratory values outside the reference limits suspected to be of any clinical significance will be re-checked. Subjects in whom the suspected clinical significance is confirmed at the repeated sampling will either not be included or, if already included, will be followed until normalisation or for as long as the investigator considers necessary.

At screening visit, all subjects will be tested for HIV, hepatitis B and C. Samples for C-peptide and GAD antibodies will also be taken as well as FSH for women unless age >50 years and 2 years without menstruation periods.

Urine will be tested for the following drugs of abuse: amphetamine, barbiturates, benzodiazepines, cannabinoid, cocaine, opiates and methadone.

If a subject tests positive for drugs of abuse they will be excluded from entering, or continuing in, the study.

For blood volume see Section 8.1.

7.3.6 Physical examination

For timing of individual measurements, see Table 2. The physical examination should include general appearance, skin, lymph nodes, thyroid, musculoskeletal/extremities, neurological (reflexes) cardiovascular and respiratory systems and abdomen.

For AE reporting in relation to physical examination, see Section 7.3.3.

7.3.7 ECG

7.3.7.1 Resting 12-lead ECG

Twelve-lead ECGs will be obtained after the subject has been lying down for 10 minutes. The ECG will be recorded at a paper speed of 50 mm/s and will be saved as paper printouts. The paper ECGs will be evaluated by the PI, or his/her delegate, and entered as normal, or specified if abnormal, in the CRF.

For AE reporting in relation to abnormal ECG finding, see Section 7.3.3.

7.3.7.2 Real time display (telemetry)

A 2-lead real-time ECG will be displayed from pre-dose until 6 hours post dose.

7.3.8 Vital signs

The following variables will be measured: weight, height (only at enrolment visit), pulse and BP.

Supine BP and pulse will be measured using a semi-automatic blood pressure recording device with an appropriate cuff size. The subjects will have to rest in bed for 10 minutes before BP and pulse rate measurements.

For timing of individual measurements see Table 2.

7.4 Efficacy (Not applicable)

7.5 Pharmacokinetics

7.5.1 Collection of biological samples

Blood samples (1.2 mL) for determination of AZD1656 and the metabolite AZ12555623 in plasma will be taken at the times presented in the Table 2. Blood samples will be collected, labelled, stored and shipped as detailed in the Appendix D. The date and time of collection will be recorded on the appropriate CRF.

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

Samples will be shipped to the appointed bioanalytical laboratory on pre-determined dates during the course of the study.

For blood volume see Section 8.1.

7.5.2 Determination of drug concentration in biological samples

Samples for determination of AZD1656 and its metabolite, AZ12555623, in plasma will be analysed by York Bioanalytical Solutions, U.K. on behalf of Clinical Pharmacology & DMPK, AZ R&D Mölndal, using liquid chromatography with mass-spectrometric detection after solid phase extraction. The lower limit of quantification (LLOQ) of AZD1656 and AZ12555623 in plasma is 5 nmol/L. The method will be referred to in the clinical study report (CSR).

7.6 Pharmacodynamics

Blood samples for determination of Glucagon, Epinephrine, Norepinephrine, growth hormone (GH), Cortisol (CR blood samples), C-peptide, plasma glucose and insulin (PD blood samples) will be taken at time points presented in Table 2. The date and time of collection will be recorded on the appropriate section of the CRF.

The PD samples will be analysed using standard methods at the contracted laboratory, Covance.

For blood volume see Section 8.1.

7.6.1 Collection of biological samples

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

For blood volume see Section 8.1.

7.7 Pharmacogenetics

7.7.1 Collection of samples

The blood sample for genetic research will be obtained from the subjects after randomisation. Samples will be collected, labelled stored and shipped as detailed in the Laboratory Manual for genetic sampling.

For blood volume see Section 8.1.

7.8 Health economics (Not applicable)

8. BIOLOGICAL SAMPLING PROCEDURES

8.1 Volume of blood

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

Table 4 Volume of blood to be drawn from each subject

Assessment		Sample volume (mL)	No. of samples	Total volume (mL)
Safety	Clinical chemistry	5 mL,V1 and 3.5 mL, V3&V4	5 mL x 1 3.5 ml x 6	5 21
	Haematology	3	7	21
Determination of AZD1656 and its metabolite (AZ12555623) in plasma		1.2	8x2=16	19.2
HbA1c		Incl in haematology	1	0
Fasting plasma Glucose, V1		2	1	2
GAD antibodies and C-peptide, V1		3.5	1	3.5
HIV and hepatitis B and C		7	1	7
INR		1.8	2	3.6
24h glucose (bedside)		0.5	18x2=36	18
7-point glucose (bedside) Visit 2 & Visit 3 Day 1, 2, 3, 4, 6, 7 and 9		0.5	45[a]	22.5
S-insulin and C-peptide (PD)		Incl in CR	12x2=24	0
GH, Epinephrine, Norephinephrine, glucagons, insulin, cortisol (CR)		14	12x2=24	336
Genotyping (optional)		9	1	9
Total				467.8

8.2 Handling, storage and destruction of biological samples

The samples will be used up or disposed after analyses.

