Clinical Study Protocol

Proteomic prediction and Renin angiotensin aldosterone system Inhibition prevention Of early diabetic nephRopathy In TYpe 2 diabetic patients with normoalbuminuria

- PRIORITY -

Study Design:	Prospective, multicenter, double-blind, randomized, placebo-controlled, phase II-III study
Version:	3.0, dated 12.10.2015
Study Code of the Sponsor:	3004
EudraCT-Number:	2012-000452-34
Sponsor:	Steno Diabetes Center Niels Steensens Vej 2 DK-2820 Gentofte Denmark
International Coordinating Investigator:	Senior Investigator, Chief Physician, Prof., Peter Rossing, MD, DMSc Steno Diabetes Center Niels Steensens Vej 2 DK-2820 Gentofte Denmark
Study Centers:	PRIORITY investigators: Approx. 17 centers throughout Europe

- Confidential -

STATEMENT OF COMPLIANCE/ CONFIDENTIALITY

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1 LIST OF ABBREVIATIONS

ACE	Angiotensin converting enzyme inhibitors
AE	Adverse event
ALT	Alanine transaminase
AR	Adverse reaction
ARB	Angiotensin II receptor blocker
ASAT	Aspartat aminotransferase
ATC	Anatomical Therapeutic Chemical
BMI	Body mass index
BP	Blood pressure
BW	Body weight
CE	Capillary electrophoresis
CI	Confidence interval
CF	Classification factor
CABG	Coronary artery bypass grafting
CKD	Chronic kidney disease
CKD273	Proteomic urine biomarker including 273 peptides significant for CKD
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CRO	Contract research organization
CRP	C-reactive protein
CTCAE	Common terminology criteria for adverse events
CVD	Cardiovascular disease
DCCT	The diabetes control and complications trial
DM	Diabetes mellitus
DN	Diabetic nephropathy
DSUR	Development safety update report
EC	Ethics committee
eCRF	Electronic case report form
EDTA	Ethylenediaminetetraacetic acid
eGFR	Estimated glomerular filtration rate
EMA	European medicines agency
ESI-TOF	Electrospray-ionization-time of flight
ESRD	End-stage renal disease
EudraCT	European Union Drug Regulating Authorities Clinical Trials
FP7	Framework Program 7
GLA	Glasgow team
GCP	Good clinical practice
GMP	Good Manufacturing Practices

GFR	Glomerular filtration rate
GP	General practitioner
hCG	Human chorionic gonadotropin
HCTC	Hannover Clinical Trial Center GmbH
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine
IEC	Independent ethics committee
IIT	Investigator initiated trial
IMP	Investigational medicinal product
IPR	Intellectual property rights
IWRS	Interactive web response system
MARVIN	The electronic case report form software program
MDRD	Modification of diet in renal disease
MedDRA	Medical Dictionary for Regulatory Activities
MI	Myocardial infarction
MS	Mass spectrometry
NCA	National competent authority
NYHA	The New York heart association classification for heart failure
PI	Principal Investigator
PTCA	Percutaneous transluminal coronary angioplasty
R	Randomization
RAAS	Renin angiotensin aldosterone system
RAS	Renin angiotensin system
SAE	Serious adverse event
SAP	Statistical analysis plan
SAR	Serious adverse reaction
SD	Standard deviation
SEM	Standard error of the mean
SmPC	Summary of product characteristics
SOC	System organ classes
SOP	Standard operating procedure
SSAR	Suspected serious adverse reaction
SUSAR	Suspected unexpected serious adverse reaction
T	Treatment
UACR	Urine albumin creatinine ratio
UAER	Urine albumin excretion rate
WHO	World Health Organization

2 SYNOPSIS

Study title	Proteomic prediction and Renin angiotensin aldosterone system Inhibition prevention Of early diabetic nephRopathy In TYpe 2 diabetic patients with normoalbuminuria
Short term	PRIORITY
Study code of the sponsor	3004
EudraCT number	2012-000452-34
Sponsor	Steno Diabetes Center Senior Investigator, Prof. Peter Rossing MD. DMSc Niels Steensens Vej 2 DK-2820 Gentofte Denmark Phone: +45 3079 5510 Fax: +45 4443 8160
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Indication	Patients with type 2 DM and normoalbuminuria
Objectives	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 2000 type 2 diabetic patients with normal urinary albumin excretion. 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 Assessment of safety in the intervention group. Additional scientific objectives: To compare the rate of change in urinary albumin excretion rate in high- vs. low-risk patients (based on the proteomic test), and to compare the effect of spironolactone on rate of change in UACR in the intervention study.

	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. To establish a biobank that will allow testing of additional putative markers for progression of diabetic nephropathy, development of cardiovascular events and death
Study design/	Prospective, multicenter, randomized, double blind, placebo-controlled phase II-III trial and a prospective observational study.
Study centres	Approx. 17 centres in Europe.
Schedule	Duration of the entire study (first patient in to last patient out): approximately 4.4 years Study-related: Recruitment period: From March 2014 to August 2016 Patient-related: Duration of interventional/observational period including: 104-230 weeks
Number of patients	Screening: Estimated to be n = 2700 To be included: n = 2000 To be randomized: n = 300 n = 300 patients will be randomized into intervention group with placebo or spironolactone and enter the double-blind treatment period if a CKD273 specific (high risk) proteomic pattern is observed. 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). The remaining patients with a negative (low risk) proteomic pattern will be observed regarding the development of microalbuminuria and will receive standard of care treatment (observational group).
Investigational medicinal products	Investigational medicinal product (IMP): Administration: orally Active medication: Spironolactone (potassium sparing diuretic) Dosage: 25 mg per day (One tablet once daily) Placebo: Dosage: matched placebo (One tablet once daily)
Study endpoints	Primary study endpoint: 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). Secondary study endpoints: Changes in albuminuria throughout the study period in all patients by assessing the slope of albuminuria. Predictive value of the urinary proteomics test in regards to renal and cardiovascular events. Changes in eGFR including development of eGFR < 60 ml/min/1.73m^2 Changes in retinopathy and frequency of laser treatment. Additional scientific endpoints: The analysis will consider age and gender effects on the predictive value of the proteomics test and treatment effect. Assessment of safety in the intervention group.
Inclusion criteria	1) Written informed consent must be provided before participation. Patient information and consent form must be approved by relevant independent EC. Specifically, all participating patients will be asked to give informed consent for long-term follow-up and collection of follow-up data 2) Male or female patients ≥ 18 years and < 75 years of age at Screening visit 3) Type 2 DM (WHO criteria) 4) Persistent normoalbuminuria (at least 2 of 3 UACR < 30 mg/g samples from ”run in”-period) 5) eGFR >45 ml/min/1.73m^2 at Screening visit 6) The patient must be willing and able to comply with the protocol for the duration of the study 7) Female without child-bearing potential at the screening visit. Defined as one or more of following: 7.1) Female patients ≥ 50 years of age at the day of inclusion, who have been postmenopausal for at least 1 year 7.2) Female patients < 50 years of age at the day of inclusion, who have been postmenopausal for at least 1 year and serum FSH levels > 40 mIU/mL as well as serum estrogen levels < 30 pg/ml or a negative estrogen test. 7.3) 6 weeks after surgical sterilization by bilateral tubal ligation or bilateral ovariectomy with or without hysterectomy. OR a negative urine pregnancy test at the Screening visit AND one or more of following: 7.4) Correct use of reliable contraception methods. This includes one or more of the following: hormonal contraceptive (such as injection, transdermal patch, implant, cervical ring or oral) or an intrauterine device (IUD) OR correct use of double barrier with one of the following: barrier methods (diaphragm, cervical cap, Lea contraceptive, femidom or condom) AND in combination with a spermicide. 7.5) General sexual abstinence from the time of screening/ baseline, during the study until a minimum of 30 days after the last administration of study medication if this is already established as the patient’s preferred and usual lifestyle. 7.6) Having only female sexual partners.

	7.7) Sexual relationship with sterile male partners only
Exclusion criteria	1) Average of systolic BP< 110 or >160 mm Hg at baseline 2) Average of diastolic BP > 100 mm Hg at baseline 3) Type 1 DM (WHO criteria) 4) HbA1c <6.5% (48 mmo l/ mol) AND > 5 years of known duration of diabetes type 2 AND never treated with an antidiabetic drug of any kind. 5) Current in treatment with more than one RAAS blocking agent (Angiotensin Converting Enzyme inhibitor, Angiotensin Receptor Blocker or Direct Renin Inhibitor) 6) Current lithium treatment(ATC: N05AN) 7) Known or suspected hypersensitivity to Spironolactone or to any of its excipients. 8) Current use of potassium sparing diuretics (ATC: C03D, C03E), such as: Spironolactone, Eplerenone or Amiloride etc. 9) Hyperkalemia at Screening: plasma potassium level >5.0 mmol/L or serum potassium level >5.4 mmol/L. 10) Hyponatriemia determine by the investigator 11) Current cancer treatment or within five years from baseline (except basal cell skin cancer or squamous cell skin cancer) 12) Any clinically significant disorder, except for conditions associated with type 2 DM history, which in the Investigators opinion could interfere with the results of the trial 13) Cardiac disease defined as: Heart failure (NYHA class III-IV) and/or diagnosis of unstable angina pectoris and/or MI, stroke, PTCA or CABG within the last 3 months 14) Diagnosis of non-Diabetic CKD current or in the past 15) Diagnosis of liver cirrhosis with current impaired liver function within the last 3 years. 16) Diagnosis of Addison's disease. 17) Being lactating. 18) Intend to become pregnant within the duration of the study or not use adequate birth control. 19) Known or suspected abuse of alcohol or narcotics 20) Not able to understand informed consent form 21) Participation in any other intervention trial than PRIORITY or a related sub-study is not allowed within 30 days before inclusion or concurrent to this study
Statistical rationale	The aims of the study are to investigate the time to development of the primary endpoint in the included patients. The patients will based on the urinary proteomic test be divided into low risk patients who will enter an observation study without specific intervention, and high risk patients who will berandomized in a double blind parallel trial comparing placebo with active treatment with spironolactone. It is our hypothesis that high risk patients will develop the endpoint more frequent than low risk patients, and that treatment with spironolactone can mitigate the increased risk in high risk patients. The null hypotheses are that high and low risk patients develop the endpoint with the same frequency, and that the development is similar in patients treated with placebo or spironolactone <u>Sample size calculation:</u>

