Statistical Analysis Plan

Version:	1.0, dated 03.12.2018
Study title:	Proteomic prediction and Renin angiotensin aldosterone system Inhibition prevention Of early diabetic nephRopathy In TYpe 2 diabetic patients with normoalbuminuria
Acronym:	PRIORITY
Trial number:	EudraCT no.: 2012-000452-34
Clinical Trial Gov. No	NCT02040441
Study protocol version:	3.0, dated 12.10.2015
Sponsor:	Steno Diabetes Center Copenhagen Niels Steensens Vej 2 DK-2820 Gentofte Denmark
Chef investigator:	Chief Physician, Prof., Peter Rossing, MD, DMSc Steno Diabetes Center Copenhagen Niels Steensens Vej 2 DK-2820 Gentofte Denmark
Statistical responsible:	Tine Willum Hansen, MD, PhD Steno Diabetes Center Copenhagen Niels Steensens Vej 2 DK-2820 Gentofte Denmark
SAP writer / Contact person:	Morten Lindhardt, MD, PhD Steno Diabetes Center Copenhagen Niels Steensens Vej 2 DK-2820 Gentofte Denmark Morten.Kofod.Lindhardt@regionH.dk Phone: +45 51922907

TABLE OF CONTENTS

1 LIST OF ABBREVIATIONS

ACE	Angiotensin converting enzyme inhibitors
AE	Adverse event
ARB	Angiotensin II receptor blocker
CE	Capillary electrophoresis
CI	Confidence interval
CABG	Coronary artery bypass grafting
CKD	Chronic kidney disease
CKD273	Proteomic urine biomarker including 273 peptides significant for CKD
CVD	Cardiovascular disease
eCRF	Electronic case report form
EDTA	Ethylenediaminetetraacetic acid
eGFR	Estimated glomerular filtration rate
ESI-TOF	Electrospray-ionization-time of flight
ESRD	End-stage renal disease
EudraCT	European Union Drug Regulating Authorities Clinical Trials
GCP	Good clinical practice
GFR	Glomerular filtration rate
ICF	Informed Consent Form
IMP	Investigational medicinal product
MI	Myocardial infarction
MS	Mass spectrometry
PTCA	Percutaneous transluminal coronary angioplasty
RAAS	Renin angiotensin aldosterone system
RAS	Renin angiotensin system
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Standard deviation
UACR	Urine albumin creatinine ratio
WHO	World Health Organization

2 INTRODUCTION

2.1 Background

Diabetes mellitus affects 9% of the European population and the cost of caring for patients with DM accounts for 15% of the European health care budget expenditure. Almost 90% of patients have type 2 DM, and absolute numbers are expected to rise in parallel to the current obesity and metabolic syndrome epidemic. Improved treatment has reduced mortality, but the prolonged duration of DM increases the likelihood of development of late diabetic complication.

Diabetic nephropathy is one of the major late complications of diabetes and is associated with substantial cardiovascular morbidity and mortality and is a leading cause of end stage renal disease (ESRD) in the Western world. In clinical practice, renal impairment is diagnosed by albuminuria or proteinuria and/or changes in serum creatinine/creatinine clearance indicating alterations of the glomerular filtration rate (GFR). However, the inter-individual variability is high, and as a consequence, these standard tests have a moderate specificity and sensitivity at early stages of disease, with major limitations in the diagnosis of the early stages of diabetic nephropathy (DN).

Development of DN is generally characterized by an increase of urinary albumin excretion rate (>300 mg/24 h or 200 µg/min). Microalbuminuria (30-300 mg/24 h or 20-200 µg/min) is considered a risk factor and as an early indicator of future onset of DN. Microalbuminuria is regarded as the earliest clinical marker of renal damage. However, structural changes to the kidney have already occurred at the stage of microalbuminuria and patients with microalbuminuria have a high risk for development of renal disease, but also increased morbidity and mortality due to cardiovascular disease.