8.2.1 Pharmacokinetic and/or pharmacodynamic samples

Pharmacokinetic samples will be disposed of after the CSR has been finalised.

8.2.2 Pharmacogenetic samples

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

For all samples irrespective of the type of coding used the DNA will be extracted from the blood sample. The DNA sample will be assigned a unique number replacing the information on the sample tube. Thereafter, the DNA sample will be identifiable by the unique DNA number only. The DNA number is used to identify the sample and corresponding data at the

AZ genetics laboratories, or at the designated contract laboratory. No personal details identifying the individual will be available to any AZ employee working with the DNA.

The blood 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 AZ. 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.

8.3 Labelling and shipment of biohazard samples

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

Any samples identified as Infectious Category A materials are not shipped and further samples taken from the patient unless agreed with AZ and appropriate labelling, shipment and containment provisions are approved.

8.4 Chain of custody of biological samples

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

The PI at each centre keeps full tractability of collected biological samples from the subjects while in storage at the centre until shipment and keeps documentation of receipt of arrival.

The sample receiver keeps full tractability of the samples while in storage and during use until used or disposed.

AZ 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 is registered in AZ bio bank system during the entire life cycle.

8.5 Withdrawal of informed consent for donated biological samples

If a subject withdraws consent to the use of biological samples donated the samples will be disposed/destroyed, if not already analysed and documented.

If collection of the biological samples is an integral part of the study then the subject is withdrawn from further study participation.

If collection of the biological samples is a voluntary part of the study then the subject may continue in the study.

The PI:

- Ensures subjects withdrawal of informed consent is notified immediately to AZ
- Ensures that biological samples from that subject, if stored at the study site, are immediately identified, disposed/destructed and the action documented
- Ensures the laboratory(ies) holding the samples is/are informed about the withdrawn consent immediately and that samples are disposed/destructed and the action documented returned to the study site

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

In the event that analysis/research has already been performed, AZ will retain the results and associated data for regulatory reasons but these will not be used in any subsequent analyses.

9. ETHICAL AND REGULATORY REQUIREMENTS

9.1 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 International Conference on Harmonisation (ICH)/Good Clinical Practice (GCP), applicable regulatory requirements and the AZ policy on Bioethics and Human Biological Samples.

9.2 Subject data protection

The Informed Consent Form (ICF) will incorporate (or, in some cases, be accompanied by a separate document incorporating) wording that complies with relevant data protection and privacy legislation.

AZ 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 AZ SDT Physician or an investigator might know a subject's identity and also have access to his or her genetic data. Also RA may require access to the relevant files, though the subject's medical information and the genetic files would remain physically separate.

9.3 Ethics and regulatory review

An EC must approve the final study protocol, including the final version of the ICF and any other written information to be provided to the subjects. The investigator will ensure the distribution of these documents to the applicable EC, and to the study site staff.

The opinion of the EC must be given in writing. The investigator must submit the written approval to AZ before enrolment of any subject into the study.

The EC must approve all advertising used to recruit subjects for the study.

AZ must approve any modifications to the ICF that are needed to meet local requirements.

If required by local regulations, the protocol must be re-approved by the EC annually.

Before enrolment of any subject into the study, the final study protocol, including the final version of the ICF, is approved by the national RA or a notification to the national RA is done, according to local regulations.

The distribution of any of these documents to the national RA will be handled by AZ.

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

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

9.4 Informed consent

The PI(s) at each centre will:

- Ensure that the subject is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study
- Ensure that the subjects are notified that they are free to discontinue from the study at any time
- Ensure that the subject are given the opportunity to ask questions and allowed time to consider the information provided
- Obtain and document the subject’s signed and dated informed consent before conducting any procedure specifically for the study
- Ensure the original, signed ICF is stored in the Investigator’s Study File (ISF)
- Ensure a copy of the signed ICF is given to the subject

9.5 Changes to the protocol and informed consent form

Study procedures will not be changed without the mutual agreement of the co-ordinating investigator and AZ.