	The expected relative proportions of diabetes type 2 patients developing microalbuminuriain 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 samples size formula for two proportions test (α= 0.05 β=0.80), randomized (1:1), n=129 in each arm of the intervention group. To account for drop-outs we plan to randomize 300 in all. It is expected that approx. 15% of the population will show a pattern of high-risk of CKD, therefore 2000 patients have to be included. Equal drop-out frequency is expected I both groups and there will be no replacement of drop-out. Discontinuation prior randomization is categorized screening failure. Analysis populations: The primary analysis will be conducted for all patients included (intent-to-treat population) in the observational and intervention study groups. In addition, all relevant efficacy endpoints will be also analyzed for the per protocol population in both study groups. Analysis of primary endpoint: Cox regression analysis of development of the primary endpoint adjusted for age, gender, study site will be conducted in both study groups (observational and intervention) and for study intervention in the interventional group. Secondly adjusted in addition for baseline UACR, HbA_{1c} and BP. Analysis of secondary endpoints: Cox regression analysis of development of secondary endpoints in the both study groups (observational and intervention) as well within the interventional group (high- vs. low-risk), adjusted for age, gender, study site and study intervention with secondary adjustment for baseline UACR, HbA_{1c} and BP. Assessment of safety: The assessment of safety will be based primarily on the frequency of eCRF-documented AEs, laboratory abnormalities, and SAEs (see section 13.3). Occurrence and frequency of eCRF-documented AEs, SAEs and laboratory abnormalities will be summarized by treatment group. In addition AEs leading to death and AEs leading to discontinuation of study medication and/or withdrawal will be summarized for the two treatment groups. Final analysis plan: Statistical analysis of the data will be performed by Steno Diabetes Center. A statistical analysis plan will be written and signed before database lock and unblinding of the entire treatment code. Methods for imputation and type of censoring will be clarified in the statistical analysis plan prior to database lock and unblinding.

3 SUMMARY

This study aims to:

1. Confirm in a prospective multicenter study of normoalbuminuric type 2 DM patients that the urinary proteome test identifies patients with a high risk for development of microalbuminuria.

2. Demonstrate the clinical utility of the test by showing that aldosterone blockade in high-risk patients can reduce progression to microalbuminuria in comparison to placebo, on the top of standard treatment in a randomized double-blind, placebo-controlled multicenter study.

Patients: After screening 2700 patients, 2000 normoalbuminuric type 2 DM patients will be included and will be stratified into an observational- and an intervention group using urinary proteomic test.

Patients who have contraindications to aldosterone blockade will not be included in the study.

Observational group: Patients who are classified as being at low risk for progression to diabetic nephropathy (approx. 85%) will enter observational period of at least 104 weeks, dependent on time of baseline visit. These patients will continue to receive usual care at their hospital diabetes clinic or GP with treatment according to standard guidelines

Intervention group: Patients who are classified as being at high risk (approx. 15%) will be randomly assigned to the mineralocorticoid receptor antagonist spironolactone 25 mg once daily or placebo therapy in addition to optimal standard therapy. These patients will enter a treatment period of at least 104 weeks, dependent on time of baseline visit.

Endpoint: The primary endpoint will be the development of confirmed microalbuminuria (UACR >30 mg/g) in at least two out of three urine tests, with at least 30% increase in UACR from “run-in” period. We will also examine changes in albuminuria throughout the study period in all patients by assessing the slope of albuminuria changes and absolute changes from inclusion to the end of study. We will compare the observational group and the treated intervention group in order to assess the predictive value of the urinary proteomics test. Furthermore, within the intervention group we will compare changes in albuminuria between patients randomized to placebo or the mineralocorticoid receptor antagonist spironolactone. The analysis will consider age and gender effects on the predictive value of the proteomics test and treatment effect.

4 BACKGRUND INFORMATION AND SCIENTIFIC RATIONALE

4.1 Background Information

Diabetes mellitus (DM) 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 complications (1;2).

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 (1). 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) (3). 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) (4).

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 (5;6). 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 (7;8).

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 (9;10). 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 (11;12). 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 (13). 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 (14). 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 (15;16). 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 (17). Urine holds several advantages over blood in clinical proteomics. It can be collected non-invasively and its proteome is relatively stable (18). Members of the consortium have successfully identified a urinary biomarker pattern including 273 peptides significantly associated with chronic kidney disease (CKD273) (19).

Importantly, the biomarker panel has been validated in a multicentric approach involving >1000 blinded samples (20;21). 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 (22). 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 (22;23). 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 (24).

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 (25).

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.

4.2 Rationale

1. Urinary proteomics predicts development of microalbuminuria (as a surrogate marker for the development of overt nephropathy) in a cohort of 2000 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

5 OBJECTIVES AND ENDPOINTS

5.1 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 2000 type 2 diabetic patients with normal urinary albumin excretion.

5.2 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.

5.3 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 (26) 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.

A research biobank will be established at Steno Diabetes Center containing biological material in the form of blood and urine samples.This will allow testing of additional putative markers for progression of diabetic nephropathy and development of cardiovascular events and death (such as but not limited to): Endothelial dysfunction (vWF, ADMA, VCAM, ICAM, E-selectin), inflammation (hs-CRP, IL-6, TNF-alpha, YKL40, IL-8, IL10), vascular riskmarkers (NT-proBNP, adiponectin, ghrelin), and bonemarkers (vitamin D, p-PTH, p-calcium, p-phosphat, p-magnesium, osteoprotegerin (OPG), osteopontin (OPN), RANKL, bone morphogenic protein (BMP), p-FGF23, osteocalcin, bone-specific alkaline phosphatase).

5.4 Primary endpoint

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).

5.5 Secondary endpoints

5.5.1 In the total population:

In the following paragraphs patients in the intervention group, (group B) and observation group (group A) will be compared.

- 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.

- Comparison of incidence of retinopathy and frequency of laser treatment. Data collected from self-reported AEs
- In addition to the categorical analysis of urinary albumin excretion, an analysis will be performed with changes in geometric mean albuminuria throughout the study period in all patients by assessing the slope of albuminuria changes and absolute changes from inclusion to end of trial
- 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
- Development of macroalbuminuria (UACR >300 mg/g) in 2 out 3 first morning void urine samples)
- For patients with eGFR ≥ 60 at baseline, development of eGFR<60 ml/min/1.73m^2.
- Change in eGFR (slope and absolute from baseline and from 3 month post-baseline to end of study)

5.5.2 In the intervention group (group B) / High-risk population:

In the following paragraphs patients receiving active medication will be compared to placebo treatment.

- 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.
- Comparison of incidence of retinopathy and frequency of laser treatment. Data collected from AEs
- 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
- Development of macroalbuminuria (UACR > 300 mg/g in 2 out 3 first morning void urine samples)
- For patients with eGFR ≥ 60 at baseline, development of eGFR <60 ml/min/1.73m^2
- Change in eGFR (slope and absolute from baseline and from 3 month post-baseline to end of study)

5.6 Assessment of Safety

The assessment of safety will be based primarily on the frequency of eCRF-documented AEs, laboratory abnormalities, and SAEs (see section 13.2.). Occurrence and frequency of eCRF-documented AEs, SAEs and laboratory abnormalities will be summarized by treatment group.

In addition AEs leading to death and AEs leading to discontinuation of study medication and/or withdrawal will be summarized for the two treatment groups.

5.7 Calculation of eGFR

Calculation is based on locally measured creatinine from a blood sample. At the time of screening the local eGFR will be used for evaluation of inclusions criteria no. 5 only.

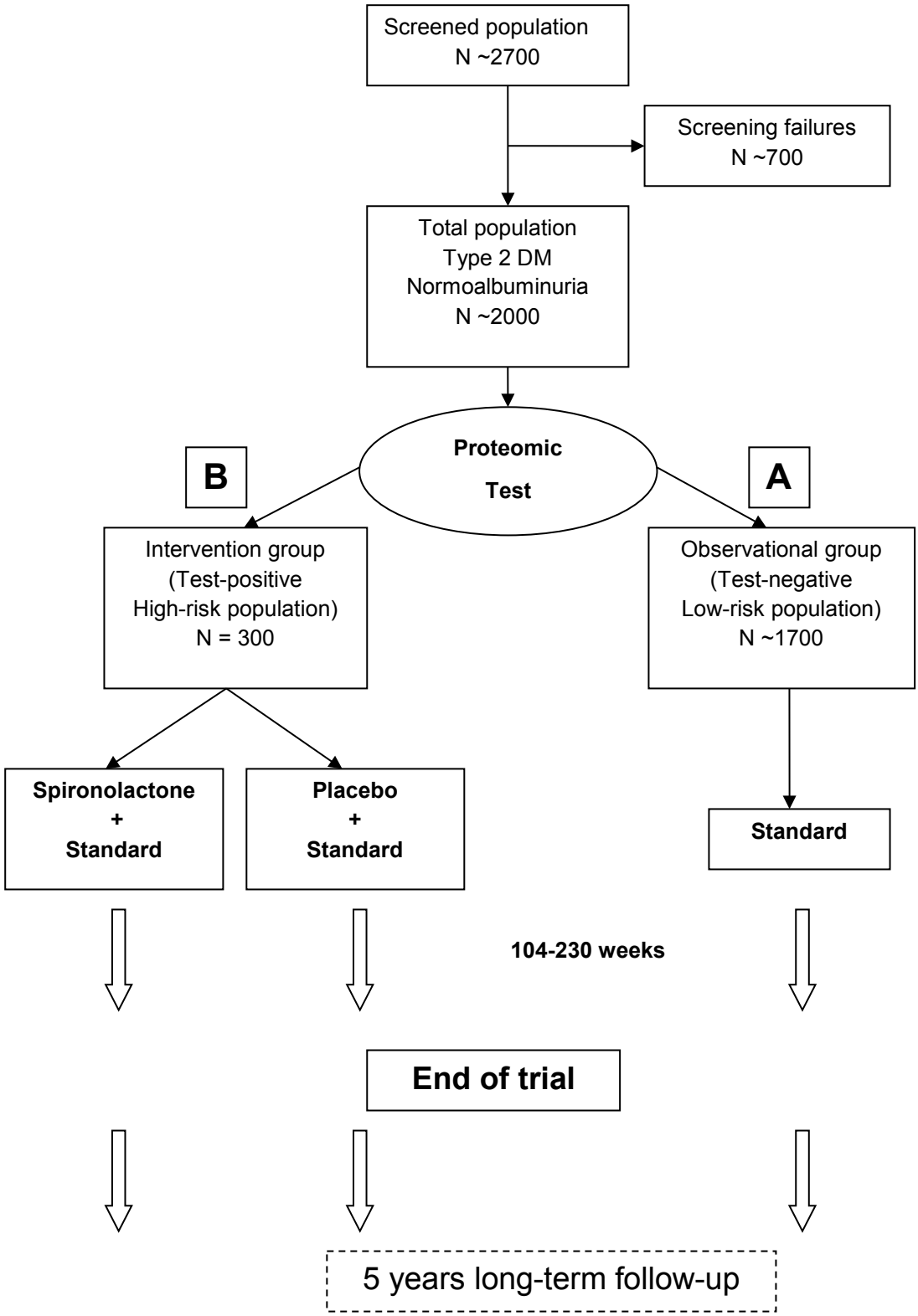
Information in the eCRF is based on the creatinine measurement method in the local laboratory. The central calculation of eGFR will be made with CKD-EPI equation. The estimation will be made centrally in the eCRF after entry of creatinine data by the investigators.

CKD-EPI equation: GFR = 141 X $\min(Scr/\kappa,1)^{\alpha}$ x $\max(Scr/\kappa,1)^{-1.209}$ x 0.993^{Age} x 1.018 [if female] x 1.159 [if black]. Where Scr is serum creatinine (mg/dL), κ is 0.7 for females and 0.9 for males, α is –0.329 for females and –0.411 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ or 1.

The information in the eCRF on eGFR will be used as safety measures throughout the study.

6 STUDY DESIGN

Figure 1: Trial flow chat



6.1 Overall study design / Total study population

Prospective, multicenter, randomized, double blind, placebo-controlled phase II-III trial and a prospective observational study. With screening of approximately 2700 patients aiming to included 2000 type 2 DM patients with normoalbuminuria. All patients included in the study will be screened for the presence of a CKD273-specific proteomic pattern at time of inclusion in the study (screening visit). All patients will be monitored for development of microalbuminuria.

Patients with a high-risk CKD273 proteomic pattern (approx. 300 patients) will enter the intervention group and randomized to receive 25 mg spironolactone once daily or placebo in a 1:1 ratio. These patients will enter a treatment period of at least 104 week, dependent on time of baseline visit.

Patients without a low-risk proteomic pattern will not be randomized, but remain in the observational group (approx. 1700 patients). These patients will enter an observational period of at least 104 week, dependent on time of baseline visit.

Patients included prior to October 2016 will have a treatment/ observation period longer than 104 week, dependent on time of baseline visit. The treatment/ observational period can't exceed 230 weeks (4.4 years). All patients will have final assessment performed in the period from 1st July 2018 to 30th September 2018.

All patients have to sign informed consent prior any study specific procedures were performed.

6.2 Observation group (group A) / Low-risk population

It is expected that 85% of the total population (approx. 1700 patients) will show a low-risk pattern. Patients should be followed by the investigator in routine clinics or seen once yearly, for standard diabetes care according to local guidelines. Annual measurements of UACR, BP, medication, and locally measured creatinine, sodium, potassium, and lipids (cholesterol, HDL, LDL, triglycerides) will be performed. HbA_{1c} will be measured in DCCT-aligned quality control programs or IFCC. During the study, the medical history regarding vascular events, current retinopathy status (by history) and information on laser treatment, as well significant medical events (see section 10.14.), including hospital admissions, are registered.

Secondary endpoints and significant medical events will be registered at the annual visits.

After the last study visit, patients will continue in the observational long-term follow-up in accordance with section 6.4.

6.3 Intervention group (group B) / High-risk population

Randomized, double-blind, placebo-controlled, multicenter study in the high-risk population based on urinary proteomic risk pattern assessment. It is estimated that 15% of the total population (approx. 300 patients) will show a high-risk pattern.

Patients will be invited to participate if the urinary proteomic pattern after the screening visit is showing a high-risk pattern, and if at least two of the three samples from the "run-in"-period show normoalbuminuria (UACR <30 mg/g) analyzed at the central laboratory.

Patients in the intervention study will be randomized (1:1) to spironolactone 25 mg daily or matching placebo in addition to standard treatment according to the investigators discretion. 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).

Patients are seen according to Table 1, with a study visit every 13th weeks. Patients must be treated by the investigator for standard diabetes care according to local guidelines. Dependent on time of baseline the patients will be treated for at least 104 weeks with measurements of UACR, BP, medication, and locally measured creatinine, sodium, potassium, and lipids (cholesterol, HDL, LDL, triglycerides). HbA_{1c} will be measured in DCCT-aligned quality control programs or IFCC. At screening and in the duration of the study, the medical history regarding vascular events, current retinopathy status (by history) and information on laser treatment, as well as adverse events (see section 13.3), including hospital admissions, are registered.

Potassium levels will be measured locally as indicated in table 1.

Secondary endpoints and adverse events (see section 13.3) will be registered at the visits every 13 weeks.

After the last study visit patients will continue in the observational long-term follow-up in accordance with section 6.4.

6.4 Long-term follow-up

A long-term follow-up study will be conducted 5 years after the end of the clinical trial. The purpose of this follow-up is to evaluate progression to later stages of DN (macroalbuminuria, deterioration of renal function) and/or CVD and/or death based on register data.

After the end of the clinical study no study visit will be conducted and no information will be recorded in the eCRF.

The long term follow-up will be carried out in accordance with the applicable national regulations.

6.5 Biobank

A research biobank will be established at Steno Diabetes Center containing biological material in the form of blood and urine samples. The material will be stored in coded form for later analysis of markers of development and progression of nephropathy mortality and cardiovascular disease.

Biological material from the biobank will be stored after the end of the study for 15 years if the patient accepts this. Thereafter, stored material will be destroyed.

At least at the baseline visit (day 0), the patients will sign and date a study-specific informed consent regarding participation in the research biobank with additional scientific potential. Patients who are not willing to participate in the additional research biobank can still participate in the PRIORITY-study.

6.6 Study visit description

For all participants, the screening visit will be the first visit in the study. No procedures related to the study may be performed before the written informed consent is obtained. The screening visit must be performed prior to both the run-in phase and the baseline visit. In accordance with table 1, the screening visit must be performed within a window of 4 to 12 weeks prior to baseline visit.

The run-in phase starts after the screening visit. The study procedures related to the run-in phase must be at least 2 weeks before the baseline visit.

The baseline visit can only take place after results on UACR and urinary proteomics risk assessment are displayed in the eCRF.

Table 1: Frequency of study visits at each participating center

Assessment procedures										
		Total Population			**Intervention Group (Group B,high-risk population)**				**Observational Group (Group A,low-risk population)**	
Visit		**Screening**	**Run in - period**	**Baseline**	Visit 1	Periodic study visits		End of study/ Early Termination Visit	Periodic study visits	End of study/ Early Termination Visit
Study week		Week -12 to -4	Week -11 to -2	Day 0	Week 2 (± 3 days)	Week 13, 26, 39, 52, 65, 78, 91, 104,117, 130, 143, 156, 169, 182, 195, 208 and 221 (± 14 days)	Week 52, 104, 156 and 208 (± 14 days)	Planned end (July to September 2018) or early Termination	Week 52, 104, 156 and 208 (± 14 days)	Planned end (July to September 2018) or early Termination
Informed consent		x		x[1]						
Inclusion/ Exclusion criteria		x		x[2]						
Medical history				x						
Tobacco/alcohol usage				x						
Concomitant medication		X[9]		x	x	x		x	x	x
Physical examination[3]				x		x		x	x	x
Height		X								
Weight		x					x	x	x	x
Vital signs (BP/Heart rate)[4]		x		x		x		x	x	x
Study medication				x		x		x		
Local clinical laboratory examinations clinical chemistry (blood samples)	Creatinine, sodium, potassium,	x		x	x	x		x	x	x
	HbA1c	x		x[10]		x		x	x	x
	Cholesterol, LDL, HDL, Triglyceride			x			x	x	x	x
3 consecutive first morning void urine samples for albuminuria assessment	Central Analyses		x[6]			x		x	x	x
	Central Storage[5]		x				x	x	x	x
Urine sample for proteomic analysis		x								
Biobank sampling (after informed consent)				x				x		x
Randomization (applicable for group B)				(R)						
Adverse Events					x	x		x		
Significant medical event									x	x
Urine dipstick analysis[7]		x	x			x		x	x	x
Pregnancy test[8]		x						x		
Final assessment								x		x

1) Informed consent for the biobank participation.
2) Only the inclusion criteria regarding UACR and exclusion criteria regarding BP will be evaluated.

3) The physical examination performed at the Baseline visit will serve as the baseline for clinical assessment. At baseline the examination will include: General appearance, heart, lungs, abdominal, lymph nodes and extremities. At all other visit, a symptom-directed physical exam will be performed. Any new clinically significant physical exam findings after the Baseline/Randomization Visit will be recorded as adverse events.
4) Blood pressure: Average of three measurements in sitting position after at least 10 min rest
5) One of three samples for storage after analyses, description in section 10.9.
6) Samples from excluded patients are not shipped to central laboratory.
7) Test for urinary tract infections: Blood, leucocyte and nitrate.
8) Pregnancy test, description in section 10.16.
9) Only in regards to ACE or ARB treatment.
10) Optional based on investigators decision.

7 STUDY POPULATION

All patients that are eligible according to the inclusion and exclusion criteria relevant at study entry can be included in the trial.

7.1 Inclusion Criteria

1) Written informed consent must be provided before participation. Patient information and consent form must be approved by relevant independent EC. Specifically, all participating patients will be asked to give informed consent for long-term follow-up
2) Male or female patients ≥ 18 years and < 75 years of age at Screening visit
3) Type 2 DM (WHO criteria)
4) Persistent normoalbuminuria (at least 2 of 3 UACR < 30 mg/g samples from ”run in”-period)
5) eGFR >45 ml/min/1.73m^2 at Screening visit
6) The patient must be willing and able to comply with the protocol for the duration of the study
7) Female without child-bearing potential at the screening visit. Defined as one or more of following:

   7.1) Female patients ≥ 50 years of age at the day of inclusion, who have been postmenopausal for at least 1 year

   7.2) Female patients < 50 years of age at the day of inclusion, who have been postmenopausal for at least 1 year and serum FSH levels > 40 mIU/mL as well as serum estrogen levels < 30 pg/ml or a negative estrogen test.

   7.3) 6 weeks after surgical sterilization by bilateral tubal ligation or bilateral ovariectomy with or without hysterectomy.

   OR a negative urine pregnancy test at the Screening visit AND one or more of following:

   7.4) Correct use of reliable contraception methods. This includes one or more of the following: hormonal contraceptive (such as injection, transdermal patch, implant, cervical ring or oral) or an intrauterine device (IUD) OR correct use of double barrier with one of the following: barrier methods (diaphragm, cervical cap, Lea contraceptive, femidom or condom) AND in combination with a spermicide.

   7.5) General sexual abstinence from the time of screening/ during the study until a minimum of 30 days after the last administration of study medication if this is already established as the patient’s preferred and usual lifestyle.