Blood pressure and glycemic control with pharmacotherapeutic intervention as well as life style interventions are the cornerstones of type 2 DM management aiming at prevention of microvascular complications. Specific therapy, particularly treatment with angiotensin converting enzyme inhibitors (ACE) and angiotensin receptor antagonists (ARB) to prevent progression to overt proteinuria and advanced stages of diabetic nephropathy is recommended if microalbuminuria is present. Studies aiming for earlier prevention of nephropathy by starting renin angiotensin aldosterone system (RAAS) blocking treatment in normoalbumuric patients have given mixed and often disappointing results. This might reflect that a large fraction of normoalbuminuric patient may not be at risk for progression thereby reducing the event rate or power in previous studies. Early identification of normoalbuminuric patients at high risk for development of diabetic nephropathy could identify patients who might benefit of intervention with increased blockade of the RAAS. Furthermore, blockade of the RAAS with aldosterone blockade has been demonstrated to reduce urinary albumin excretion with 20-30% on top of standard antihypertensive treatment including ACE or ARB in proteinuric type 1 and 2 diabetic patients, and a 60% reduction was seen in microalbuminuric type 1 diabetic patients. Therefore, it may also hold the potential to reduce the risk of development of microalbuminuria in high risk normoalbuminuric patients.

CKD Biomarker panel

Proteomics is the analysis of large number of proteins or polypeptides in tissue and body fluids. Capillary electrophoresis-mass spectrometry (CE-MS) enables reproducible and robust high-resolution analysis of several thousand low-molecular-weight urinary proteins/peptides in about one hour. Urine holds several advantages over blood in clinical proteomics. It can be collected non-invasively and its proteome is relatively stable. Members of the consortium have successfully identified a urinary biomarker pattern including 273 peptides significantly associated with chronic kidney disease (CKD273).

Importantly, the biomarker panel has been validated in a multicentric approach involving >1000 blinded samples. The accuracy was high (96% sensitivity and 98% specificity), when evaluating only the diabetic patients in the test-set. To test the CKD273 pattern as a tool for early detection of DN, we recently performed an independent longitudinal study of normoalbuminuric diabetic patients at inclusion. The urinary CKD273 pattern distinguished progressing patients from non-progressing patients. The corresponding receiver operating characteristic (ROC) analysis resulted in an area under the curve (AUC) of 0.925 assuming a prevalence of 30% for DN. The positive predictive value was 97% and the negative predictive value was 88%. The specificity of the CKD273 pattern was further evaluated in patients without any evidence for renal impairment based on clinical history, creatinine, or urinary protein levels resulting in an overall specificity of 98%.

The used CKD273 pattern showed that these biomarkers can detect initiation and progression of DN earlier than the currently used indicators, well preceding increases in urinary albumin levels. While the CKD273 pattern detected DN with >90% accuracy four years before clinical diagnosis, serum creatinine and/or UAER did not detect DN earlier than one and two years before clinical manifestation, respectively. In addition, diagnostic accuracy was significantly lower compared to the CKD273 pattern. In addition, two independent studies on type 1 and type 2 DM patients, on longitudinally collected samples over a period of 10 years demonstrate that CKD273 markers of kidney disease were altered 3 to 5 years prior to manifestation of albuminuria, and 1 to 2 years prior to development of microalbuminuria. Thus, the performance of the CKD273 pattern is better than prediction based on urinary albumin values and represents potentially a significant improvement over the current state of the art in assessing DN, enabling earlier detection with higher accuracy than urinary albumin.

Finally, the proteome analysis and application of the CKD273 pattern indicated a positive scoring for CKD in microalbuminuric type 2 diabetic patients, which showed persistent improvement during long-term renoprotective treatment with Irbesartan, while placebo treated patients showed a slight deterioration of kidney damage markers likely reflecting disease progression in the absence of pre-emptive intervention.

Collectively, our existing data strongly indicate that the urinary proteomics based test appears ideal to identify patients who will develop microalbuminuria and ultimately DN and thereby facilitates targeting intensified preventative therapy to this group.

2.2 Rationale

1. Urinary proteomics predicts development of microalbuminuria (as a surrogate marker for the development of overt nephropathy) in a cohort of 1811 type 2 diabetic patients with normal urinary albumin excretion at screening.
2. Early initiation of preventive therapy with spironolactone reduces risk of transition to microalbuminuria in those identified by urinary proteomics to be at high risk, and thereby delays progression to overt nephropathy. Treatment can be spared for those with low risk according to urinary proteomics, paving the way of personalised medicine

2.3 Objective

2.3.1 Primary objective

To confirm that urinary proteomics can predict development of microalbuminuria (as a surrogate marker for the development of overt nephropathy) in a cohort of 1811 type 2 diabetic patients with normal urinary albumin excretion.

2.3.2 Secondary objectives

To investigate if early initiation of preventive therapy with spironolactone 25 mg once daily reduces risk of transition to microalbuminuria in those patients identified by urinary proteomics to be at high risk.

2.3.3 Additional scientific objectives

To compare the rate of change in urinary albumin excretion rate in high vs. low-risk population (based on the proteomic test), and to compare the effect of spironolactone on rate of change in UACR in the intervention group.

In addition, the objective is to study the rate of change in eGFR in relation to urinary marker pattern (CKD 273) and the intervention with spironolactone.

To study the ability of urinary proteomic patterns, to predict cardiovascular or renal events during the study as well as response to intervention, in relation to study endpoints.

2.4 Hypothesis

Hypothesis 1: It is hypothesised that participants with a “low”-risk proteomic pattern experience a significantly lower event rate of the primary endpoint in comparison with the “high”-risk proteomic pattern.

Hypothesis 2: It is hypothesised that participants with a “high”-risk proteomic pattern allocated to active treatment (spironolactone) experience a significantly lower event rate of the primary endpoint as compared to participants with a “high”-risk proteomic pattern allocated to placebo treatment

3 STUDY METHODS

3.1 Trial design

Prospective, multicenter, double-blind, randomized, placebo-controlled, clinical trial.

3.2 Randomization

At baseline eligible patients with a high-risk proteomics pattern will be included in the intervention part of the study. Randomization lists will be prepared by an independent statistician at the Robertson Centre, University of Glasgow, who is not involved into other tasks of the trial.

The patients will be randomly assigned in a 1:1 ratio to one of the two treatment arms using a block randomization stratified by study center.

Patients will be stratified in the two treatment arms (1:1) based on whether or not the patients is treated with RAS blocking agents at the time of entry (Screening) in order to have the number on RAS blocking agents balanced in the two treatment arms.

3.3 Blinding/ Masking

Doubled masked as both participants and study team are unaware of treatment allocation.

Placebo and active study drug will not be distinguishable from each other in terms of appearance, odour, labelling or instructions for use.

3.4 Sample size

The expected relative proportions of diabetes type 2 patients developing microalbuminuria in our study population are: 24% in patients at high-risk for diabetic nephropathy in the treatment group, 40% in those patients at high-risk for diabetic nephropathy in the placebo group and 8.5% in those therapy-naive patients at low-risk for diabetic nephropathy. Using the sample size formula for two proportions test (α= 0.05 β=0.80), randomized (1:1), n=129 in each arm of the intervention group are required. To account for an expected drop-out rate of 10 %, we plan to include 300 participants in the intervention part of the study.

3.5 Framework

Superiority of both proteomic test (CKD273) and active comparator.

3.6 Interim analysis/ Stop rules

No statistical interim analysis was planned or conducted. An independent safety monitoring committee conducted annual safety analysis in case of unbalanced SAE/death between placebo and comparator allocation. In total, four planned and no unscheduled DMC reviews were conducted and no unbalance or concern wasidentified.

3.7 Timing of final analysis

The final analysis will be performed after data-lock. Data lock will be permed by Data management at Hannover Clinical Trial Center, holder and responsible for eCRF. Data lock will be done after written confirmation from the sponsor and after entry of all available data.

4 STATISTICAL PRINCIPALS

4.1 Confidence limits and significance level

Any p-value below 0.05 will be considered statistically significant. A p-value will be calculated for the primary analysis. P-values for the secondary analyses will only be calculated if the primary analysis yields a p-value below 0.05. For all primary and secondary analyses 95 % confidence limits will be calculated.

Continuous values fulfilling terms of normal distribution will be displayed as mean values and 95 % confidence limits of the mean. Variables not fulfilling terms of normal distribution will be displayed as median and inter quartile range from p25 to p75.

4.2 Adherence and protocol deviations

The study is conducted in accordance with GCP and monitoring. All minor protocol deviations are recorded in the eCRF. All major protocol deviations were brought to the attention of the sponsor, recorded in the eCRF and site-specific trial master file.

4.3 Analysis populations

4.3.1 Screening population

All participants with a valid informed consent and data entry at the screening visit.

4.3.2 Observational cohort

All participants with valid proteomic score and data at baseline visit.

4.3.3 Intention to treat cohort

All participants with a valid proteomic score with a high-risk pattern who was provided with study medication

4.3.4 Per protocol cohort

All include in the intention to treat cohort AND with an accountability between 80 % and 110 % at more than 95 % of the study period

5 TRIAL POPULATION

5.1 Screening data

Patients who discontinue prior randomization for any reason are considered screening failures.

If a patient are considered a screening failure, demographic information (sex, age, onset of diabetes and race) are to be documented, as well as primary reason for discontinuation.

5.2 Eligibility

All participants who fulfil all inclusion and no exclusion criteria and with a valid signed informed consent were eligible.

Summary of inclusion criteria:

- Age 18 to 75, Type 2 diabetes,
- Persistent normoalbuminuria (at least 2 of 3 UACR < 30 mg/g)
- eGFR > 45 ml/ min/ 1.73 m^2
- Not pregnant or intent to become pregnant in the trial period

Summary of exclusion criteria:

- Uncontrolled hypertension
- Type 1 diabetes
- Current in dual blockade with both ACE and/or ARB and/or direct renin inhibition
- Current use of MRA
- Hyperkalemia: Plasma potassium level >5.0 mmol/L or serum potassium level >5.4 mmol/L.
- Current cancer treatment or within five years
- Diagnosis of non-Diabetic CKD current or in the past
- Known or suspected abuse of alcohol or narcotics
- Participation in any other intervention trial than PRIORITY

5.3 Recruitment

5.3.1 Recruitment period

Recruitment started in March 2014. The recruitment period will run from approval by REC and NCA in the participating countries and until the 31st of August 2016.

5.3.2 Patients

It is intended to observe/ treat each patient for at least two years after baseline. Patients included prior to October 2016 will be observed/ treated for more than two years depending on the time of inclusion. The observation/ treatment period can't exceed 4.4 years. All patients will have final assessment performed in the period from 1st July 2018 to 15th October 2018.

5.3.3 Site

15 sites in 10 countries were active recruiting.

5.4 Withdrawal/ follow-up

Time of follow-up will be estimate from baseline to end – exposure time measure in day/years. Participants who withdraw will be included in the analysis with exposure time from baseline to last observation. Participants who withdraw consent are included in the statistical analysis unless they specifically also withdrawal consent to use of data already entered in the eCRF.

5.5 Base characteristics / data description

Variable (reporting methods, [unit])

- Gender (N, % male)
- Medical history
    - Diabetes duration (mean, SD, [years])
    - Cardiovascular disease, hypertension, dyslipidemia [%]
    - Retinopathy/ maculopathy (grade, [%])
    - Smoking (grade, [%])
- Concomitant medication
    - Use of antidiabetic medication (active substance group, [%])
    - Use of antihypertensive agents (active substance group, [%]).
- Vital signs
    - Systolic and diastolic blood pressure (Mean, SD, [mmHg]).
- Blood sample
    - Creatinine, sodium, and potassium, HbA_{1c}, cholesterol, HDL, LDL, and triglycerides. (mean, SD)
- Urine sample
    - Proteomic score (CKD273) (high, low-risk) (N, mean, [%])
    - Urine albumin to creatinine level. (median, IQR, [mg/g])

6 ANALYSIS

6.1 Outcome definition

6.1.1 Primary outcome

Development of confirmed microalbuminuria (UACR >30 mg/g) in at least two out of three first morning voids with ≥ 30% increase (geometric mean) in UACR from “run-in” period samples OR > 40 mg/g (geometric mean).

6.1.2 Secondary outcome

A. Comparison of composite fatal and non-fatal cardiovascular outcome (MI, stroke, CABG, PTCA, hospitalization for heart failure and CVD) and all-cause mortality during the study.

B. Comparison of incidence of retinopathy and frequency of laser treatment. Data collected from self-reported AEs.

C. In addition to the categorical analysis of UACR, an analysis will be performed with changes in geometric mean of UACR throughout the study period in all patients by assessing the slope of albuminuria changes and absolute changes from inclusion to end of trial.

D. Development of microalbuminuria (UACR >30 mg/g) in at least one morning void urine sample will be used as a secondary outcome instead of confirmed microalbuminuria.

E. Development of macroalbuminuria (UACR >300 mg/g) in 2 out 3 first morning void urine samples).

F. For patients with eGFR ≥ 60 ml/ min/ 1.73 m^2 at baseline, development of eGFR<60 ml/min/1.73m^2.

G. Development of CKD stage 4 (eGFR <30 ml/min/1.73m^2).

H. Change in eGFR (slope) from baseline and from three-month post-baseline to end of study.

I. Change in eGFR (40 % reduction) from baseline and from three-month post-baseline to end of study.

J. Safety: Adverse Events and Serious Adverse Events. Adverse Events leading to death or discontinuation of study medication and/or withdrawal.

K. Event of hyperkalaemia with potassium level in plasma >= 5.5 mmol and/or >= 5.8 mmol in serum at any time point.

6.2 Missing Data

No assumption or statistical method will be used in case of missing data. No censoring of data will be performed.

6.3 Analysis methods

6.3.1 Observational study (Observational cohort)

6.3.1.1 Primary analysis of primary endpoint

In the observation cohort a comparison between the high- and low-risk stratum will be conducted using an unadjusted Cox-regression model with log-rank test.

In addition, adjustment for age, gender, Hba1c, systolic blood pressure, retinopathy eGFR and UACR at baseline will be conducted.

6.3.1.2 Analysis of secondary endpoints

Based on a 2x2 table included the primary endpoint (event/ event free) vs. risk stratification ("high"-/ "low"-risk) we will calculate sensitivity, specificity and numbers need to screen for CKD273 and tested for significance using Chi-square test. In a logistic model we will added value using AUC and ROC with comparison between "high"- and "low"-risk vs. event and event-free for both primary endpoint. The logistic models will be performed both unadjusted and adjusted for age, gender, HbA_{1c}, systolic blood pressure, retinopathy, eGFR and UACR at baseline.

For the Cox-regression model a model, including adjustment for age, gender, Hba1c, systolic blood pressure, retinopathy eGFR and UACR at baseline will be comprised for secondary endpoints (see section 6.1.1 and 6.1.2, excluding 6.1.2 C, H and J).

For further clarification of the added value for discrimination, the rIDI will be calculated based on all above described Cox-regressions models with a significant HR for CKD273 in the unadjusted model for each endpoint.

To evaluate changes in UACR and eGFR over time (outcome measurement C and H in section 6.2.1) a linear mix model will be applied with adjustment for eGFR and UACR at baseline.

6.3.1.3 Sensitivity analysis

In order to out-balance the fact that the "high"-risk stratum has primary outcome measures every third month we will perform a sensitivity analysis on the primary endpoint. Any primary endpoint in the "high"-risk stratum between annual visits will be carried forward to the date of the following annual visit. This analysis is most likely underpowered because of exclusion of endpoints in the high-risk stratum, however, the trend of the primary analysis is expected.

As in the "high"-risk group we have participants allocated to either active IMP or placebo we potentially limit the number of endpoints if the active IMP is beneficial. To adjust for this potential effect, we will perform a sensitivity analysis on the primary endpoint only in "low"-risk participants and in "high"-risk participants allocated to placebo treatment. This analysis is most likely also underpowered because of exclusion of endpoints in the "high"-risk stratum, however the trend of the primary analysis is expected.

ANOVA models for each continuous variable measured at baseline: age, HbA_{1c}, systolic blood pressure, Study site, eGFR and UACR will be tested within each stratum "high"- and "low"-risk as well as interaction in the Cox-model between strata with the primary endpoint as determinant. Chi square tests will be used for categorical variables: gender, use of antihypertensive agents, known cardiovascular disease and retinopathy.

We expect a correlation between the “high”-risk stratum and events of the primary endpoint for participants with known risk factors for overt kidney disease (higher age, HbA_{1c}, systolic blood pressure, UACR, and lower eGFR, male gender, use of antihypertensive agents, known cardiovascular disease and retinopathy).

6.3.2 Intervention study (Intention to treat cohort)

6.3.2.1 Primary analysis of primary endpoint

In the intention to treat cohort, a comparison between active and placebo treatment will be performed in a Cox-regression model including data on the primary outcome. Adjustments in the model will only be performed for eGFR and/or UACR in the case of a significant unbalance in the baseline value (significance level for unbalance between treatment allocation groups is a p-value < 0.01).

6.3.2.2 Analysis of secondary endpoints

Based on a 2x2 table included the primary endpoint (event/ event free) vs. treatment allocation (active/ placebo) we will calculate numbers needed to treat and tested for significance using Chi-square test.

For Cox-regression models both unadjusted and adjusted using treatment allocation as determinant. Adjustment for age, gender, HbA_{1c}, systolic blood pressure, retinopathy, study site, eGFR and UACR at baseline will be conducted for both primary and secondary endpoints (see section 6.1.1 and 6.1.2, excluding 6.1.2 C, H and J).

For further clarification of the added value for discrimination, the rIDI will be calculated for all above described Cox-regression models with a significant HR for treatment allocation in the unadjusted model for each endpoint.

For safety outcome (section 6.2.1 J) only AE/SAE with the term hypotension, hyperkalaemia or gynaecomastia will be analysed using chi-square test.

To evaluate changes in UACR and eGFR over time (outcome measurement C and H in section 6.2.1) a linear mix model will be applied with adjustment for eGFR and UACR at baseline.

6.3.2.3 Sensitivity analysis

In a forest plot each baseline continuous variable: Age, HbA_{1c}, systolic blood pressure, eGFR and UACR will be divided based on the median value and tested for heterogeneity between treatment allocation. Of the categorical variables we will evaluate the effect of: Gender, and presence of retinopathy at baseline also in a forest plot. We expect the largest effect of active treatment in participants with known risk factors of overt kidney disease (high age, HbA_{1c}, systolic blood pressure, UACR and low eGFR, male gender and presence of retinopathy).

6.4 Additional analysis

For the per protocol cohort Cox-regression models will be performed both unadjusted and adjusted using treatment allocation as determinant and testing the primary outcome. Adjustment will include: Age, gender, HbA_{1c}, systolic blood pressure, retinopathy, eGFR and UACR at baseline. To examine added value for discrimination rIDI will be calculated for on all above described Cox-regression models.

In the observational cohort excluding all participants allocated to active treatment Cox-regression models will be performed to evaluate the hazard ratio between the “high”-risk without potential influence of the active treatment and the “low”-risk stratum. The model will be performed both on all available data and on censured data only (including annual samples). The models will be achieved unadjusted and adjusted for Age, gender, HbA_{1c}, systolic blood pressure and presence of retinopathy, eGFR and UACR at baseline. To examine added value for discrimination the rIDI will be calculated for all above described Cox-regression models.

6.5 Statistical software

SAS enterprise Guide version 7.11 by SAS institute Inc. Carry. NC. USA.

7 SIGNATURES

This Statistical Analysis Plan has been approved by the Sponsor. The following signature documents this approval.

Prof. Peter Rossing MD, DMSc	Date