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Effects on subclinical heart failure in type 2 diabetic subjects on liraglutide treatment versus glimepiride both in combination with metformin

Product:	Victoza®
Substance:	Liraglutide
EudraCT Number:	EudraCT nr 2010-022695-31
Sponsor:	Karolinska Institutet, Södersjukhuset AB
Coordinating Investigator:	Johan Jendle
Principal Investigators:	Thomas Nyström, Johan Jendle, Åke Sjöholm, Leif Bojö, Bo Nilsson, Lars-Åke Brodin

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Protocol Summary

PROTOCOL IDENTITY AND OBJECTIVES	
EudraCT Number:	2010022695-31
Protocol Title:	Effects on subclinical heart failure in type 2 diabetic subjects on liraglutide treatment versus glimepiride both in combination with metformin
Trial Objectives:	To investigate whether liraglutide 1.8 mg QD improves left ventricle longitudinal functional reserve. An 18 week, open, assessor-blinded and active-controlled, parallel-group trial.

INVESTIGATIONAL MEDICINAL PRODUCTS (IMP)	
Test Product:	Liraglutide
Pharmaceutical Form:	Solution for injection in prefilled pen
Route of Administration:	Subcutaneous (s.c)
Test Product:	Glimepiride
Pharmaceutical Form:	Tablet
Route of Administration:	Oral (p.o)

METHODOLOGY	
Trial Design:	Open, assessor-blinded and active-controlled, parallel-group trial, in combination with metformin
Dose/Duration:	1.8 mg liraglutide QD vs. glimepiride 4 mg QD in total 18 weeks
Primary Endpoint:	Increase in left ventricle longitudinal function and/or functional reserve during rest and after exercise
Efficacy Parameters:	Improvement in left ventricle longitudinal functional reserve, as measured by tissue Doppler echocardiography
Safety Parameters:	

POPULATION OF TRIAL SUBJECTS	
Description of Trial Subjects:	Type 2 diabetes subjects with an HbA1c above 60 mmol/mol
Number of Subjects:	Eighty (80)

TRIAL TIMETABLE	
First Subject In:	2011
Last Subject In:	2011
Last Subject Out:	2012

Abbreviations

Abbreviation	Explanation
AE	Adverse Event
ADR	Adverse Drug Reaction
CRF	Case Report Form
IB	Investigator's Brochure
ICH	International Conference of Harmonisation
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Products
MPA	Medicinal Product Agency
SADR	Serious Adverse Drug Reaction
SAE	Serious Adverse Event
SPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction

1 Administrative Information

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2 Background Information

Heart disease, often presenting as cardiomyopathy, is the leading cause of death among patients with diabetes mellitus [1]. Heart failure in diabetic patients might be due to metabolic disturbances even in the absence of ischemia, *e.g.* heart failure and cardiomyopathy may be accompanied by the development of insulin resistance, both in whole-body and myocardial glucose uptake disturbances [2]. The spectrum of diabetic heart disease involves a progression from the normal heart, to subclinical left ventricular (LV) diastolic and systolic dysfunction, followed by clinically overt symptomatic heart failure. Notably, sub-clinical LV dysfunction is common in patients with diabetes [3], and the detection of sub-clinical LV dysfunction in these patients may provide an approach for identifying high-risk individuals who may benefit from earlier and more active intervention to prevent heart failure [4]. Although overt LV diastolic and systolic dysfunction can be readily identified by conventional diagnostic techniques including echocardiography, the initial stage of myocardial dysfunction may be concealed by various compensatory mechanisms.

Glucagon-like peptide-1 (GLP-1) is a naturally occurring incretin with insulinotropic properties [5]. Apart from the glycemic actions, cardiovascular effects by GLP-1 have recently been reviewed [6]. Receptors for GLP-1 are expressed in the rodent and human heart and acute activation of GLP-1 signalling has been shown to influence *e.g.* heart rate and blood pressure [6]. In a knock-out mouse model, GLP-1R-/- mice exhibited a defective cardiovascular contractile response together with left ventricular hypertrophy [7]. GLP-1 improves severe left ventricular heart failure in humans suffering from a myocardial infarction [8]. Hence, it has been demonstrated that GLP-1 exerts direct functional effects through both GLP-1 receptor dependent and independent pathways in the heart [9].

Native GLP-1 is an extremely short acting peptide, with a half-time breakdown of 1-2 minutes, a feature that makes it unsuitable as a drug treatment for type 2 diabetes. To this end, several long-acting GLP-1 analogues, drugs for treating type 2 diabetes, have been tested for this purpose. The analogue liraglutide exerts its effects via the native GLP-1 receptor, localized not only on the pancreatic β-cells, but also in the human heart. Interestingly, liraglutide has been demonstrated to have beneficial effect on heart function in mice [10]. Taken together, recent data shows that GLP-1 and its stable analogue liraglutide exert beneficial cardiovascular effects.

2.1 Trial Rationale

Acutely beneficial cardiovascular effects of GLP-1 on the heart have recently been demonstrated. Therefore the rational for this study is to investigate liraglutide effect on the heart in type 2 diabetic subjects. The 18 week treatment period is expected to be sufficient to evaluate the efficacy and safety of the treatment to be studied. Also, the comparator was chosen due to the fact that glimepiride 4 mg QD has an equal anti-diabetic effects in terms of lowering plasma glucose as for liraglutide 1.8 mg QD , during a 18 week period [11]. In order to obtain the necessary power in the trial, 80 subjects are expected to complete the trial (further described under Determination of sample size, section 9.2).

3 Endpoints

3.1 Primary Endpoints

Subjects achieving an absolute increase in left ventricle longitudinal function and/or functional reserve during rest and/or after exercise of 0.7 cm/s, i.e., $\Delta E'$ $[1-(1/E'_{base})]$ or $\Delta S'$ $[1-(1/S'_{base})]$ after 18 weeks of liraglutide + metformin, compared with glimepiride + metformin, using tissue Doppler echocardiography.

3.2 Secondary Endpoints

The changes from baseline for 18 weeks of liraglutide + metformin compared with glimepiride + metformin treatment will improve or affect;

- Global LV function (echocardiography) expressed as ejection fraction (EF)
- Exercise ECG, including working capacity
- 24-hour blood pressure
- Energy delivering from the carotid artery
- N-terminal pro b-type natriuretic peptide (NT-proBNP) levels in serum over time and symptoms of dyspnea or fatigue as assessed by patient and clinician using established scoring systems.
- Gene expression (Affymetrix)
- Plasma markers of inflammation *i.e.* hsCRP, IL-6, TNF-α and PAI-1
- Plasma markers of endothelial activation *i.e.* E-selectin, VCAM-1, ICAM-1 and plasma levels of nitrate/nitrite
- Lipids
- HbA_{1c}
- Body weight
- Adverse events in terms of hypoglycaemia
- Quality of life (SF 36)
- Blood test (venipuncture)

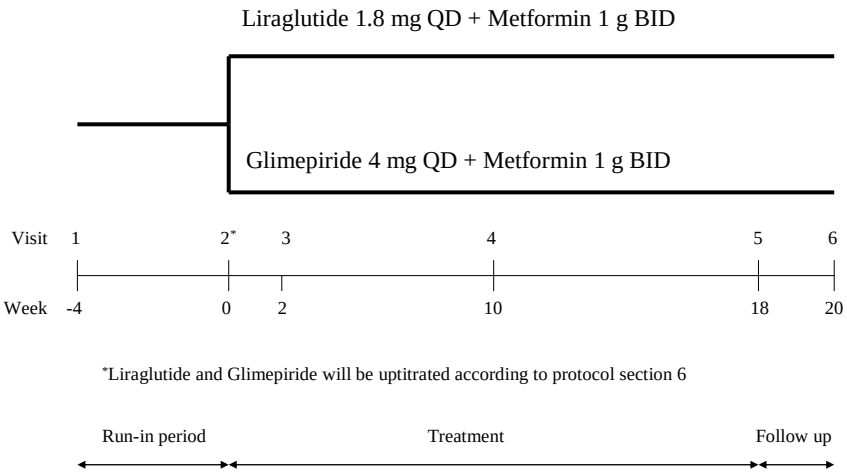
4 Design

4.1 Outline

The present trial is a two centre, open, assessor-blinded and active-controlled, parallel-group trial, in combination with metformin. The trial will compare the treatment with liraglutide 1.8 mg (s.c) QD + metformin up to 1 g BID, with that of glimepiride 4 mg QD (comparator) + metformin up to 1 g BID, on LV function in subjects with type 2 diabetes.

Patients are asked to participate in the study and after informed and written consent has been obtained, subjects will be screened based on the inclusion and exclusion criteria. If needed, subjects will have metformin up-titrated to a maximal daily dose of 2g, or the highest tolerated dose in the run-in period. Total trial duration for the individual subject will be 18 ± 4 weeks. A population of 80 type 2 diabetic subjects will be investigated (Figure 1).

Fig 1.



4.2 Assessments and Procedures

The subjects will attend a screening visit (Visit 1) in order to assess their eligibility. If found eligible, the subjects will return at Visit 2 within approximately 4 weeks, after Visit 1, with an up-titration with metformin 1 g BID or the maximal tolerated dosage of metformin (Run-in period).

At Visit 2 patients will be tested for;

- Heart function at rest and during an exercise ECG Stress Test with tissue Doppler echocardiography
- 24-hour blood pressure
- Antropometric assessment
- Symptoms of dyspnea or fatigue (scoring system, classified as NYHA).
- Quality of life (SF 36)
- Blood test (venipuncture)
- U-HCG (in fertilized women)

Subsequently thereafter, subjects will during visit 2 be randomized to receive either liraglutide 1.8 mg s.c. (initial dose of 0.6 mg with an up-titration of 0.6 mg every week, final dose 1.8 mg QD) or glimepiride 4 mg p.o (initial dose of 2 mg, with an up-titration of 1 mg every week, final dose 4 mg QD).

At Visit 2, subjects will be supplied with a glucose meter (Abbot Contour) and instruction on use of the device including regular calibration according to the manufacturer's instruction. Subject will also be provided with written instruction. The glucose meter use test strips calibrated to

plasma values. Therefore all glucose measurements performed with drawn capillary blood are automatically calibrated to plasma equivalent glucose values, which will be shown on the display and are the values to be used.

Subjects will be instructed on how to record the results of the self measured plasma glucose (SMPG) values in the meter. Subjects will then ask to monitor a 7 point profile glucose curve consecutively in three days before visit 3, at visit 4 and at the end of treatment (visit 5) and at the end of the trial (visit 6). SMBG values will be transferred via a computerized system (Diasend®).

Visit 3. Telephone visit. Self-reporting glucose measurements.

Visit 4. Telephone visit. Self-reporting glucose measurements.

At week 18 (Visit 5), subjects will be re-tested for:

- Heart function at rest and during an exercise ECG Stress Test with tissue Doppler echocardiography
- 24-hour blood pressure
- Antropometric assessment
- Symptoms of dyspnea or fatigue (scoring system, classified as NYHA).
- Quality of life (SF 36)
- Blood test (venipuncture)
- U-HCG (in fertilized women)

The physician will decide what treatment patients will be continued on Visit 6. Follow up and re-evaluate the anti-diabetic treatment.At all contacts Investigators will ask subject for signs or symptoms of an AE.

4.3 Schedule of Investigational Events

Procedures for the scheduled visits and phone contacts are described in the section above and in the flowchart (Schedule of Investigational Events, Appendix 17. 1). Each subject will be followed approximately 18 ± 4 weeks after randomisation.

5 Selection and Withdrawal of Subjects

5.1 Inclusion Criteria

1 Type 2 diabetes.
2 Heart Failure, visualized with echocardiography, one of the following (2.1, 2.2 or 2.3).
2.1 Ejection Fraction ≤ 50%.
2.2 Decreased systolic velocity (four chamber view) where two, out of four segments (Septum, Lateral, Inferior and Anterior Wall) has a relative decrease in velocity of 20% compared to a normal population.
2.3 Evidence of diastolic dysfunction as shown by abnormal left ventricular relaxation, filling, diastolic distensibility or stiffness. An E/E' ratio (ratio of early diastolic velocities of mitral inflow derived Doppler imaging and myocardial movement derived by tissue Doppler

imaging) >15 is considered diagnostic of diastolic dysfunction and an E/E′ ratio < 8 as diagnostic of the absence of diastolic heart failure. An increased left atrial size (>49 ml/ m^2) and an increased left ventricular mass (>122 g/m^2 in women and >149 g/m^2 in men) are considered sufficient evidence of diastolic dysfunction when the E/E′ ratio is inconclusive.

3 HbA_{1c} (accordingly to IFCC) 60 mmol/mol – 95 mmol/mol.

4 If antihypertensive treatment, the medication has to be stable, no change, for the last 1 month.

5 Male and female subjects, 18-70 years of age.

6 Signed informed consent form.

5.2 Exclusion Criteria

1. Type 1 diabetes (autoantibody positive).
2. Any history of receiving GLP-1 analogues or dipeptidyl peptidase inhibitors (DPP-IV inhibitor) or glimeperid.
3. Previous treatment with glitazones within 6 months.
4. Previous treatment with other sulphonylurea within 3 months.
5. Previous treatment with insulin (any regimen) within 1 month.
6. Known severe heart failure, classified as NYHA 3-4.
7. Significant ischemic heart disease (defined as angina-limited exercise or unstable angina); documented acute myocardial infarction (MI) within the previous 8 weeks.
8. Active myocarditis; malfunctioning artificial heart valve.
9. Atria fibrillation or flutter
10. History of ventricular tachycardia within 3 months before study entry; second- or third-degree atrioventricular block.
11. Implanted pacemaker.
12. Supine systolic blood pressure <85 mm Hg or >200 mm Hg.
13. Primary renal impairment (creatinine clearance < 30 ml/min), or creatinine clearance < 60 ml/min if treated with metformin.
14. Uncorrected hypokalemia or hyperkalemia (potassium <3.5 mmol/l or >5.5 mmol/l).
15. Significant anemia (Hb < 90 g/l)
16. Treatment with another investigational agent within 30 days before study entry, judged by the investigator.
17. Severe gastrointestinal disease, including gastroparesis. As judged by the investigator.
18. Body mass index (BMI) > 40 kg/m^2.
19. Malignant neoplasm requiring chemotherapy, surgery, radiation or palliative therapy in the previous 5 years. Patients with intraepithelial squamous cell carcinoma of the skin treated with topical 5FU and subjects with basal cell skin cancer are allowed to enter the trial.
20. Females of child bearing potential who are pregnant, breast-feeding or intend to become pregnant or are not using adequate contraceptive methods (adequate contraceptive measures as required by local law or practice).
21. Current drug and alcohol abuse.
22. History of acute or chronic pancreatitis
23. Subjects considered by the investigator to be unsuitable for the study.

5.3 Criteria for Withdrawal

The subjects may withdraw at will at any time. The subjects may be withdrawn from the trial at the discretion of the Investigator due to safety concerns or if judged non-compliant with trial procedures. Subjects randomised in error must be withdrawn from the trial

A subject must be withdrawn if the following applies:

1. Pregnancy or intention of becoming pregnant
2. Participation in other trials throughout the trial
3. Serious heart failure or a cardiovascular event

5.4 Subject Log

All subjects in the screening process will be documented in a medical chart accordingly to their inclusion criteria or exclusion criteria.

6 Treatment

All subjects have been instructed to up-titrate metformin to 1 g BID, or maximal tolerated dose of metformin, before randomisation (run-in period). At randomisation (Visit 2) subjects will be randomised into one of the two treatment groups; liraglutide 1.8 mg (s.c) QD (initial dose of 0.6 mg with an up-titration of 0.6 mg every week, final dose 1.8 mg) or glimepiride 4 mg (p.o) QD (initial dose of 2 mg, with an up-titration of 1 mg every week, final dose 4 mg), both in combination with metformin 1 g BID.

Subjects will be instructed to take metformin meanwhile the breakfast and the dinner meal.

Subjects will be instructed to take glimepiride before the breakfast meal and liraglutide at the same time point every day.

6.1 Description of Investigational Medicinal Products

Liraglutide will be available as a solution for injection at a concentration of 6.0 mg/ml, supplied in a 3 mL pre-filled disposable pen.

Glimepiride will be available as tablet for oral administration, each tablet containing 1 mg, 2 mg or 4 mg of glimepiride.

6.2 Packaging, Labelling, Storage and Handling of Investigational Medicinal Products

The trial products will be packed and labelled by an external clinical supply contractor, which can be done since both trial products are commercially available. Labelling will be in accordance with Annex 13, local law and trial requirements.

Each investigator site will be supplied with sufficient trial products for the trial.

Trial product will be packed in dispensing units and will be distributed to the sites according to enrolment and randomisation.

The trial products will be dispensed to each subject at Visit 2, as required according to treatment group. The randomisation procedure will allocate trial product Dispensing Unit Number (DUN) to the subject at the dispensing visit. The correct DUN must be dispensed to the subject at the dispensing visit.

Instruction to the subject on how to use the trial liraglutide pre-filled disposable pen should be provide by the Investigator at Visit 2.

The trial products should be stored in accordance with the storage conditions as stated in the Summary of Product Characteristics for each product, see Appendices 17.2 and 17.3.

The Investigator must ensure availability of proper storage conditions, and record and evaluate the temperature (at least every working day). Storage facilities should be checked frequently. A log to document the temperature must be kept.

Storage and in-use conditions:

Liraglutide

Not in use: The liraglutide pre-filled pen must be stored in a refrigerator at a temperature between +2°C and + 8°C. Keep away from the cooling element. Do not freeze and do not use if it has been frozen.

In-use: After first opening the liraglutide pre-filled pen can be stored for one month at temperatures below +30°C or in a refrigerator between +2°C and +8°C.

Do not freeze and do not use if it has been frozen.

The pen must be protected from all sources of light, and the pen cap should be kept on when the pen is not in use. The liraglutide should not be used if it does not appear clear and colourless.

Glimepiride

Store at temperatures below +30°C. Store in original package. Sensitive to damp.

In case of incorrect storage, the site staff should inform the Principal Investigator without delay. The trial products must be set on-hold until notified by Principal Investigator.

No trial product should be dispensed to any person not enrolled in the trial.

6.3 Treatment Assignment

The study is an open, assessor-blinded and active-controlled, parallel-group trial.

Randomisation will be carried out in a (1:1) manner using an interactive voice/web response system (IV/WRS) to randomise subjects into the treatment groups:

- Liraglutide QD or
- Glimepiride QD

both in combination with metformin.

6.4 Concomitant Medication

Type 2 diabetic patients with metformin, as a single oral anti-diabetic drug, or diet controlled, will be eligible for the study.

All other new medications in the study period will be documented in the CRF.

6.5 Compliance to Treatment

Subject compliance will be assessed by monitoring of drug accountability. The unused amount of trial product will be assessed against the dispensed amount and, in case of discrepancies, the subjects must be asked.

6.6 Product Accountability and Destruction

The person delegated by the Investigator must keep track of all received, used, partly used and unused trial products, and if possible all empty packaging, This shall be properly documented.

Used, partly used and unused trial products and empty packaging must be stored separately from non-allocated trial products. Used, partly used and unused trial products must be stored until the monitor has performed drug accountability.

The monitor is responsible for arranging destruction of trial products. The destruction will be performed in accordance with local legislation and recorded on a destruction form.

6.7 Continuation of Treatment

After the study end subjects will be referred to theirs ordinary general practitioner (GP). All subjects will be offered to contact Investigator regarding questions of the study and their treatment for diabetes.

7 Assessment of Efficacy and Safety

7.1 Clinical Efficacy Assessments

Accordingly to 20.1 Schedule of Investigational Events subjects will be investigated at two centres, Stockholm South General Hospital and Karlstad Central Hospital, respectively.

7.1.1 Blood pressure

Systolic and diastolic pressure and pulse will be recorded in the patient's both arms while the subject is sitting in an upright position, after a 15 min rest. For blood pressure at Visit 2 and Visit 5, three measurements need to be performed and all three values should be entered into the CRF.

7.1.2 24 hours blood pressure

An ambulatory 24-hour blood pressure recording will be performed using an ABPM-05 system (Meditech Ltd, Budapest, Hungary).

7.1.3 Tissue Doppler Echocardiography

Transthoracic echocardiography will be performed, with the subject in a lying position, using a Vivid 9 system (GE, Vingmed Ultrasound, Horten, Norway) with a 3,5 MHz phased-array transducer, Cine-loops including at least 3 consecutive heart beats will be saved and transmitted to a work station (EchoPAC-PC version 60; GE Medical systems) for off-line analysis. Parasternal long- and short axis views, apical long axis, 2- and 4-chamber views are obtained. Measurements of cardiac dimensions are made in accordance with recommendations of the American Society of Echocardiography Committee [12]. The diastolic mitral inflow velocities are recorded by pulsed wave Doppler from the apical 4-chamber view with the sample volume located at the tips of the mitral leaflets. Blood flow velocities during early diastole, and during atrial contraction, will be obtained. From apical views, Tissue Velocity Imaging (TVI) will be performed and loops are saved for analysis of long-axes contraction and relaxation velocities.

7.1.4 Carotid artery energy delivering

The carotid artery measurements will be performed, with the subject in a lying position, using a Vivid 9 system (GE, Vingmed Ultrasound, Horten, Norway) with a high-resolution 10 MHz transducer. Artery diameter and blood flow during at least 3 consecutive pulse waves will be saved and transmitted to a work station (EchoPAC-PC version 60; GE Medical systems) for off-line analysis.

7.1.5 Exercise ECG Stress Test and Tissue Doppler Echocardiography

A bicycle exercise stress test, following a standard protocol, will be performed using a Case 5 system (GE Medical Systems, WI, USA) immediately after the work. A stepwise increase of 20 W in working-load capacity will be changed every other minute. Subjects' will be asked to finish the work accordingly to the Borg scale 14-15. The electrocardiogram (ECG) will be monitored with a 12 lead ECG devices.

7.1.6 Adverse events

Adverse Events will be recorded at each visit in accordance with the procedures described in section 8. Any clinically significant worsening since baseline of a previous finding must be reported as an AE.

7.1.7 Hypoglycaemic episodes

Blood glucose should always be measured when there is a suspicion of a hypoglycaemic episode. All plasma glucose values ≤ 3.9 mmol/l when hypoglycaemic symptoms have occurred will be recorded by the subjects in the diaries and transcribed into the CRF.

A hypoglycaemic episode will be defined as treatment emergent if the onset of the episode is on or after first day of trial product. The recording should include:

- Date
- Time of hypoglycaemic episode
- Time of last liraglutide/glimepiride dose prior to episode
- Amount of last liraglutide/glimepiride dose prior to episode
- Time of last main meal prior to episode
- Whether the episode was symptomatic
- Whether the episode was in relation to exercise
- Whether hypoglycaemic re-occurred in the 24-hrs after initial recovery
- If yes, approximately number of hours since the first hypoglycaemic episode
- Whether the subject was able to treat him/herself, whether he/she recovered with oral administration of carbohydrates
- The plasma glucose level before treating the episode

Hypoglycaemic episodes will be defined as nocturnal if the time of onset is between 00:01 (included) and 05:59 am (included).

Documentation of hypoglycaemia by data from the Glucometer being downloaded at the next visit using Diasend®.

A hypoglycaemic episode form must be filled in for all hypoglycaemic episodes. If the hypoglycaemic episodes fulfil the criteria of a serious AE, a hypoglycaemic episode Form, an AE form and a Safety Information Form must be filled in.

ADA definitions and classification of hypoglycaemia will be applicable:

Severe hypoglycaemia: an episode requiring assistance of another person to actively administer carbohydrate, glucagons, or other resuscitative actions.

Documented hypoglycaemia: an episode during which typical symptoms of hypoglycaemia are accompanied by measured plasma glucose concentration ≤ 3.9 mmol/l.

Asymptomatic hypoglycaemia: an episode not accompanied by typical symptoms hypoglycaemia, but with a measured plasma glucose concentration ≤ 3.9 mmol/l.

7.1.8 Physical examination

Physical examination will include:

- Head, ears, nose, throat, neck
- Lungs
- Cardiovascular system
- Gastrointestinal system
- Vital signs

In the case of an abnormal clinical significant finding, the Investigator must comment in the subject notes.

Any clinically significant worsening from baseline must be reported as an AE.

7.2 Laboratory Efficacy Assessments

For laboratory analysis of efficacy parameters a total of approximately 100 ml blood will be drawn during the 18 weeks of the trial. The laboratory analyses will be performed by a central laboratory unless otherwise specified. Description of assays methods, laboratory supplies and procedures for obtaining samples, handling and storage of samples and information on who will perform the assessments, will be described in a trial specific laboratory manual provided by a central laboratory.

Laboratory samples can be drawn on a day other than the day the visit is performed as long as it is within the visit window stated in the flowchart. For some of the samples drawn during the trial it is required for the sensitivity of the analysis that the subject is fasting.

Samples will be coded such that the subject identity will remain anonymous.

Blood samples will be drawn for the efficacy assessments to determine levels of following:

- HbA_{1c}
- Fasting plasma glucose
- Lipids – Subject must be fasting
  - Total Cholesterol
  - HDL Cholesterol
  - LDL Cholesterol
  - Triglycerides
- Plasma markers of inflammation
  - hsCRP
  - IL-6
  - TNF-α
  - PAI-1

- Plasma markers of endothelial activation
  - E-selectin
  - VCAM-1
  - ICAM-1
  - Plasma levels of nitrate/nitrite

- Brain natriuretic peptide (NT-proBNP)

- Whole blood for Gene Expression (Affymetrix)

- Proteomics

7.3 Laboratory Safety Assessments

For laboratory analysis of safety parameters a total of approximately 16 ml blood will be drawn during the 18 weeks of the trial. The laboratory analyses will be performed by a central laboratory unless otherwise specified. Description of assays methods, laboratory supplies and procedures for obtaining samples, handling and storage of samples and information on who will perform the assessments, will be described in a trial specific laboratory manual provided by a central laboratory.

Laboratory samples can be drawn on a day other than the day the visit is performed as long as it is within the visit window stated in the flowchart. For some of the samples drawn during the trial it is required for the sensitivity of the analysis that the subject is fasting. Samples will be coded such that the subject identity will remain anonymous.

Laboratory results will be sent by the central laboratory to the Investigator on an ongoing basis. Data from the analysis will be included in the clinical trial report and the trial database. All laboratory printouts must be dated and signed by the Investigator on the day of evaluation. If a result is outside the normal range, the investigator must judge whether the abnormality is clinically significant. If considering clinically significant the results must be reported as an AE according to section 8, Proceedings for Adverse Events.

Blood samples will be drawn for safety assessments to determine levels of following:

- Haematology
  - Erythrocytes
  - Haematocrit
  - Haemoglobine
  - Leucocytes
  - Thrombocytes
- Biochemistry
  - Creatinine
  - ALAT (Alanine aminotransferase)
  - ASAT (Aspartate aminotransferase)
  - Alkaline phosphate (AP)
  - Sodium

- Potassium
- Calcium
- Calcitonin (TBD might be a demand)
- Albumin
- Amylas
- Bilirubin (total)

- Pregnancy test will be performed as a urine sample (U-HCG) for females of childbearing potential, at visit 1 and visit 5. For those of childbearing potentials the required use of contraceptives will be discussed.

- Urine samples
  - Albumin/creatinine ratio by urine spot

8 Proceedings for Adverse Events

8.1 Definition of Adverse Events

8.1.1 *Definition of Adverse Events*

An Adverse Event (AE) is any untoward medical occurrence in a subject administered Investigational Medicinal Products (IMP) and which does not necessarily have a causal relationship with this product. An AE can be any unfavourable and unintended sign, abnormal laboratory finding, symptom or disease temporally associated with the use of IMP, whether or not related to the product.

8.1.2 *Definition of Adverse Reactions*

Each AE is to be classified by the investigator as related or not related to the IMP. An Adverse Reaction (AR) is a noxious and unintended medical response to a medical product related to any dose. For an AE to be an AR the suspected association between the product and the unwanted medical condition should be at least a reasonable possibility.

8.1.3 *Definition of Serious Adverse Events*

Each AE is to be classified by the investigator as serious or non-serious. Seriousness is not defined by a medical term; it is a result or an outcome. An AE is defined as a Serious Adverse Event (SAE) if it:

- results in death
- is life-threatening
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity
- results in a congenital anomaly/birth defect
- Important medical events that may not result in death, be life-threatening, or require hospitalisation may be considered an SAR when, based upon appropriate medical judgement, they may jeopardise the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

Medical and scientific judgement should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalisation; or development of drug dependency or drug abuse.

8.1.4 *Definition of Suspected Unexpected Serious Adverse Reactions*

Each SAE that is at least possibly related to IMP is to be classified by the investigator as expected or unexpected. A SAE that is at least possibly related to IMP, and ***unexpected***, is defined as a Suspected Unexpected Serious Adverse Reaction (SUSAR). It is expected if it is already known from earlier trials or is mentioned in relevant documents (SPC, summary of product characteristics).

8.2 Assessment of Adverse Events

8.2.1 *Assessment of Intensity*

Each AE is to be classified by the investigator as mild, moderate or severe.

Mild: Acceptable. The subject is awareness of symptoms or signs, but they are easy tolerated.

Moderate: Disturbing. The AE is discomfort enough to interfere with usual daily activity.

Severe: Unacceptable. The subject is incapacity to work or to do usual daily activities.

8.2.2 *Assessment of Causality*

Unlikely: The event is most likely related to aetiology other than the IMP.

Possible: A causal relationship is conceivable and cannot be dismissed.

Probably: Good reason and sufficient documentation to assume a causal relationship.

8.3 Methods for Eliciting Adverse Events

All adverse effects regardless of relationship to study drug or protocol procedure will be recorded in the adverse event CRF, including date and time of symptoms.

The Investigator should record the diagnosis, if available. If no diagnosis is available then the Investigator should record each sign and symptom as Individual SAEs.

8.4 Reporting of Adverse Events

8.4.1 *Reporting of Adverse Events*

All AEs will be recorded on a separate AE form in the CRF.

8.4.2 *Reporting of Serious Adverse Events*

In addition will SAEs also be reported by the investigator to the sponsor on a separate SAE form within 24 hours after the SAE have been communicated to the investigator. Follow-up information describing the outcome of the SAE and action taken will be reported as soon as it is available. The original SAE form must be filed with the CRF. The sponsor shall report all cases of death to the MPA within 7 days, and if necessary complete the report to the MPA within 8 days and all cases of death to the Independent Ethics Committee (IEC).

8.4.3 *Reporting of Suspected Unexpected Serious Adverse reactions*

The sponsor must report all SUSARs that resulted in death or was life threatening to the authority through the EudraVigilance database and to the IEC within 7 days, and if necessary complete the report within the following 8 days. Other SUSAR should be reported within 15 days. In addition will the sponsor report all SUSAR to all Principal Investigators involved in trials with the IMP.

8.5 Follow-up of Adverse Events

Follow-up information (corrections, new or additional information) should be reported within the timeline of obtaining knowledge of the information for SAEs, and if previously non-serious AEs become SAEs to the Sponsor. The Investigator must ensure that the worst case severity and seriousness is kept consistent.

During and following a subject's participation in a clinical trial, the Investigator/institution should ensure that adequate medical care is provided to the subjects for any AE(s), including clinically significant laboratory values related to the trial. The investigator should inform the subject when medical care is needed for AE(s) of which the Investigator becomes aware.

The follow up information should only include new (updated and/or additional) information that reflects the situation at the time of the Investigator's signature.

8.6 Pregnancy

Subjects must be instructed to notify the Investigator immediately if they or their partner become pregnant during the trial.

The Investigator must report any pregnancy reported during the trial except for pregnancies occurring in the screening period. Trial subjects will give consent on enrolment that the Investigator will document any pregnancy during the trial, and that she will be asked to provide information about her pregnancy, delivery and the health of her infant until age one month. If the pregnancy results in an abnormal outcome, such as congenital anomalies, foetal death, spontaneous abortion, or SAEs in the neonate then this should be regarded as an SAE with the same reporting requirements and timelines as for SAE.

If an SAE occurs in relation to a pregnancy, either to the mother or the newborn, then follow the same reporting requirements and timelines as for SAE.

9 Statistics and Data Management

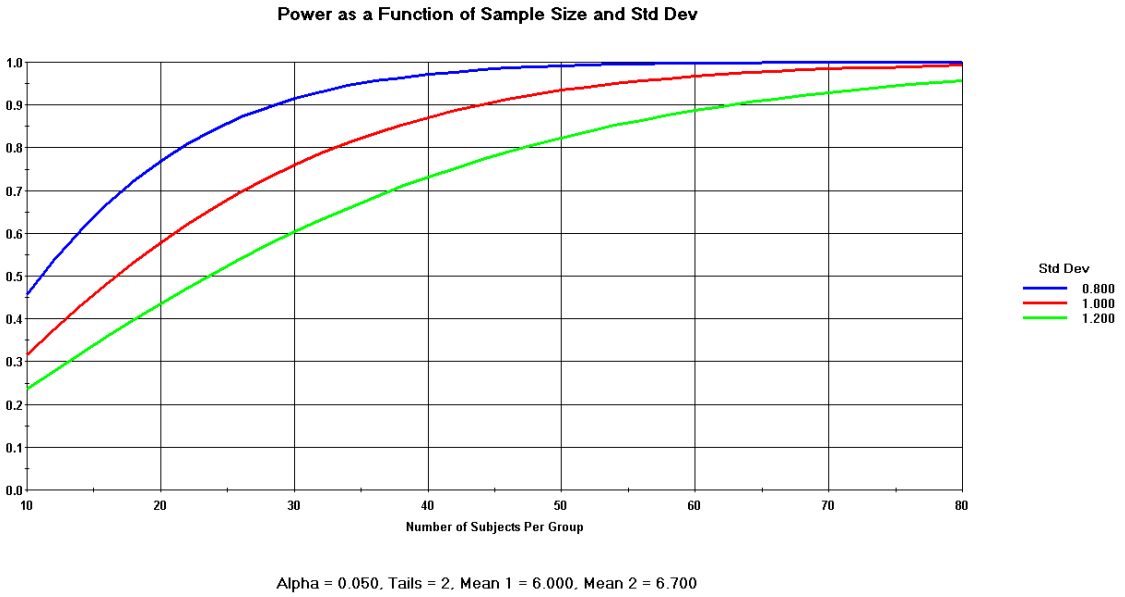
9.1 Data Management

A case report form (CRF) will be obtained and completed for each included patient. Investigators will ensure completion and review of the CRF. Investigators have personal responsibility for the accuracy and authenticity of all clinical and laboratory data that are entered into the CRF. Also, electronic charts will be transferred to paper charts and stored for each included patient. CRFs, including paper charts, will be in locked in a safe place. However, in case of emergency, CRFs will be made readily available.

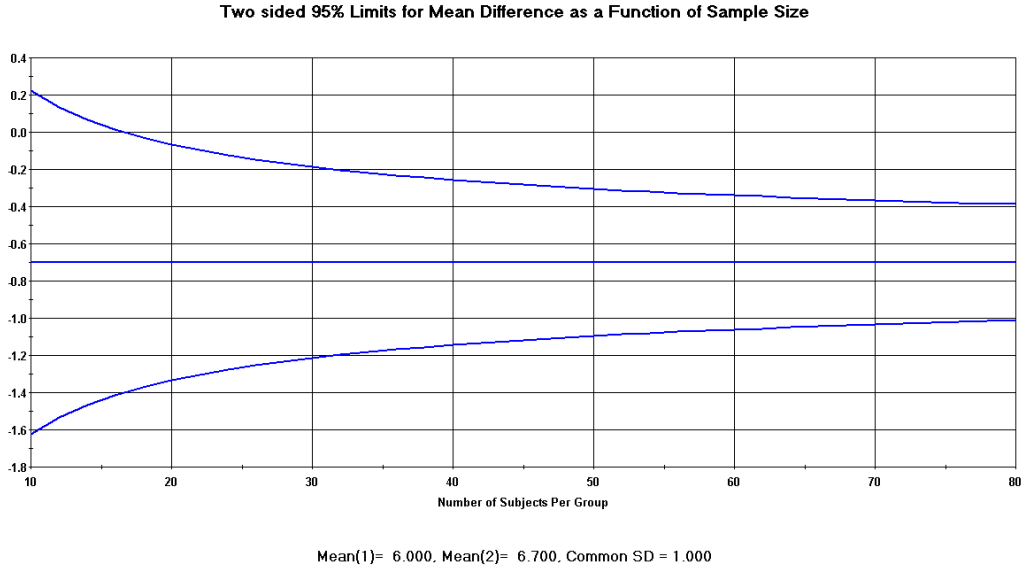
9.2 Determination of Sample Size

The primary endpoint is defined as an improvement of LV longitudinal contraction measured with tissue Doppler echocardiography at rest and at ECG Stress Test. Longitudinal diastolic function reserve index = $\Delta E'[1-(1/ E'_{base})]$ and Longitudinal systolic function reserve index = $\Delta S'[1-(1/ S'_{base})]$. Patients with an absolute increase in $\Delta E'[1-(1/ E'_{base})]$ = cm/s or $\Delta S'[1-(1/ S'_{base})]$ cm/s of 0.7 cm/s, i.e., a 15 % in relative increase in cm/s from an assumed mean value of 6 cm/s) [13,14]. This assumption is estimated in accordance with previous human data [3, 4]. In that case we need to investigate 68 patients to demonstrate a mean absolute difference of 0.7 cm/s with an alpha error of 5 % and a beta error of 80 %, in an open labelled parallel trial. This calculation is done with a standard deviation for the method assumed to be 1.0 cm/s (absolute). We estimate that some subject will drop-out, therefore we will investigate 80 subjects (40 in each group). See attached figures. These figures (A and B), below.

A) With the proposed sample size of 34 and 34 for the two groups, the study will have power of 81.2% to yield a statistically significant result. This computation assumes that the mean difference is -0.7 (corresponding to means of 6.0 versus 6.7) and the common within-group standard deviation is 1.0.



B) The precision estimated here is the median precision. Precision will vary as a function of the observed standard deviation (as well as sample size), and in any single study will be narrower or wider than this estimate.



10 Quality Control and Quality Assurance

10.1 Monitoring

During the course of the trial, the Monitor will visit the trial site to ensure that the protocol has been adhered to, that all issues have been recorded, perform source data verification, monitor drug accountability etc. The study will be monitored by a boarded monitor (Christina Häll, Metabollab Södersjukhuset). All adverse effects regardless of relationship to study drug or protocol procedure will be recorded in the adverse event CRF.

The monitor must be given direct access to source documents (original documents, data and records). Direct access includes permission to examine, analyse, verify and reproduce any record(s) and report(s) that are important to the evaluation of the clinical trial. In order to that, a written consent from patient should be fulfilled. In addition the monitor should be available for discussions by telephone.

An investigator Source Document Verification Agreement will be completed and signed for each site in order to describe where source data for each data item can be found.

The monitor will ensure that the CRFs are completed by site staff.

11 Ethics

11.1 Ethical Conduct of the Trial

The protocol will be reviewed and approved by an IEC. The trial will be performed in accordance with the protocol guidelines in full accordance with Good Clinical Practice and applicable local regulatory requirements and laws. The investigator must insure that each trial

subject is fully informed about the nature and the objectives of the trial and possible risks associated with participation. The investigator will obtain written informed consent from each subject before any trial-specific activity is performed. The investigator will retain the original of each subject's signed consent form.

11.2 Subject Information and Informed Consent

In obtaining and documenting informed consent, the Investigator must comply with applicable regulatory requirement and adhere to the ICH GCP and the requirements in the Declaration of the Helsinki

Prior to any trial related activity, the Investigator must give the subject oral and written information about the trial in a form that the subject can read and understand.

A voluntary, signed and personally dated, including time, informed consent must be obtained from the subject before any trial related activity. The subject must be provided with a copy of his/her signed informed consent.

The responsibility for obtaining informed consent must remain with that of a medically qualified person and cannot be delegated to a non-medical qualified person. The written informed consent must be signed and personally dated, by the person who obtained the informed consent.

If information becomes available that may be relevant to the subject's willingness to continue participating in the trial, the Investigator must inform the subject in a timely manner, and a revised written informed consent must be obtained.

Once a subject's participation in the trial has ended no additional care different from what is normally provided according to subjects medical condition is required.

12 Data Handling and Record Keeping

12.1 Case Report Forms

A case report form (CRF) will be obtained and completed for each included patient. Investigators will ensure completion and review of the CRF. Investigators have personal responsibility for the accuracy and authenticity of all clinical and laboratory data that are entered into the CRF. CRFs, including paper charts, will be stored in locked and secure place. However, in case of emergency, CRFs will be made readily available.

13 Financing and Insurance

Separate financially protocol will be set up. Subjects are insured accordingly to the Swedish patient insurance scheme and the Swedish Drug Insurance Scheme.

14 Publication Policy

The results of the trial will be published by the investigators in an international scientific journal.

15 Supplements

15.1 Amendments

Before the Investigator starts the trial, the following documents must be available:

- Regulatory approval and/or notification as required (independent ethics committee and the Medical Products Agency – Sweden)
- Signed and dated agreement on the final protocol
- Curricula vitae of the Investigator and Sub-Investigator(s) (current, dated and signed and/or supported by an official regulatory document)

15.2 Personnel Information

The Investigator is accountable for the conduct of the trial. If any tasks are delegated, the Investigator should maintain a list of appropriately qualified persons to whom he/she has delegated specified significant trial-related duties

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Appendices

16.1 Schedule of Investigational Events

	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6
	Screening	Randomisation	(Telephone)	(Telephone)	End of treatment	End of trial
	Week -4 to -1	*Week 0*	*Week 2*	*Week 10*	*Week 18*	*Week 20*
Informed consent	X					
Inclusion/exclusion criteria	X					
Randomisation		X				
Dispensation of trial drug		X				
Echocardiogram (Tissue Doppler)		X			X	
Exercise ECG		X			X	
Blood chemistry		X			X	
Body weight/length		X			X	
Waist and hip circumferences		X			X	
Quality of life (scoring)		X			X	
24-hour blood pressure		X			X	
Vital signs		X			X	
Pregnancy test	X				X	
AE/SAE		X	X	X	X	X

17.2 Summary of Product Characteristics Glimepiride

17.3 Summary of Product Characteristics Liraglutide