If there are any substantial changes to the study protocol, then these changes will be documented in a study protocol amendment anywhere required a new version of the study protocol (Amended Protocol).

The amendment must be approved by each EC and if applicable, also the national RA, before implementation. Local requirements must be followed for amended protocols.

AZ will distribute any subsequent amendments and new versions of the protocol to the PI(s). For distribution to EC see Section 9.3.

If a protocol amendment requires a change to a centre's ICF, AZ and the centre's EC must approve the revised ICF before the revised form is used.

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

9.6 Audits and inspections

Authorised representatives of AZ, a regulatory authority, or an EC 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, GCP, guidelines of the ICH, and any applicable regulatory requirements. The investigator will contact AZ immediately if contacted by a regulatory agency about an inspection at the centre.

10. STUDY MANAGEMENT BY ASTRAZENECA

10.1 Pre-study activities

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

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

10.2 Training of study site personnel

Before the first subject is entered into the study, an AZ representative 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 system(s) utilised.

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

10.3 Monitoring of the study

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

- Provide information and support to the investigator(s)
- Confirm that facilities remain acceptable
- Confirm that the investigational team is adhering to the protocol, that data are being accurately and timely recorded in the CRFs, and that investigational product accountability checks are being performed
- Perform source data verification (a comparison of the data in the CRFs with the subject's medical records at the hospital or practice, and other records relevant to the study) incl. verification of informed consent of participating subjects. This will require direct access to all original records for each subject (eg, clinic charts)
- Ensure withdrawal of informed consent to the use of the subject's biological samples is reported and biological samples are identified and disposed/destructed accordingly, and the action is documented, and reported to the subject

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

10.3.1 Source data

For the purpose of this study the CRF will serve as source data for some of the variables in the study. This will be specified in a separate source data verification (SDV) plan before the start of the study and stored in the Study Master File (SMF).

10.4 Study agreements

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

Agreements between AZ and the PI must be in place before any study-related procedures can take place, or subjects be enrolled.

10.5 Study timetable and end of study

The end of the entire study is defined as ”the last visit of the last subject undergoing the trial”.

The study is expected to start in Q1 2009 and to be completed by the end of Q1 2009.

The study may be terminated at individual centres if the study procedures are not being performed according to GCP, or if recruitment is slow. AZ may also terminate the entire study prematurely if concerns for safety arise within this study or in any other study with AZD1656.

11. DATA MANAGEMENT BY CRO

Paper CRFs will be used to record all data not captured electronically. The CRFs will be monitored by appropriate skilled person on an ongoing basis. The database is set up by the CRO using appropriate version of SAS.

Medical coding will be performed on an ongoing basis using the Medical Dictionary for Regulatory Activities (MedDRA) and AstraZeneca Drug Dictionary (AZDD).

Data associated with biological samples will be transferred from the CRO laboratory to AZ via the CRO.

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

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

12. EVALUATION AND CALCULATION OF VARIABLES BY CRO

12.1 Calculation or derivation of safety variable(s)

12.1.1 Other significant adverse events (OAE)

During the evaluation of the AE data, an AZ medically qualified expert will review the list of AEs that were not reported as SAEs and discontinuation due to adverse events (DAEs). Based

on the expert's judgement, significant adverse events of particular clinical importance may, after consultation with the Global Drug Safety Physician, be considered OAEs and reported as such in the CSR.

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.

12.2 Calculation or derivation of efficacy variable(s) (Not applicable)

12.3 Calculation or derivation of pharmacokinetic variables

The PK analyses will be performed by Profil. The actual sampling times will be used in the PK calculations. Plasma concentrations below LLOQ will be excluded from the calculations. However, at time points before C_{max}, plasma concentrations below LLOQ will be taken as zero in the calculations.

If data permits, the PK variables of AZD1656 defined below will be calculated by non-compartmental methods using WinNonlin professional or other appropriate software. AUC will be determined using lin-log trapezoidal rule. The variables AUC_{0-24}, C_{max} and t_{max} will also be calculated for AZ12555623, a metabolite of AZD1656. Other PK variables may also be calculated if judged appropriate.

