THE EFFECTS OF GLP-1 IN MATURITY-ONSET DIABETES OF THE YOUNG (MODY)

CLINICAL TRIAL PROTOCOL

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1 INTRODUCTION

1.1 Clinical experience

Maturity-onset diabetes of the young (MODY) is a monogenic form of diabetes responsible for approximately 1-2% of all cases of diabetes. The disease is clinically defined by: 1) autosomal dominant inheritance (diabetes for at least two consecutive generations), 2) non-insulin dependent diabetes at onset (or measurable serum C-peptide three years after onset), and 3) diagnosis in a young age (at least one family member with onset before the age of 25 years) (1). Clinically, MODY-patients resemble patients with type 2 diabetes (T2DM) more than patients with type 1 diabetes mellitus (T1DM). MODY is genetically heterogeneous, with known mutations in eight different genes and mutations in either of these genes leads to specific forms of MODY. Based on a national epidemiological survey, we know that in Denmark, approximately 50% of patients who are diagnosed with MODY have mutations in the hepatocyte nuclear factor (HNF) 4 alpha (HNF4A) (MODY1), glucokinase (GCK) (MODY2), or HNF1A (MODY3) genes (2-4).

MODY3 is the most common form of MODY in Denmark (approximately 60% of all patients with MODY)(2). Patients with MODY3 are often diagnosed around puberty, more than 50% of mutation carriers will develop diabetes before the age of 25, and the lifetime risk of developing diabetes is higher than 95%. The typical course of disease is characterised by a rapid progression from impaired glucose tolerance to diabetes. After the diagnosis of diabetes, the glucose tolerance is further impaired due to a continuous loss of beta cell function (4). MODY3 often develops abruptly with classic hyperglycaemic symptoms such as polyuria and polydipsia, which is why this form of diabetes is often misclassified as T1DM (5). Patients with MODY3 have the same risk of developing microvascular and macrovascular late diabetic complications as patients with T2DM, and, strict glycaemic control combined with proper screening for diabetic late complications is crucial for a good prognosis.

About half of MODY3 patients are treated with diet or oral antidiabetic agents, the latter mostly in the form of sulphonylureas (SU), which, if possible is preferred to insulin injections. Due to a high sensitivity to SU combined with normal or even increased insulin sensitivity (MODY3 patients are more insulin sensitive than age- and body mass index (BMI)-matched patients with T2DM), this treatment is often associated with hypoglycaemia even when rather low doses of SU are used (4,10). Although SU treatment offhand seems to constitute a logical choice of treatment in MODY, due to beta cell dysfunction, the risk of hypoglycaemia is a clinical drawback due to potential suboptimal glycaemic control and decreased patient compliance. In a recent study, in which patients with MODY3 were exposed to physical activity (light cycling for 30 minutes approximately 2 hours after meal ingestion), hypoglycaemia was observed in 40% of subjects treated with short-acting SU (glibenclamide) with one patient experiencing hypoglycaemia for 12 hours (10).

Glucagon-like peptide-1 (GLP-1) is an incretin hormone, which is secreted from endocrine L cells of the small intestine in response to nutrients in the gut lumen (6,7). GLP-1 conveys an insulinotropic effect through GLP-1 receptors (GLP-1R) on pancreatic beta cells thereby

decreasing plasma glucose (PG). Moreover, GLP-1 inhibits the secretion of glucagon from pancreatic alpha cells, which further contributes to lowering of the PG levels (6,7). Both of these effects are strictly glucose-dependent (more pronounced at higher PG levels) and the effects cease as PG levels reaches values below 4-5 mM (8). Therefore, the hormones keep PG at normal levels without increasing the risk of hypoglycaemia. In addition, GLP-1 inhibits gastrointestinal motility including gastric emptying and leads to a centrally-mediated inhibition of appetite resulting in reduced food intake. Thus, GLP-1 is essential for glycaemic control. The GLP-1R agonist, liraglutide (Victoza®), has 97% homology to the naturally occurring GLP-1 hormone, but has a longer half-life (11-15 hours).

Since the effects of the incretin hormones are strictly glucose-dependent, treatment with GLP-1R agonists is rarely associated with hypoglycaemia. Thus, the current study aims to elucidate whether liraglutide (Victoza®) could be a safe and efficacious new treatment modality for patients with MODY.

1.2 Benefits and risks

Liraglutide (Victoza®) is approved and marketed as non-insulin, once-daily medication for the treatment of patients with T2DM. Besides control of PG levels, liraglutide may provide the additional benefit of body weight loss. The patients in the present study suffer from MODY3 and we expect to see improved glycaemic control in subjects treated with liraglutide. The risks attributed to liraglutide are mainly related to gastrointestinal symptoms. The most common adverse events include nausea, vomiting, headache and diarrhoea, which most often cease with time (weeks). Less commonly, the patients may experience stomach pain, constipation, fever, reflux, gastritis or dizziness. Liraglutide can be injected subcutaneously in the abdomen, in the thigh or in the upper arm (11).

Patients will receive detailed information in writing and orally about the risk of adverse events. If the patients find the adverse events unacceptable, they are free to withdraw from the study without any further explanation.