   7.6) Having only female sexual partners.

   7.7) Sexual relationship with sterile male partners only.

7.2 Exclusion criteria

1) Average of systolic BP < 110 or >160 mm Hg at baseline
2) Average of diastolic BP > 100 mm Hg at baseline
3) Type 1 DM (WHO criteria)
4) HbA1c <6.5% (48 mmo l/ mol) AND > 5 years of known duration of diabetes type 2 AND never treated with an antidiabetic drug of any kind.

5) Current in treatment with more than one RAAS blocking agent (Angiotensin Converting Enzyme inhibitor, Angiotensin Receptor Blocker or Direct Renin Inhibitor)
6) Current lithium treatment (ATC: N05AN)
7) Known or suspected hypersensitivity to Spironolactone or to any of its excipients.
8) Current use of potassium sparing diuretics (ATC: C03D, C03E), such as: Spironolactone, Eplerenone or amiloride etc.
9) Hyperkalemia at Screening: Plasma potassium level >5.0 mmol/L or serum potassium level >5.4 mmol/L.
10) Hyponatriemia determine by the investigator
11) Current cancer treatment or within five years from baseline (except basal cell skin cancer or squamous cell skin cancer)
12) Any clinically significant disorder, except for conditions associated with type 2 DM history, which in the Investigators opinion could interfere with the results of the trial
13) Cardiac disease defined as: Heart failure (NYHA class III-IV) and/or diagnosis of unstable angina pectoris and/or MI, stroke, PTCA or CABG within the last 3 months
14) Diagnosis of non-Diabetic CKD current or in the past
15) Diagnosis of liver cirrhosis with current impaired liver function within the last 3 years.
16) Diagnosis of Addison's disease.
17) Being lactating.
18) Intend to become pregnant within the duration of the study or not use adequate birth control.
19) Known or suspected abuse of alcohol or narcotics
20) Not able to understand informed consent form
21) Participation in any other intervention trial than PRIORITY or a related sub-study is not allowed within 30 days before inclusion or concurrent to this study

7.3 Screening failures

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

It is not necessary to complete all inclusion/exclusion criteria unless it is medically indicated. 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.

7.4 Replacement of participants

Rescreening of patients is allowed with at least one week between screening visits. Any patient who is withdrawn from the study for any reason, after study medication has been initiated may not re-enter the study at any time. There will be no replacement of participants who discontinue the IMP or withdraw from the entire study.

8 RANDOMIZATION/BLINDING AND UNBLINDING PROCEDURES

8.1 Patient Identification

All patients included into the screening process will receive a consecutive patient number, starting with patient number 0001. The patient identifier is the combination of the study site number (e.g. 01) and the patient number (e.g. 0001).

8.2 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 institute, 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.

8.3 Assignment to treatment

The IWRS will assign each patient to treatment.

8.4 Blinding procedure

Placebo and active study drug will not be distinguishable from each other in terms of appearance, labeling or instructions for use. All IMP will be labelled with a unique pack identifier number.

8.5 Unblinding procedures

8.5.1 Emergency unblinding

Emergency unblinding should only be done if emergencies occur that can be directly linked to the study medication, where it is essential to know if the patient was actually treated with spironolactone. If hyperkalemia occurs, it is not in all case necessary to break the code, the patient is to be treatment according to guidance as described in the Appendix.

Unblinding will be performed via a telephone menu system. Several prompts in the system warn the user that they require to be a health professional and to record their name and other pertinent information. For each unblinding an email alert is generated to the sponsor, HCTC and the provider of IWRS. Requests are set at a maximum of 2-3 per 24 hours in case of malicious unblinding.

In case unblinding is needed a rapport has to be filed in the TIF and patient file and documented in the eCRF and HCTC must be contacted in accordance with section 13.

8.5.2 Planned unblinding

Planned unblindling will be performed by the Robertson Institute after finalizing the SAP and with the consent from the steering committee on science.

9 STUDY SCHEDULE

9.1 Involvement of patient

9.1.1 Recruitment period

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

9.1.2 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 dependent 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 30th September 2018.

9.2 Data management and statistics

According to section 15 and 19 data management will be performed in the period from establishment of protocol after end of study.

The statistical assignment for the primary publications will be carried out after last patient visit.

9.3 Publication

According to section 21 three publications are foreseen.

10 STUDY PROCEDURES

All study data will be included in the patients' source documentation and provided in the appropriate eCRF. Study procedures planned for a specific study visit must be performed in accordance with table 1. In any case all participants must sign and date a study-specific informed consent before any study procedures can be performed.

10.1 Informed consent

At the Screening visit the patients will sign and date a study-specific informed consent form before any study procedures are performed. Included in the informed consent will also be information regarding the long-term follow-up in accordance with section 6.4.

At the Baseline visit (day 0), the patient will sign and date a study-specific informed consent regarding participation in the research biobank with additional scientific potential. Patients who are not willing to participate in the additional research biobank can still participate in the PRIORITY-study.

10.2 Medical history

A complete medical history, including history of tobacco and alcohol use, will be obtained from each patient as indicated in Table 1. The medical history will be updated prior to randomization at the baseline/randomization visit and will serve as baseline for clinical assessment.

10.3 Concomitant medication

At the Baseline visit and every visit thereafter, information about usage of concomitant medication will be documented for each patient. Patients are allowed to take any form of concomitant medication during the study except Lithium or treatment with more than one RAAS blocking agent. For concomitant use of ACE, ARB or Spironolactone see section 12.1.1.

10.4 Physical examination

A physical examination will be performed as indicated in Table 1. Height will be measured only at the Screening Visit. Body weight will be measured at the Screening Visit and yearly thereafter, , upon patient discontinuation or at end of study. The physical examination performed at the baseline visit will serve as the baseline for clinical assessment. At Baseline the examination will include: General appearance, heart, lungs, abdominal, lymph nodes and extremities. At all other visit, a symptom-directed physical exam will be performed. Any new clinically significant physical exam findings after the Baseline/randomization will be recorded as adverse events. Significance will be adjusted by an investigator after consultation of CTCAE version 4.0 AE-intensity grading.

10.5 Vital signs

Heart rate and BP will be measured as indicated in Table 1. BP and heart rate will be measured in the sitting position after 10 min rest, as an average of 3 measurements with a validated automatic or manual device and appropriately sized cuff will be used. The vital signs measured prior to randomization at the Baseline/Randomization Visit will serve as the baseline for clinical assessment.

Whenever possible, the study personnel should use the same instrument and measure BP and heart rate in the same arm. When the timing of these measurements coincides with a blood collection, vital signs should be assessed prior to blood collection or at least 10 minutes after.

10.6 Dispensing of investigational medicinal products

Study medication will be provided to the patients of the high-risk population at baseline and at each study visit. Prior to receiving the IMP patients will be informed orally and in writing of the contents of the IMP as describes in section 11.1 and the potential side effect described in section 11.3.

At every visit after baseline (every 13 week) patients has to return the unused IMP for accountability and destruction of unused tablets according to section 11.2.

10.7 Laboratory examination

The laboratory manual holds instructions about the blood- and urine sample collection.

10.7.1 Labelling of samples

All samples will be labeled with the study acronym (PRIORITY), name of sponsor and the unique patient identifier (visit number, study site number, patient number, and the date when the sample was obtained). Labels will be provided by Steno Diabetes Center.

10.8 Blood samples

Clinical chemistry has to be performed according to table 1 with measurement of creatinine (traceable standardized method), sodium, and potassium at every visit. HbA_{1c} (DCCT-aligned method or IFCC) has to be measured at Screening and every periodic visit in both groups. Lipids (cholesterol, HDL, LDL, and triglycerides) have to be measured at least every year in all patients.

All blood samples are to be analysed locally.

10.9 Urine samples for albuminuria

Urine samples are to be analysed centrally for UACR. Three consecutive first morning void urine samples are collected for UACR. The samples are collected two days prior any study visit, and at the day of the visit as indicated in table 1. The urine samples of the “run-in”-period are collected the three days following the screening visit and brought or sent to the study site. Sample shipment from patient to the study center is done using any authorised material for shipment of biological material in regular mail.

Every effort should be made to ensure collection of three consecutive first morning voids for analysis of UACR. This is especially important in the “run in” period. If a patient attends a visit and has collected only one or two first morning voids, the patient must collect supplemental first morning voids samples on the following two mornings after the visit and send or bring these to the study site.

The most recently performed morning void is tested for urinary infection with dipstick test. Samples are discarded if infection is detected and a new sample must be performed preferably within two weeks of the planned visit, or as soon as possible thereafter.

Samples are frozen immediately at -20^{o}C and are stored until shipment. Shipment to Steno Diabetes Center will be performed by using a local courier and samples are to be sent on dry ice.

Urine samples from the “run-in” period are to be sent to Steno Diabetes Center at least every third weeks for screening analyses. Urine samples from all other visits than the screening visit and the “run-in” period are to be shipped at least every sixth months.

Urine sample from the “run-in” period and every annually visit one urine sample will be stored at Steno Diabetes Center. The most recently performed urine sample will be preferred. The samples will be stored at least at -20 ^{o}C upon arrival.

10.10 Urine samples for proteomic analysis

Urine samples for proteomic analysis will be obtained at the screening visit. Samples are tested for urinary infection with urine dipstick test. Samples are discarded if infection is detected and a new sample must be performed preferably within two weeks of the planned visit, or as soon as possible thereafter.

Samples are to be frozen at -20 ^{o}C until shipment. Shipment to Steno Diabetes Center will be performed by using a local courier and samples are to be sent on dry ice. Urine sample from the screening visit is to be sent to Steno Diabetes Center at least every third weeks. From Steno Diabetes Center the sample will be sent for analysis at Diapat GmbH in Hannover.

Upon arrival at Diapat GmbH the samples are catalogued, prepared for measurement and immediately analyzed. The result of the analysis will be displayed in the eCRF.

10.11 Biobank

Samples are to be obtained at Baseline and at Final assessment visit from, all patients who agreed to biobank sampling.

Urine sample for biobank will be tested for sign of infection with dipstick test. In case of infection this has to be treated according to national guidelines in terms of antibiotics and the patient has to reschedule for a new baseline visit within two weeks. If the incident occurs at the baseline it has to reported as medical history. If the incident occurs at the final assessment visit it must be reported as an AE according to section 13.3.

At baseline and at end of study, the following samples will be stored **(DNA only at Baseline)**

Material	Collection container	Centrifuge		Storage container	Freezer
DNA Buffy-coat	2 X 10.0 ml K-EDTA	Time	10 min.	2 X 3.6 ml Cryo tubes	-80°C
		Temp	20°C		
		RCF	1500G		
EDTA plasma (ICE)	1 X 4.0 ml K-EDTA	Time	10 min.	1 X 1.8 ml Cryo tybe	-80°C
		Temp	4°C		
		RCF	1500G		
EDTA plasma	2 X 4.0 ml K-EDTA	Time	10 min.	2 X 1.8 ml Cryo tubes	-80°C
		Temp	20°C		
		RCF	1500G		
Serum	3 X 6.0 ml Dry glass	Time	10 min.	2 X 1.8 ml Cryo tubes	-80°C
		Temp	20°C		
		RCF	1500G		
Citrate Plasma (ICE)	2 X 4.5 ml Na-Citrate	Time	10 min.	2 X 1.8 ml Cryo tubes	-80°C
		Temp	4°C		
		RCF	1500G		
Urine (morning)	20 ml plastic tubes			2 X 3.6 ml Cryo tubes	-80°C

Following pre-processing and aliquoting, blood and urine samples will be stored locally, at least at -70 °C prior to shipping to the central laboratory at Steno Diabetes Center. Shipment to Steno Diabetes Center will be performed by using a local courier and samples are to be sent on dry ice. All samples will be stored upon arrival at -80 °C.

10.12 Randomization

Randomization will be performed at the baseline visit. If the patient participates in the biobank the randomization will be performed after collection of samples. Only patients with a high-risk pattern in the proteomic analysis from the screening visit will be randomized. Patients will be randomized in a 1:1 ratio to either active medication or placebo in addition to standard treatment as described in section 8. 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).

10.13 Adverse Events

Documentation of all adverse events will be performed at all visits as indicated in table 1 and as described in section 13.1.

10.14 Significant medical event

Documentation of significant medical events will be conducted at the annual visit for the low-risk participants. Any medical untoward occurrence after baseline can be documented as a significant medical event. Of special interest are events described in section 5.5.1 as secondary end points.

10.15 Urine dipstick test

Urine dipstick test are to be performed on urine samples at the study sites to confirm that urine tract infection is present. Urine dipstick test is performed before the samples are frozen.

If the patients has a urinary tract infection or menstrual bleeding at the time of a planned study visit, urine collection should be postponed and the visit can be rescheduled, preferably within two weeks of the planned visit, or as soon as possible thereafter.

10.16 Pregnancy test

All female patients with child-bearing potential will have a pregnancy test performed at the Screening visit, and at the end of study or discontinuation, as indicated in Table 1. The assessment will be according to local standard based on blood or urine analysis.

10.17 Final assessment

At the Final assessment standard diabetic care according to local guidelines will be performed, as well as the study procedures indicated in table 1.

10.18 Clinical procedure after end of the study/ Early termination

After the end of the study or early termination, the patients will receive standard care according to national guidelines under the discretion of the treating physician.

10.19 Unscheduled visit

Unscheduled visit is possible any time after screening on the opinion of the investigator. The content of such visit is on the discretion of the investigator. The main issue for the unscheduled visit must be documented in the eCRF.

11 INVESTIGAL MEDICAL PRODUCTS

11.1 IMPS

All IMPs will be manufactured in accordance with EU Good Manufacturing Practice requirements and will be supplied and distributed by Mawdsleys Clinical Services UK.

Tablets and bottles containing active IMP or placebo will be indistinguishable from each other in terms of taste, appearance, durability, packaging, labeling and instruction for use.

Only patients of the intervention group will receive IMP. Only those supplies intended for use in the study may be dispensed to study participants.

11.1.1 Formulation and preparation

Spironolactone: Tablet for oral use containing spironolactone and additional excipients

Placebo: matched placebo tablet for oral use. (Placebo tablets have the same constituents as the active with the exception of spironolactone content.)

11.1.2 Administration

Patients will be advised to take the IMP tablets once each day orally, with or just after food in the morning.

Dosage Treatment: 25 mg spironolactone once daily.

Dosage titration is prohibited.

11.1.3 Packaging

Packages with active or place study drug will be indistinguishable from each other.

Primary packaging will contain IMP for 13 weeks of treatment plus a small overage (110 tablets).

11.1.4 Labeling

Labeling will be performed according to national regulatory requirements and in accordance with current annex 13 EU Good Manufacturing Practice requirements.

11.1.5 Storage and stability

The IMPs will be provided to each participating study center from the study manufacturer in a blinded fashion.

The tablets should be stored in a dry place at room temperature below 25 ^{o}C in a secure location.

Study supplies must be stored under the correct storage conditions at all times.

11.2 Accountability procedures for the IMP

After randomization, patients of the intervention group will receive IMP from the study site they attend.

At every 13th week after the Baseline the patients should be asked to return the IMP for accountability.

Patient should take between 80-110% of IMP to be compliant. The process for capturing the compliance information is the assessment by tablet counts at each site.

A record of all IMP movements including drug dispenses must be performed and documented by each site.

11.3 Main side effects of spironolactone

Gynecomastia may develop in association with the use of spironolactone. Development appears to be related to both dosage level and duration of therapy and is normally reversible when the drug is discontinued. In rare instances some breast enlargement may persist.

The following adverse events have been reported in association with spironolactone therapy:

Organ system	Side effect
General disorders and administration site conditions	malaise
Neoplasms benign, malignant and unspecified (including cysts and polyps)	benign breast neoplasm
Gastrointestinal disorders	gastrointestinal disturbances, nausea
Blood and lymphatic system disorders	leukopenia (including agranulocytosis), thrombocytopenia
Hepatobiliary disorders	hepatic function abnormal
Metabolism and nutrition disorders	electrolyte disturbances, hyperkalemia
Musculoskeletal disorders	leg cramps
Nervous system disorders	dizziness
Psychiatric disorders	changes in libido, confusion
Reproductive system and breast disorders	menstrual disorders, breast pain
Skin and subcutaneous tissue disorders	Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), drug rash with eosinophilia and systemic symptoms (DRESS), alopecia, hypertrichosis, pruritus, rash, urticaria
Renal and urinary disorders	acute renal failure

11.4 Overdose

Acute overdosage may be manifested by drowsiness, mental confusion, nausea, vomiting, dizziness or diarrhoea. Hyponatremia, or hyperkalemia may be induced, but these effects are unlikely to be associated with acute overdosage. Symptoms of hyperkalemia may manifest as paresthesia, weakness, flaccid paralysis or muscle spasm and may be difficult to distinguish clinically from hypokalemia. Electrocardiographic changes are the earliest specific signs of potassium disturbances. No specific antidote has been identified. Improvement may be expected after withdrawal of the drug. General supportive measures including replacement of fluids and electrolytes may be indicated. For hyperkalemia**,** specific procedures are to be followed as described in the Appendix.

11.5 Risks in connection with the investigation procedures

The collection of the urine samples is without. The blood withdrawals for research purposes will be performed in relation to standard investigational procedures. As with any venepuncture, pain, bleeding and/or bruising may occur due to penetration of the needle; more rarely, infection can occur at the entry spot. Very rarely, there may be arterial puncture, nerve damage or phlebitis.

12 CONMEDICATION AND TREATMENT

12.1 Treatment in general

Treatment of any medical condition aside from hypertension, albuminuria and hyperkalemia must be in accordance with local guidelines. Treatment of hypertension, albuminuria and hyperkalemia should be carried out in accordance with the study protocol, section 12.2, 12.3 or 24.

All participants in the trial can receive anti-diabetic treatment in accordance with local guidelines and standard of care. The treatment regimen can include dietary advice, oral- and/or injection of anti-diabetic medication.

12.2 Prohibited medication

Participants are prohibited to use the following medical treatment in the duration of the study:

ATC-code	Drug class	Drug example
C03D	Potassium-sparing diuretics	Spironolactone, Eplerenone, Amilorid
C03E	Diuretics and potassium-sparing agents in combination	C03D in combination with other drugs
N05AN	Lithium salt	Lithium

12.3 Hypertension treatment

It can be expected that the included study population will be heterogeneous at baseline in terms of antihypertensive treatment, as this may reflect different individual and local treatment considerations. Antihypertensive treatment prior to inclusion, especially ACE or ARB treatment, should be continued unchanged during “run-in” period and throughout the study if possible.

Definition: Hypertension, for the purpose of this study, is defined as systolic pressure >130 mm HG and/or diastolic pressure >80 mm Hg.

All patients should be treated according to local guidelines. In any case the care team should be encouraged to initiate or intensify treatment in case of repeated (or confirmed) blood pressure > 140 mm Hg systolic and or diastolic > 90 mmHg. If local guidelines do not recommend a specific first line antihypertensive agent it is suggested to start with a calcium channel blocker or thiazide diuretic to reduce interference with UACR.

In any case, choice of therapy is left to the discretion of the investigator. RAS blocking agents are accepted, if indicated. If blood pressure cannot be managed consideration of a secondary cause should be studied. If patients are assigned to IMP is started on ACE, ARB or renin-inhibitors the investigator is advised to measure potassium according to local guidelines, to minimize the risk of hyperkalemia.

12.4 Albuminuria treatment

All participants included in the trial are normoalbuminuric (UACR <30 mg/g). If a participant reaches the primary endpoint during the course of the study, the information will be flagged in the eCRF and the investigator will be notified.

Participants not in treatment with ACE or ARB at the time of screening should not start treatment before the primary endpoint is reached.

13 ADVERSE EVENT MONITORING AND REPORTING

13.1 Definitions

13.1.1 Adverse Events

An AE as defined in this study is any untoward medical occurrence in a patient or clinical trial patient after baseline. The event does not necessarily have to have a causal relationship with that treatment. AEs can be diseases, signs of disease or symptoms. Among others, AEs include the following:

- All suspected medication adverse reactions
- Apparently unrelated illnesses, including the worsening of a preexisting illness, injury or accidents
- Laboratory abnormalities that require clinical intervention
- Accidents and complications from diagnostic interventions

The following events do not fulfil the definition of an AE:

- An elective hospitalization during participation in a trial for an intervention or diagnostic procedure for a condition which was present before entering the trial
- A pre-existing disease or condition which does not deteriorate during the participation in the trial

13.1.2 Adverse Reaction

An AR is any untoward and unintended response to the IMP related to any dose administered. A response to the medicinal product is given when a causal relationship between the adverse event and one of the IMPs is at least a reasonable possibility.

13.1.3 Serious Adverse Events/ Reaction

A SAE or SAR is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening
  (i.e., in the opinion of the investigator, the patient is at immediate risk of death from the event)
- Requires in-patient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity
  (i.e. a substantial disruption of a person's ability to conduct normal life functions)
- Is a congenital anomaly/birth defect

Important AEs that may not result in death, may not be life-threatening, or do not require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

The following events do not fulfill the definition of an SAE:

- Events which might have resulted in a life-threatening situation but did not

- Elective hospitalization during participation in a trial for an intervention or diagnostic procedure for a condition which was present before entering the trial

13.1.4 Suspected Unexpected Serious Adverse Reaction

A SAE, the nature or severity of which is not consistent with the applicable product information.

13.2 Reference document / Applicable product information

The applicable product information for this trial is the summary of medicinal product characteristics from http://www.medicines.org.uk/emc/medicine/9136/SPC/of 13/12/2012 respectively its updates (actual version - http://www.medicines.org.uk/emc/medicine/9136/SOC/of 03/03/2015).

13.3 Documentation and reporting of AE

13.3.1 Documentation requirements

13.3.1.1 AEs

AE will be collected by the investigator either based on the information provided spontaneously by the patient or evaluated by non-suggestive questions.

The following information is necessary:

- Type of AE (disease, symptoms)
- Classification (serious vs. nonserious)
- Start and termination date of the AE
- Grading of AE-intensity (grade 1-5).The descriptions and grading scales found in the revised NCI CTCAE version 4.0 will be used for adverse event reporting.
- Causality to the study drug
- Measures taken with regard to study medication and treatment of the AE
- Outcome of the AE

The AE documentation period for this trial begins upon first administration of the IMP. In case of continuation of any AE the documentation period will be prolonged until all AEs are resolved or until the investigator assesses the adverse events as “chronic” or “stable”.

Site investigator is responsible for monitoring the safety of patients enrolled into the study at the study sites. All AEs will be documented in the patient’s medical record and managed according to standard practice.

The following AEs will additionally be documented in the eCRF:

- AEs leading to study drug discontinuation and/or withdrawal
- Serious AEs (SAEs, see Section 13.1.3)
- AEs related to cardiovascular and/or renal disease
- AEs related to retinopathy (and corresponding laser treatment)

- Laboratory abnormalities suspected by the investigators to be related to the study drug including the potassium and sodium value

At each patient visit the investigator will review the patient's medical record and determine if there have been any serious adverse events (SAEs) and/or AEs to be documented.

All eCRF-documented AEs, SAEs and laboratory abnormalities must be graded for severity and relationship to study drug and will be coded using the MedDRA via MARVIN.

Documentation of AEs must be performed in a timely manner on the respective AE forms in the eCRF.

13.3.1.2 SAEs

The same documentation responsibilities as described for AEs apply to SAEs as well. In addition, SAEs must be documented on the paper SAE-form and reported to the sponsor. Documentation of SAE must be as complete and detailed as possible. In case of death, an autopsy should be aimed for and the results should be forwarded to the principal investigator and the sponsor.

13.3.2 Reporting requirements

13.3.2.1 Reporting period

The reporting period for AEs and SAEs begins with the first application end ends 30 days after the last application of the IMP.

13.3.2.2 SAE reporting period by the investigator

The investigator has to report all SAEs by faxing the SAE form immediately (within 24 hours) to the authorized representative of the sponsor:

Institute of Clinical Pharmacology
Hannover Medical School
Carl-Neuberg-Str. 1
D-30625 Hannover
Phone: +49-511-532-3959
Fax: +49-511-532-16-2794
E-Mail: sae-reporting@mh-hannover.de

13.3.2.3 Exceptions to the SAE reporting requirements

No exceptions.

13.3.2.4 Pregnancies

Any pregnancies occurring in the intervention group within the reporting period have to be reported to:

Institute of Clinical Pharmacology
Hannover Medical School
Carl-Neuberg-Str. 1
D-30625 Hannover
Phone: +49-511-532-3959
Fax: +49-511-532-16-2794

E-Mail: sae-reporting@mh-hannover.de

Pregnancies have to be followed until an outcome of the pregnancy is documented with information about the status of both, mother and child. Any preterm termination of pregnancy has also to be reported.

13.3.2.5 Events associated with pharmaceutical quality

Any defects or events that are encountered with the IMP that may be attributed to shortcomings of pharmaceutical quality must be reported to:

Institute of Clinical Pharmacology
Hannover Medical School
Carl-Neuberg-Str. 1
D-30625 Hannover
Phone: +49-511-532-3959
Fax: +49-511-532-16-2794
E-Mail: sae-reporting@mh-hannover.de

And to IMP management :

Elizabeth Douglas or Pamela Surtees
Research & Development
NHS Greater Glasgow & Clyde
1st Floor Tennent Institute, Western Infirmary
Church Street, Glasgow, UK G11 6NT
Phone: +44 (0) 141 211 8554
E-mail: Elizabeth.Douglas@ggc.scot.nhs.uk

13.3.2.6 Overdosing

Significant overdosing must be reported as a SAE by the Investigator to the sponsor. Overdose of IMP is defined by the Sponsor as administration of > 110%. E.g. ≥ 100 tablets over a period of 91 days OR > 2 tablets at any single day.

13.3.2.7 SUSAR-Reporting by the Sponsor

In line with the “CT-3” guideline and EU directive 2001/20/EC, it is the responsibility of the sponsor to report all SUSARs that occur in this trial to the respective competent authorities, the leading IEC, and to all investigators of the trial.

Fatal or life-threatening SUSARs must be reported within 7 days, all other SUSARs need to be reported within 15 days.

Also, all events which can change the benefit-risk ratio of the investigational medicinal product have to be reported within 15 days in the same way as SUSARs. This includes:

- Case reports of expected SARs with an unexpected outcome
- Increased incidence of SARs which is considered clinically relevant
- Cases of suspected SUSARs which occurred in patients after completion of the trial participation
- Occurrences in relationship to the trial or the IMP which might affect the safety of the patients

13.3.2.8 Development safety update report (DSUR)

On an annual basis throughout the clinical trial, the sponsor or his authorized representative will provide the NCA and the leading IEC with a written safety report. The safety report's format will adhere to the ICH-guideline E2F (developmental safety update report, DSUR) and include a listing of all suspected serious adverse reactions which have occurred over this period as well as a report of the patient's safety.

13.4 Data monitoring committee and stopping rules

The sponsor has the right to terminate the study at any time for reasonable medical or administrative reasons.

Stopping rules for the whole study: The Steering Committee on samples, data and ethics has the right to terminate the study at any time for reasonable medical safety reasons. The Data Monitoring Committee, which has access to safety data, will provide the Steering Committee on samples, data and ethics with recommendations on the continuation or discontinuation of the study as well as on recommendations for amendments to the study protocol.

The rules about decision finding and meeting procedures for the Data Monitoring committee will be laid out in a separate Data Monitoring Committee Charter.

14 RISK ASSESSMENT AND WITHDRAWAL RULES

14.1 Potential risks

For the patients, sample collection bare minuscule risks of infection at the venipuncture site.

Patients in the intervention groups will be randomized to placebo or spironolactone on top of standard treatment with the potential common site effects like fatigue, hyperkalemia, hyponatremia, gynecomastia and disorder of menstruation (see section 11.3)

14.2 Known potential benefits

Spironolactone has known diuretic effects and antihypertensive effects, although small in this dose. The applied dose has demonstrated significant beneficial effects on urinary albumin excretion in patients with elevated levels, and a beneficial effect on survival in patients with heart failure.

14.3 Potential benefits

The observational study will determine whether the proteomic analysis can identify patients at high risk of developing renal complications in patients with type 2 diabetes. The intervention study will determine whether spironolactone on top of conventional treatment reduces development of renal and cardiovascular complication to diabetes in normoalbuminuric type 2 diabetic patients.

14.4 In concurrent illness

In case of in concurrent illness or acceleration of chronic disease it will of the investigators opinion to either pause or stop the IMP permanently. In case of hyperkalemia action must be taken in accordance with appendix 1, section 24.

In case of hypotensive symptoms or measured BP below 100/50 consider temporary or permanent reduction in antihypertensive or diuretic treatment, while taking concomitant disease or dehydration into consideration.

In any case of in concurrent illness it will on the investigators opinion to pause or stop the IMP. In that case a rapport must be made in accordance with section 13.

14.5 Patient withdrawal

Patients have the right to withdraw voluntarily from study treatment or the entire study at any time for any reason. The investigator also has the right to withdraw patients from the study treatment and the entire study if he or she considers this to be in the best interest of the patient. Should a patient decide to withdraw, all efforts will be made to complete and report the observations as thoroughly as possible. The investigator will make every reasonable attempt to contact the patient to determine as completely as possible the reason for the withdrawal. If the reason for withdrawal of a patient from the study is an adverse event or an abnormal laboratory test result, the adverse event or test will be recorded in the eCRF.

A participant must be withdrawn if any of the following is present:

- Withdrawal of informed consent.

- Lack of adherence to the protocol as judged by the investigator.
- Simultaneous participation in another intervention study.

14.6 Criteria for discontinuation

A participant must be discontinued in the IMP treatment if any of the following is present:

- Female patients in the intervention study who become pregnant, intend to become pregnant or do not use adequate birth control (according to inclusion criteria 7, in section 7.1).
- Experience of research-related injury or unacceptable side effects to the study medication.
- Safety considerations assessed by the investigator
- Treatment with Lithium (ATC: N05AN).
- Treatment with potassium sparing diuretics (ATC: C03D, C03E).
- Confirmed eGFR below 15 ml/min/1.73 m^2.
- Confirmed elevated liver enzyme (ASAT and/or ALT) 3 times above normal range.

Discontinued patients will be offered tests corresponding to the final study visit. Data on all patients will be included in the study and will be used in safety analysis.

After discontinuation of study medication in the randomized population, observation should continue with visits as planned in the observational group (if the patient accepts), or if that is not possible/acceptable, evaluation of vital status and development of endpoints should be evaluated through registries, information of hospitals and GPs, or interviews with the patient as appropriate and possible in order to monitor development of endpoints in all included patients as thoroughly as possible, provided that the patient agrees.

15 STATISTICAL CONSIDERATIONS

15.1 Sample size considerations

The expected relative proportions of diabetes type 2 patients developing microalbuminuria within the planned study period are: 24% in patients at high-risk for diabetic nephropathy in the treatment group receiving active study medication, 40% in those patients at high-risk for diabetic nephropathy in the placebo treated group and 8.5% in those therapy-naive patients at low-risk for diabetic nephropathy. Using the samples size formula for two proportions test (α= 0.05 β=0.80), randomized (1:1), n=129 in each arm of the intervention group. To account for an expected drop-out frequency of approx. 10% we plan to randomize 300 high-risk patients to double blind treatment with spironolactone treatment or matched placebo.

High-risk patients are expected to comprise 15% of the screened and included patients. In order to identify 300 high-risk patients the study requires 2000 patients to be included. To take screen failures of approx. 25% into account, we expect to screen 2700 patients.

Recruitment will continue until a minimum of 300 high risk patients are enrolled in order to preserve statistical power. Furthermore, at least 2000 patients in the overall study cohort will be included.

15.1.1 Considerations of risk stratification

The study population will be stratified based on the CKD273 score at the screening visit. Patients with a CKD273 biomarker score above the level of 0.154 are classified as high-risk patients. The level of the cut-off is based on previous publications and unpublished data from >800 subjects with type 2 diabetes (20;22).

15.2 Study hypotheses

The null hypothesis of the study is that the probability for development of microalbuminuria is equal in both CKD273 pattern risk populations (low and high):

P(microalbuminuria | high-risk population) = P(microalbuminuria | low-risk population).

The alternative hypothesis is that the probability for microalbuminuria is lower in the low-risk population as judged by the CKD273 urinary proteomics pattern in comparison to patients in the high-risk population.

P(microalbuminuria | high risk group) > P(microalbuminuria | low risk group).

The significance level (type I error = probability of false rejection of the null hypothesis) for establishing the superiority of the CKD273 urinary proteomics pattern is 0.05 using a two-sided Cochran-Mantel Haenszel test.

15.3 Patient enrollment

Screening for eligible patients will be stopped, when at least 300 high-risk patients are enrolled in the interventions group and at least 2000 patients in the overall study.

15.4 Planned interim analyses

15.4.1 Safety review

The SAE and cases of death during IMP treatment in both arms of the intervention group will be reviewed by the Institute of Clinical Pharmacology of Hannover Medical School on an on-going basis. The Data Monitoring Committee will be provided with safety data to assess the overall safety of the study.

15.4.2 Efficacy review

No interim analysis for efficacy is planned.

15.5 Final analysis plan

Statistical analysis of the data will be performed by Steno Diabetes Center. A SAP will be written and signed before database lock and unblinding of the entire treatment code. Methods for imputation and type of censoring will be clarified in the SAP.

The primary efficacy analysis will be performed for all patients randomized, which is the intent-to-treat population. In addition, the primary and secondary efficacy endpoints will also be analyzed for the per-protocol population. The per-protocol analysis population includes all patients who were randomized into the double-blind study received study medication for at least 3 days.

For all analyses a p value <0.05 is considered significant.

Tables will be created for baseline variables, and comparisons performed between low- and high-risk population, and in the intervention group for active vs. placebo treatment. Furthermore, tables with observations from study visits will be provided for the observational part and for the intervention study.

Analyses not described here or in the SAP will be considered exploratory/post hoc analyses.

15.6 Analysis of primary endpoint

The primary endpoint is defined as time to the first occurrence of microalbuminuria (UACR >30 mg/g and <300 mg/g) in 2 out 3 sterile first morning void urine samples with a geometric mean of UACR >30% above initial geometric mean UACR OR geometric mean > 40 mg/ g in three samples. The baseline urinary albumin-to-creatinine ratios are the geometric mean of three measurements obtained during the “run-in" period.

It is expected, that the number of patients who will drop out between randomization and occurrence of microalbuminuria will be low (<15%).

If those cases where drop out occurs before sampling during the study period, prior to unblinding the treatment code it will be specified in the statistical analysis plan, whether these patients have to be considered as a treatment failure in the analysis of the primary endpoint.

The predictive value of a positive and negative test, specificity and sensitivity of the test in relation to prediction of development of the primary endpoint will be evaluated. To assess whether CKD273 classifier (the marker) improved risk prediction of the primary outcome we determined the concordance statistic (c-statistic) and Integrated Discrimination Improvement (IDI). Both parameters measure the discriminative ability of a marker in a model to distinguish between people who will or will not develop the outcome (in case of predictive methods).

The primary endpoint will be analyzed in the total population with comparisons of high- vs. low-risk pattern and active vs. placebo treatment using a Cox proportional-hazards regression model with treatment as a fixed effect; the baseline urinary albumin-to-creatinine ratio will be logarithmically transformed (base 10) as a covariate, and a two-tailed Wald chi-square test with an alpha level of less than 0.05; hazard ratios and two-sided 95 % confidence intervals will be calculated. Kaplan Meier plots of the groups will be made.

Secondly, the analyses will be adjusted for age, gender, center, HbA_{1c} and BP. Thirdly, further adjustment for any baseline demographic variables which are significantly different between the groups and related to albuminuria at baseline will be calculated. In addition adjustment will be made for significant changes in RAAS blockade or treatment with aldosterone receptor antagonist as concomitant medication during the study period.

A third analysis will plot and compare all three groups (low, high placebo and high active treatment) using a log-rank test.

15.7 Analysis of secondary endpoints

All secondary endpoints as described in section 5.5 will be analyzed to test if there is a difference in development of endpoints over time between a) active vs. placebo treated patients, and b) high-risk and low-risk population based on CKD273. As described in section 15.5.1 the analyses will initially compare low-risk with high-risk placebo treated patients, and secondly all high-risk patients. Adjustments will be performed as for the primary endpoint.

In addition to time to event (survival) analyses for discrete endpoints, analyses will evaluate the change over time in geometric mean UACR as slope analyses unadjusted as well as adjusted for confounders as described for the primary endpoint, comparing slope in the high-risk population with active vs. placebo and between low-risk and high-risk population the total population. Similar analyses will be performed with change in eGFR with time as the endpoint.

In the analyses with time to cardiovascular events, only time to first event in case of more than one event in a patient will be included.

In addition to the above described analyses comparing endpoints or slopes in high- vs. low-risk population or active vs. placebo, explorative analyses will also evaluate if the CKD 273 pattern and other baseline variables or changes in variables from baseline to 13 weeks visit in the high-risk population are predictive of development of endpoints.

Further analyses may be described in the SAP before database lock.

15.8 Analysis of safety endpoints

The exposure of study treatment will be characterized by number of administrations and the cumulative dose for both treatment groups.

AEs will be coded using the MedDRA via MARVIN.

An overview of all eCRF-documented AEs will be prepared. For all these categories of AEs (see section 13.3) separate frequency tables will be provided presenting the AEs by MedDRA preferred terms, SOC and treatment groups.

In addition AEs leading to death and AEs leading to discontinuation of study medication will be summarized for the two treatment groups.

Safety laboratory results will be classified by grade according to CTCAE version 4.0. The worst on-study grades after the first dose of study medication will be summarized. Only patients with post-baseline laboratory values will be included in these analyses. Furthermore, summary statis-

tics (mean, median, SD, SEM, range) will be calculated for the laboratory values at the various visits and for the changes from baseline. Baseline values are defined as the last available value prior to start of study treatment.

Corresponding descriptive summaries will be generated for vital signs.

16 QUALITY ASSURANCE

16.1 Site monitoring

To ensure compliance with the protocol, to legal and regulatory requirements applicable for clinical trials and with the ethical principles of Declaration of Helsinki and ICH-GCP, monitoring visits for source data verification by members of HCTC will be scheduled to take place during the study.

Before the start of the study, pre-study visits will be conducted and each participating center will be trained via initiation visits. Further visits during the study will be performed, according to patient recruitment rate. Finally, after the last patient has completed the study a close-out visit will be conducted.

During site visits, the monitor should review drug accountability records and document retention (investigator's study file) as well. Additionally, the monitor should observe clinical study procedures and discuss any problems with the investigator(s) and/or the trial staff.

All original data should be readily available for review during scheduled monitoring visits. Reviewing the eCRFs for completeness and clarity, and cross-checking with source documents will be required, and the investigator assures HCTC of support at all times.

16.2 Site audit an inspections

Audits (by the sponsor) and inspections (by regulatory authorities) may be performed in order to verify that the clinical study is performed according to the study protocol as well as to other applicable regulatory requirements. The auditor or inspector is independent in regard to personnel involved in the conduct of this clinical trial. This may occur at any time from start to after closure of the study.

17 ETHICAL, REGULATORY AND ADMINISTRATIVE ASPECTS

17.1 Investigator responsibilities

Investigator responsibilities are set out in ICH-GCP and in the Consortium Agreement (project no. 279277).

Investigators must enter study data in eCRFs within 2 weeks after the visit. The investigator will permit study-related monitoring visits and audits by the sponsor or its representatives as well as regulatory inspection(s). The investigator must provide direct access to the study center's facilities, to source documents, and to all other study documents.

The investigator or a designated member of the investigators staff must be available at some time during monitoring visits to review data and resolve any queries and to allow direct access to the patient's records (e.g., medical records, office charts, hospital charts, and study related charts) for source data verification. The data collection must be completed prior to each monitoring visit so that the accuracy and completeness may be checked.

The investigators reporting responsibilities regarding AEs are described in section 13.

17.2 Regulatory requirements

The study will be performed under ICH-GCP requirements and national laws and regulations depending on each country.

17.3 Justification for selection criteria

The justification of the second inclusion criteria limiting the age of eligible patients to 75 years is due to the expected prevention potential of the trial. Patient included in the trial must have persistent normoalbuminuria and therefor no sign of diabetic nephropathy at the time of inclusion. The time course of diabetic nephropathy will usually be >10 years from the onset of microalbuminuria to overt kidney disease. Since the aim of the study is to prevent kidney disease (onset of microalbuminuria) we consider it unethical to start preventive medical treatment in patient where the potential unset of disease is after the expected life time.

17.4 IEC and NCA

This protocol and any accompanying material provided to the patient (such as patient information sheets or informed consent) as well as any advertising will be submitted by the sponsor to a country specific IEC. Written favorable opinion from the IEC must be obtained before starting the study in the respective country. Furthermore, written approval of the respective NCA and / or other regulatory authorities, if applicable, must be available before study-specific procedures.

17.5 Patient information and informed consent

It is the responsibility of the investigator to obtain written informed consent from each individual participating in this study after adequate explanation of the aims, methods, objectives and potential hazards of the study. It should be made clear that refusal to participate or withdrawal from the trial at any stage is without any prejudice to the patient's subsequent care.

No patient should be obliged to participate in the trial.

The patient must be given ample opportunity to enquire about details of the trial. If there is any doubt as to whether the patient has understood the written and verbal information, the patient should not enter the study.

The patient must be made aware that the monitors, auditors and regulatory authorities will be granted direct access to the study patient source medical records without violating patient confidentiality, and to the extent permitted by applicable regulations.

If the patient agrees to participate in the study he will be asked to sign and date a consent form which will be kept by the investigator. The patient information leaflet and a copy of the signed informed consent will be provided to the patient.

The signed consent forms will be retained by the investigator and made available (for review only) to the study monitors, auditors and inspectors.

If new safety information results in significant changes in the risk/benefit assessment, in the duration of the intervention study, the consent form should be reviewed and updated if necessary. All patients (including those already being treated) should be informed of the new information, given a copy of the revised form and asked to give their consent to continue in the study.

17.6 Insurance

Every patient participating in the study will be insured according to national laws and regulations. All patients will be informed about their rights and obligations in regard to insurance policies before participating in the study.

Insurance company:

HDI-Gerling Industrial Insurance Company
1 Great Tower Street
London, UK, EC3 R5AA
Contact person: Vicky Cockayne
Tel: 020 7696 8099
Fax: 020 7696 8444

17.7 Financial aspects

The study is supported by EU under the FP7 program. This will be made clear to patients in the consent form.

No financial compensation will be given to the patients for participating in the study.

18 PROTOCOL AMENDMENTS AND DEVIATIONS

18.1 Protocol amendments

Any amendment to this protocol must be approved by the sponsor and coordinating investigator. Favorable opinion of leading IEC and NCA approval will be obtained before any substantial amendment is implemented.

18.2 Protocol deviations

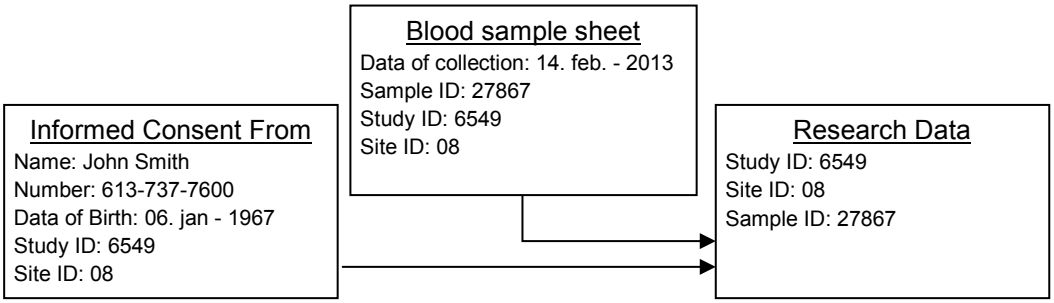
When an emergency occurs that requires a deviation from the protocol for a patient, a deviation will be made only for that patient. A decision will be made by the PI, the coordinating investigator and the sponsor as soon as possible to determine whether or not the patient (for whom the deviation from protocol was effected) is to continue in the study. The patient's medical records will completely describe the deviation from the protocol and state the reasons for such deviation.

19 DATA MANAGEMENT

All study data will be collected by the investigator and/or other study personnel. A clinical trial data base is provided, in which the data are entered via an eCRF. Authorized and trained staff of the study centers will enter the data in the eCRF. Only SAEs will be additionally documented and notified on paper forms.
Verification of the data in the eCRF occurs by monitoring as well as via range, validity, and consistency checks programmed in the system. In certain cases, queries can be detected by the study software or by authorized study staff. Based on the queries, the investigator can review and answer the found discrepancies directly in the system. All changes of data entered in the eCRF can be followed by an audit trail.
A quality control will be performed before the database is closed. This procedure is documented. Finally, data transfer takes place for statistical evaluation.

19.1 Data retention

We will pseudonymize (de-identify) rather than permanently (and irreversibly) anonymize data in the data retention process. Depersonalization means that data with identifying information is collected, but the identifying information is then severed from the personal health information data in the research database and is stored separately. Identifying information linking to the research database will be kept at the local site. No identifying information will be stored in an electronic way.



19.2 Security

Data transferred between all local sites and the central data repository is encrypted. This will be realized with the help of HTTPS (HTTP over SSL). To prevent unauthorized access to the Research Database, each user has to login with his personal user name and password. Passwords have to be changed in a regular manner and have to meet a defined complexity (minimum length, mix of characters, numbers and special characters).

19.3 Data protection

All study staff has to give due consideration to data protection and medical confidentiality. The collection, transfer, storage and analysis of personal study-related data are performed pseudonymized according to national regulations.

20 DATA HANDLING AND RECORD KEEPING

20.1 Analyses and reporting

Data are captured in a web-based eCRF (MARVIN). For each patient enrolled an eCRF must be completed after the patients visit. This also applies to records for those patients who fail to complete the study.

The investigator should ensure the accuracy, completeness, legibility, and timelines of the data reported in the eCRFs and all required reports.

Data will be analyzed and reported as soon as the data of the treatment period have been collected and cleaned.

20.2 Study records retention

The sponsor must ensure that the records and documents pertaining to the conduct of the study and the distribution of the study drug, that are source documents (original documents, data, and records [e.g., hospital records; clinical and office charts; laboratory notes; memoranda; patient's diaries or evaluation checklists; pharmacy dispensing records; recorded data from automated instruments; copies or transcriptions certified after verification as being accurate copies; microfiches; photographic negatives, microfilm, or magnetic media; x-rays; patient files; and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical study; documents regarding patient treatment and study drug accountability; original signed informed consents, etc.]) be retained by the investigator for as long as needed to comply with national regulations (minimum 15 years). The investigator agrees to adhere to the document/records retention procedures by signing the protocol.

21 PUBLICATION POLICY

21.1 General terms

The data of the study will be published in international, peer reviewed journals in accordance with Consortium Agreement section 8 and Grant Agreement section II 30. All publications related to the study will acknowledge the support of EU FP7 Proposal No: 279277-2 in accordance with Grant Agreement section II. 30.4.

At the annual assembly of the study group, membership of the writing group will determined and will depend on number of patients included, or other commitment to the study.

21.2 Study publications

The primary publications are foreseen, one presenting the results of the randomized study, and one presenting the results of the observational study. In addition two other publication will are foreseen. One regarding design and rationale of the study and one concerning the baseline condition.

The papers will be written by a writing group with the coordinator as chairman. Authors will be members of the writing group on behalf of the PRIORITY study group, with acknowledgement of all relevant site personnel as the PRIORITY study group.

The trial will also be registered at the EU Clinical Trials Register according to current publication guidelines.

21.3 Substudy publications

Any publication from a substudy must be conducted in accordance with Consortium Agreement section 8 and Grant Agreement section II 30.

22 SUBSTUDIES

Substudies are encouraged, but must not interfere with the main study. The Scientific Committee must approve all substudies and any additional funding (amendment fee, lab and personnel cost, monitoring etc.) required must be covered by the study centre(s) undertaking the substudy.
Suggestions (1 page synopsis) should be forwarded to the Scientific Committee (Christian.Delles@glasgow.ac.uk). This also applies for suggestions regarding additional analyses of data available from the main study datasets.

23 REFERENCE

Scientific references are available upon request to the sponsor.

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PROTOCOL SIGNATURES

This protocol has been approved by the Sponsor. The following signature documents this approval.

Prof. Peter Rossing MD, DMSc Date

This protocol has been approved by the International Coordinating Investigator. The following signature documents this approval.

Prof. Peter Rossing MD, DMSc Date

This protocol has been approved by the study statistician. The following signature documents this approval.

Tine Willum Hansen MD, Ph.D. Date

INVESTIGATOR STATEMENT

By my signature, I agree to personally supervise the conduct of this study and to ensure its conduct in compliance with the protocol, informed consent, IEC procedures, the Declaration of Helsinki, ICH-Good Clinical Practices guidelines, and the applicable parts of the European Regulations or local regulations governing the conduct of clinical studies.

Name of Study Center

Printed Name of (Principal) Investigator

Signature of (Principal) Investigator Date

24 APPENDIX 1: TREATMENT GUIDELINES FOR HYPERKALEMIA

24.1 General principles

Patients will have their plasma (or serum) potassium checked by a local laboratory at every visit and no patient can be included with hyperkalemia at Screening based on plasma potassium level >5.0 mmol/L or serum potassium level >5.4 mmol/L according to exclusion criteria. All patients not receiving IMP will be treated according to local guideline. All events of hyperkalemia must be recorded in the eCRF for all patients in the intervention group, in accordance with section 13.

24.2 Potassium level > 0.4 and < 0.9 mmol/L above upper reference range

24.2.1 Confirmation

Confirm potassium concentration in a non-haemolysed sample.

24.2.2 Change of dietary intake

Patients will be given advice to lower dietary intake of potassium and restriction of food/drinks with high potassium content. (e.g. fruit juice, melon, bananas etc.)

24.2.3 Review of medical treatment

Review of medical regimens for agents known to cause hyperkalemia (including over-the-counter medication). Consider reduction or discontinuation of these agents, *only if it is clinically acceptable*.

- Potassium supplements
- Non-Steroidal anti-inflammatory drugs (NSAIDs)
- Cyclo-oxygenase-2 (COX-2) inhibitors
- Trimethoprim
- Herbal supplements

24.2.4 Additional treatment

Consider reduction in ACE or ARB or increase in diuretics.

24.2.5 Follow-up

Repeat measurement of potassium within 5 days.

If potassium remains in the level of > 0.4 and < 0.9 mmol/L above upper reference range regularly monitoring of plasma potassium to ensure stability shout be done. (Suggested once monthly).

24.3 Plasma potassium level ≥ 0.9 and < 1.4 mmol/L above upper reference range

Apply all instruction listed in section 24.2. In addition:

24.3.1 Study medication

Temporarily discontinue study medication.

Evaluate continuation of the study medication after 10 days (according to section 24.5).

24.3.2 Additional treatment

Consider treatment with non-potassium sparing diuretics (or increased doses of ongoing treatment).

Consider treatment with natriumpolystyrensulfonat.

Reduce treatment dose of ACE inhibitor or Angiotensin II receptor blocker (ARB)

24.3.3 Follow-up

Repeat measurement of potassium within 3 days.

24.4 Plasma potassium level ≥ 1.4 mmol/L above upper reference range

Apply all instruction listed in section 24.2. In addition:

24.4.1 Study medication

Immediately discontinue study medication.

24.4.2 Follow-up

Urgently evaluate the patient and treat hyperkalemia as clinically indicated.

24.5 Continuation of study medication

If the potassium level is < 0.9 mmol/L above upper reference range the patient can continue taking study medication.

Patients who continue study medication after temporary discontinuation of study medication must have the potassium measured according to section 24.2.5

In a patient who has experienced a potassium level of ≥ 1.4 mmol/L above upper reference range, and in whom the potassium fails to decrease to < 0.9 mmol/L above upper reference range after the procedures described above have been tried, the study medication must be discontinued permanently and it should be recorded in the eCRF.

In case of repeated episodes of confirmed hyperkalemia after the attempt to re-start study medication following discontinuation the study medication must be discontinued permanently and it should be recorded in the eCRF.

Patients, who discontinue the study medication, will remain in the study and be followed for the full duration of the study.