AstraZeneca

Revised Clinical Study Protocol

Drug Substance	AZD1656
Study Code	D1020C00009
Edition Number	2
Date	

A 4-Month, Randomized, Double-Blind, Placebo- and Active-Controlled, Multi-Centre, Parallel-Group Study, with an Optional 2-Month Extension, to Evaluate Efficacy, Safety and Tolerability of AZD1656 as Add-on Treatment to Metformin in Type 2 Diabetes Mellitus Patients

Sponsor:

AstraZeneca AB, 151 85 Södertälje, Sweden.

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

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

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

AstraZeneca

PROTOCOL SYNOPSIS

A 4-Month, Randomized, Double-Blind, Placebo- and Active-Controlled, Multi-Centre, Parallel-Group Study, with an Optional 2-Month Extension, to Evaluate Efficacy, Safety and Tolerability of AZD1656 as Add-on Treatment to Metformin in Type 2 Diabetes Mellitus Patients

Study centre(s) and number of patients planned

This study will be conducted in approximately 130 study centres in 12 countries in Europe, South America and Mexico. It is planned to have approximately 540 patients in totally 7 arms. One arm with open-label AZD1656 up to 90 patients, 4 arms with approximately 90 patients randomized per arm and 2 "fixed dose" arms with approximately 45 randomized patients per arm.

Study period		Phase of development
Estimated date of first patient enrolled	Q4 2009	Phase II
Estimated date of last patient completed	Q1 2011	

Objectives

Primary objective

- The primary objective is to compare the effect on glucose control of 4 different AZD1656 dosing regimens with placebo in type 2 diabetes mellitus patients on metformin treatment, as evaluated by the difference in HbA1c between baseline and final visit at 4 months.

Secondary objectives

- To characterise the population pharmacokinetic and pharmacodynamic properties of AZD1656 in type 2 diabetes mellitus patients treated with metformin by utilisation of non-linear mixed effects modelling methodology.

- To evaluate other variables of glucose control (fasting plasma glucose, mean daily self-monitored plasma glucose [7-point measurements], oral glucose tolerance test data and number of responders in terms of HbA1C ≤ 7% and ≤ 6.5%, respectively) after 4 months in patients receiving AZD1656 as add-on treatment to metformin compared to placebo as add-on treatment to metformin.

- To evaluate other cardiovascular risk factors, such as total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides and C-reactive protein (CRP), after 4 months in patients receiving AZD1656 as add-on treatment to metformin compared to placebo as add-on treatment to metformin.

- To investigate the safety and tolerability of AZD1656 compared to placebo and glipizide by assessments of adverse events occurring during the study (including hypoglycaemic events), blood pressure, pulse, physical examination, body weight, safety laboratory variables and electrocardiogram

- To explore the safety and tolerability of AZD1656 in patients with severely impaired glucose control during a 4 months treatment period.

Exploratory objectives

- To compare the effect on glucose control of the included AZD1656 dosing regimens with the comparator glipizide.

- To investigate the effect on glucose control of patients in the open label AZD1656 treatment with severely impaired glucose control (baseline HbA1c between >10 % and ≤12 %) during a 4 months treatment period.

- To collect and store DNA for future exploratory research into genes/genetic variation that may influence response (ie, distribution, safety, tolerability and efficacy) to AZD1656, metformin and glipizide.

Extension Objectives

- To investigate the safety and tolerability of AZD1656 compared to placeb and glipizide after 6 months by assessments of adverse events occurring during the study (including hypoglycaemic events), blood pressure, pulse, physical examination, body weight, safety laboratory variables and electrocardiogram.

- To evaluate other cardiovascular risk factors, such as total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides and c-reactive protein, after 6 months in patients receiving AZD1656 as add-on treatment to metformin compared to placebo as add-on treatment to metformin.

Exploratory objectives (extension)

Potential variables of efficacy and /or pharmacokinetic properties, which may be explored in patients treated with AZD1656, glipizide and/or placebo:

- Change in HbA1c between baseline, 4 months and 6 months
- Change in FPG between baseline, 4 months and 6 months
- Change in self-monitored plasma glucose (SMPG) between baseline, 4 months and 6 months
- Number of responders (HbA1c $\leq$7% and $\leq$6.5) between baseline, 4 months and 6 months
- PK/PD modelling: to characterise the population PK and PD properties by utilisation of non-linear mixed effects modelling methodology

Study design

This will be a 4-months double-blind, with an optional 2-month extension, randomized, placebo- and active-controlled, multi-centre study of AZD1656 as add-on therapy to metformin compared to placebo, in approximately 540 type 2 diabetes mellitus patients. The sponsor and CRO will be unblinded during the extension, but the treatment will continue to be blinded for investigator, monitor and patients. Treatment with glipizide will be included in a separate treatment arm as add-on treatment to metformin, in order to generate data on a comparator in the same study setting. The study consists of 5 parallel arms with 90 patients in each arm except for the two fixed dose arms that will have 45 patients each.

The study includes 2 cohorts of patients. In the main treatment cohort (cohort 1) patients can be randomized to one of 6 different treatment arms with AZD1656, glipizide or placebo. Patients in this cohort should have HbA1c between $\geq$7.5 % and $\leq$10% at enrolment. The patients will be randomized to 1 of 4 different dose regimens of AZD1656 (a minimum dose of 10mg daily titrated to maximum dose of 140mg daily, a minimum dose of 20mg daily titrated to a maximum dose of 200mg daily, 20mg daily or 40mg daily fixed dose), glipizide regimen (minimum dose of 5mg daily to a maximum dose of 20mg daily), or placebo, as add-on to an unchanged dose of metformin. The double-blind AZD1656/glipizide/placebo will be given twice daily, two tablets AZD1656/AZD1656 placebo together with 2 capsules glipizide/glipizide placebo at each administration and metformin will be given once, twice or three times daily. In cohort 2, eligible patients with HbA1c between >10 % and $\leq$12 % at enrolment will be assigned (no randomization) to an open-label treatment with AZD1656, which will be titrated from the minimum dose 20mg to the maximum dose 200mg. Two tablets open label AZD1656 are given twice daily without glipizide capsules. Recruitment will be completed regardless of reaching the number in the open-label AZD1656 arm. The termination of the recruitment will be linked to cohort 1 (450 randomized patients).

Target patient population

Male or female patients of non-childbearing potential, of at least 18 years of age with a diagnosis of type 2 diabetes mellitus, not adequately controlled on a maximum tolerated dose of metformin (≥ 1500mg) as single therapy and with stable metformin treatment for at least 10 weeks prior to enrolment (screening).

Investigational product, dosage and mode of administration

All treatments in this study are investigational products (ie AZD1656/placebo, glipizide/placebo), except metformin which is additional drug.

AZD1656 tablets will contain AZD1656 corresponding to the strengths 5mg, 20mg or 50mg of the active compound, respectively, or placebo. The patients will take half of the daily dose orally in the morning with breakfast and half of the daily dose orally in the evening with dinner.

Glipizide will be encapsulated into orange hard gelatine capsules and contain glipizide tablets corresponding to a dose of 5mg glipizide or no glipizide tablets if a placebo capsule. The total daily dose of glipizide/ placebo will be composed of 4 capsules, of which up to 4 capsules will contain active compound and rest placebo, depending on the daily dose.

Two tablets AZD1656 and two capsules glipizide will be administrated orally in the morning with breakfast and 2 tablets AZD1656 and 2 capsules glipizide will be administrated orally in the evening with dinner. Patients randomized to the blinded treatment will take both AZD1656/placebo and glipizide/placebo. Patients assigned to the open dose of AZD1656 will only take 2 tablets of AZD1656 at each dosing event.

Two strengths of metformin tablets, 500mg and 850mg respectively, will be provided by the sponsor. The metformin will be packed in to blister cards, open-label, 100 tablets per package for the 500mg metformin and 56 tablets per package for the 850mg metformin. The dose will be individual but will stay between 1.5 and 3 g per day, administrated orally as tablets once, twice or three times daily together with meal.

Duration of study and randomized treatment period

An enrolment visit (screening) will take place approximately 4 weeks before the start of randomized study treatment. Eligible patients will receive randomized treatment for 4 month and those patients consenting also to the 2-month extension will receive randomized treatment for 6 months in total. During the randomized treatment period, visits to the clinic are scheduled on a weekly basis during the initial titration period, followed by visits each month during the maintenance phase up to 4 months, with an optional 2 months extension. A follow-up visit will be scheduled 2 weeks ± 5 days after the end of treatment visit (or last visit if premature discontinuation).

Outcome variable(s):

Primary outcome variable

- The primary efficacy variable is defined as the change in HbA1c from baseline to the final visit at 4 months. Baseline will be defined as the last non-missing value on or before the first treatment dose received (the HbA1c sample at Visit 3 will be collected prior to the first dose intake). If the value is missing at the randomization visit, the enrolment (screening) result will be used.

Secondary outcome variables

- The time-course of drug concentrations for AZD1656 and its active metabolite AZD12555623 in plasma.
- Glucose Control variables:
  - Change from baseline to final visit (at 4 months) in terms of fasting plasma glucose, as assessed at the central laboratory.
  - Change from baseline to final visit (at 4 months) in mean plasma glucose over a day, calculated from the 7-point measurements at home.
  - Change from baseline to final visit (at 4 months) in oral glucose tolerance test results: area under the curve (0-120 min) for plasma glucose, insulin, C-peptide and pro-insulin
  - Number of patients with Hb1Ac $\leq$ 7% and $\leq$ 6.5%, respectively, at the final visit (4 months).
- Cardiovascular risks factors: change from baseline to final visit (at 4 months) in:
  - low-density lipoprotein cholesterol
  - high-density lipoprotein cholesterol
  - total cholesterol
  - triglycerides
  - C-reactive protein
- Safety, both for full safety analysis set and for safety analysis set excluding post-rescue treatment:
  - Adverse events.

- Adverse events related to hypoglycaemic symptoms.
- Classification of hypoglycaemic events.
- Number of plasma glucose measurements in defined ranges from glucometer assessment at home, analysed at the clinic and at the central laboratory, respectively.
- Vital signs (diastolic and systolic blood pressure and pulse rate) and body weight.
- Electrocardiograms.
- Safety laboratory analysis.

Statistical methods

Efficacy: A hierarchical closed testing procedure will be adopted for the efficacy analyses. Treatment group comparisons will be tested in dose span order whereby comparisons with placebo will commence with the highest dose regimen (randomized patient on 20mg daily titrated to maximum of 200mg daily) tested first, next dose regimen 10mg daily titrated to maximum of 140mg daily, fixed dose 40mg daily and finally the fixed dose 20mg daily. Estimates of the treatment effect and corresponding 95% confidence intervals will be presented together with the p-values for the treatment effect. All tests will be performed at the 5% level of significance.

- Primary analysis: an analysis of covariance will be performed on the change from baseline to month 4 with treatment as a factor variable. A factor with five different levels. One level for each dose regimen of AZD1656, one level for each fixed dose 20mg and 40mg, and placebo as reference group. The primary analysis will be on the last observation carried forward and will be based on the full analysis set. As an additional analysis to the primary analysis, the same model will be fitted to data but with treatment as a factor with six levels, one level for each randomized arm.

- Secondary analyses: for the continuous secondary efficacy variables (self-monitored plasma glucose, fasting plasma glucose), an analysis of covariance will be performed as for the primary analysis. The categorical efficacy variables including HbA1c responders and hypoglycaemia incidence (see definition in Section 3.1) will be compared across treatment groups utilising a chi-square test. Analyses of the secondary variables will be conducted on the full analysis set (last observation carried forward) only unless otherwise stated.

Safety: All adverse events will be categorised by body system organ class and preferred term using MedDRA dictionary, and be listed for each patient. In the safety analysis set, adverse events will be reported as frequencies in each treatment group.

Haematology and clinical chemistry data will be listed for each patient and summarised for each treatment group at each visit. Shift tables will also be presented.

Summary of value and change from baseline for electrocardiogram, vital signs, weight and waist and hip measurements will be presented. In addition, summary and shift tables for physical examination will be provided. All data will be presented in listings. Summary of adverse events of special interest, hypoglycaemic events and need for additional glucose lowering treatment will also be presented.

Extension

Safety data obtained from the extension period will be analysed in the same way as data from the 4-month treatment period. All safety data will be presented in the clinical study report. Any efficacy data from the extension period explored by AstraZeneca may or may not be included in the clinical study report.

TABLE OF CONTENTS PAGE

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

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

Abbreviation or special term	Explanation
AE	Adverse event (see definition in Section 6.4.1)
ALT	Alanine Aminotransferase (also known as SGPT, serum glutamic pyruvic transaminase)
AST	Aspartate Aminotransferase (also known as SGOT, serum glutamic oxaloacetic transaminase)
BP	Blood Pressure
CD	Candidate Drugs
CRF	Case Report Form (electronic/paper)
CRP	C-Reactive Protein
CSA	Clinical Study Agreement
CSP	Clinical Study Protocol
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Event
DAE	Adverse Event leading to discontinuation of investigational product
DMC	Data Management Committee
DNA	Deoxyribonucleic acid
DPP-4	Dipeptidyl peptidase-4
ECG	Electrocardiogram
EC	Ethics Committee, synonymous to Institutional Review Board (IRB) and Independent Ethics Committee (IEC)
EMEA	European Medicines Agency
FAS	Full Analysis Set
FDA	Food and Drug Administration
FPG	Fasting Plasma Glucose
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
GK	Glucokinase
GKA	Glucose Kinase Activator
GLP-1	Glucagon-like peptide-1
GMP	Good Manufacturing Practice

Abbreviation or special term	Explanation
HDL	High-Density Lipoprotein
ICH	International Conference on Harmonisation
International Co-ordinating investigator	If a study is conducted in several countries the International Co-ordinating Investigator is the Investigator co-ordinating the investigators and/or activities internationally.
IP	Investigational Product
IUD	Intra-Uterine Device
IVRS/IWRS	Interactive Voice Response System
IWRS	Interactive Web Response System
LDL	Low-Density Lipoprotein
LIMS	Laboratory Information Management System
LLOQ	Lower Limit of Quantification
LOCF	Last Observation Carried Forward
MAD	Multiple Ascending Dose
MDRD	Modification of Diet in Renal Disease Study Group
MODY2	Maturity Onset Diabetes of the Young Type 2
NYHA	New York Heart Association
PP	Per Protocol
OAE	Other Significant Adverse Event
OGTT	Oral Glucose Tolerance Test
PGx	Pharmacogenetic research
PD	Pharmacodynamic
PK	Pharmacokinetic
PI	Principal Investigator
PT	Preferred Term
SAD	Single Ascending Dose
SAE	Serious adverse event (see definition in Section 6.4.2).
SMPG	Self-Monitored Plasma Glucose
SOC	System Organ Class
SU	Sulphonylureas
T2DM	Type 2 Diabetes Mellitus
ULOQ	Upper Limit of Quantification
WBDC	Web Based Data Capture

1. INTRODUCTION

1.1 Background

The aim of this study is to evaluate the efficacy, safety, and tolerability of different dosing regimens of AZD1656, a glucokinase activator, after 4 months, with an optional 2 months extension, compared to placebo, as add-on treatment to metformin, in type 2 diabetes mellitus (T2DM) patients not adequately controlled on metformin. One active comparator (glipizide) as add-on to metformin is included in the study.

Currently, there are 6 different classes of oral or injectable antidiabetic drugs available on the market except for insulins: sulphonylureas (SU), biguanides (metformin), alpha glucosidase inhibitors, thiazolidinediones and just recently glucagon-like peptide-1 (GLP-1) analoges, and GLP-1 providers (dipeptidyl peptidase-4 [DPP-4] inhibitors) have been launched. Despite different mechanisms of action, with sometimes potent glucose lowering effects, current agents do not provide optimal treatment for T2DM. Each of the existing therapies is associated with adverse effects on T2DM itself (eg, weight gain and β-cell dysfunction) and/or has adverse effects that limit 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 the liver and in 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 2 processes significantly contribute to the development of hyperglycaemia in T2DM. AZD1656 is a new potent glucose kinase activator (GKA), which, due to its dual-compartment mode of action, 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. In phase I and IIa studies including both healthy volunteers and T2DM patients, AZD1656 has been well tolerated and no safety signal has been identified. A glucose-lowering effect has been demonstrated when given as monotherapy, on top of insulin and on top of metformin during up to 4 weeks of treatment. The results merit further clinical development of AZD1656 for the treatment of T2DM.

Due to its novel, dual compartment, mode of action AZD1656 should deliver anti-hyperglycaemic efficacy through increased hepatic glucose uptake and increased glucose-dependent insulin secretion. Therefore, AZD1656 has the potential to provide superior glycaemic control to existing oral agents. Additionally, AZD1656 may be further differentiated from existing oral glucose lowering agents on the basis of sustained long-term glycaemic control and better tolerability and safety.

Data from the initial clinical studies have demonstrated that AZD1656 is rapidly absorbed and maximum concentration (C_{max}) is reached within 1 hour for most patients. AZD1656 is eliminated with a half-life ($t_{1/2}$) of approximately 3 to 5 hours. AZD1656 is metabolised to the active metabolite AZD12555623 with a C_{max} at approximately the same time as AZD1656. The half-life of AZD12555623 is approximately 8-12 hours. AZD1656 has no or negligible accumulation while AZ12555623 levels are approximately 50% higher at steady state. The excretion of AZD1656 and AZ12555623 into urine is small (<10% of the dose found as AZD1656 and AZ12555623). AZD1656 is metabolised by CYP2C8 and is a weak inhibitor of CYP2C8 and CYP2C9 *in vitro* (IC50= 12 µmol/L and 30 µmol/L, respectively) otherwise neither AZD1656 nor the metabolite inhibits the major CYP450 enzymes, therefore, the risk for clinically relevant drug-drug interactions are considered to be low.

Several mutations in the GK gene are described, including variants resulting in mild diabetes mellitus such as maturity onset diabetes of the young, type 2 (MODY2). AstraZeneca intends to collect and store DNA during the AZD1656 development programme, in order to have the opportunity to perform possible future exploratory genetic research, evaluating how genetic variations may affect the clinical parameters associated with AZD1656 treatment. Collection of DNA samples from populations with well described characteristics may lead to improvements in the design and interpretation of clinical trials and, possibly, to genetically guided treatment strategies. The exploratory, genetic part of this study is optional for the study participants. Any genetic analyses may be performed and reported separately from the main study.

1.2 Research hypothesis

This study will investigate if AZD1656 on top of metformin is superior to placebo on top of metformin, in terms of the change in HbA1c from baseline to the final visit at 4 months in T2DM patients not adequately controlled on metformin alone.

Furthermore, the study will evaluate the safety profile of AZD1656 in the investigated dose ranges and whether AZD1656 has a predictive pharmacokinetic (PK) profile in a patient population similar to future target populations.

In addition, this study will investigate that AZD1656 on top of metformin has favourable effects on other parameters of glucose control (eg, fasting and post-prandial glucose levels) compared to placebo, and if AZD1656 has no negative effects on cardiovascular risk factors such as lipid metabolism.

The safety and efficacy (only explorative analysis) of AZD1656 on top of metformin will also be compared to treatment with glipizide on top of metformin. In addition, the safety and tolerablity of AZD1656 in patients with severely impaired glucose control will be explored during a 4 months, with an optional 2-month extension, treatment period. The results of the active comparator arms will also indicate the validity of the study outcome.

Genetic samples will also be collected in the study to enable future exploratory research in to genes/genetic variation that may influence response (ie, distribution, safety, tolerability and efficacy) to AZD1656, metformin, and glipizide.

1.3 Rationale for conducting this study

This study seeks to evaluate the effect of AZD1656 on HbA1c in T2DM patients not adequately controlled on metformin, in order to plan future clinical development in this target patient group. In order to support further clinical development and to guide the selection of a safe and effective dose span of AZD1656, the safety profile of AZD1656, effects on other glucose control parameters, cardiovascular risk factors and data on comparators from the same study setting need to be evaluated. In order to explore the glucose-lowering effects of a glucokinase activator (GKA) in patients with severely impaired glucose tolerance and high glucose levels, and also have early indications of safety and tolerability of AZD1656 in this population, a cohort 2 is included in the study. In addition, the PK of AZD1656 needs to be evaluated in order to plan future clinical development, with the potential influence of covariates on PK (eg, gender and renal function) also to be determined.

The proposed amendment to protocol D1020C00009 is to allow extending the duration of subject participation by 2 months (from 4 to 6 months) in order to provide a longer-term evaluation of safety and tolerability and to explore efficacy of AZD1656 given to patients with type 2 diabetes mellitus treated with metformin.

1.4 Benefit/risk and ethical assessment

AZD1656 is a GKA to be developed as a glucose-lowering agent for the treatment of T2DM patients. Due to its novel, dual compartment, mode of action AZD1656 is expected to deliver anti-hyperglycaemic efficacy through increased glucose-dependent insulin secretion and increased hepatic glucose uptake. Therefore, AZD1656 has the potential to provide superior glycaemic control to existing oral agents.

1.4.1 Pre-clinical information

A strong and predictive PK/pharmacodynamic (PD) relationship has been established between AZD1656 levels and the magnitude and duration of glucose lowering in animal models. AZD1656 induced hypoglycaemia (blood glucose <2.5 mM) at higher doses/exposures in normoglycaemic animals but not in diabetic animals.

The safety and tolerability of AZD1656 has been investigated in toxicology studies of up to 6 months duration in rats and dogs. AZD1656 showed a potent glucose lowering effect and the results of these, and preceding toxicology studies, in healthy animals were confounded by severe hypoglycaemia at higher doses. Towards the end of the 6-month rat toxicology study, some animals in the high-dose group (20mg/kg/day) were found dead with muscular wastage in their hind legs. Dose related increased incidence and severity of neuropathy (Wallerian type nerve degeneration) and myopathy (skeletal muscle fibre degeneration) were seen in rats at the mid and high-dose levels, but not in the low-dose group (5mg/kg/day). These changes seen in the mid and high-dose groups were considered to be associated with hypoglycaemia.

Even though, a direct effect on myocytes cannot completely be excluded, there are reliable biomarkers for skeletal muscle damage available. Thus, this potential effect can be monitored in the clinical programme, and is not considered to be exposure limiting for the proposed clinical studies. Changes considered to be secondary to hypoglycaemia were also seen in the liver (loss of hepatocellular glycogen). There were no treatment related effects on male fertility. Data on effects on the female reproductive system are not yet available.

Prolonged hypoglycaemia is known to result in nerve degeneration (Leow and Wyckoff 2005, Levey et al 2003, Potter et al 1988, Tabata 2000, Yasaki and Dyck 1990). A study comparing AZD1656 to insulin glargine in rats showed that both treatments resulted in an increase in the incidence of Wallerian type degeneration at doses causing similar blood glucose profiles. AZD1656 causes hypoglycaemia in wild type mice but not in heterozygous glucokinase knockout mice model ($gk^{del/wt)}$ mice. A study comparing the effects of AZD1656 in $gk^{del/wt}$ mice and wild-type mice demonstrated increased incidence and severity of Wallerian-type nerve degeneration in the wild-type mice compared to control animals, but not in the $gk^{del/wt}$ mice at the same plasma exposure. Both these studies support the hypothesis that the peripheral nerve damage observed in AZD1656-dosed healthy animals is related to repeated episodes of hypoglycaemia and not simply related to the AZD1656 plasma concentration. In addition, exposures in the knockout mice could be increased significantly, compared to wild-type mice (as indicated by $AUC_{(0\text{-}24h)}$ up to 480 mM*h for 14 days) without revealing any other toxicity finding.

AZD1656 is metabolised by CYP2C8 into an active metabolite (AZ12555623). AZD1656 is a weak inhibitor of CYP2C8 and CYP2C9 *in vitro*, otherwise neither AZD1656 nor the active metabolite inhibits the major CYP450 enzymes, therefore, the risk for clinically relevant drug-drug interactions by inhibition of CYP450 enzymes considered to be low.

1.4.2 Clinical information from Phase I and IIa studies

More than 60 healthy, male volunteers and 120 diabetic patients (males and non-fertile females) have been exposed to AZD1656 in the phase I and IIa studies so far. These studies include evaluation of AZD1656 as monotherapy, as add-on to metformin and as add-on to insulin glargine in T2DM patients. Single doses from 2mg up to 180mg AZD1656 have been given to healthy volunteers during euglycaemic clamp conditions. Regarding patients with T2DM, repeated doses of 10mg up to 300mg daily during 1 week and up to 180mg daily during 4 weeks, have been given. The Phase I and IIa studies also include evaluation of AZD1656 on top of metformin in doses up to 100mg daily for 4 weeks and up to 180mg daily on top of insulin for 4 weeks. AZD1656 has been well tolerated and no safety concerns have been raised during Phase I-IIa. No clinically significant laboratory abnormalities have been observed. There have been no deaths. Two serious adverse events (SAEs) have been reported following AZD1656 exposure in Phase I to IIa; one hospitalisation due to atrial fibrillation and one self-limiting, 16-beat ventricular tachycardia after a hypoglycaemic clamp procedure.

There have been 4 hypoglycaemic events, i.e. events with a plasma glucose below 3 mmol/L (54mg/dL). The events were either asymptomatic, scheduled measurements or associated with mild symptoms. No impaired consciousness or need for assistance from another person to

rescue the patient from the hypoglycaemia have been reported. In addition to these episodes, there have been symptoms suggestive of hypoglycaemia reported but with plasma glucose above 3 mmol/L (54mg/dL). All events with low plasma glucose have responded rapidly to oral carbohydrates. In a study of forced hypoglycaemia in T2DM patients on metformin treatment, the individual symptoms of hypoglycaemia were shown to be similar when induced with AZD1656 + insulin or insulin alone and glucose levels could rapidly be restored by glucagon injections in both groups.

The other reported adverse events (AEs) during treatment with AZD1656 or placebo have not revealed any emerging pattern of side effects related to AZD1656 exposure.

1.4.3 Safety monitoring in the study

Safety will be carefully monitored in this study. Low plasma glucose levels and hypoglycaemia will be avoided by a careful stepwise increase of AZD1656 during monitoring of plasma glucose and visits to the clinic on a weekly basis. Patients will receive glucometers and are being asked to check their plasma glucose on a daily basis and in case of symptoms suggestive of hypoglycaemia. AZD1656 will be given together with meals. Furthermore, frequent visits are scheduled to the clinic for monitoring of safety laboratory variables (including liver function tests and muscle enzymes) and adverse events will be collected with special focus on hypoglycaemic events.

Criteria for rescue treatment in case of hyperglycaemia are included in this study to ensure safe glucose levels in all arms during the randomized treatment period.

Glipizide will be used in accordance with the approved labels and clinical practice and should not be associated with significant risk within the context of this clinical trial.

All participants may benefit from the life-style advice included in the study and the intensified glucose monitoring. The chance of receiving active treatment is 5 out of 6. The chance of receiving treatment with a drug approved for use in T2DM on top of metformin and with proven efficacy and well-known safety profile (glipizide) is 1 out of 6.

The clinical findings to date give confidence that the known risks can be mitigated, and that the proposed 4-month trial, as well as the optional 2-month extension, can be conducted without undue risk to patient safety (provided that patients' safety is monitored in accordance with this clinical study protocol) and meets the standard of ethics and patient safety.

2. STUDY OBJECTIVES

2.1 Primary objective

- The primary objective is to compare the effect on glucose control of 4 different AZD1656 dosing regimens with placebo in type 2 diabetes mellitus patients on metformin treatment, as evaluated by the difference in HbA1c between baseline and final visit at 4 months.

2.2 Secondary objectives

- To characterise the population PK and PD properties of AZD1656 in type 2 diabetes mellitus patients treated with metformin by utilisation of non-linear mixed effects modelling methodology.

- To evaluate other variables of glucose control (fasting plasma glucose, mean daily self-monitored plasma glucose [7-point measurements], oral glucose tolerance test data and number of responders in terms of HbA1C ≤ 7% and ≤ 6.5%, respectively) after 4 months in patients receiving AZD1656 as add-on treatment to metformin compared to placebo as add-on treatment to metformin.

- To evaluate other cardiovascular risk factors, such as total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides and c-reactive protein, after 4 months in patients receiving AZD1656 as add-on treatment to metformin compared to placebo as add-on treatment to metformin.

- To investigate the safety and tolerability of AZD1656 compared to placebo, glipizide by assessments of adverse events occurring during the study (including hypoglycaemic events), blood pressure, pulse, physical examination, body weight, safety laboratory variables and electrocardiogram.

- To explore the safety and tolerability of AZD1656 in patients with severely impaired glucose control during a 4 months treatment period.

2.3 Exploratory objectives

- To compare the effect on glucose control of the included AZD1656 dosing regimens with the comparator glipizide as add-on treatment to metformin.

- To investigate the effect on glucose control of patients in the open label AZD1656 treatment with severely impaired glucose control (baseline HbA1c between >10 % and ≤12 %) during a 4 months treatment period.

- To collect and store DNA for future exploratory research into genes/genetic variation that may influence response (ie, distribution, safety, tolerability and efficacy) to AZD1656, metformin and glipizide.

2.4 Extension Objectives

- To investigate the safety and tolerability of AZD1656 compared to placebo and glipizide after 6 months by assessments of adverse events occurring during the study (including hypoglycaemic events), blood pressure, pulse, physical examination, body weight, safety laboratory variables and electrocardiogram.

- To evaluate other cardiovascular risk factors, such as total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides and

c-reactive protein, after 6 months in patients receiving AZD1656 as add-on treatment to metformin compared to placebo as add-on treatment to metformin

Exploratory objectives

Potential variables of efficacy and /or pharmacokinetic properties, which may be explored in patients treated with AZD1656, glipizide and/or placebo:

- Change in HbA1c between baseline, 4 months and 6 months
- Change in FPG between baseline, 4 months and 6 months
- Change in self-monitored plasma glucose (SMPG) between baseline, 4 months and 6 months
- Number of responders (HbA1c ≤7% and ≤6.5) between baseline, 4 months and 6 months
- PK/PD modelling: to characterise the population PK and PD properties by utilisation of non-linear mixed effects modelling methodology

3. STUDY PLAN AND PROCEDURES

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

3.1 Overall study design and flow chart

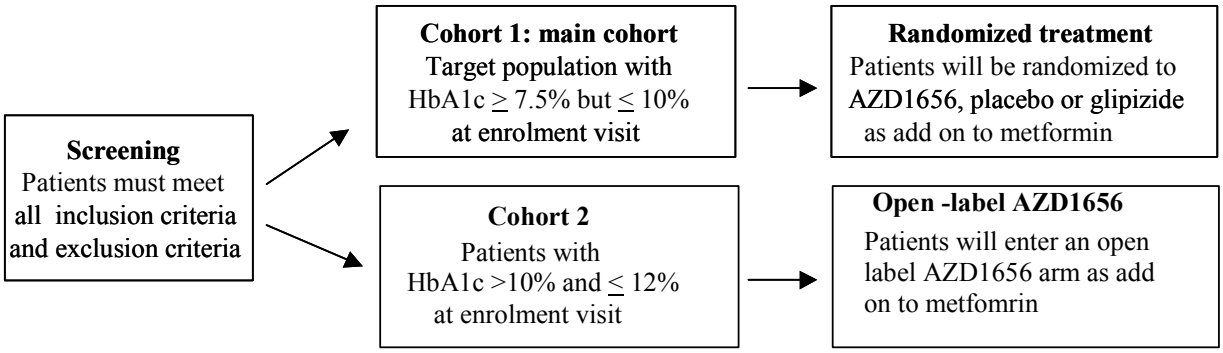
This will be a 4-month, randomized, double-blind, placebo- and active-controlled, multi-centre study of AZD1656 as add-on therapy to metformin compared to placebo, in approximately 540 T2DM patients. Glipizide will be included in separate treatment arm as add-on treatments to metformin. After 4 months, an optional 2 months extension will follow. The study consists of 7 parallel arms with 90 patients in each arm except for the 2 fixed dose arms with AZD1656 that will consist of 45 patients in each arm.

The study includes 2 cohorts of patients, see Figure 1. In the main treatment cohort (cohort 1) patients can be randomized to one of 6 different treatment arms with AZD1656, glipizide or placebo. Patients in this cohort should have HbA1c between ≥7.5 % and ≤10 % at enrolment. The patients will be randomized to 1 of 4 different dose regimens of AZD1656 (a minimum dose of 10mg daily titrated to maximum dose of 140mg daily, a minimum dose of 20mg daily titrated to a maximum dose of 200mg daily, 20mg daily or 40mg daily fixed doses), an glipizide regimen of glipizide (minimum dose of 5mg daily to a maximum dose of 20mg daily) or placebo, as add-on to an unchanged dose of metformin. The double-blind AZD1656/glipizide/placebo will be given twice daily.

Cohort 2:

In cohort 2, up to 90 eligible patients with HbA1c between >10 % and ≤12 % at enrolment will be assigned (no randomization) to an open-label treatment with AZD1656, which will be titrated from 20mg up to 200mg. For safety reasons, patients in cohort 2 must meet all eligibility criteria and after the initial titration period, the same criteria for hyperglycaemic rescue medication will apply also in this cohort. Recruitment will be completed regardless of reaching the number in the open-label AZD1656 arm. The termination of the recruitment will be linked to cohort 1 (450 randomized patients).

Figure 1 Study design overview



Target patient population is T2DM patients not adequately controlled on maximum tolerated metformin dose (≥ 1500mg) as single therapy and with stable metformin treatment for at least 10 weeks prior to enrolment (screening).

An enrolment visit (screening) will take place 4 weeks before the start of randomized study treatment. Two weeks prior to randomization, eligible patients will be switched to the study metformin provided by AstraZeneca (see Section 5.5) but keeping their initial dose unchanged, and receive glucometers for self-monitored plasma glucose (SMPG) and the patient diary. Eligible patients will then enter the study and receive randomized treatment for 4 months, with an optional 2 months extension period. During the randomized treatment period, visits to the clinic are scheduled on a weekly basis during the initial 4-week titration period, followed by visits each month during the maintenance phase up to 4 months as well as during the optional 2 months extension phase. A follow-up visit will be scheduled 2 weeks ± 5 days after the end of treatment visit (or last visit if premature discontinuation/early termination). All visits, except for Visit 2, will require fasting.

3.1.1 Study visits

Visit 1 (Enrolment visit)

At the enrolment visit 18 to 28 days before randomization and after written informed consent is given, eligible patients will enter the study. Each patient will be allocated a strictly sequential enrolment code (E-code) by accessing the IVRS/IWRS system.

The following enrolment assessment will be performed: demography, relevant medical and surgical history, smoking history, alcohol use, physical examination, ECG, vital signs (BP and pulse), weight and height, concomitant medications.

All patients need to be fasting, see Section 5.1. A safety laboratory screen will be done by central lab. Additional blood samples will be taken such as FPG, HbA1c, Insulin, C-peptide. For women whose last menstruation was >1 year ago , serum FSH and LH will be tested to ensure that they are within the postmenopausal range.

Visit 2 (Run-in period)

At visit 2, eligible patients will be switched to the study metformin, but keeping their initial dose unchanged. Patients will be given a Patient Diary and receive glucometers for self-monitored plasma glucose (SMPG). For more information on SMPG se section 3.1.2. A fasting morning plasma glucose and a bed-time plasma glucose assessment are needed on a daily basis from the run-in period until the end of the titration period. At this visit, patients do not need to be fasting.

Visit 3 (Randomization visit)

At visit 3, eligible patients will be randomized to study medication (AZD1656, glipizide or placebo treatment) via the IVRS/IWRS system. The eligible patients in cohort 2 will be assigned (no randomization) to an open-label treatment with AZD1656. Patients will be fasting and undergo a physical examination, a safety laboratory screen, ECG, vital signs (BP and pulse), weight, waist and hip circumference measurement and concomitant medications. Advice will be given for diet and life style during the visit. Additional blood samples will be taken such as FPG, HbA1c, Insulin, C-peptide, Lipids and CRP. The OGTT test will be performed in a subgroup of patients.

The patient diary will be reviewed for compliance. The site will check that the patient has taken their metformin as planned. The first dose of the study medication will be taken together with breakfast at the site or at home/work. The investigator will also record if any Adverse Event (AE) occurred since the last visit.

After signing the optional genetic informed consent, a blood sample can be taken at any visit after randomization.

Visit 4 to Visit 7 (Titration period)

One week after randomization (visit 4) and thereafter one visit per week (visit 5 to visit 7) will be scheduled in the morning before dose. Patients will come in fasting. Diet and lifestyle

advice will be given at all visits. New study medication will be allocated to the patients through the IVRS/IWRS system at each visit.

The following assessments will be done: Diary compliance review, PK sampling of AZD1656 (visit 4 and visit 7), physical examination (visit 7), safety laboratory screen, ECG (visit 4 and visit 7), vital signs (BP and pulse), weight (visit 7) and concomitant medications. Additional blood samples will be taken at visit 7 such as FPG, HbA1c, Insulin, C-peptide, Lipids and CRP. Drug accountability will be recorded and new study medication will be dispensed to cover the need for the coming week. The investigator will also record if any AE occurred since the last visit.

The patients needs to do a 7-point glucose assessment at home within 3 days before Visits 4, 5, 6 and 7 and register the results in the Patient Diary.

Visit 8 to Visit 10 (Maintenance period)

Patient will come for visits each month starting from 2 month after randomization (visit 8) until four months after randomization (visit 10). Patients will come in fasting and undergo a physical examination, a safety laboratory screen, ECG, vital signs (BP and pulse), weight (visit 9 and visit 10), waist and hip circumference (visit 10) and concomitant medications. Advice will be given for diet and life style during all visits. Additional blood samples will be taken at visit 10 such as Insulin, C-peptide, Lipids and CRP. Fasting plasma glucose and HbA1c will be taken at all visits. PK will be taken at visits 9 and 10. The OGTT test will be performed in a subgroup of patients at visit 10. All left over study medications needs be recorded. The investigator will also record if any AE occurred since the last visit.

The patients need to do a 7-point glucose assessment at home within 5 days before Visit 8 to Visit 10 and register the results in the Patient Diary. Patients not entering the extension period will be advised to re-start their pre-study metformin treatment during Visit 10.

Visit 12 and Visit 13 (Extension period)

Patients will have to provide a new informed consent for the extension of the study. Patients will come for monthly visits, Visit 12 at 5 months and visit 13 at 6 months.

Visit 11 (Follow-up visit) and Visit 14 (Follow-up visit -Extension)

Two weeks (±5 days) after the last visit, the patient will be scheduled in the morning to a final visit. Any ongoing AEs will be followed up as long as medically indicated. Patients will come in fasting and undergo a physical examination, a laboratory screen, ECG, vital signs (BP and pulse) and weight measurement, advice for diet and life style will be given.

Genetic informed consent and blood samples

Patients who take part in the genetic component of the study will be required to give written informed consent prior to blood being drawn. The blood sample for genetic research will be obtained from the patients at Week 0 (Visit 3) after randomization. If for any reason the sample is not drawn at Visit 3, it may be taken at any visit until the last study visit. Only

1 sample should be collected per patient for genetics during the study. The blood draw should not take place prior to randomization.

Unscheduled visit

Extra visits may be necessary for safety reasons. These visits will be recorded in the eCRF as unscheduled visits, necessary safety samples sent to the central lab and AE/SAE section completed as deemed appropriate by the Investigator.

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

Extension of the 4-month study:

At the 4 months visit, the following two options will be offered to study participants:

- Continue as per the original study protocol, with the scheduled follow-up visit 2 weeks after the last dose at 4 months
- Patients can provide informed consent for optional treatment for 2 additional months, ie up to 6 months randomized treatment. Patients consenting to the extension will have their follow-up visit 2 weeks after having taken the last IP at the 6-month visit (or earlier if pre-matured stopped IP) .

All patients consenting to the extended study will continue on the same blinded (or open in cohort 2) treatment as during the 4-month study. Patients, investigators and site monitors will be kept blinded during the whole study. After finalising and cleaning the data in the 4-month study, the sponsor and CRO will be unblinded.

3.1.2 Self-monitoring of plasma glucose

All patients will be asked to check their plasma glucose at home on a regular basis during the study from visit 2 and record results in a Patient Diary. Patients will also be instructed to measure plasma glucose if they experience symptoms of hypoglycaemia or hyperglycaemia and register plasma glucose results and event-related information in the Patient Diary. Patients will be instructed to contact the trial site if a self measured plasma glucose is <3 mmol/L (54mg/dL) at any time, or if fasting plasma glucose is >11 mmol/L (198 mg/dL) at 2 consecutive mornings. The Patient Diary will be reviewed by the investigator at each visit from baseline (Visit 3) and onwards.

Between Visit 2 and 3, and during the titration weeks, at least a fasting morning plasma glucose and a bed-time plasma glucose assessment are needed on a daily basis. Within 3 days before Visit 3, 4, 5, 6 and 7, a 7-point glucose assessment (see Section 6.3.1.3) will be required at home. After the titration period, ie, after Visit 7, plasma glucose assessments will not be required on a daily basis, but patients are requested to collect a 7-point glucose assessment within a 5-day window before Visits 8, 9 and 10 and register the results in the Patient Diary. Patients entering the extension period are requested to collect a 7-point glucose assessment within a 5-day window before extension visit 12 and visit 13 and register the results in the Patient Diary. However, the protocol-mandated frequency of self-monitoring of

glucose levels should be seen as a minimum. The investigator may increase the test frequency or ask for testing also at other time-points, as considered medically necessary, eg, in case of inadequate glucose control, infections or addition of any new concomitant medications that may influence glucose levels.

3.1.3 Treatment with AZD1656/placebo/glipizide and titration overview

The investigational products (IPs) in this study for patients in cohort 1are the blinded, randomized treatments of AZD1656/placebo, glipizide/placebo and placebo/placebo. Patients in cohort 2 will be assigned the open treatment of AZD1656.

For patients in cohort 1, the dose of glipizide and of AZD1656 in the titrated arms will be increased stepwise on a weekly basis in a blinded fashion during the first 4 weeks after randomization. On Visits 4, 5 and 6 the dose of blinded treatment will be increased if the fasting morning plasma glucose analysed at the clinic at the specific visit is >6.1 mmol/L (110mg/dL), and no conditions (eg hypoglycaemic risk) oppose a dose increase according to investigator's judgement, See Figure 2. For patients in cohort 2, the open-label dose of AZD1656 will be increased in the same way, but the increase will be open to both the investigator and the patients. See Table 1 for dose steps during the titration period. Once the dose of AZD1656/glipizide/placebo is established, patients will continue on the dose achieved for the rest of the study, unless reduction is needed due to hypoglycaemia, as judged by the investigator (dose reductions will be simulated in the fixed AZD1656 arm and the placebo arm).

There will be a simulated dose increase in the placebo arm and the fixed dose arms with AZD1656.

Figure 2 Dosing overview during titration

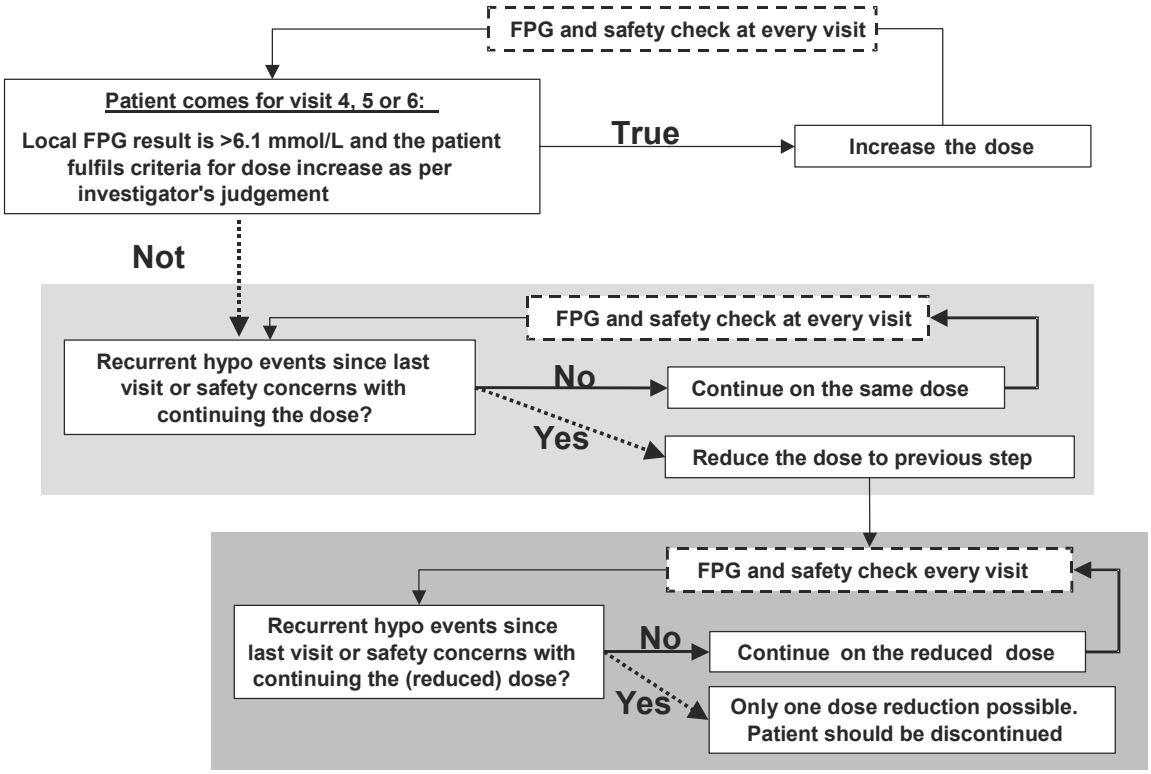


Table 1 Schematic overview of possible daily doses of AZD1656 and glipizide during the initial titration period

	Visit 3	Visit 4	Visit 5	Visit 6
	0 = baseline	After 1 week	After 2 weeks	After 3 weeks
AZD1656 open label dose span	20mg (10mg bid)	50mg (25mg bid)	100mg (50mg bid)	200mg (100mg bid)
Randomized treatment				
AZD1656 arm higher dose span	20mg (10mg bid)	50mg (25mg bid)	100mg (50mg bid)	200mg (100mg bid)
AZD1656 arm lower dose span	10mg (5mg bid)	20mg (10mg bid)	50mg (25mg bid)	140mg (70mg bid)
AZD1656 fixed dose arm	40mg (20mg bid)	40mg (20mg bid)	40mg (20mg bid)	40mg (20mg bid)
AZD1656 fixed dose arm	20mg (10mg bid)	20mg (10mg bid)	20mg (10mg bid)	20mg (10mg bid)
Glipizide arm	5mg (5 + 0mg)	10mg (5mg bid)	15mg (10 + 5mg)	20mg (10mg bid)
Placebo arm	Placebo	Placebo	Placebo	Placebo

3.1.4 PK sampling

One blood sample for PK analysis will be taken prior to the morning dose at Week 1 (Visit 4), Week 4 (Visit 7) and Month 3 (Visit 9). During Month 4 (Visit 10), blood samples for PK analysis will be taken before the morning dose and up to 5 hours post-dose. Patients entering the extension will take one blood sample for PK analysis prior to the morning dose at visit 13.

3.1.5 OGTT

In order to study effects of AZD1656 on post-prandial glucose control, an OGTT, with collection of blood samples over 2 hours, will be performed at baseline (Week 0, Visit 3, no IP at this visit) and 4 months (Visit 10). This test will only be performed in a subgroup of the study population (approximately half the patients in the study) at selected sites. For details regarding the OGTT, see section 6.3.1.4.

3.1.6 Hyperglycaemia during study

After randomization, definitions of hyperglycaemia and the use of rescue medication will apply, see Section 5.6.1. Patients will receive written instructions on the need for plasma glucose assessments in case of hyperglycaemia symptoms, and to contact the investigator if two consecutive fasting morning plasma glucose values are >11 mmol/L (198 mg/dL). The investigator must also at every study visit consider extra plasma glucose monitoring, especially if the patient's glucose levels start to deteriorate. Patients meeting criteria for hyperglycaemia should receive treatment with basal night-time insulin in addition to metformin and the randomized IP treatment.

3.1.7 Hypoglycaemia

Patients will receive instructions regarding hypoglycaemia symptoms and treatment, and to contact the clinic if plasma glucose is <3 mmol/L. Patients experiencing hypoglycaemia during the titration period (until Visit 7) may have their dose of AZD1656/placebo/glipizide reduced as judged by the investigator (open dose reduction in the open-label AZD1656 arm and simulated dose reduction in the AZD1656 fixed-dose arms and placebo arm). The reduction in dose can be done if the patient experience two or more hypoglycaemic events with documented plasma glucose below 3.0 mmol/L (54mg/dL) within a week or if the investigator judged that there is a safety concern for the patient to stay on the same dose as before. Such a dose reduction can only be done once, if safety concerns remain despite a dose reduction, the patient must be discontinued.

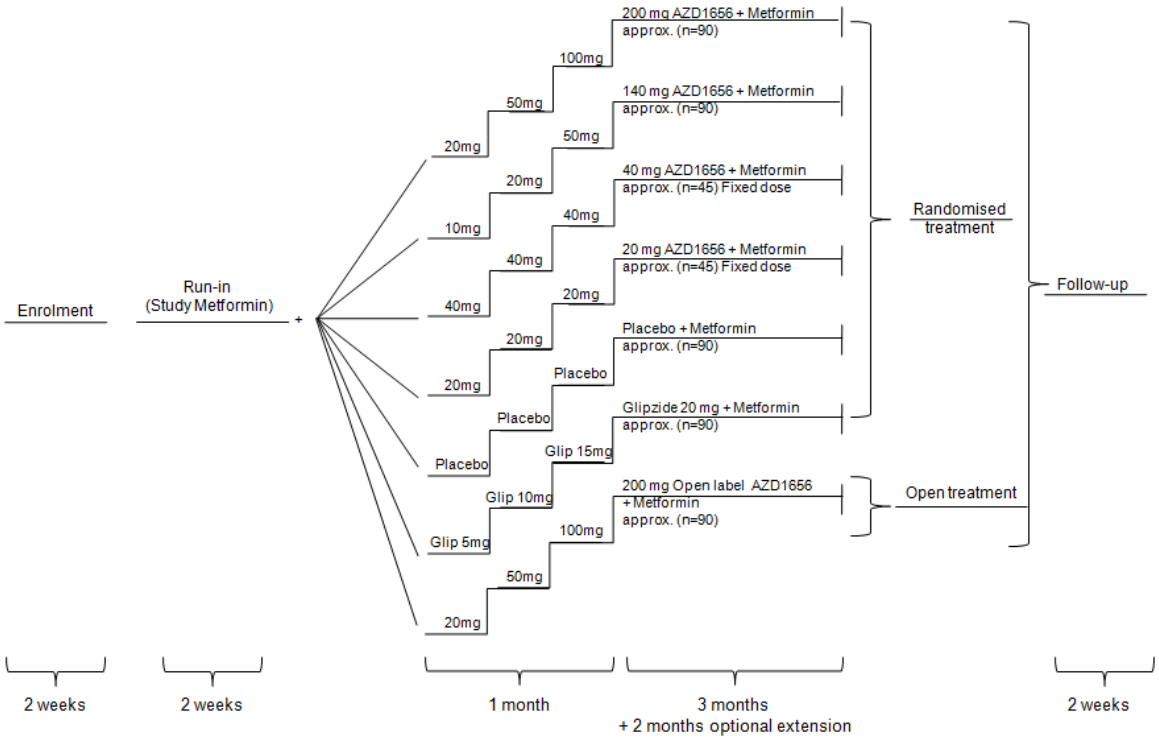
The investigator must continuously evaluate if study participation is safe for the individual patient and if recurrent hypoglycaemia despite dose reduction, if applicable, patient may have to be discontinued from study (for study specific discontinuation criteria see Section 5.8).

3.1.8 Adjudication of cardiovascular events

An Adjudication Committee will be appointed to evaluate all deaths and suspected cardiovascular events from the time point of randomization until study completion and perform an event categorisation. The adjudication procedure will be defined in a specific adjudication charter and will include events of death, myocardial infarction/acute coronary

syndrome, stroke, events of cardiac heart failure and arrhythmia. This evaluation will be performed on blinded data.

Figure 3 Study flow chart



Glip. = Glipizide.

Table 2 Study Plan

Activity	Enrolment period	Run-in period	Randomized treatment period								Extension		Follow-up period
Visit number	1	2	3	4	5	6	7	8	9	10	12	13	11 or 14 [o]
Study week / month	*W -4*	*W -2*	*W0*	*W1*	*W2*	*W3*	*W4*	*M2*	*M3*	*M4*	*M5*	*M6*	*2 weeks ± 5 days after last visit*
Study day and visit window	-28 to -18	-16 to -12	0	7(±2)	14(±2)	21(±2)	28(±2)	60(±5)	90(±5)	120(±5)	150 (±5)	180(±5)	
Informed Consent	x					(x [n])	(x [n])	(x [n])	(x [n])	x [n]			
Genetic informed consent [b]	(x)	(x)	(x)										
Randomization			x										
Inclusion/exclusion criteria	x	x	x										
Demography and nicotine/alcohol use	x									x [c]			
Medical/surgical history	x												
Physical examination	x		x				x	x	x	x		x	x
12-lead digital ECG	x		x	x			x	x	x	x	x	x	x
Height	x												
Vital signs (pulse, BP)	x		x	x	x	x	x	x	x	x	x	x	x
Weight	x		x				x		x	x		x	x
Waist and hip circumference			x							x		x	
Test for HIV, Hepatitis B and C	x												
Drug screening test	x												
FSH, LH [a]	x												
Safety blood and urine sampling	x		x	x [d]	x [d]	x [d]	x	x	x	x	x	x	x
Fasting plasma glucose (FPG) [e]	x		x	x	x	x	x	x	x	x	x	x	x
HbA1c	x		x				x	x	x	x	x	x	
Efficacy blood samples (Insulin, C-peptide, lipids and CRP)	x [f]		x				x			x	x	x	
SMPG [g]		x	x	x	x	x	x	x	x	x	x	x	
Distribution and review of Patient Diary		d	d/r	d/r	d/r	d/r	d/r	d/r	d/r	d/r	d/r	r	
PK blood sampling for AZD1656				x [h]			x [h]		x [h]	x [i]		x [h]	
Diet and lifestyle advice		x	x	x	x	x	x	x	x	x	x	x	x

Activity	**Enrolment period**	**Run-in period**	**Randomized treatment period**								**Extension**		**Follow-up period**
Visit number	1	2	3	4	5	6	7	8	9	10	12	13	11 or 14 [o]
Study week / month	*W -4*	*W -2*	*W0*	*W1*	*W2*	*W3*	*W4*	*M2*	*M3*	*M4*	*M5*	*M6*	*2 weeks ± 5 days after last visit*
Study day and visit window	-28 to -18	-16 to -12	0	7(±2)	14(±2)	21(±2)	28(±2)	60(±5)	90(±5)	120(±5)	150 (±5)	180(±5)	
Contact IVRS/IWRS	x	x	x	x	x	x	x	x	x	x	x		
Dispense of blinded IP (AZD1656, placebo, or glipizide)			x	x	x	x	x	x	x	x	x		
Dispense of open metformin [m]		x		x	x	x	x	x	x	x	x		
Dispense of open AZD1656			x	x	x	x	x	x	x	x	x		
Drug accountability, IP (AZD1656, placebo or glipizide)				x	x	x	x	x	x	x	x	x	
Drug accountability, metformin [m]			x	x	x	x	x	x	x	x	x	x	
Concomitant medication	x	x	x	x	x	x	x	x	x	x	x	x	x
Genetic blood sampling [b]			(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)
AE / SAE collection [k]	x	x	x	x	x	x	x	x	x	x	x	x	x
OGTT [l]			x							x			

d: distribution, r: review, d/r: distribution and review

a For females where postmenopausal status needs to be confirmed, see Section 4.1
b After the patient has signed the genetic informed consent at any of visits 1, 2 or 3, the blood sample can be taken at any visit after randomization.
c Nicotine use only.
d Excluding p-lactate and cystatin-C.
e FPG to be analysed immediately locally at the clinic. A venous sample will also be sent to the central laboratory.
f No lipid status or CRP at enrolment visit.
g Self-monitoring of Plasma Glucose at home. Section 3.1.2 and Section 6.3.1.3.
h One blood sample for PK analysis before morning dose (trough sample).
i PK samples will be taken before morning dose and up to 5 hours post-dose. See Section Section 6.6.
k All AEs will be collected from the time of the first administration of IP until the end of the study (including follow-up visit). Before the first administration of IP, only SAEs will be collected.
l OGTT will be performed in a subgroup of patients at selected sites. Blood sampling before and during 2 hours after glucose intake. See Section 6.3.1.4.
m Depending on the individual dose, and hence the individual consumption of different tablet strengths, metformin dispensing and drug accountability occasions may differ between patients.
n The informed consent form for the optional extension period should be signed at any visit but no later than at visit 10.
o For patients completing the study at 4 months, the follow-up visit no 11 will be used. For patients consenting to the extension, the follow-up visit no 14 will be used

3.2 Rationale for study design, doses and control groups

The purpose of this dose finding study is to provide information on efficacy and safety to support the selection of the optimal dose range(s) of AZD1656 to be used in the confirmatory phase III programme.

Study design, choice of control group(s) and treatment duration

A randomized, double-blind, parallel-group, multi-centre, placebo-controlled study design is standard in dose finding studies and is considered the best choice to achieve the objectives of the study, from both safety and efficacy perspectives. The study is designed to demonstrate superior efficacy versus placebo; a placebo control arm in the trial will enable placebo corrected analysis of safety and efficacy.

Comparing the efficacy of new drugs for treatment of T2DM by comparing the results with data in the literature is difficult, since baseline characteristics and the way AEs are collected and defined, especially hypoglycaemia, which may differ between studies and influence the result. To generate data on approved anti-diabetic drugs in the same specific study setting, one arm with glipizide is included in the study as add-on treatment to metformin. Data from these combinations will enhance understanding of the performance of AZD1656 from both an efficacy and safety perspective. The blinded, titrated SU arm will be used for safety and efficacy comparisons since both SUs and AZD1656 are insulin-providing drugs with the possibility to induce low glucose values. From the SU group, glipizide has been chosen due to possessing PK properties similar to AZD1656 and the possibility to titrate this drug on a weekly basis. Furthermore, it is a second generation SU and perceived to have a less likelihood to induce hypoglycaemia than the older agents. In addition, the open arm with AZD1656 is included to explore the safety and tolerability of AZD1656 during a 4 months treatment period, with an optional 2 months single-blind extension, in patients with severely impaired glucose control.

The pre-defined rescue treatment in case of hyperglycaemia will ensure safe glucose control in all patients, regardless of randomization, during the study.

Glipizide and AZD1656 stimulate insulin secretion and, in order to mitigate the risk for hypoglycaemia, the dose of these drugs will be increased stepwise based on the glucose response during the initial titration period. A stepwise dose increase is in line with the current clinical use of SU drugs and also in line with the anticipated future use of AZD1656. Considering this initial titration period, a total treatment period of 4 months has been selected with the aim to ensure appropriate duration of the maintenance period to generate robust safety and efficacy data to support a Phase III programme. However, an optional 2-month extension period is added to provide safety data up to 6 months. A double-dummy technique will be used with simulated titration in the fixed dose arms of AZD1656 and the placebo arm, in order to keep the blind (see Section 5.4).

Patient population and background anti-diabetic treatment

Metformin will be the drug of choice for the vast majority of newly diagnosed T2DM patients for the foreseeable future according to T2DM treatment guidelines. However, many patients with T2DM do not reach glycaemic control goals with metformin monotherapy and the addition of a glucose-lowering agent, with a different mechanism of action, is indicated. The AZD1656 development programme is initially focused on second line treatment and consequently, the patient population for this dose ranging study will be TD2M patients not adequately controlled on metformin. According to regulatory guidelines for add-on studies, criteria for background treatment should ensure that maximally tolerated dose have been reached as well as the maximal efficacy that could be expected (ref European Medicines Agency [EMEA] and Food and Drug Administration [FDA] guidelines for DM therapies). However, it is recognised that local clinical practice sometimes add the second drug already at lower doses of metformin. To ensure standardised condition in this multi-centre study and in line with regulatory guidelines, the patients must be on a maximally tolerated metformin dose of at least 1500mg daily. To allow evaluation of randomized treatment effects, the dose of metformin must be stable. The other eligibility criteria aim at recruiting a study population, which is representative of the future target population, but without significant or unstable medical conditions that could compromise patient safety, influence the ability to comply with study procedures or interfere with the interpretation of efficacy and safety data.

Choice of dosing regimens

AZD1656 is given twice daily. The suggested dose range of AZD1656 to be evaluated in this study have been defined based on data from T2DM patients in the Phase I and IIa studies where studies of 100mg AZD1656 daily on top of metformin and 180mg daily on top of insulin during 4 weeks as well as 300mg on top of insulin for 1 week were included. The dose range is believed to include safe starting doses (10mg to 40mg daily), as well as safe and effective top doses in the titrated arms (140mg and 200mg daily, respectively). Two arms with a fixed dose of AZD1656 are included in order to thoroughly evaluate the effects of AZD1656 over time.

The glucose-lowering effect of AZD1656 corresponds with the plasma concentrations of AZD1656. The half-lives of AZD1656 and its active metabolite AZ12555623 are approximately 4 and 12 hours, respectively. These half-lives suggest that steady states for AZD1656 and AZ12555623 are achieved during the first day and after 3-4 days, respectively. Moreover, data from 1-month studies of AZD1656 indicate that most of the PD effect is achieved within the first week. Thus, the dose will be increased weekly based on the glucose response. The dose of glipizide will also be increased weekly, which is in line with the approved dosing instructions.

Primary and secondary variables

HbA1c is considered the standard surrogate outcome measure in diabetes trials and thus used for evaluation of the primary objective in this study. Use of the secondary variables related to safety and tolerability defined in Section 6.4 are standard. Fasting plasma glucose, insulin and

the OGTT will be used to evaluate effects of AZD1656 on fasting and post-prandial glucose control as well as beta-cell function and insulin resistance over time.

Pharmacokinetic sampling

Pharmacokinetic blood samples will be collected in order to characterise the PK properties of AZD1656 and to link the PD response to drug exposure, using population PK/PD modelling. Employing the non-linear mixed effects modelling approach, a sparse PK sampling design is enabled.

Genetic sampling

Several genetic variants in the GK gene have already been identified, eg, variants leading to a less functional enzyme as in MODY2. Thus, genetic factors affecting the GK enzyme itself or other mechanisms involved in the PD effects and/or the PK properties of the drug might potentially influence inter-individual variability in efficacy and safety during treatment. Therefore, blood samples for DNA extraction will be taken (optional for participants) and DNA will be saved for potential future genetic research of genes/genetic variants influencing drug response (ie, distribution, safety, tolerability and efficacy) to AZD1656, metformin and glipizide.

4. PATIENT SELECTION CRITERIA

Investigator(s) should keep a record of patients who were pre-screened and screened for the study via patient tracking records.

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

4.1 Inclusion criteria

For inclusion in the study patients should fulfil the following criteria:

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

2. Male or female of non-childbearing potential (postmenopausal, and/or have undergone hysterectomy and/or bilateral oophorectomy or salpingectomy/tubal ligation) aged ≥ 18. Women will be defined as postmenopausal if last menstruation period was >1 year ago and serum FSH and LH are within the postmenopausal range, or if age >50 years and with last menstruation period >2 years ago.

3. Body mass index between ≥ 19 and ≤ 42 kg/m^2.

4. Clinical diagnosis of T2DM.

5. Treated with maximally tolerated dose of metformin (≥ 1500mg/day) for at least 10 weeks prior to enrolment.

6. Patients with HbA1c ≥ 7.5 but ≤ 10% at enrolment visit (Visit 1) (HbA1c value according to international DCCT standard and obtained from the central laboratory used in the study) can enter cohort 1. Patients with HbA1c between >10 % and ≤12 % can enter the open-label arm with AZD1656 (cohort 2)

7. Patients must have taken at least 75% of their metformin tablets during the run-in period (ie, from Visit 2 to Visit 3).

For inclusion in the optional genetic research, patients must fulfil all of the inclusion criteria described above and:

- Provide informed consent for the genetic sampling and analyses.

4.2 Exclusion criteria

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

1. Clinical diagnosis of Type 1 diabetes.

2. Positive test for Hepatitis B surface antigen or antibodies to HIV virus or antibodies to Hepatitis C virus. Results obtained from the central laboratory will be utilised.

3. Participation in weight loss programme (other than the lifestyle advice normally given to T2DM patients) or use of weight loss drugs (OTC or prescriptions) in the last 3 months

4. Participation in another clinical trial within 30 days prior to enrolment.

5. Unwilling or unable to perform self-monitoring of plasma glucose at home according to protocol.

6. Significant cardiovascular event within the last 6 months prior to enrolment (eg, myocardial infarction/acute coronary syndrome, revascularisation procedure, stroke or transient ischaemic attack) or heart failure New York Heart Association (NYHA) class III-IV.

7. Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) laboratory results >3xULN at enrolment, obtained from the central laboratory used in the study.

8. Haemoglobin laboratory results <115 g/L at enrolment, obtained from the central laboratory used in the study.

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

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

11. Known allergy to glipizide.

12. 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.

13. Impaired renal function in terms of GFR<60 ml/min, based on Modification of Diet in Renal Disease Study Group (MDRD) calculation[a].

14. Past or present alcohol or drug abuse within the last 5 years or positive test in drugs of abuse screens.

15. SBP >160 mmHg or DBP >95 mmHg at enrolment/screening.

16. Treatment with any other anti-diabetic agent than metformin during the last 10 weeks prior to enrolment/screening.

17. Proliferative retinopathy or other significant diabetes complications as judged by the investigator.

18. Use of warfarin or amiodarone within 3 months prior to enrolment (screening) and use of potent CYP450 inhibitors, eg, ketoconazole and/or macrolide antibiotics within 14 days before randomization.

19. Use of anabolic steroids and systemic treatment with glucosteroids within 3 months before enrolment. Inhalation steroid treatment allowed.

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

21. Intake of another investigational drug within 30 days (or at least 5 $t_{1/2}$ of the drug, if longer than 30 days) before enrolment.

22. Prior exposure to GKAs.

23. Involvement in the planning and conduct of the study or staff at the investigational site.

24. Any other condition prohibiting the patient from participating in the study according to the protocol as judged by the investigator.

25. Previous bone marrow transplant (applicable only for genetic sampling).

26. Whole blood transfusion within 120 days of the date of genetic sample collection (applicable only for genetic sampling).

27. Unable or unwilling to use basal night-time insulin as rescue treatment during the study period in case of hyperglycaemia.

Procedures for withdrawal of incorrectly enrolled patients are presented in Section 5.3.

a GFR (mL/min per 1.73m2) = 186 x (SCr)-1.154 x (Age) –0.203 x (0.742 if female) x (1.210 if African-American).
SCr = serum creatinine concentration inmg/dL, age is in years, and weight is in kg.

5. STUDY CONDUCT

5.1 Restrictions during the study

Patients will have to comply with the following restrictions:

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

2. Fast, except for water, from the night before each visit to the clinic (not applicable to Visit 2), including enrolment visit (Visit 1). During the randomized treatment period, patients should not take the IP morning dose until after arrival at clinic and after blood samples have been collected.

3. Concomitant medication: No other anti-diabetic agents than defined in the CSP according to criteria for rescue medication for hyperglycaemia are allowed during the study. However, acute treatment, up to 7 days, with any kind of insulin is allowed in all patients in acute situations, eg, infections or surgery. Treatment with certain drugs during the randomized treatment period will result in discontinuation of the patient (amiodarone, warfarin, rimfampicin or potent CYP450 inhibitors, such as, but not limited to, ketokonazole and macrolide antibiotics). All other drugs will be allowed during study and changes in concomitant medications will be registered in the CRF.

4. Patients must not participate in any weight-loss programme, other than the life-style advice given according to Study Protocol, and should not initiate pharmacological weight-loss treatment during the study.

5. In case of event of suspected hypoglycaemia, patients should record the details of the event, including plasma glucose values if possible, in their Patient Diary.

6. Male patients must refrain from fathering a child during the study and for 3 months after the dose of study drug. They should not donate sperm and ensure that their partners of child bearing potential use a reliable method of contraception as well as themselves using a barrier method for this period. Acceptable methods used by female partners of child bearing potential include the oral contraceptive pill, hormonal implants, intra-uterine devices (IUDs) or diaphragms with spermicide.

Female partners should use this additional contraception for at least 3 months after the follow-up visit.

5.2 Patient enrolment and randomization

The Principal Investigator or appropriate delegate, will be responsible for the patient enrolment and randomization procedures. Signed, written informed consent must be obtained from potential patients before any study-specific procedures are performed. Once a patient has signed the written informed consent, they will be assigned with a unique identifying number (enrolment number, beginning with "E"). The enrolment number will be assigned by the site and will be used as the identification number for the patient throughout the study. Once allocated, it will not be re-used. The enrolment number will be a combination of the country number, site number and the sequential patient at that site.

Patient eligibility will be checked at Visit 1 at the start of the enrolment phase (see Sections 4.1 and 4.2) and will be confirmed at Visit 3 (before treatment randomization). If a patient withdraws from participation in the study, the patient will not be allowed to re-enter the study and the patient's enrolment or randomization number cannot be re-used. The IVRS/IWRS will also be used to assign study medication for the open-label treatment with AZD1656.

The Principle Investigator, or appropriate delegate, will be required to enter information regarding each patient's study visit in to the ICON ICOPhone Interactive Voice/ Web Response System (IVRS/IWRS). During Week –4 (Visit 1), the patient identifier information and visit date information will be entered. Additional information may be requested to track participation in the OGTT substudy. After Week –4 (Visit 1), the site will be requested to enter the E-code, visit number and additional information. Information regarding use of IVRS/IWRS will be provided in the ICOPhone IVRS/IWRS study manual.

Blinded treatment during the extension

All patients will continue on the same blinded treatment as during the 4 months study.

5.2.1 Procedures for enrolment/randomization

Procedures for enrolment

The Principle Investigator or delegate will:

- Obtain signed informed consent from the potential patient before any study-specific procedures are performed.
- Assign potential patients with a unique enrolment number, beginning with E….001.

Procedures for randomization

The Principle Investigator or delegate will:

- Determine patient eligibility (see Section 4).

- Assign eligible patients with a unique randomization code (patient number) through IVRS/IWRS.

Patients who satisfy the entry criteria at Week 0 (Visit 3) will be randomized to receive either AZD1656 (10mg daily titrated to maximally 140mg daily, 20mg titrated to maximally 200mg daily (blinded or open), 20mg daily or 40mg daily fixed dose), placebo or glipizide (5mg daily titrated to maximally 20mg daily) in combination with background metformin. The randomization scheme will be generated by AstraZeneca, using the global randomization system (GRand). Randomization codes will be assigned as patients become eligible for randomization and each patient will be assigned a unique randomization code.

Nobody outside the group generating the randomization scheme will have knowledge of the scheme. Hence, investigators will have no knowledge beforehand of the treatment the patient will receive. If a patient is provided incorrect treatment by mistake (ie, treatment not consistent with that assigned by ICOPhone IVRS/IWRS), attempts should not be made to remedy the error once study medication has been dispensed and ingested by the patient. An ICON Clinical Research representative should be immediately notified as soon as any error is discovered, in order to determine the patient status (ie, allowance to continue or need for immediate withdrawal). If a patient is provided with the wrong treatment, this will be noted as a protocol violation, regardless of whether the patient is allowed to continue or not.

5.2.2 Run-in period and study metformin

An enrolment visit will take place 4 weeks before the start of randomized study treatment. Two weeks prior to randomization, eligible patients will be switched to study metformin, but with their metformin dose unchanged (ie 1.5 – 3.0 g). Patients will receive glucometers and a diary for recording glucose measurements at home, as well as any suspected hypoglycaemic events. Eligible patients will then enter the study and receive randomized treatment for 4 months, with an optional 2-month extension.

5.3 Procedures for handling patients incorrectly enrolled or randomized

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

Incorrectly randomized patients must be excluded. Correctly randomized patients can never retrospectively fail enrolment criteria. Discontinuation post-randomization is regulated by study-specific discontinuation criteria or investigator judgement.

The ICON Medical Monitor or designee is to ensure all such decisions are appropriately documented. In situations where an agreement cannot be reached, the patient should have their study therapy stopped and be discontinued from the study.

5.4 Blinding and procedures for unblinding the study

5.4.1 Methods for ensuring blinding

All packaging and labelling will be done in such a way as to ensure blinding, except for the open-label AZD1656 arm. AZD1656 tablets will be identical in appearance, smell and taste to the matching placebo. Glipizide will be over-encapsulated to match the placebo capsules. Members of the study 9team, at investigational centres or third part vendors conducting study or handling data will not have access to randomization schedule, with the exception of AstraZeneca IPS, ICOPhone IVRS/IWRS and CRO for drug packaging and distribution. This documentation will be kept in a secure location until the end of the study. A double-dummy technique will be used with simulated titration in the fixed dose arms of AZD1656 and the placebo arm in order to keep them blind.

5.4.2 Methods for unblinding the study

Individual treatment information, indicating the treatment randomization for each randomized patient, will be available to the investigator(s) at the study centre and the personnel who are independent to the study evaluation at the Patient Safety Department from the IVRS/IWRS , in case of a need for emergency unblinding. IVRS/IWRS will provide 24 hours/day 7 days a week unblinding service. Routines for this will be described in the IVRS/IWRS user manual that will be provided to each centre.

The treatment information must not be unblinded except in medical emergencies when the appropriate management of the patient necessitates knowledge of the treatment randomization. If the treatment information is unblinded then the investigator(s) must document and report to ICON.

AZ retains the right to unblind the patient for SAEs that are unexpected and are suspected to be causally related to an investigational product and that potentially require expedited reporting to regulatory authorities. The data management committee (DMC) will keep treatment information strictly confidential and will not reveal any information to the study and project team.

5.4.3 Blinded treatment during the extension:

All patients will continue on the same blinded treatment (or open in cohort 2) as during the first 4 months of the study.

The sponsor and CRO personnel will have access the treatment codes after all patients in the 4-month study have been cleaned and database is locked (DBL) to allow for the planned analyses. However, the treatment codes will be strictly kept within the sponsor and CRO to safeguard the blinding of the monitors, investigators and patients and hence avoid possible bias in data handling. Thus, except for safety reasons, patients, investigators and study monitors in the field will have no access to the individual treatment codes until the study has been completed.

5.5 Treatments

5.5.1 Identity of investigational products and metformin

AZD1656 and placebo will be administrated as white, round tablets. The tablets will contain AZD1656 corresponding to the strength 5mg, 20mg, 50mg of the active compound respectively or placebo. The tablets will be packed into blister cards to achieve the intended dose. Each dose of AZD1656 will be composed of 2 tablets. The patient will take half of the daily dose in the morning and half of the daily dose in the evening.

Glipizide will be encapsulated into orange hard gelatine capsules and contain glipizide tablets corresponding to a dose of 5mg glipizide. The capsules will be packed into blisters of 5, 10, 15 or 20mg of glipizide or placebo. Each daily dose of glipizide will be composed of 4 capsules. When 5mg or 15mg is given as the total daily dose, the largest dose should be taken in the morning.

Patients randomized to either of the blinded arms of AZD1656 or glipizde will take two tablets AZD1656 and two capsules glipizide orally in the morning with breakfast and 2 tablets AZD1656 and 2 capsules glipizide will be administrated orally in the evening with dinner. Patients fulfilling the criteria for open-label AZD1656 arm will only take two tablets of AZD1656 at each dosing event.

A wallet, consisting of a blister card of AZD1656 dose 10, 20, 40, 50, 100, 140 , 200mg or placebo and a wallet consisting of a blister card of glipizide 5, 10, 15, 20mg or placebo, will be created. In total, 13 different wallet combinations will be created. AZD1656 and glipizide will be administrated twice daily together with breakfast and dinner. The patient will take 2 tablets of AZD1656 and 2 capsules glipizide at each administration. Patients randomized into either of active arms of AZD1656 will take glipizide placebo and vice verse.

The number of kits to be distributed for patients receiving AZD1656 and glipizide is presented in Table 3.

Table 3 Number of kits per visit for patients on AZD1656 and glipizide

Visit	AZD1656	Glipizide
2	-	-
3	1	1
4	1	1
5	1	1
6	1	1
7	4	4
8	4	4
9	4	4
10	4	4
Extension: visit 12	4	4

Two strengths of metformin tablets, 500mg and 850mg respectively, will be provided by the sponsor. The metformin will be packed in to blister cards, open-label, 100 tablets per package for the 500mg metformin and 56 tablets per package for the 850mg metformin . The dose will be individual but will stay between 1.5 and 3 g per day, administrated orally as tablets once, twice or three times daily together with meal. See Table 4 below for dosing regimes for metformin.

Table 4 Doses and number of metformin tablets for different doses

Daily dose metformin (mg)	Metformin Tablet strengths	No of 500mg metformin tablets	No of 850mg metformin tablets
1500	500	3	
1700	850		2
1850	500 + 850	2	1
2000	500	4	
2500	500	5	
2700	500 + 850	2	2
2850	500 + 850	4	1
3000	500	6	

All medication should be taken at approximately the same time of the day during the study period.

Primary and secondary packaging of AZD1656 and glipizide will be carried out by a CRO. Commercial metformin (study metformin) is primary packed by Merck and secondary packed by a CRO. The packaging will be carried out in accordance with Good Manufacturing Practice (GMP).

Table 5 Product Information

Investigational product	Dosage form and strength	Manufacturer
AZD1656	Tablets 5mg	AstraZeneca
AZD1656	Tablets 20mg	AstraZeneca
AZD1656	Tablets 50mg	AstraZeneca
AZD1656 placebo	Tablets	AstraZeneca
Investigational product	**Dosage form and strength**	**Marketing Authorisation Holder**
Glipizide	Capsule 5mg	Generics
Glipizide placebo	Capsule	Generics
Metformin*	Tablets 500mg	Merck
Metformin*	Tablets 850mg	Merck

*Metformin is additional drug.

5.5.2 Doses and treatment regimens

Treatment during run-in

During the run-in period (Weeks –2 [Visit 2] to Week 0 [Visit 3]) each patient will switch to study metformin and they will receive a box containing 100 tablets of 500mg and/or a box containing 56 tablets 850mg study metformin depending on individual dose metformin. Patients' initial dose of metformin will remain unchanged.

Randomized treatment period

The randomized treatment period starts at Week 0 (Visit 3) with a titration period of 4 weeks, where the patients will receive 1 double-blind kit containing either of AZD1656 starting dose of 10mg, 20mg, 20mg (fixed dose), 40mg (fixed dose), placebo, glipizide 5mg or 20mg open AZD1656. The patients will be titrated stepwise in a blind fashion to the target dose and the dose will be increased, if appropriate, weekly as they visit the clinic. Each week they will receive a new set of AZD1656 and glipizide with a higher strength. For patients in the fixed dose AZD1656 and placebo arms, the titration will be simulated and no increase in dose will take place. If a dose increase is not considered appropriate by the investigator, they will instead receive another set of IP that corresponds to the previous tolerated dose.

Randomized treatment period

After 4 weeks of titration, each patient will continue on the highest dose achieved up to 4 months as well as during the optional 2-month extension. The patients will receive a new kit of IP once a month, ie, Visit 8 and Visit 9. Patients entering the extension period will receive a new kit of IP once a month, ie, visit 10 and extension visit 12.

One kit of IP consists of:

- A wallet consisting of 1 blister of AZD1656, dose 10mg, 20mg, 40mg, 50mg, 100mg, 140mg, 200mg or placebo and a wallet consisting of a blister of glipizide 5mg, 10mg, 15mg or 20mg or placebo. Each blister card of AZD1656 will have 10 rows, where 1 row is 2 doses consisting of 2 tablets each. The glipizide blisters will also consist of 10 rows where 1 row is 2 doses consisting of 2 capsules each.

Study medication should be returned by the patients for drug accountability.

Treatment during the extension period:

The patients in the extension will continue on the same blinded (cohort 1) or open treatment (cohort 2) as during the 4 months study.

5.5.3 Labelling

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

Each wallet will be labelled with a single panel booklet label with a front page with variable text (ie, kit ID, expires end visit, E-code, daily dose (if open label AZD1656), order number and investigator). The inner pages will be country specific and translated into each language.

The labels will include the following information:

- Name, address and phone number of the sponsor (AstraZeneca).
- Dosage form, route of administration and quantity of dosage units.
- Study code.
- Kit ID.
- Direction for use.
- The name of the investigator (to be added on the label when the IP is dispensed).
- E-code (to be added on the label when the IP is dispensed).
- Storage condition.
- Batch/Lot ID number.
- Expiry date.
- The following statements:
  - "for clinical study use only".
  - "keep out of reach of children".

5.5.4 Storage and shipment

All study drugs should be kept in a secure place under appropriate storage conditions. The IP label on the box or wallet specifies the appropriate storage conditions. See each label for more specific information.

5.6 Concomitant and post-study treatment(s)

Other concomitant medication or therapy will be allowed during the study, from the time of signing informed consent to the follow-up visit and recorded in the appropriate section of the CRF, except for warfarin, amiodarone and potent CYP450 inhibitors such as (but not limited to) ketoconazole and macrolide antibiotics. No other anti-diabetic treatment than defined in this study protocol will be allowed. However, in addition to the defined hyperglycaemia rescue treatment described in Section 5.6.1, occasional use of insulin in case of acute illness is allowed. Such occasional use is limited to maximally 7 continuous days on 1 occasion.

Changes in concomitant medication should be avoided during study participation, with the exception of situations defined in this protocol, but medication, which is considered necessary for the patient's safety and well-being, may be given at the discretion of the investigator, who must decide if the patient should remain in study or need to be dismissed from study due to patient's safety or interference with study objectives. The administration of all medication must be recorded in the appropriate sections of the electronic case report form (eCRF) with trade names, dosages and dates of starting and ending of medication.

5.6.1 Hyperglycaemia rescue treatment

After randomisation, patients with confirmed fasting hyperglycaemia on at least 2 consecutive assessments (either SMPG measurements, samples analysed at the clinic or at the central laboratory) or an HbA1c value above 8%, should receive rescue treatment with basal night-time insulin.

The randomized treatment given to patients in **cohort 1** includes placebo and the glucose-lowering effect of AZD1656 has not been documented for periods longer than 4 weeks. Thus, criteria for hyperglycaemia requiring additional treatment with basal night-time insulin (rescue treatment) are as described below.

After randomization, hyperglycaemia will be defined in cohort 1 as follows:

- FPG greater than 15 mmol/L (270mg/dL) from baseline up to the 4-week visit (Visit 7).
- FPG greater than 13.3 mmol/L (240mg/dL) after the 4-week visit up to the 3-month visit (Visit 9).
- FPG greater than 11.1 mmol/L (200mg/dL) or HbA1c greater than 8.0 % at any time after the 3-month visit (Visit 9).

Although all patients in cohort 2 will receive active treatment with AZD1656, efficacy beyond 4 weeks is not yet documented, so criteria for hyperglycaemia requiring additional treatment with basal night-time insulin (rescue treatment) have been defined also for this cohort as described below.

After the initial titration period, hyperglycaemia will be defined in **cohort 2** as follows:

- FPG greater than 13.3 mmol/L (240mg/dL) after the 4-week visit (Visit 7) up to the 3-month visit (Visit 9).
- FPG greater than 11.1 mmol/L (200mg/dL) or HbA1c greater than 8.0 % at any time after the 3-month visit (Visit 9).

The type and brand of insulin, as well as doses used, will be according to local clinical practice and the insulin will be procured by the trial site. Initiation and subsequent dose

changes of any hyperglycaemia rescue treatment must be recorded in the appropriate sections of the eCRF.

5.7 Treatment compliance

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

Treatment compliance, both regarding background metformin treatment and IP, will be assessed by tablet count. To be eligible for randomization, patients must have taken at least 75 % of their metformin tablets during the run-in period. In the statistical analysis, acceptable compliance is defined as having taken at least 75 % of the metformin doses and at least 75% of the IP doses during the randomized treatment period. Treatment compliance will be calculated as follows:

$$\textbf{Compliance} = \frac{\text{number of tablets dispensed - number of tablets returned}}{\text{(date of last dose – date of first dose +1) x number of tablets}}$$

The number of tablets in the calculation is the number of tablets that patients should have received each day, as per protocol.

5.7.1 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 study drugs dispensed to and returned from the patient.

The study personnel will account for all received study drugs and return all unused study drugs. At the end of the study, all used and unused study drug will be returned for destruction by the study monitor. Documentation of drug accountability and drug destruction return will be maintained by the site.

All sites will receive training regarding appropriate procedures for drug ordering, accountability and return.

5.8 Discontinuation of investigational products

Patients may be discontinued from IP in the following situations:

1. Patient decision. The patient is at any time free to discontinue treatment, without prejudice to further treatment.

2. AE.

3. Severe non-compliance to study protocol as judged by the investigator and/or AZ/ICON Clinical Research.

4. Risk to patients as judged by the investigator and/or AZ

5. Patient lost to follow-up.

6. Incorrectly randomized patient, see Section 3.1.

Development of any study specific criteria will lead to discontinuation from IP:

- Patient uses amiodarone, warfarin, rimfampicin or potent CYP450 inhibitors, such as, but not limited to, ketokonazole and macrolide antibiotics during the study should be discontinued from the study.

- Patients who despite dose reduction of IP once, experiences more than one major hypoglycaemic event with need for external assistance due to impaired consciousness, or with other hypoglycaemic event(s) resulting in safety concerns with continued IP treatment, should be discontinued from the study. The hypoglycaemic event should be recorded in the appropriate CRF modules.

- Patients with remaining hyperglycemia despite maximum tolerable dose of basal night-time insulin, according to the investigator, should be discontinued from the study and receive appropriate glucose corrective treatment. For the definition of hyperglycemia at different time points during study, see section 5.6.1

- Patient who develops a condition contraindicating treatment with metformin or glipizide during the study should be discontinued. Conditions include, but are not limited to, renal impairment with GFR <60 ml/min, shock and diabetic ketoacidosis. However, temporary discontinuation of metformin and/or IP (up to 7 days) due to e.g. acute illness or radiological investigations with iodinated contrast media is allowed

- Increase in plasma CK levels to more than 10 x ULN on two consecutive measurements that cannot be ascribed to a benign condition or situation.

- Increase in AST and or ALT to more than 5 x ULN

- ALT or AST more than 3 x ULN and total bilirubin more than 1.5 x ULN.

5.8.1 Procedures for discontinuation of a patient from investigational product

All discontinuation due to hypoglycaemia should be according to the judgement of the investigator, ie, the investigator should be able to allow the patient to continue (even after a major hypoglycaemic event) if there are clear circumstances explaining the event, and the risk for a recurrence is low.

If the patient is discontinued from IP, the patient is also discontinued from the study. As far as possible they will be seen and assessed by the investigator at an end of study visit and then at a follow-up visit scheduled according to the study protocol and including the procedures described in Section 5.8.1. If the patient does not agree to this option, a modified follow up (eg, telephone contacts) should be arranged, if agreed to by the patient and in compliance with local data privacy laws/practices. The approach taken should be registered in the eCRF. Follow-up is not applicable if the patient is lost to follow-up.

For patients discontinuing due to liver enzyme criteria, special evaluation will be performed, see Appendix E.

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

5.9 Withdrawal from study

Patients are at any time free to withdraw from study (IP and assessments), without prejudice to further treatment (withdrawal of consent). Such patients will always be asked about the reason(s) and the presence of any adverse events. If possible, they will be seen and assessed by an investigator. Adverse events will be followed up (See Sections 6.4.3 and 6.4.4); the Patient Diary and study drug should be returned by the patient. The investigator and AstraZeneca/ICON may withdraw patients from the study (IP and assessments) due to safety reasons. Patients withdrawn from study will not be replaced.

6. COLLECTION OF STUDY VARIABLES

6.1 Recording of data

The Rave Web Based Data Capture (WBDC) system will be used for data collection and query handling. The investigator will ensure that data are recorded on the electronic Case Report Forms (eCRF) as specified in the study protocol and in accordance with the instructions provided.

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

6.2 Data collection

For full details please see the study plan (Table 2) and the eCRF manuals.

6.3 Efficacy

6.3.1 Efficacy variables related to glucose control

6.3.1.1 HbA1c

Samples for HbA1c analysis will be collected at the enrolment visit (screening; Visit 1), the randomization visit (Visit 3), and monthly after the titration period (from Visit 7), see Table 2.

6.3.1.2 FPG

Samples for fasting plasma glucose will be collected and analysed immediately at the clinic at every visit (except for Visit 2), see Table 2. At each visit, a venous sample for analysis of FPG will also be sent to the central laboratory. The FPG samples, both the 1 analysed immediately at the clinic and the 1 for the central laboratory, should be taken when the patient is fasting, and before the IP is administered.

6.3.1.3 SMPG measurements

All patients will receive life-style advice and glucometers from 2 weeks prior to randomization onwards and will be asked to check their plasma glucose on a regular basis at home during the study, registering the results in a Patient Diary. The diary will be utilised from Visit 2 before randomization onwards. Between Visit 2 and 3, and during the titration weeks, at least 1 fasting morning plasma glucose and 1 bed-time plasma glucose assessment are needed on a daily basis and within 3 days prior to each of Visit 3, 4, 5, 6 and 7, a 7-point glucose assessment should be completed and results registered in the Patient Diary. During the maintenance period, after Visit 7 and onwards, plasma glucose checks are not required on a daily bases at home, but patients are requested to collect a 7-point glucose assessment within a 5-day window before each visit to the clinic (ie, before Visits 8-10). Patients entering the extension period are requested to collect a 7-point glucose assessment within a 5-day window before the extension visits.

Patients will be carefully instructed to register any episode with symptoms suspected being related to low plasma glucose levels. The Patient Diary will be reviewed by the investigator at each study visit and any low plasma glucose values and episodes considered as hypoglycaemic events will be transferred into the CRF. The 7-point assessment should include the following time points:

- Fasting before breakfast.
- 2 hrs after breakfast.
- Before lunch.
- 2 hrs after lunch.
- Before dinner.
- 2 hrs after dinner.

- At bed-time.

During the titration period, the decision to increase the dose will be based on SMPG data and the FPG measured at the clinical visit (see Section 3.1). The study staff will transfer the 7-point data and any hypoglycaemic event from the Patient Diary to the eCRF. The morning and bedtime glucose results and any additional glucose checks requested by the investigator will not be transferred to the eCRF, those measurements are used for the investigator's safety monitoring of the patient.

6.3.1.4 OGTT

An oral glucose tolerance test with collection of blood samples during 2 hours will be performed at baseline (Week 0, Visit 3) and 4 months (Visit 10) in a subgroup of the study population. At baseline (Week 0, Visit 3), after an overnight fast, patients will receive 75 g glucose orally, dissolved in water . Blood samples will be collected pre-dose and at 30 - 60 - 90 - 120 minutes post glucose intake for the analyses of plasma glucose, insulin, C-peptide and pro-insulin. IP will taken together with breakfast after the last OGTT sample at 120 minutes. At 4 months (Visit 10), after an overnight fast and 15 minutes after administration of the IP morning dose (no IP at baseline visit) at the clinic, patients will receive 75 g glucose orally, dissolved in water. Blood samples will be collected pre-dose and at 30, 60, 90 and 120 minutes post glucose intake for the analyses of plasma glucose, insulin, C-peptide and pro-insulin. Breakfast will be served immediately after the last OGTT sample at 120 minutes. The OGTT will be performed at selected sites. Countries that not will take part of the OGTT are Chile, Hungary, UK and Sweden.

6.3.1.5 Insulin, C-peptide

In addition to the measurements during the OGTT procedure, levels of fasting insulin and C-peptide will be analysed also at time points defined in Table 2.

6.3.2 Assessment of lipid status and marker of inflammation

Samples for analysis of total cholesterol, HDL cholesterol, LDL cholesterol, triglyceride and CRP will be collected at time points defined in Table 2.

6.4 Safety

The Principal Investigator is responsible for ensuring that all staff involved in the study is familiar with the content of this section. For timing of all safety assessments please see Table 2.

6.4.1 Definition of adverse events

An adverse event is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product. An undesirable medical condition can be symptoms (eg, nausea, chest pain), signs (eg, tachycardia, enlarged liver) or the abnormal results of an investigation (eg, laboratory findings, electrocardiogram). In clinical

studies, an AE can include an undesirable medical condition occurring at any time, including run-in or washout periods, even if no study treatment has been administered.

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

6.4.2 Definitions of serious adverse event

A serious adverse event is an AE occurring during any study phase (ie, run-in, treatment, washout, follow-up), that fulfils 1 or more of the following criteria:

- Results in death.
- Is immediately life-threatening.
- Requires in-patient hospitalisation or prolongation of existing hospitalisation.
- Results in persistent or significant disability or incapacity.
- Is a congenital abnormality or birth defect.
- Is an important medical event that may jeopardise the patient or may require medical intervention to prevent 1 of the outcomes listed above.

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

6.4.3 Recording of adverse events

Time period for collection of adverse events

All AEs will be collected from the time of the first administration of IP until the end of the study (including follow-up visit). Before the first administration of IP, only SAEs will be collected.

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. AstraZeneca retains the right to request additional information for any patient with ongoing AE(s)/SAE(s) at the end of the study, if judged necessary.

Variables

The following variables will be collect for each AE:

- AE (verbatim).
- The date and time when the AE started and stopped.
- Maximum intensity.

- Whether the AE is a hypoglycaemic symptom or not.
- Whether the AE is serious or not.
- Investigator causality rating against the IP (yes or no).
- Action taken with regard to IP.
- Outcome.

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

- Date AE met criteria for serious AE.
- Date Investigator became aware of serious AE.
- Seriousness criteria.
- Hospitalisation start/stop dates.
- In cases of fatal AE, probable cause of death.
- Date of death.
- Autopsy result, if performed.
- Causality assessment in relation to study procedure(s).
- Causality assessment in relation to additional drug (metformin)
- Causality assessment in relation to additional drug (insulin)
- Causality assessment in relation to Other medication.
- Description of AE.

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria in Section 6.4.2. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not a SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke but would be a SAE.

Causality collection

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

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

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

Adverse Events based on signs and symptoms

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

Adverse Events based on examinations and tests

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

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

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

Assessment of hypoglycaemic events

From Visit 2 the patient will be asked to document details of hypoglycaemic episodes in a diary. At each visit the investigator must review any such entries with the patient and a corresponding module in the CRF should be completed based on the investigator's medical

interpretation of the event. Any symptoms described for a hypoglycaemic episode will be reported as AE.

6.4.4 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 eCRF.

If any SAE occurs in the course of the study, then investigators or other site personnel inform appropriate ICON Clinical Research representatives within 1 day ie, immediately but **no later than the end of the next business day** of when he or she becomes aware of it.

The designated ICON Clinical Research representative will work with the investigator to ensure that all the necessary information is provided to the AstraZeneca Patient Safety data entry site **within 1 calendar day** of initial receipt for fatal and life threatening events **and within 5 calendar days** of initial receipt for all other SAEs.

For fatal or life-threatening adverse events where important or relevant information is missing, active follow-up is undertaken immediately. Investigators or other site personnel inform ICON Clinical Research representatives of any follow-up information on a previously reported SAE within 1 calendar day, ie, immediately but **no later than the end of the next business day** of when he or she becomes aware of it.

Once the Investigators or other site personnel indicate an AE is serious in the RAVE WBDC system, an automated email alert will be sent to the designated ICON Clinical Research representatives.

If the RAVE WBDC system is not available, then the Investigator or other study site personnel shall report a SAE to the appropriate ICON Clinical Research representative by completing the appropriate paper documentation and then faxing to the appropriate fax number. In addition, an ICON Clinical Research representative should be notified by telephone to inform/confirm receipt.

Procedures regarding reporting of AEs and SAEs will be provided in the investigator manual provided to the site; eCRF completion guidelines will be provided in the eCRF manual.

6.4.5 Laboratory safety assessment

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

The following laboratory variables will be analysed at the central laboratory for all visits from enrolment to follow-up:

Table 6 Measurement of Laboratory Variables

Haematology	B-Haemoglobin, B-Leukocyte count, B-Leukocyte differential count (absolute count), B-platelet count
Clinical Chemistry	S-Albumin, S-ALT, S-AST, S-Alkaline phosphatase, S-Bilirubin total, S -Calcium total, S-Creatinine, S–CRP, S-g-GT, S-Potassium, S-Sodium, S-Creatine kinase, Glucose, P-Lactate [a], Cystatin-C [a]
Urinalysis	U-Hb, U-Protein, U-Glucose

[a] P-lactate and cyctatin-c are excluded on the titration visits.

Urine samples will be analysed at the central laboratory. Laboratory values outside the reference limits suspected to be of any clinical significance will be re-checked but without further recording in the CRF. Patients 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. If there is post-study follow-up, AstraZeneca retains the right to request additional information for any patient with ongoing AE(s)/SAE(s) related to laboratory values, if judged necessary.

At the enrolment visit, all patients will be tested for HIV, hepatitis B and C. Samples for FSH and LH will also be taken from women if last menstruation period was >1 year ago to ensure that the serum FSH and LH are within the postmenopausal range.

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

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

Glomerular filtration rate (GFR) will be calculated by the central laboratory, see exclusion criteria 13, Section 4.2.

For blood volume see Section 7.1

6.4.6 Physical examination

A complete physical examination will be performed and will include an assessment of the following: general appearance, skin, head and neck (including ears, eyes, nose and throat), lymph nodes, thyroid, abdomen, musculo-skeletal (including spine and extremities), cardiovascular, respiratory and neurological systems (reflexes only).

6.4.7 Resting 12-lead ECG

Digital 12-lead ECGs will be recorded using a Mortara ELI-150/250 ECG unit.

A central ECG laboratory service provider will provide the site with study specific ECG instructions and manuals, except for at Visit 3 (baseline), when triple ECGs will be recorded; single ECGs will be recorded at all other visits.

A 12-lead ECG will be obtained after the patient has been lying down for 5 minutes (for details, please see separate ECG manual). The ECG print-outs will be reviewed at the visit by the investigator. The results will be entered as normal or specified if abnormal in the CRF by the investigator. In addition, a centralised reading and analysis of the following will be performed: interval Duration Measurement (PR, QRS, QT, R-R), rhythm, ectopy, Conduction, morphology, myocardial infarction, ST Segment, T and U wave observations.

6.4.8 Vital signs and anthropometric measurements

6.4.8.1 Pulse and blood pressure

Pulse and blood pressure will be measured after 10 minutes rest on a bed. Pulse will be recorded and assessed according to local clinical practice.

6.4.9 Anthropometric assessments

Weight measurements should be taken pre-dose in the fasting state in the morning after a visit to the lavatory. Patients are only allowed to wear light clothing (ie, t-shirt and underwear) during the measurement and each patient should use the same scale during all measurements.

Height will be measurement at baseline only and will not be recorded/monitored at any subsequent visits.

Waist circumference will be measured in centimetres (cm) at the level midway between the most caudal part of the lateral costal arch and the iliac crest at the end of a normal expiration.

6.5 Patient reported outcomes (PRO) (Not applicable)

6.6 Pharmacokinetics

6.6.1 Collection of samples

Blood samples (2 mL) for trough concentration determination of AZD1656 and its metabolite AZ12555623 in plasma will be drawn before morning dose at days 7 (±2 days), 28 (±2 days), 90 (±5 days) and 120 (±5 days), ie at visits 4, 7, 9 and 10. In addition, samples will be taken on day 120 (±5 days), ie visit 10, post-dose at 1 (±0.5), 3 (±1) and 5 (±1) hours. Patients entering the extension period will take one pre-dose PK sample at visit 13. Each sample should be separated by at least 1 hour. Note, PK sampling will take place in all subjects; thus, the PK sampling will be done in parallel to the OGTT in the subjects in the OGTT subgroup. All samples from patients given active treatment of AZD1656 will be analysed, but no analysis of AZD1656 is planned in samples from patients allocated to glipizide or placebo

treatment. However, if considered necessary, PK samples from the glipizide and placebo treatment groups may be analysed for determination of AZD1656.

The patients will be instructed to record the exact time of morning and evening dose intake the day before each study visit including PK sampling. The times will be recorded by the patient on a diary card and transferred to the eCRF by the site personnel. The exact time of dose intake in the morning at visit 10 and times for breakfast intake during the 5-hr PK sampling will be recorded in the eCRF by the site personnel. Subjects will be served breakfast at the site during the 5-hr PK sampling: subjects not doing OGTT will have breakfast immediately after the IP intake, and subjects in the OGTT subgroup will be served breakfast immediately after the last OGTT sample after 2 hours. The actual blood sampling time will be entered into the lab requisition form on all occasions and transferred to the eCRF by the site personnel.

Plasma samples should be stored at -20ºC 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 central laboratory during the course of the study. For more details of labelling, storage and shipment see Appendix D and the Lab manual.

For blood volume see Section 7.1.

6.6.2 Determination of drug concentration

Samples for determination of drug concentration in plasma will be analysed on behalf of Clinical Pharmacology & DMPK, AstraZeneca R&D, Mölndal, Sweden 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 methods will be referred to in the CSR.

6.7 Pharmacodynamics

6.7.1 Collection of pharmacodynamic markers

Not applicable. See Section 6.3.1 for efficacy variables related to glucose control.

6.8 Pharmacogenetics

6.8.1 Collection of pharmacogenetic samples

The blood sample for genetic research will be obtained from the patients at Week 0 (Visit 3) after randomization. If for any reason the sample is not drawn at Visit 3, it may be taken at any visit until the last study visit. Only 1 sample should be collected per patient for genetics during the study. The blood draw should not take place prior to randomization. Although genotype is a stable parameter, early sample collection is preferred to avoid introducing bias through excluding patients who may withdraw due to an AE, such patients would be important to include in any genetic analysis. Samples will be collected, labelled, stored and shipped as detailed in the Laboratory Manual.

For blood volume see Section 7.1.

6.9 Health economics (Not applicable)

7. BIOLOGICAL SAMPLING PROCEDURES

7.1 Volume of blood

The maximum volume of blood that will be drawn from each patient in this study is shown in Table 7. Additional blood might be taken if laboratory values outside the reference limits suspected to be of any clinical significance.

Table 7 Volume of blood to be drawn from each patient

Assessment	**Sample volume**	**No. of samples (4-month)**	**Total volume (4-month)**	**No. of samples (2-month extension)**	**Total volume (6-month)**
	(mL)		mL		mL
Chemistry, Lipids, HIV, Hepatitus Screen, Total Insulin, Proinsulin, C-Peptide, C-Reactive Protein, Follicle Stimulating Hormone, Luteinizing Hormone, Serum B-hCG	8.5	29	246.5	2	263.5
Haematology w/ differential, HbA1c	4	10	40	2	48
OGTT	8.5	10	85	0	85
Plasma Glucose	4	12	48	2	56
Lactic Acid	4	12	48	2	56
Pharmacokinetic	1.2	7	8.4	1	9.6
Pharmacogenetics	9	1	9	0	9
Total			484.9 ml		527.1 ml

Note: Additional blood will be taken as finger sticks or as a drop of blood for plasma glucose both at home at several occasions and as FPG at site at each visits.

7.2 Handling, storage and destruction of biological samples

Safety samples will be disposed of after analysis.

7.2.1 Pharmacokinetic and/or pharmacodynamic samples

Samples will be disposed after the clinical study report has been finalised, unless retained for future analyses, see below.

Key samples for investigation of long-term stability, metabolite identification and/or analysis can be retained on behalf of Clinical Pharmacology, Drug Metabolism and Pharmacokinetics (CPD), AstraZeneca, for a maximum of 1 year following the finalisation of the Clinical Study Report. The results from the investigation will not be reported in the Clinical Study Report but separately in a bioanalytical/metabolism report.

7.2.2 Pharmacogenetic samples

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

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

The samples and data for genetic analysis in this study will be coded. The link between the patient enrolment/randomization code and the DNA number will be maintained and stored in a secure environment, with restricted access WITHIN the Clinical Genotyping Group Laboratory Information Management System (LIMS) at AstraZeneca. The link will be used to identify the relevant DNA samples for analysis, facilitate correlation of genotypic results with clinical data, allow regulatory audit and to trace samples for destruction in the case of withdrawal of consent when the patient has requested disposal/destruction of collected samples not yet analysed.

7.3 Labelling and shipment of biohazard samples

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

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

7.4 Chain of custody of biological samples

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

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

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

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

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

7.5 Withdrawal of informed consent for donated genetic samples

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

As collection of the genetic samples is an optional part of the study, then the patient may continue in the study.

The Principal Investigator:

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

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

8. ETHICAL AND REGULATORY REQUIREMENTS

8.1 Ethical conduct of the study

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

8.2 Patient data protection

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

AstraZeneca will not provide individual genotype results to patients, 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 patient. In exceptional circumstances, however, certain individuals might see both the genetic data and the personal identifiers of a patient. For example, in the case of a medical emergency, an AstraZeneca Physician or an investigator might know a patient's identity and also have access to his or her genetic data. Also regulatory authorities may require access to the relevant files, though the patient's medical information and the genetic files would remain physically separate.

8.3 Ethics and regulatory review

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

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

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

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

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

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

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

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

Each Principal Investigator is responsible for providing the Ethics Committees/IRB with reports of any serious and unexpected adverse drug reactions from any other study conducted with the IP. AstraZeneca will provide this information to the Principal Investigator so that he/she can meet these reporting requirements.

8.4 Informed consent

The Principal Investigator(s) at each centre will:

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

8.5 Changes to the protocol and informed consent form

Study procedures will not be changed without the mutual agreement of the International co-ordinating Investigator, National Co-ordinating Investigator, and the Principal Investigator and AstraZeneca.

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

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

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

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

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

8.6 Audits and inspections

Authorised representatives of AstraZeneca, a regulatory authority, or an Ethics Committee may perform audits or inspections at the centre, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents, to determine whether these activities were conducted, and data were recorded, analysed, and accurately reported according to the protocol, Good Clinical Practice (GCP), guidelines of the International Conference on Harmonisation (ICH), and any applicable regulatory requirements. The investigator will contact AstraZeneca immediately if contacted by a regulatory agency about an inspection at the centre.

9. STUDY MANAGEMENT BY ICON CLINICAL RESEARCH

9.1 Pre-study activities

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

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

9.2 Training of study site personnel

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

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

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

9.3 Monitoring of the study

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

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

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

9.3.1 Source data

For the purpuse of this study eCRF and medical journals will act as the source data for all information collected in the study.

9.4 Study agreements

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

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

9.4.1 Archiving of study documents

The Investigator will follow the principles outlined in the Clinical Study Agreement (CSA).

9.5 Study timetable and end of study

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

The study is expected to start in Q4 2009 and to end by Q1 2011.

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

10. DATA MANAGEMENT

Data management will be performed by ICON Clinical Research. Data collected through third party sources (eg, laboratory assessments, ECGs) will be obtained and reconciled against study data.

Adverse events and medical/surgical history will be classified according to the terminology of the latest version the Medical Dictionary for Regulatory Activities (MedDRA). Medications will be classified according to the AstraZeneca Drug Dictionary. All coding will be performed by ICON Data Management.

Data queries will be raised for inconsistent, impossible or missing data. All entries to the study database will be available in an audit trail.

The data will be validated as defined in the Data Management Plan. Quality control procedures will be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly.

When all data have been coded, validated, signed and locked, clean file will be declared. Any treatment revealing data may thereafter be added and the final database will be locked.

Data associated with biological samples will be transferred to laboratories internal or external to AstraZeneca.

Any genotype data generated in this study will be stored in the AstraZeneca genotyping LIMS database, or other appropriate secure system within AstraZeneca and/or ICON Clinical Research to analyse the samples.

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

11. EVALUATION AND CALCULATION OF VARIABLES

11.1 Calculation or derivation of efficacy variable(s)

All data derivations will be performed by ICON Biostatistics.

The mean of SMPG for each day will be computed only if more than half the data collected that day are available (ie, if 4 or more collection point are missing on the 7-point scale the daily mean will be set to missing).

If the assessment (CRF or lab data) is collected more than once for the same visit/time-point, the first assessment will be used in the analysis. If the assessment is outside the visit window then it will still be considered of interest and won't be excluded from the analysis.

For the lab parameters (C-peptide, LDL, HDL, trygliceride, etc.), value reported as <LLOQ will be analysed as LLOQ/2, value reported as > upper limit of quantification (ULOQ) will be analysed as ULOQ.

Change from baseline will be computed as the value at each visit post-baseline – baseline result. Baseline value will be defined as the last non-missing value on or before first dose of treatment. If the value is missing at the randomization visit, the enrolment (screening) result will be used.

Percentage will be based on the number of patient in the respective analysis set.

11.2 Calculation or derivation of safety variable(s)

Events of hypoglycaemia will be categorised into 1 of the following main categories, based on the investigator reported data:

- Major hypoglycaemia: Hypoglycaemic episode where the patient absolutely needed help from another person to recover, due to impaired consciousness or altered behaviour. There should also be documented prompt recovery after treatment with glucose or glucagon, and pre-treatment plasma glucose should be below 3.0 mmol/L or missing.

- Minor hypoglycaemia: Hypoglycaemic episode that the patient was able to manage by self-administration. There should also be a documented plasma glucose below 3.0 mmol/L before treatment.

- Episode suggestive of hypoglycaemia: Any hypoglycaemic episode that the patient was able to manage by self-administration but where pre-treatment plasma glucose is either missing or greater than 3.0 mmol/L.

ECG (12-lead and telemetry): QTc will be derived as:

Bazett: $$QTc = \frac{QT}{\sqrt{RR}}$$

Frederica QTc $$QTc = \frac{QT}{(RR)^{1/3}}$$

Where RR (interval from the onset of 1 QRS complex to the onset of the next QRS complex) will be derived as 60/HR with HR been the heart rate.

The GFR will be calculated as:

GFR (mL/min per 1.73m2) = 186 x (SCr)-1.154 x (Age) –0.203 x (0.742 if female) x (1.210 if African-American) where SCr = serum creatinine concentration inmg/dL, age is in years, and weight is in kg.

11.2.1 Other significant adverse events (OAE)

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

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

11.3 Calculation or derivation of patient reported outcome variables

Not applicable.

11.4 Calculation or derivation of pharmacokinetic variables

Not applicable, see Section 11.5.2.

11.5 Calculation or derivation of pharmacodynamic variable(s)

Not applicable, see Section 11.5.2.

11.5.1 Calculation or derivation of the relationship between pharmacokinetic and pharmacodynamic variables

Not applicable, see Section 11.5.2.

11.5.2 Population analysis of pharmacokinetic/pharmacodynamic variables

Population PK/PD modelling of the data will be performed at AstraZeneca R&D, using non-linear mixed effects regression in NONMEM. A semi-mechanistic drug-disease model, describing the interplay between drug concentrations, glucose, HbA1c and insulin levels as well as their time-course, will be applied and fit to data to estimate mean population PK/PD parameters with associated between- and within-patient variability. Also, the influence of patient-specific covariates such as gender, age, body weight, creatinine clearance and disease status on the PK and PD parameters may be investigated. A population PK/PD analysis plan will be written prior to clean file. The modelling results will be provided in a separate population PK/PD report.

11.6 Calculation or derivation of pharmacogenetic variables

Provision of a blood sample for genetic research is optional to study participants. For all obtained samples, the DNA will be extracted from the blood sample. DNA samples will be handled in the coded format and stored for a maximum of 25 years.

The decision to perform a genetic analysis of archived DNA will be based on potential scientific and clinical indication. The potential exploratory genetic analysis will be performed by AstraZeneca R&D, Alderly Park, United Kingdom or at the designated contract laboratory. The genotypic data generated from the study will be stored in the AstraZeneca LIMS database, clinical database or other appropriate system. Accordingly, patients placed into genotype groups will be compared for their respective phenotype (such as response to AZD1656, metformin and glipizide distribution, safety, tolerability and efficacy or susceptibility for T2DM). The results from this genetic research will be reported in a separate report. 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.

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

12. STATISTICAL METHODS AND SAMPLE SIZE DETERMINATION

12.1 Description of analysis sets

The following study analysis sets will be defined.

12.1.1 Full analysis set

All randomized patients with at least 1 dose administered, and with at least 1 post-baseline efficacy data, will be included in the Full Analysis Set (FAS).

Patients will be analysed according to the randomized treatment, irrespective of the treatment received.

This will be the primary analysis set used for all efficacy analysis variables.

The Per-Protocol (PP) analysis set will consist of the patients in the FAS who do not violate any major entry condition and do not violate the protocol between randomization and study completion. Major deviations from the protocol procedures include:

- Violation of inclusion/exclusion criteria.
- Disallowed concomitant medication.
- Missing key efficacy data. (ie, Hb1Ac at 4 months or last visit).
- Patient is not compliant

This will be the secondary efficacy analysis set and will be applied to all key efficacy analysis variables.

12.1.2 Safety analysis set

All patients who receive at least 1 dose of randomized investigational product, and for whom any post-dose data are available will be included in the safety analysis set. Safety results will be grouped according to randomization, and not the actual dose taken.

12.1.3 PK and PD analysis set

Data from all included patients receiving at least 1 dose, contributing with at least 1 PK or PD measurement and providing reliable time-points for sampling and dose intakes, will be used for PK/PD analysis.

12.2 Methods of statistical analyses

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

The statistical analysis will be performed using SAS® version 9.1 or later.

All tests will be performed at the 5% significance level unless otherwise is stated. The study is not powered to demonstrate a statistically significant difference between glipizide and AZD1656. Nevertheless, comparisons will be performed for the comparator versus AZD1656 and placebo. For the comparison the comparator versus AZD1656, a one-sided 90% confidence interval will be calculated for the treatment effect. One-sided 90% confidence intervals for the treatment effect should also be provided for the comparison between AZD1656 and placebo.

12.2.1 Data Handling Considerations

Last observation carried forward methods (LOCF) will be used for any missing efficacy data; baseline data will not be carried forward. Analyses based on LOCF data will only be performed using the FAS.

Genotype data might be analysis if needed and a separate report will be prepared where appropriate.

12.2.2 Multiple Treatment Comparisons

For conclusion a hierarchical closed testing procedure will be adopted for the efficacy analyses. Treatment group comparisons will be tested in dose span order whereby comparisons with placebo will commence with the highest dose regimen (randomized patient on 20mg daily titrated to maximum of 200mg daily) tested first, next dose regimen 10mg daily titrated to maximum of 140mg daily, fixed dose 40mg daily and finally the fixed dose 20mg daily. Estimates of the treatment effect and corresponding 95% confidence intervals will be presented together with the p-values for the treatment effect. All tests will be performed at the 5% level of significance.

12.2.3 Demographic, baseline and background characteristics

All variables will be presented with descriptive statistics within each treatment group.

12.2.4 Concomitant Medication

Concomitant medication is defined as a medication that is taken prior or after the first day of study treatment.

For incomplete or partial concomitant medication start and/or stop dates, where it is not possible to classify the concomitant medication as before or during, using available date information, it will be assumed that the concomitant medication was taken during double blind treatment.

Concomitant medication will be summarised by treatment group for the Safety analysis set using drug class and preferred term. Patients will only be counted once within a drug class, and once for a medication.

All medication, taken both prior to and during the double blind treatment phase, will be included in the supportive data listing. A flag will be added to indicate where a medication was taken prior to the start of double blind treatment.

12.2.5 Compliance and Study Drug Exposure

The compliance of study drug will be completed for overall exposure per patient.

12.2.6 Efficacy Analysis

Individual data will be listed. Where appropriate, descriptive statistics or table of frequencies will be presented.

12.2.6.1 Primary variable

An analysis of covariance (ANCOVA) will be performed on the change in HbA1c from baseline to the final visit at 4 months, with treatment as a fixed factor and the corresponding baseline HbA1c as a covariate.

The FAS will be applied as the primary analysis set on the LOCF approach whereby for missing assessments (visits), the last observation will be carried forward. Baseline values will not be carried forward .

The PP analysis set based on Observed Case will be applied as supportive analyses to the FAS.

The following additional sensitivity analyses will be performed on the primary variable:

- Observed Case analyses will be performed on the FAS whereby missing intermediate assessments (visits) will be excluded.

- If the primary analyses are significant, an additional analysis adjusting for centre/regional effects will be conducted. The primary efficacy variable will be analysed applying the ANCOVA model including additional covariate for centre/region on both the FAS and PP analysis sets.

- Further exploratory and subgroup investigations will be performed on the primary variable considering effects of other factors including gender. Exploratory analysis methodology will be detailed further in the statistical analysis plan.

12.2.6.2 Secondary variables

Analyses of the secondary variables will be conducted on the FAS (LOCF) only unless otherwise stated. The secondary variables are:

- Proportion of patient responders with HbA1c ≤ 7% after 4 months.
- Proportion of patient responders with HbA1c ≤ 6.5% after 4 months.
- Mean FPG levels after 4 months
- Mean SMPG after 4 months.
- OGTT after 4 months in a subgroup of the patients.
- Plasma glucose profiles
- Cardiovascular risk factors, such as total cholesterol, LDL, HDL, triglycerides and CRP, after 4 months.

For the continuous secondary efficacy variables (SMPG, FPG), an ANCOVA will be performed as for the primary analysis.

The categorical efficacy variables including HbA1c responders and hypoglycaemia incidence will be compared across treatment groups applying χ^2-test methodology.

The remaining secondary efficacy variables are deemed exploratory. Statistical comparisons will be performed at the 5% level of significance on the FAS (LOCF) only:

- All of the above secondary variables and the change from baseline for HbA1c will also be summarised and analysed at other scheduled visits eg, Week 4, Month 2, month 3 as applicable.
- The primary and secondary variables for the reference treatment group will also be compared to placebo and AZD1656 doses.

12.2.7 Safety Analysis

Safety variables will be summarised for the safety analysis set and the safety analysis set excluding post rescue treatment.

12.2.7.1 Adverse events

Individual AEs will be listed per patient and sorted by treatment group. Number of patients with adverse events (AEs), serious and leading to discontinuation of IP will be summarised by System Organ Class (SOC) and preferred term (PT) for each treatment group. AE will be considered to be treatment emergent if the onset date occurs, on or after the date of the first date of a treatment period and up to and including 1 day after the last dose is taken (or the last day of the period, if a non-IP period). A separate SOC/PT table will summarize the AEs that have been marked as hypoglycaemic symptoms.

12.2.7.2 Plasma glucose measurements

Number of plasma glucose measurements in defined ranges from glucometer assessment at home, analysed at the clinic and at the central laboratory, respectively.

12.2.7.3 Vital signs

Vital signs will be summarised at each visit and also for change from baseline for each treatment group.

12.2.7.4 ECG

ECG abnormalities will be summarised at each visit for each treatment group.

12.2.7.5 Clinical laboratory data

Descriptive statistics will be presented for the quantitative parameters overall and for all sites (since all clinical laboratory tests will be performed by a central laboratory). Changes from baseline will be summarised at each visit and shift tables produced for each treatment group.

Laboratory abnormalities will be flagged and listed, as appropriate.

12.2.8 PK/ PD Analysis

PK parameter estimates will be estimated using the NONMEM software. Parameter estimates expressing population mean, between-subject variability and random variability will be presented with their associated precision in a separate population PK/PD report. The PK/PD analysis and the report will be performed by AstraZeneca.

12.2.9 PG Analysis

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

12.2.10 Extension data

Safety data obtained from the extension period will be analysed in the same way as data from the 4-month treatment period. All safety data will be presented in the clinical study report.

Any explorative work analyzing the efficacy data or PK data obtained from the extension period will be done at the discretion of the sponsor and may be included in the clinical study report or reported elsewhere.

12.3 Determination of sample size

The primary efficacy variable in this study is the change from baseline in HbA1c to final visit at 4 months. In previous studies standard deviation for change in HbA1c have varied between 1.1 and 1.4.

A sample size of 86 patients in each group will have 90% power to detect a difference of 0.7 between AZD1656 and placebo treatment arms at 4 months, assuming that the common standard deviation is 1.4 using a 2-group t-test with a 5% 2-sided significance level. Allowing for an approximate 4 to 5% drop-out rate from the primary analysis, a minimum of 90 patients will be randomized to receive study medication in each treatment group.

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

12.4 Data monitoring committee (Not applicable)

13. IMPORTANT MEDICAL PROCEDURES TO BE FOLLOWED BY THE INVESTIGATOR

13.1 Medical emergency contacts

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

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

13.2 Overdose

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 treated with oral or iv glucose to counteract hypoglycaemia as prolonged episodes of hypoglycaemia may have detrimental effects on CNS, peripheral nerves and other tissues.

In order to collect more information concerning excessive doses of AZD1656 a suspected drug intake of more than 4 tablets of AZD1656/placebo at one occasion or a total daily intake of more than 6 tablets of AZD1656/placebo needs to be reported to AZ as an overdose, regardless of clinical consequences.

- An overdose with associated AEs is recorded as the AE diagnosis/symptoms on the relevant AE modules in the CRF and on the Overdose CRF module.

- An overdose without associated symptoms is only reported on the Overdose CRF module.

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

13.3 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 3 months following the last dose. If the investigator receives information that a pregnancy has occurred during the study (including pregnancy in the partner of a male patient) despite these restrictions, the designated ICON Clinical Research representative should be informed.

14. LIST OF REFERENCES

Leow and Wyckoff 2005
Leow M.K.S., Wyckoff J. Under-recognised paradox of neuropathy from rapid glycaemic control. Postgrad. Med J. 2005;81:103-7

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

Potter et al 1988
Potter C.G., Sharma A.K., Farber M.O., Peterson R.G. Hypoglycaemic neuropathy in experimental diabetes,. Journal of the Neurological Sciences 1988;88:293-301

Tabata 2000
Tabata H. Peripheral neuropathy in B6C3F1 mice and SD rats induced by chronic intermittent insulin hypoglycaemia. Drug and Chemical Toxicology. 2000;23: 485-96

Yasaki and Dyck 1990
Yasaki S, Dyck P.J. Duration and severity of hypoglycaemia needed to induce neuropathy. Brain Research. 1990;531:8-15

Clinical Study Protocol Appendix B

Drug Substance	AZD1656
Study Code	D1020C00009
Edition Number	1.0

Appendix B
Additional Safety Information

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

Life threatening

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

Hospitalisation

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

Important medical event or medical intervention

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

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

Examples of such events are:

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

A GUIDE TO INTERPRETING THE CAUSALITY QUESTION

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

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

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

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

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

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

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

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

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

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

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

- Is there a known mechanism?

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

Clinical Study Protocol Appendix C

Drug Substance	AZD1656
Study Code	D1020C00009
Edition Number	1.0

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

LABELLING AND SHIPMENT OF BIOHAZARD SAMPLES

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

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

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

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

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

Exempt - all other materials with minimal risk of containing pathogens

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

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

Clinical Study Protocol Appendix D

Drug Substance	AZD1656
Study Code	D1020C00009
Edition Number	1

Appendix D
Instructions for Sampling, Handling, Storage and Shipment of Pharmacokinetic Samples

TABLE OF CONTENTS PAGE

Instruction for blood sampling for determination of AZD1656 and 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 determination of AZD1656, 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 AZD1656 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
. The plasma samples must be immediately frozen in upright position

3. SAMPLE LABELLING

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

3.1 Sample list

Sample list, with the same information as on the tube labels and comments important for the analytical work, eg, additional and missing samples, must accompany the samples to the central lab/CRO as a delivery note. Sample lists should also be sent to the central lab/CRO.

3.2 Additional samples

In case the label is not available for a certain sample or when an additional sample is taken, additional labels are to used.

4. STORAGE – NOT APPLICABLE

5. THE FROZEN SAMPLES MUST BE STORED AT -20°C. THE SAMPLES MUST BE TRANSPORTED ON DRY ICE.DELIVERY

Every batch of samples, accompanied with the sample lists, will be shipped via an agreed upon overnight courier to the central laboratory/ CRO. 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.

Clinical Study Protocol Appendix E

Drug Substance	AZD1656
Study Code	D1020C00009
Edition Number	1

Appendix E
Liver Enzyme Criteria Special Evaluation

1. LIVER ENZYME CRITERIA SPECIAL EVALUATION

Initial evaluation and follow up of abnormal results

- See the subject within 48 hours to instigate enhanced follow-up.
- Obtain a complete history and examine after to rule out non-DILI, and other causes. This should include the following:
- Full medical history including cardiac disease, surgery, blood transfusions, iv drug abuse, tattoos, travel, work, sexual history, alcohol intake.
- Details of all medicines taken (including oral contraceptives, prescribed and "overthe-counter" medicines, complementary and alternative medicines, recreational drugs) and chemicals exposed to within one month of the onset of the liver injury.
- Full clinical examination looking for evidence of acute or chronic liver disease, cardiac disease and infection.

Additional tests that can be used to identify possible causes of the liver injury include the following:

- Clotting screen (particularly prothrombin time) and serum albumin
- Serology (and molecular virology) for Epstein-Barr virus (EBV), Cytomegalovirus
- (CMV), hepatitis A, B, C, D and E.
- Antinuclear antibodies
- Serum α-1-antitryspin
- Iron studies (serum iron, total iron binding capacity, serum ferritin)
- Caeruloplasmin in patients ≤40 years
- Liver ultrasound/ Computed Tomography (CT) scan if cholestatic or mixed type of liver injury is indicated

Monitor the patient every 48 hours until the peak values or reached and/or until the subject is feeling better. The frequency of retesting can decrease to once per week or less if abnormalities stabilize and the patient is asymptomatic. The patient should be followed until resolution. This is very important, not only for arranging subject well being, but also for helping to assess causality.