AUC_{0-24} Area under the plasma concentration-time curve from time 0 to 24 hours post dose

C_{max} Observed maximum plasma concentration

t_{max} Time to reach C_{max}

$t_{½}$ Terminal half-life in plasma, calculated by $\ln 2/\lambda$ (λ is the elimination rate constant estimated from individual linear regression of the terminal part of the log concentration-time curve)

CL/F Oral clearance, calculated as dose/AUC_{0-24}

12.4 Calculation or derivation of pharmacodynamic variable(s)

12.4.1 Plasma glucose level 20 minutes post release of the hypoglycaemic clamp

The primary efficacy of the study is the plasma glucose level 20 minutes post release of the hypoglycaemic clamp, where a statistically significant difference is anticipated between glucagon treatment and spontaneous recovery from hypoglycaemia.

12.4.2 Time to recovery from hypoglycaemia

The secondary outcome variables of the study are defined as the time (in minutes) from release of the hypoglycaemic clamp, after 30 minutes at plasma glucose 2.7 mmol/L, to the time point where two consecutive plasma glucose levels ≥3.5 mmol/L and ≥5.0 mmol/L respectively, have been measured.

12.5 Calculation or derivation of the relationship between pharmacokinetic and pharmacodynamic variables (Not applicable)

12.6 Calculation or derivation of pharmacogenetic variables (Not applicable)

12.7 Calculation or derivation of health economics variables (Not applicable)

13. STATISTICAL METHODS AND SAMPLE SIZE DETERMINATION BY CRO

13.1 Description of analysis sets

The PK and PD analysis will be based on an as-treated approach (erroneously treated subjects, eg, those randomised to treatment A but actually given treatment B, will be accounted for in the actual treatment group).The PK analysis set will consist of all evaluable PK data appropriate for the analysis of interest. Correspondingly the PD analysis set will consist of all evaluable PD-data appropriate for the analysis of interest. Data affected by major protocol deviations or violations thought to significantly affect PK or PD of the drug will be excluded.

13.1.1 Analysis of Safety population

Safety analyses will be based on an as-treated approach (erroneously treated subjects, eg, those randomised to treatment A but actually given treatment B, will be accounted for in the actual treatment group) and based on the safety analysis set consisting of all subjects who received at least one dose of investigational product, and for whom any post-dose data are available. Post-dose data implies that there was contact during which an opportunity was given to report any health problem.

13.2 Methods of statistical analyses

Descriptive statistics includes n, mean, standard deviation (SD), min, median, and max, and for variables log transformed in the analysis, also geometric mean and coefficient of variation (CV).

13.2.1 Baseline characteristics

Baseline characteristics (age at consent, weight, height, BMI, race, HbA1c, fasting p-glucose, number of OADs and duration of T2DM) will be presented with descriptive statistics for all subjects.

13.2.2 Pharmacokinetics

All PK parameters derived for Day 5 and 8 (AUC_{0-24}, C_{max}, t_{max}, $t_{½}$ and CL/F for AZD1656 and AUC_{0-24}, C_{max} and t_{max} for AZ12555623) will be presented with individual values and descriptive statistics. All presentations will be done for both AZD1656 and AZ12555623.

13.2.3 Pharmacodynamics

For the PD efficacy variables (glucose after 20 min and time to reach 3.5 and 5 mmol/L) confidence intervals (95%) will be presented. A mixed model ANOVA with fixed effects for sequence, period and treatment and a random effect for subject within sequence will be used..

All PD-variables will be presented with individual values and descriptive statistics by treatment (with and without glucagon).

For mean values of the PD-variables by time will be presented graphically together in the same plot for with and without glucagon period.

13.2.4 Safety

AEs will be presented as number of patients with at least one AE, and will be further detailed by presenting the categories fatal AEs, serious non-fatal AEs and AEs leading to treatment discontinuation. All AEs should be summarized by the preferred term, assigned to the event using MedDRA vocabulary, for the periods on treatment, during follow-up and during study. Treatment period is defined as from date for start of study drug to date of last study drug or, when time is available, from time for start of study drug to 24:00 the date of last study drug or until the start of the next study drug if there are multiple exposures without a washout period. A follow-up period should capture any event occurring after the last treatment period up to the end of study for the patient, eg, the patient's last visit in the study. AEs will be represented in each period where there is overlapping occurrence.

Actual values and change from baseline for laboratory variables will be presented descriptively by treatment and time. Shift-tables will be used to classify the subjects in the categories below, within and above upper limit of normal (ULN), pre-treatment versus post-treatment. Pre-entry versus follow-up for laboratory variables will be presented in shift plots. A list with abnormal (out of reference range) laboratory values will be presented. For each abnormal value all values for that specific laboratory variable will be presented for the subject with the abnormal value. If a subject has more than one observation on the same measurement occasion, the results from the earliest draw should be used in all summaries.

Actual values and change from baseline for vital signs (BP, pulse and weight) will be presented descriptively by treatment and time.