1.3 Clinical trial regulations

The GCP unit at University of Copenhagen will monitor the trial. The clinical trial will be conducted in compliance with the protocol, according to ICH E6: Good Clinical Practice: Consolidated guideline, CPMP/ICH/135/95, and national guidelines including Clinical Trials Directive 2001/20/EC. The trial can be subjected to quality audit.

2 OBJECTIVES OF THE TRIAL

The objective of this study is to investigate long term (6 weeks) effects of liraglutide compared to glimepiride on FPG and the risk of hypoglycaemia in patients with MODY3 in a double-blind, randomised cross-over trial.

3 INVESTIGATIONAL TRIAL DESIGN

3.1 Study endpoints

3.1.1 Primary endpoints

Fasting PG (FPG)
Glycaemic control will be evaluated by FPG monitored twice weekly, 7-point PG profiles every two weeks and 3 blinded 48-hour continuous PG profiles (before randomisation and at the end of both treatment periods). The patients who will be their own controls, will randomly be assigned (after one week washout of usual antidiabetic treatment) to receive either liraglutide or glimepiride for 6 weeks, and after another one-week washout period treated with the opposite treatment for 6 weeks.

3.1.2 Secondary endpoints

Secondary endpoints include: serum fructosamine, number and severity of hypoglycaemic events, estimation of endocrine pancreas function (plasma concentrations of insulin, C-peptide and glucagon) and plasma concentrations of incretin hormones. Postprandial responses of incretin hormones and beta cell function (assessed as fasting proinsulin-to-insulin ratio) will be evaluated during three standardised 4-hour meal tests (at baseline and in the end of each treatment period). Hypoglycaemic events will be reported by the patient in a diary. During cycling tests patients will be tested further according to hypoglycaemia. *Mild hypoglycaemia* is defined as episodes with symptoms of hypoglycaemia familiar to the patient and managed solely by the patient. Events of *severe hypoglycaemia* are defined as episodes with symptoms of hypoglycaemia with need for assistance from another person. Such events must be reported to the investigator within 24 hours after the event. Episodes of severe hypoglycaemia will be validated as follows: 1) symptoms of hypoglycaemia; 2) PG <3.0 mM; and 3) adequate response to glucose/glucagon treatment. Episodes fulfilling all three criteria will be classified as definite; those fulfilling two criteria with no other explanation of the symptoms will be classified as probable, and those only fulfilling one criterion will be classified as possible. Severity will be sub-classified according to level of assistance and consciousness during the episode: 1) conscious during the event, but with need of assistance to treat orally with glucose; 2) coma or seizure during the event and/or if parenteral treatment is necessary. *Biochemical hypoglycaemia* is defined as PG <3.0 mM.

Fructosamine
Fructosamine is a time-averaged indicator of PG levels, which is used to assess the glycaemic status of patients with diabetes. It reflects the total amount of glycated proteins such as glycohaemoglobin and glycoalbumin in a blood sample. The concentration of glycated proteins is generally recognized to be valuable in evaluating the glycaemic status of patients with diabetes. It is simple compared to other methods used for such determinations such as affinity

chromatography and the thiobarbituric acid method, which are labour-intensive and time consuming and results obtained from different laboratories are difficult to compare. The turnover of serum proteins (albumin has a half-life of 19 days) is less than that of haemoglobin (lifespan of erythrocytes is approximately 120 days), and therefore fructosamine determinations provide a means of monitoring patient blood glucose status over a shorter period (1-3 weeks) than glycohaemoglobin (6-8 weeks). As a result, changes in fructosamine values alert the physician to deteriorating glycaemic control earlier than changes in HbA_{1c} values. In addition, fructosamine levels decrease more quickly than HbA_{1c} when antidiabetic treatment is optimised (12).

Cycling test
Patients will be evaluated during a cycling test. The number of patients experiencing hypoglycaemia during a 30-minute bicycle exercise test 2½ hours after ingestion of a standardised test meal will be noted. PG will be evaluated at predefined time intervals throughout the study in order to evaluate biochemical hypoglycaemia. All patients will be tested three times: at baseline (after one week washout of usual antidiabetic treatment and in the end of each treatment period. Two and a half hours after ingestion of a test meal (375 kcal, 46 g carbohydrate, 14.5 g fat and 15 g protein) all patients will be subjected to a 30-minute cycling test (50-60 revolutions/min with adjustment of the bicycle resistance to fix the heart rate at 100-120 beats/min throughout the test) (10). Patients will ingest the meal 30 minutes after administration of study medication.

Endocrine measures and hypoglycaemia
Endocrine pancreas function will be estimated by analyses of plasma concentrations of insulin, C-peptide and glucagon. Other hormone analyses include cortisol, epinephrine, nor-epinephrine, growth hormone and incretin hormones. Furthermore, observation of symptoms of hypoglycaemia including sweating, tremor, confusion, nausea, nervousness, weakness, hunger, trouble speaking, palpitations, anxiety and irritability will be noted. *Mild hypoglycaemia* is defined as episodes with symptoms of hypoglycaemia familiar to the patient and managed solely by the patient. *Biochemical hypoglycaemia:* is defined as PG <3 mM without symptoms of hypoglycaemia. *Severe hypoglycaemia* is defined as episodes with symptoms of hypoglycaemia with need for assistance from another person. Severity will be sub-classified according to level of assistance and consciousness during the episode: 1) conscious during the event, but with need of assistance to treat orally with glucose; 2) coma or seizure during the event and/or if parenteral treatment is necessary.

3.2 Study design

A double-blind, randomised, clinical cross-over trial has been chosen in accordance with the trial objectives.

3.3 Comparative treatment regimens

Treatment with: 1) liraglutide injections/placebo tablets, or 2) glimepiride tablets/placebo injections

3.4 Randomisation and blinding

Patients will be randomised to treatment order with liraglutide and glimepiride. The patients who will be their own controls, will randomly be assigned after one week washout (of usual antidiabetic treatment) to receive either liraglutide or glimepiride for 6 weeks, and after another one-week washout period treated with the alternative treatment for 6 weeks.
The randomisation will be carried out by drawing sealed opaque envelopes with the randomization code.

The supplier of Victoza® and placebo pens (Novo Nordisk) will be responsible for labelling and blinding of the liraglutide and placebo pens before the beginning of the treatment period and for generating the randomisation code. The supplier of glimepiride and placebo tablets (*Region Hovedstadens Apotek*) will be responsible for labelling and blinding of the glimepiride capsules. An emergency code will be kept at Gentofte Hospital. If a patient develops adverse events that demand knowledge of the treatment, the code may be broken.

3.5 Description of investigational drugs and placebo drugs

Victoza® (liraglutide) is supplied in pens for injection containing 18 mg of the GLP-1R agonist liraglutide in 3 ml sterile water with disodium-phosphate and propylenglycol, and phenol for conservation (pH 8.15). Commercial pens will be used and the information given in the packaging will be applicable. Amaryl® (glimepiride) is supplied in gelatine capsules containing ½ mg and 1 mg of glimepiride, respectively. Commercial tablets will be used and sealed in a gelatine capsule by *Region Hovedstadens Apotek*. The information given in the packaging will be applicable.

Liraglutide
The initial daily dose will be 0.6 mg for one week, 1.2 mg the following week and then 1.8 mg for the remaining treatment period. Patients who, due to adverse events, do not tolerate up-titration to 1.8 mg liraglutide will remain on 1.2 mg of liraglutide. The injection is administered once daily in the morning.

Glimepiride
At randomisation patients will be initiated on their pre-study daily dose of glimepiride minus 0.5 mg. After one week the dose will be titrated (see below). Drug naïve patients will be initiated on an initial dosage of glimepiride of 0.5 mg for one week. Thereafter, glimepiride is increased to 1.0 mg and after another one week to 1.5 mg, and there after further up to 3 mg (if the average FPG during one week is above 6 mM). The dose of glimepiride can be increased up to 4 mg if average FPG is above 6 mM and no symptoms of hypoglycaemia are observed.

Placebo pens/tablets
The placebo pens contain “Victoza-vehicle” (no active drug) and are administered in the same way and volume as Victoza®. The placebo pens are specially prepared for this study and will be used in the present study only. Placebo tablets are administered similar to the glimepiride

tablets as mentioned above. The placebo tablets are specially prepared for this study and will be used in the present study only.

3.6 Drug ordering and storage

The pens are delivered in separate boxes. Storage conditions: When not in use the liraglutide pre-filled pen must be stored in a refrigerator at a temperature between +2°C and + 8°C. The pens must be kept away from the cooling element. The pens may not freeze (they may not be used if they have been frozen). After first opening, the liraglutide pre-filled pen can be stored for 30 days at room temperature (15°C to 30°C) or in a refrigerator (2°C to 8°C). The pens may still not freeze. 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. Liraglutide should not be used if it does not appear clear and colourless. Glimepiride and placebo capsules have a durability of 6 months from sealing at *Region Hovedstadens Apotek* and must be stored at room temperature. All trial medication will be supplied free of charge to the patients.

3.7 Drug accountability

One investigator will be responsible for drug accountability. For each patient treated, the batch number of the pen and glimepiride/placebo must be documented and the patients will be asked to return the pens after usage. Non-used glimepiride/placebo capsules must also be returned at end of study period. After verification of the drug accountability, proper destruction of the used pens will be ensured.

3.8 Study duration

The full study period constitutes 14 weeks (two wash-out periods of 1 week and two treatment periods of 6 weeks), preceded by a pre-treatment evaluation approximately 14 days before start of the first wash-out period. At the end of each treatment period each patient will be monitored with regard to efficacy parameters (FPG, fructosamine and HbA_{1c}) and adverse events. The rationale for choosing treatment duration of 6 weeks is that this time period allegedly is sufficient to ensure a possible clinical effect on diabetes.

3.9 Trial timetable

The anticipated timetable for the trial is:

Start of recruitment	August 2012
End of recruitment	June 2013
Last treatment	October 2013

4 PATIENT SELECTION

4.1 Number of patients and target population

Sixteen patients with MODY3 will be included in this study. The patients will be recruited through *Steno Diabetes Center*, Gentofte, Denmark, as all MODY patients in Denmark are diagnosed and initially treated here. Recruitment will take place through contact by mail or telephone. Inclusion will stop when 16 patients have completed the two treatment periods and the associated examinations. The expected number of dropouts is none or low.

4.2 Patient screening

Eligible patients will be informed about the possibility to participate in this study. Before any trial related procedures are performed, the patient must be thoroughly informed about the study and he/she must sign and date the informed consent form. A pre-treatment evaluation will be carried out to screen the patients according to inclusion and exclusion criteria (see Treatment Procedures).

4.3 Inclusion criteria

- Caucasian above 18 years of age
- Well characterised MODY3
- *Body mass index* (BMI) >19 kg/m^2
- Normal haemoglobin (males >8.2 mM, females >7.2 mM)
- Normal blood pressure (<160/100 mmHg)
- Informed consent
- Capability to perform a light cycling test (heart rate 100-120 beats per minute during 30 minutes)
- Females: use of anticonception (IUC or hormonal)

4.4 Exclusion criteria

- Heart failure: New York Heart Association class III-IV
- Uraemia, end-stage renal disease, or any other cause of impaired renal function with s-creatinine >130 µM and/or albuminuria
- Liver disease (alanine amino transferase (ALAT) and/or aspartate amino transferase (ASAT) >2 × upper normal serum levels)
- Anaemia
- Acute or chronic pancreatitis
- Struma or thyroid cancer
- Pregnancy or breast feeding
- Inability to complete the study
- Treatment naïve patients with HbA_{1c} <7.0 %

- Treatment with medicine that can not be paused for 12 hours
- Known allergic reaction to study medication
- Intention to become pregnant
- Unwillingness to complete the protocol

4.5 Patient withdrawal

Completion or trial termination for any reason will be fully documented in the clinical record form (CRF) pages. Patients are free to withdraw from the trial at any time without providing reason(s) for withdrawal and without prejudice to further treatment. The reason for withdrawal may be withdrawal of consent, treatment failure, adverse event(s), pregnancy discovered during the trial, or loss to follow-up. The reason(s) will be recorded in the CRF. Dropouts will be replaced until 16 patients have completed both treatment periods. Data from dropouts will be included in data processing. Patients withdrawing from the trial should be encouraged to go through the same final evaluations as patients completing the trial according to the protocol with special focus on safety. The aim is to record data in the same way as for patients who complete the trial. Otherwise data will be recorded as consented by the patient. This will be documented in the CRF.

5 TREATMENT PROCEDURE

The study consists of a pre-treatment evaluation and a one-week wash-out period followed by a 6-week treatment period where patients are randomised to treatment with either 1) liraglutide (subcutaneous injection) and placebo capsule, or 2) glimepiride (capsule) and subcutaneous placebo injection. This period is followed by another 1 week wash-out period which is followed by the second treatment period, where the patients are crossed over to the other treatment.

5.1 Pre-treatment evaluation

Before enrolment, after obtaining informed consent, the patients will be screened according to inclusion and exclusion criteria. Medical history will be obtained, including registration of current medication. Height, weight, abdominal- and hip circumference will be measured. Screening blood samples are collected including creatinine, electrolytes (Na^+ and K^+), ALAT/ASAT, haemoglobin, alkaline phosphatase, albumin, HbA_{1c}, PG, and urine is examined for the albumin/creatinine ratio and HCG (females).

Pre-treatment evaluation will only be performed after the patient has agreed to participate and has signed and dated the informed consent form. No treatment will be initiated before the signed consent has been given. If the patient meets all inclusion criteria, an appointment for the first visit will be set up. The clinical examinations will be conducted at Diabetes Research Division at Department of Internal Medicine F, Gentofte Hospital, where the necessary equipment is present. Regular antidiabetic medication (if any) will be paused 1 week before

randomisation in order to secure sufficient wash out. Patients treated with diet only (HbA_{1c} ≥7%) will be examined and thereafter randomised.

5.2 Treatment evaluation

The patients that fulfil the inclusion criteria are randomised to treatment sequence with liraglutide and glimepiride. In the second treatment period, the patients cross over to the alternative treatment as scheduled below.

Time (weeks)	>-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
Screening	X														
Randomisation		X													
CGM*		X						X							X
Test meal and cycling test		X						X							X
7-point PG profile				X		X		X			X		X		X

*CGM, continuous glucose monitoring

Wash-out period
Treatment period 1: liraglutide/glimepiride + placebo
Treatment period 2: the opposite of period 1 + placebo

Victoza®/placebo is administered subcutaneously one time daily in the entire treatment period (Day 8-49/57-98) and up-titrated as follows: Day 8-14/57-63: 0.6 mg Victoza®/placebo; Day 15-21/64-70: 1.2 mg Victoza/placebo; Day 22-49/71-98: 1.8 mg Victoza®/placebo). Patients who, due to adverse events, do not tolerate up-titration to 1.8 mg liraglutide will remain on 1.2 mg of liraglutide. The patients are instructed in injection technique. Compliance and adverse events are noted during the entire period. The patients have to self-inject Victoza®/placebo every morning. Glimepiride/placebo is administered orally (glimepirid, capsule ½ mg and 1 mg). Patient compliance will be recorded at each visit in the CRF and the treatment pens will be asked to be returned after use.

Study flow chart

		Screening	Day 0	Day 1-7	Day 5	Day 8-49	Day 47	Day 50-56	Day 57-98	Day 96
	Time	*Max. 14 days before Day 0*		*+/- 1 day*	*+/- 1 day*		*+/- 2 days*	*+/- 2 days*		*+/- 2 days*
Inclusion	Introduction	X								
	Informed consent	X								
	Medical history	X								
	Screening blood and urine	X								
	Physical examination	X								
Protocol	Inclusion and randomisation		X							
	Wash out			X	X			X		
	Dispensing visit				X		X			
	Administration of medicine					X	X		X	X
Safety	Adverse events				X	X	X		X	X
	Compliance					X	X		X	X
Examinations	Test meal & bike test				X		X			X
	48-hour CGM				X		X			X

Test meal and bike test (baseline, Day 47 and Day 96)

The patients meet after an overnight fast. After baseline blood sampling study medication is administered (time= -30). Thirty minutes later a standard liquid test meal (375 kcal, 46 g carbohydrate, 14.5 g fat and 15 g protein) added 1.5 g paracetamol will be ingested (time = 0 to 10 min). Between 150 to 180 min after ingestion of the meal, a 30-minute bicycle test will be performed (50-60 revolutions/min with adjustment of the bicycle resistance to fix the heart rate at 100-120 beats/min throughout the test). Time 0-240 min: the patients will be observed (symptoms of hypoglycaemia) and blood samples (PG, C-peptide, insulin, glucagon, incretin hormones, cortisone, epinephrine, nor-epinephrine and growth hormone) collected (a total of 18 blood samples during 240 minutes (see table below). Paracetamol is used as a tool to measure the gastric emptying. Compliance and adverse events will be noted.

Blood samples

Time (min)	PG	Incretin hormones	Acetaminophen	Insulin/C-peptide	Epinephrine/ Nor-epinephrine	Cortisol	Growth hormone	Total (ml)
-30	0.5	9	3	3				15.5
-15	0.5	9	3	3				15.5
0	0.5	9	3	3	4	3	3.5	26
15	0.5	9	3	3				15.5
30	0.5	9	3	3				15.5
45	0.5	9	3	3				15.5
60	0.5	9	3	3				15.5
90	0.5	9	3	3				15.5
120	0.5	9	3	3				15.5
140	0.5	9	3	3	4	3	3.5	26
150	0.5	9	3	3	4	3	3.5	26
160	0.5	9	3	3	4	3	3.5	26
170	0.5	9	3	3	4	3	3.5	26
180	0.5	9	3	3	4	3	3.5	26
240	0.5	9	3	3	4	3	3.5	26
Total (ml)	4.5	135	45	45	28	21	24.5	306

Test meal 0-10 min.
Bike test

48-hour continuous PG assessment (baseline, Day 47 and Day 96)

Continuous glucose monitoring (CGM) for 48-hours will be evaluated three times during the study. CGM provides information about the direction, magnitude, duration, frequency, and causes of fluctuations in PG levels. Compared with conventional intensified glucose monitoring, defined as three to four blood glucose measurements per day, CGM provides insight into PG levels throughout the day. In the current study these readings will supply information, which can help identify and prevent unwanted periods of hypoglycaemia.

7-point PG profiles (Day 21/70, Day 35/84 and Day 49/98)

Patients self-measure PG 7 times daily every 14 days during treatment periods.

Hypoglycaemic episodes

The patients will be instructed in symptoms of hypoglycaemia, and how to manage hypoglycaemia at each visit on the study site. *Mild hypoglycaemia*, *Biochemical hypoglycaemia, Severe hypoglycaemia* where the patient is conscious is treated with 100ml of juice or similar. Unconscious patients are treated by health care professionals with injection of glucagon and/or glucose.

6 ASSESSMENT OF EFFICACY

6.1 Clinical response

The clinical response of liraglutide compared to glimepiride is assessed by monitoring FPG twice weekly by 7-point blood glucose profiles and by a 48-hour CGM three times during the study period. Episodes of hypoglycaemia are reported in patient diaries, during examination and assessed by monitoring PG during a bicycle test. Symptoms of hypoglycaemia are also observed.

6.2 Blood samples

Glycaemic control will be assessed by FPG, fructosamine and HbA_{1c} on the days of the test meal. Endocrine pancreas function is assessed by plasma concentrations of insulin, C-peptide and plasma glucagon. Plasma levels of incretin hormones will as well be measured during the three standardised test meals. During bike tests other hormone analyses include cortisol, epinephrine, nor-epinephrine and growth hormone.

7 ASSESSMENT OF SAFETY

7.1 Serious adverse event (SAE) and serious adverse reactions (SAR)

7.1.1 Definition of SAE and SAR

SAE and SAR include any untoward medical occurrence that at any dose:

1) results in death
2) is life-threatening

NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event, which hypothetically might have caused death if it were more severe.

3) requires in-patient hospitalisation or prolongation of existing hospitalisation[1]
4) results in persistent or significant disability/incapacity[2], or
5) is a congenital anomaly/birth defect

[1] Complications occurring during hospitalisation are AEs and are SAEs if they cause prolongation of the current hospitalisation. Hospitalisation for elective treatment of a pre-existing non worsening condition is not, however, considered an AE. The details of such hospitalisations must be recorded on the medical history/physical examination page of the CRF.

[2] An AE is incapacitating or disabling if it results in a substantial and/or permanent disruption of the patient's ability to carry out normal life functions.

and is either known (SAE) or suspected (SAR).

In addition, medical and scientific judgment is required to decide if prompt notification is required in other situations, i.e. any event which the investigator regards as serious that did not strictly meet the criteria above but may have jeopardised the subject or required intervention to prevent one of the outcomes listed above, or which would suggest any significant hazard, contraindication, side effect or precaution that may be associated with the use of the drug.

7.1.2 Reporting of SAE and SAR

The investigators must immediately report any SAE and SAR to the manufacturer of study medication (Novo Nordisk and Sanofi-Aventis) occurring between the treatment with study drug and completion of last follow-up, i.e., 1 month after the treatment, whether or not considered related to study drug. All pregnancies occurring during the study, although not SAEs, should be reported using the SAE reporting procedures and will be reported directly to Novo Nordisk.

The investigator should not wait to receive additional information to fully document the event before notifying a SAE, although additional information may be requested. Where applicable, information from relevant laboratory results, hospital records and autopsy reports should be obtained. The investigators are also required to submit follow-up reports until such time as the SAE or SUSAR has resolved or in the case of permanent impairment, until the SAE or SUSAR stabilizes.

The sponsor will report all SAEs to the Ethics Committees (IECs) and all SARs to the Danish Medical Agency once a year together with a report on the safety of the study patients. All SAEs and SARs will be reported to Novo Nordisk and Sanofi-Aventis within 15 days from the investigator getting knowledge of the case. Details of SAEs and SARs will be noted on the adverse event pages in the CRF. There will be no formal follow-up after last patient visit but all patients have the opportunity to contact the investigators in case of uncertainties. Instances of death, congenital abnormality or an event that is of such clinical concern as to influence the overall assessment of safety, if brought to the attention of the investigators at any time after cessation of study medication and linked by the investigators to this study should be reported.

7.1.3 Suspected unexpected serious adverse reaction (SUSAR)

Any serious adverse event that is unexpected will immediately be reported by sponsor to the Danish Medical Agency. SUSARs that result in death or are life-threatening will be reported to the Danish Medical Agency at the latest 7 days after the sponsor has been notified about it. After reporting the SUSAR, the sponsor will provide the Danish Medical Agency all relevant further information on the course of case within 8 days. SUSARs that do not result in death or are not life-threatening will be reported to the Danish Medical Agency at the latest 15 days after sponsor has been notified about the SUSAR. Any report will be followed by comments about any consequences for the research project. Sponsor will moreover report any SUSARs to the marketing authorization holder (Novo Nordisk/ Sanofi-Aventis).

7.2 Adverse event

7.2.1 Definition of adverse event

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product. All events occurring after the subject has signed the study consent form but before receiving the drug/procedure will be registered as clinical symptoms at baseline.

7.2.2 Reporting of adverse event

AEs related to the treatment according to the investigator will be recorded in the CRF. All reported AE's will be followed up until resolved or as clinically required.

7.2.3 Assessment of adverse event

AEs may be reported spontaneously by the patient through open (non-leading) questioning during the study. As far as possible, all AEs must be described by their duration (start and stop date), severity (mild, moderate, or severe), relationship to treatment (yes, uncertain, no), and according to the need of other specific therapy. The onset of AEs will be classified relative to the stage of treatment.

7.3 Reporting at the end of the study

All SAEs, SARs, SUSARs, and AEs will be reported to the Danish Medical Agency at the end of the study.

8 STATISTICAL EVALUATION

8.1 Statistical analyses

All analyses will be performed on an intent-to-treat sample of subjects who were randomized and received at least one dose of medication or placebo. Missing data will be imputed using a last-observation-carried-forward (LOCF) method. Continuous data will be presented by descriptive statistics with the number of observations (n), mean, standard deviation, minimum, median, and maximum. Categorical data will be summarized in frequency tables using count and percentages. All patients will be presented in separate data listings. Data from patients screened, but not included in the study, will not be presented in any tables or listings.

Comparisons of data from the treatment groups will be performed using two-tailed *t* test (paired within groups, unpaired between groups) if the data are expected to follow normal distribution. For data expected not to follow a normal distribution, the significance of differences between the groups will be tested using Mann-Whitney U-test. For within-group comparisons, Wilcoxon test for paired differences will be used. One-way ANOVA will be used to compare means of several groups. The relationship between an effect of liraglutide and hip, waist and weight measures, respectively, will be examined by correlation analysis. All tests will be carried out at a significance level of 5%. Adverse events will be summarized qualitatively.

8.2 Justification of sample size

The study is a non-inferiority study which will estimate the effect of two different treatments with the hypothesis that the effect of treatment with liraglutide will result is similar glycaemic control (non-inferiority) but with a lower risk of hypoglycaemia. The sample size comprises no more than 16 patients and may introduce a type 2 error, thus missing an effect of liraglutide versus glimepiride. However, we consider only an effect that can be found within a sample size of 16 patients to be clinically relevant. The number of patients is based on a clinical evaluation and by practical and financial considerations. The study is an exploratory study. GLP-1Ra has never been used to MODY patients thus a regular sample size calculation is not possible.

8.3 Disposition of patients

Efficacy results will be presented for the per-protocol efficacy population (PP) and intent-to-treat efficacy population (ITT) population.

Per-protocol (PP) efficacy population:

This population consists of all treated patients. Only observed data will be part of the per-protocol analysis.

Intent-to-treat (ITT) efficacy population:

This population will consist of the entire population for whom any aspect of treatment was initiated. This population will be analyzed using the LOCF method to impute missing values and to avoid possible bias introduced by non-random dropout of patients.

8.4 End of the study before time

The study will be stopped for a given patient if this patient wishes to withdraw from the study or in case of extraordinary circumstances that makes it impossible for the patient to complete the study. Moreover, extraordinary events that prevent the study to be carried through will lead to interruption of the whole study for all participating patients, which will be informed about the decision and the reason for ending the study before time.

9 DATA MANAGEMENT

9.1 Source data identification and source data verification

Patient information collected in the CRF but not recorded in the patient notes is regarded as source data. However, the patient's participation and any serious adverse events related to the study treatment should be documented in the patient hospital files. In the process of ensuring data completeness and accuracy, source data verification (SDV) should be performed. The patients will be informed in writing about the need for SDV. SDV will be performed by the GCP monitors. To be able to do SDV, the investigators will require and review relevant part of the patient hospital files.

9.2 Subject data protection

Patient number, initials, date of birth and sex will identify the patients in the CRFs. The sponsor-investigator is responsible for keeping a list of all randomized patients including patient numbers, full names and date of birth. In addition, the sponsor-investigator will prepare a list of patients who were screened for participation of the trial but were not randomized and the reason for non-eligibility. The patients will be informed in writing that the results will be stored and analyzed in a computer according to national laws, as applicable, and that patient confidentiality will be maintained.

9.3 Data handling

All data obtained during the study will be documented in the individual CRF. The reasons for any missing data must be noted in the CRF. Corrections should be made legibly, dated and initialed. Incorrect entries must not be covered by correction fluid, or obliterated, or made illegible in any way. Source data, source documents, CRF, protocol and amendments, drug accountability forms, correspondence, patient identification list, informed consent forms, and other essential GCP documents will be retained for at least 15 years after the part is completed at the study site. Patient data will be entered continuously into the database by the sponsor-investigator.

10 ADMINISTRATIVE PROCEDURES

10.1 Insurance

The investigators make sure that the participation of the patients in the study is covered by insurance via the hospital.

10.2 Ethics committee (EC) / institutional review board

The trial protocol, including the patient information and informed consent to be used, must be approved by the regional EC. Written approval must be obtained before enrolment of any subjects into the trial. It is the responsibility of the sponsor-investigator to obtain the letter of approval. The investigators will ensure that this study is conducted in full conformance with the Edinburgh, Scotland (2000), amendment to the Declaration of Helsinki 1964 and with national laws and regulations for clinical research. The sponsor-investigator is responsible for informing the ethics committees and regulatory authorities of any SAE and/or major amendments to the protocol as per national requirements. The sponsor-investigator should file all correspondence and notify the ethics committees and regulatory authorities when the study is completed.

10.3 Ethical considerations

This study is not considered as having any ethical problems. The treatment is associated with minimal discomfort for the participating patients comprising blood sample collection and daily injection of liraglutide or placebo in the subcutis in the abdomen, in the thigh or in the upper arm. Common adverse events are mild to moderate transient gastrointestinal symptoms (nausea, vomiting and diarrhoea) and headache affecting around 10-15% of treated patients. Less commonly, the patients may experience stomach pain, anorexia, constipation, fever, reflux, gastritis, dizziness, tiredness and upper airway infection. Rare adverse events comprise acute pancreatitis and thyroid adenoma. Adverse events related to glimepiride are rare and include hypoglycaemia, penias of the blood cells, vasculitis, allergic reactions, impaired liver function, gastrointestinal symptoms (constipation, stomach pain, nausea, vomiting and diarrhoea) and lowering of blood sodium.

When collecting blood, some patients may experience minor discomfort when the needle penetrates the skin and rarely a small bleeding occurs. A rare complication is superficial phlebitis. The condition is self-limiting and harmless. Infectious phlebitis caused by bacteria can be treated with antibiotics. The risk of superficial phlebitis is low, and can be minimized by following clinical standards for perforating skin including disinfection and other sterile procedures. The amount of blood collected during the entire study period (14 weeks) is around 1000 ml and only patients with normal blood haemoglobin will be included. This blood loss is analogous to the volume of blood a blood donor can give in a corresponding period (time= 0 and time= 12 weeks), why this blood loss is considered secure.

Symptoms of hypoglycaemia such as sweating, tremor, confusion, nausea, nervousness, weakness, hunger, trouble speaking, palpitations, anxiety and irritability can be experienced by some patients. If such symptoms occur and biochemical hypoglycaemia is also present in bike test, the test will be stopped. Patients will be treated on highest tolerated dose of liraglutide or glimepiride. Severe systemic adverse events are not expected.

The patients will receive thorough verbal and written information about the risk of developing the mentioned adverse events. Verbal and written informed consent will be obtained from patients prior to participation in accordance with current rules. It will be emphasized in the

declaration of consent that participation in the project is voluntary and that patients may withdraw their consent to participate at any time without providing a reason and without any consequences for the patient's current or future treatment by the health service.

The participating patients will receive a study number when entering the study. All data forms and blood samples will only be labelled with the patient's initials and study number. The sponsor-investigator is responsible for keeping a list separately for all randomized patients containing patient numbers, full names and date of birth.

Extra plasma and white blood cells will be stored in a bio-bank in a freezer (-80 degrees) for up to 10 years after the end of the study for repeated measurements in case of error analysis or the need for more analyses. The use of these samples will demand a new approval. Urine samples will only be collected at the screening and will be analysed immediately. The protocol will be notified to the Danish Data Protection Agency (reference number: 2007-58-0015), The Danish Ethics Committee (reference number: H-1-2012-045) and the Danish Medicines Agency (EurdaCT number: 2012-000592-17).

The individual participating patient will not benefit on the project, besides the health examination (the screening visit), but will contribute to the assessment of the treatment potential of GLP-1R agonists for MODY patients.

Conclusively can be stated that serious adverse events are not expected, side effects are rare according to written earlier and the patients will be monitored carefully. Therefore, we consider the possible risks of adverse events to be outweighed by the probable advantages of the study.

10.4 Patient informed consent

The investigators are responsible for giving the patients complete verbal and written information about the nature, purpose, and possible risks and benefits of the trial. The patients must also be notified that they are free to withdraw from the trial at any time. The patients should have reasonable time to read and understand the information before signing. The patient will be informed about the possibility of bringing a bystander. It will be emphasized in the declaration of consent that participation in the project is voluntary and that patients may withdraw their consent to participate at any time without providing a reason and without any consequences for the patient's current or future treatment by the health service. The sponsor-investigator is responsible for obtaining signed and oral EC-approved informed consent from all subjects before performing any trial-related procedures.

A copy of the patient information and of the patient informed consent form will be given to the patients. The signed consent form will be kept by the sponsor-investigator, either in the patient hospital file or in the sponsor-investigator's study file. Participating patients will be informed about the result of the study if they express a wish for this.

10.5 Regulatory affairs

A notification will be submitted to national authorities before commencement of the trial, as applicable according to local regulations. Notifications and reports will be filed according to ICH E6(R1): GCP: Consolidated guideline, CPMP/ICH/135/95, and national guidelines including Clinical Trials Directive 2001/20/EC.

10.6 Trial monitoring

Prior to the start of the study, the sponsor-investigator will ensure that the other investigators are familiar with the protocol, CRFs and other study documents and procedures. The sponsor-investigator will be visited on a regular basis by the monitor, who will check trial procedures, including safety assessments, drug handling, data recording and SDV. The monitor will be allowed to review relevant hospital records to confirm that required protocol procedures are being followed and check consistency between patient record and CRF. Incorrect or missing entries onto the CRFs will be addressed as data queries and must be corrected immediately. Trial monitoring will not jeopardize patient confidentiality. The trial will be monitored by the GCP unit of Copenhagen University. The clinical trial will be conducted in compliance with the protocol, according to ICH E6: Good Clinical Practice: Consolidated guideline, CPMP/ICH/135/95, and national guidelines including Clinical Trials Directive 2001/20/EC. The trial can be subjected to quality audit.

10.7 Trial audits and inspections

The patients will be informed in writing about the possibility for audits and/or inspections. The audit and/or inspection might be performed by the hospital institutional review board/ethics committee or regulatory authority. In these cases, relevant part of the patient records will be required and reviewed.

10.8 Financing

The study is sponsored by Novo Nordisk A/S, who will provide study medication (Victoza® as well as placebo pens). The rest of the study is financed solely by funding.

10.9 Economic compensation for patients

Participating patients will receive a compensation of DKK 500 per test meal (including bike test) giving a total of DKK 1,500. The money will be paid after termination of the study, and must be taxed as B-income. Study medication will be provided free of charge to the patients. Documented transport expenses will be covered.

10.10 Recruitment of patients

Professor, Torben Hansen and professor, Oluf B. Pedersen (*Hagedorn Research Institute/Steno Diabetes Center*) who in cooperation with Signe Harring Østoft will be responsible of recruiting well characterized MODY 3 patients, who will be recruited through the *Hagedorn Research Institute/Steno Diabetes Center*. The patients are found in a database at *Hagedorn Research Institute/Steno Diabetes Center*. The first contact will be performed by letter containing the Participant Information. After two weeks the patient will be contacted again. If the patient do not wish to participate no more contacts are made. If the patient wishes to get more information or participate a date for the first visit is made. At this visit the patient will be informed in written and verbally, and the informed consent will be signed. Hereafter the screening visit can be performed.

11 CONFIDENTIALITY AND COMMUNICATION OF RESULTS

11.1 Publication

At the end of the trial one or more manuscripts will be prepared for publication in scientific journals. The investigators will be given 14 days to review and comment on any manuscript/abstract or other means intended for publication or presentation of the data. While it is the intention that the sponsor-investigator will be the first author, the published international guidelines for authorship (International Committee of Medical Journal Editors, 1997) will be adhered to; i.e. 'All persons designed as authors should qualify for authorship. Each author should have participated sufficiently in the work to take public responsibility for the content'.

The final decision on the order of authorship will be decided when the study has been finalized. The results from the study may moreover be presented as posters or oral presentations at national and/or international conferences. The trial will be registered at www.clinicaltrials.gov according to the requirements from the US Food and Drug Administration (FDA) and the International Committee of Medical Journal Editors.

11.2 Availability of information for participating patients

The participants can get more information about the project via sponsor-investigator MD, PhD-student Signe Harring Østoft, Diabetes Research Division (*Diabetologisk Forskningsenhed, Medicinsk afd. F*, Gentofte Hospital); mobile: 2123 0343; e-mail: s.ostoft@dadlnet.dk.

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