All abnormal physical examination findings and ECG assessments will be listed. Any new or aggravated abnormal findings as compared to the baseline assessment will be listed.

13.3 Determination of sample size

The sample size is not firmly based on statistical considerations but as most individuals are expected to respond to glucagon 8 patients give a good power to detect a response frequency above 90% with a sign test.

A statistical analysis plan will be prepared before clean file.

13.4 Interim analyses (Not applicable)

13.5 Data monitoring committee (Not applicable)

14. LIST OF REFERENCES

Levey et al 2003
Levey A.S., Coresh J, Balk E, Kausz A.T, Levin A, Steffes M.W et al. National Kidney Foundation Practice Guidelines for Chronic Kidney Disease: Evaluation, Classification, and Stratification. Ann Intern Med. 2003;139:137-47

D0280C00003
A Randomized, Open, Two-Way Cross-Over, Single-Centre, Phase I Study to Assess the Counterregulatory Response during Hypoglycemia in Healthy Male Volunteers after a Single Oral Dose of AZD6370 Suspension in Comparison with Insulin Infusion, D0280C00003, AstraZeneca R&D Mölndal, 23 May 2008

Clinical Study Protocol Appendix B

Drug Substance	AZD1656
Study Code	D1020C00018
Edition Number	01
Date	

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 (e.g., 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 (e.g., 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 jeopardise 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 (e.g., 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	AZD1656
Study Code	D1020C00018
Edition Number	01
Date	

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	AZD1656
Study Code	D1020C00018
Edition Number	01
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Appendix D
Instructions for Sampling, Handling, Storage and Shipment of Pharmacokinetic Samples

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Instruction for blood sampling for determination of AZD1656, it's metabolite AZ12555623 in plasma

This specified sampling procedure must be followed to avoid jeopardising the subsequent drug determination in plasma.

1. SAMPLING DEVICE

Blood samples (1.2 mL) for the determination of AZD1656 and it's metabolite, AZ12555623 in plasma will be collected in 1.2 mL S-Monovette tubes from Sarstedt (article No 06.1664.001) containing K3-EDTA anticoagulant. In case of repeated sampling, catheters made of stainless steel or teflon (Venflon®) are used.

2. BLOOD SAMPLING

After applying a tourniquet, venous blood is taken with a disposable needle. If a catheter is used without using an obturator, the first mL of blood on each sampling occasion must be discarded.

1.2 mL of blood for determination of AZD1940 and it's metabolite AZ12555623 are collected in the sampling tubes and K3-EDTA and blood are mixed carefully and left in room temperature

The blood samples will be centrifuged at approximately 4°C for 10 minutes at a Relative Centrifugal Force (RCF) of about 1500g within 30 minutes of the sample collection. Following centrifugation, the plasma (approx 0.56 mL) will be transferred with a fresh pipette into a 1.8-mL polypropylene tube (Nunc Cryo Tube™, Nalgene Nunc International, Rochester, New York, USA, cat no 375418. The plasma samples must be immediately frozen in upright position

3. SAMPLE LABELLING

Sample labels provided by the CRO will be used, marked with study code, subject number, drug, scheduled time after drug administration, day, sample type and unique sample identification. The label shall be used for the sample tube.

3.1 Sample list

Sample list, from Electronic Data Capture System, with the same information as on the tube labels and comments important for the analytical work, e.g., additional and missing samples, must accompany the samples to the bioanalytical laboratory as a delivery note. Electronic sample lists should also be sent to the Bioanalytical CRO before sample shipment

3.2 Additional samples

In case the label from electronic data capture system is not available for a certain sample or when an additional sample is taken, additional labels are printed from the system.

4. STORAGE

The frozen samples must be stored at -20°C. The samples must be transported on dry ice.

5. DELIVERY

Every batch of samples, accompanied with the sample lists, will be shipped via an agreed upon overnight courier to the address below. The samples must be packed (in the same order as on the packing list) to avoid breakage during transit, double-bagged to contain leaks and packed with a sufficient quantity of dry ice to ensure they remain frozen for at least 72 h. All applicable shipping regulations must be followed. An email with the time of arrival, and the electronic sample lists attached must be send to the Bioanalytical CRO

In addition the same e-mail with the time of arrival, and the electronic sample lists attached must be sent to the monitoring scientist.

Samples must only be shipped on Monday to Wednesday. Do not ship 2 days of a local holiday.

All shipping dates must be agreed upon before study start.

Plasma samples along with the corresponding documentation should be shipped to: