Randomized, controlled interventional trial to investigate the efficacy of amiloride for the treatment of edema in human nephrotic syndrome

Short Title of Clinical Study	***Amiloride in nephrotic syndrome (AMILOR)***
Version / Protocol Code:	**2.1 / AmiloridNS-01**
EudraCT-number	**2019-002607-18**
Date of Protocol	**01.03.2021**
Register-number	
Sponsor of the Study	**University Hospital Tuebingen,** **Medical Director, Prof. Dr. med. M. Bamberg** **Director of Administration, G. Sonntag** **Geissweg 3** **72076 Tuebingen** **Germany**
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Investigational drug	**Amiloride**
Comparator	**Furosemide**
Summary of the revision history (amendments)	**Version 1.1 from 10.01.2020** **Amendment 1 from 01.03.2021**

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I.b) List of Figures

II. Signature Page

The present trial protocol was subject to critical review and has been approved in the present version by the persons undersigned.

Sponsor: The University Hospital Tuebingen is sponsor for the purpose of § 4 (24) German Drug Law with complementary regulations. The internal responsibility to comply with the obligations of the sponsor in terms of these regulations stays with Prof. Dr. Ferruh Artunc.

Date: 16.3.21 Signature of the delegated Sponsor:

Name (block letters): Prof. Dr. Ferruh Artunc
Function: Delegated sponsor and person in charge to meet the obligations of the sponsor

Date: 11.3.2021 Signature:

Name (block letters): Marion Schütt
Function: Biometrician

Declaration of the Site Principal Investigator

By my signature, I agree to supervise personally the conduct of this study at my study site and to ensure its conduct is in compliance with the protocol, informed consent, the national laws, the ICH Good Clinical Practices Guidelines and the Declaration of Helsinki. I will train the involved personal accordingly.

Date: 16.3.21 Signature:

Name (block letters): Prof. Dr. Ferruh Artunc

Function: Site Principal Investigator

Date: 16/03/2021 Signature:

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IV. Abbreviations

ACE	Angiotensin converting enzyme
ADR	Adverse Drug Reaction
AE	Adverse Event
AMG	German Drug Law (Deutsches Arzneimittelgesetz)
BCM	Body Composition Monitor, Fresenius Medical Care AG & Co
BDSG	Bundesdatenschutzgesetz
BfArM	Bundesinstitut für Arzneimittel und Medizinprodukte
BGA	Blood gas analysis
CRF	Case Report Form
CTCAE	Common Toxicity Criteria for Adverse Events
CI	Coordinating Investigator
DBL	Data Base Lock
EC	Ethics Committee
ECW	Extracellular water
ENaC	Epithelial sodium channel
FCBP	Females of childbearing potential
FSI	First Subject In
GCP	Good Clinical Practice
GCP-V	Good Clinical Practice Ordinance (GCP-Verordnung)
HCT	Hydrochlorothiazide
IC	Informed Consent
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
IIT	Investigator Initiated Trial
IKEAB	Institut für Klinische Epidemiologie und angewandte Biometrie Tübingen
IMP	Investigational Medicinal Product
ISF	Investigator Site File
K	Potassium (chemical element)
LSI	Last Subject In

LSO	Last Subject Out
n.a.	Not applicable
Na	Sodium (chemical element)
NKCC2	Sodium-potassium-chloride cotransporter
OH	Overhydration
PEI	Paul-Ehrlich-Institut
RAAS	Renin-angiotensine-aldosterone system
SAP	Statistical Analysis Plan
SAE	Serious Adverse Event
SmPC	Summary of Product Characteristics
SDV	Source Data Verification
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File

V. Synopsis

Sponsor	University Hospital of Tuebingen represented by Medical Director: Prof. Dr. med. M. Bamberg Director of Administration: G. Sonntag
Title	Randomized controlled interventional trial to investigate the efficacy of amiloride for the treatment of edema in human nephrotic syndrome
Short Title	Amiloride in nephrotic syndrome
EudraCT-Number	2019-002607-18
Internal Study Code	AmiloridNS / AMILOR
Investigator and Sponsor's authorized person	Prof. Dr. med. Ferruh Artunc University Hospital Tuebingen Department of Internal Medicine IV Otfried-Mueller-Strasse 10, 72076 Tuebingen phone: 07071-29 82712; fax: 07071-29 25215 e-mail: ferruh.artunc@med.uni-tuebingen.de
Study Design:	Phase IIIb, monocenter, interventional, two-arm, randomized, controlled clinical trial
Number of Patients	n = 44 (n = 18 per group, parallel design)
Patient Population	Patients with nephrotic syndrome

Length of study/ Time Lines	Duration of total trial (estimated): 36 months Duration of clinical part (FSI – LSO) 24 months Duration for individual patient: 23 days (Study treatment: 16 days + Follow-up: 7 days) FSI (First Subject In): 06/2020 LSI (Last Subject In): 05/2022 LSO (Last Subject Out): 06/2022 DBL (Data Base Lock): 09/2022 Statistical Analyses Completed: 12/2022 Trial Report Completed: 06/2023
Aim of the Study	To prove the efficacy of amiloride for reduction of edema and overhydration in human nephrotic syndrome in comparison to standard medication with furosemide
Objectives/Endpoints	Primary endpoint: Decrease of overhydration (OH) after 8 days, compared to baseline. OH is measured using bioimpedance spectroscopy with the device Body Composition Monitor (Fresenius Medical Care AG & Co; BCM), and expressed as percent of extracellular water (% ECW). Secondary endpoints: 1. Decrease of OH after 16 days 2. Decrease of body weight after 8 and 16 days 3. Decrease of edema circumference after 8 and 16 days 4. Decrease of systolic and diastolic blood pressure after 8 and 16 days 5. Increase of urine volume and natriuresis after 8 and 16 days 6. Course of plasma renin activity and serum aldosterone concentration after 8 and 16 days 7. Number of required changes of dose of study medication 8. Need for co-medication with HCT after 8 days 9. Occurrence of adverse events

Inclusion Criteria	1. Acute nephrotic syndrome with proteinuria > 3 g/day and formation of edema. 2. Age ≥ 18 years at the time of signing the informed consent. 3. Understand and voluntarily sign an informed consent document prior to any study related assessments/procedures. 4. Ability to adhere to the study visit schedule and other protocol requirements. 5. Use of adequate thrombosis prophylaxis due to the increased risk of thrombosis in nephrotic syndrome and the expected fluctuations in volume balance during study participation 6. Subject (male or female) is willing to use highly effective methods of contraception according to the "Clinical trial fertility group" recommendations (http://www.hma.eu/fileadmin/dateien/Human_Medicines/01About_HMA/Working_Groups/CTFG/2014_09_HMA_CTFG_Contraception.pdf) during treatment and for 28 days (male or female) after the end of treatment (adequate: intrauterine device, intrauterine hormone-releasoing system, bilateral tubal occlusion, vasectomized partner[1], sexual abstinence[2]; due to the increased risk of thrombosis in nephrotic syndrome, no hormonal contraceptives are recommended). 7. Female Patients of childbearing potential (WOCBP)[3] must agree to pregnancy testing before inclusion in the study. 8. Female Patients must agree to abstain from breastfeeding during study participation and 28 days after study drug discontinuation. 9. All subjects must agree not to share medication. [1] Vasectomized partner is a highly effective birth control method provided that partner is the sole sexual partner of the WOCBP trial participant and that the vasectomized partner has received medical assessment of the surgical success [2] In the context of this guidance sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject [3] For the purpose of this document, a woman is considered of childbearing potential (WOCBP), i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.

Exclusion Criteria	1. Severe reduction of kidney function: Creatinine clearance or calculated GFR < 30 mL/min/1.73m² or acute kidney injury KDIGO stage 2 or 3 or anuria. 2. Hypovolemia or dehydration. 3. Uncontrolled diabetes mellitus. 4. Hypotension, systolic blood pressure < 90 mmHg 5. Hyperkalemia, plasma potassium concentration > 4.8 mmol/l 6. Hypokalemia, plasma potassium concentration < 3.3 mmol/l 7. Hyponatremia, plasma sodium concentration < 128 mmol/l 8. Hypercalcemia, ionized calcium > 2.0 mmol/l or total albumin corrected calcium > 3.0 mmol/ 9. Signs of cardiac decompensation (orthopnoe, dyspnoe NYHA IV) 10. Hepatic coma or precoma 11. Symptoms of gout 12. Current therapy with potassium-sparing diuretics (e.g. spironolactone) or potassium supplements 13. Women during pregnancy and lactation. 14. History of hypersensitivity to the investigational medicinal product, comparator or co-medication or to any drug with similar chemical structure or to any excipient present in the pharmaceutical form of the investigational medicinal product, comparator or co-medication. 15. Any other clinical condition that would jeopardize the patient's safety while participating in this clinical trial. 16. Active participation in other clinical trials or observation period of competing trials.

Statistics, Safety Variables and Stopping Rules	Statistics: Primary endpoint of the study is the decrease of overhydration (measured using bioimpedance spectroscopy) after 8 days, compared to baseline. Based on data from a baseline sample (n = 14) and clinical experience on reduction of overhydration, baseline overhydration is expected to be 26.5 % of extracellular water (standard deviation 12.3; normal distribution is assumed) and it is expected that the overhydration will be reduced by 90% in the amiloride group and by 50% in the furosemide group. For the final t-test for two groups, a one-tailed significance level of 0.05 and a power of 80% should be ensured. From these conditions results a sample size of n = 18 patients per group (total n = 36; calculated for a t-test with the nQuery 7.0 program). In order to take into account dropouts, the sample size is defined as n = 22 per group (total n = 44). Safety variables: - General safety parameters: adverse events, concomitant medication and check of study medication - Safety parameters for specific risks of the study: serum potassium and sodium levels, plasma creatinine concentration

Study Intervention/ Study Medication	Substance 1 (Investigational medical product): Amiloride (Modamide®), initial dose 5 mg Manufacturer: Laboratoires GERDA, 24 rue Erlanger, 75016 Paris / France Unit strength: 5 mg Route of administration: oral, once daily Substance 2 (Comparator): Furosemide (Furosemid-ratiopharm®), initial dose 40 mg Manufacturer: ratiopharm GmbH, Graf-Arco-Straße 3, 89079 Ulm, Germany Unit strength: 40 mg Route of administration: oral, once daily Substance 3 (Co-medication): Hydrochlorothiazide (HCT-Hexal®) 12,5 mg Manufacturer: Hexal AG, Industriestraße 25, 83607 Holzkirchen / Germany Unit strength: 25 mg Route of administration: oral, once daily Participants are randomized to start medication with Amiloride 5 mg (trial arm 1) or Furosemide 40 mg (trial arm 2) at day 0. Dose adjustments depending on course of overhydration, sodium and potassium level in blood gas analysis and plasma creatinine are performed on day 2, 5, 8 and 12. In case of therapy-refractory overhydration co-medication with HCT is started at day 8.
Participating Centers	University Hospital Tuebingen, Department of Internal Medicine IV

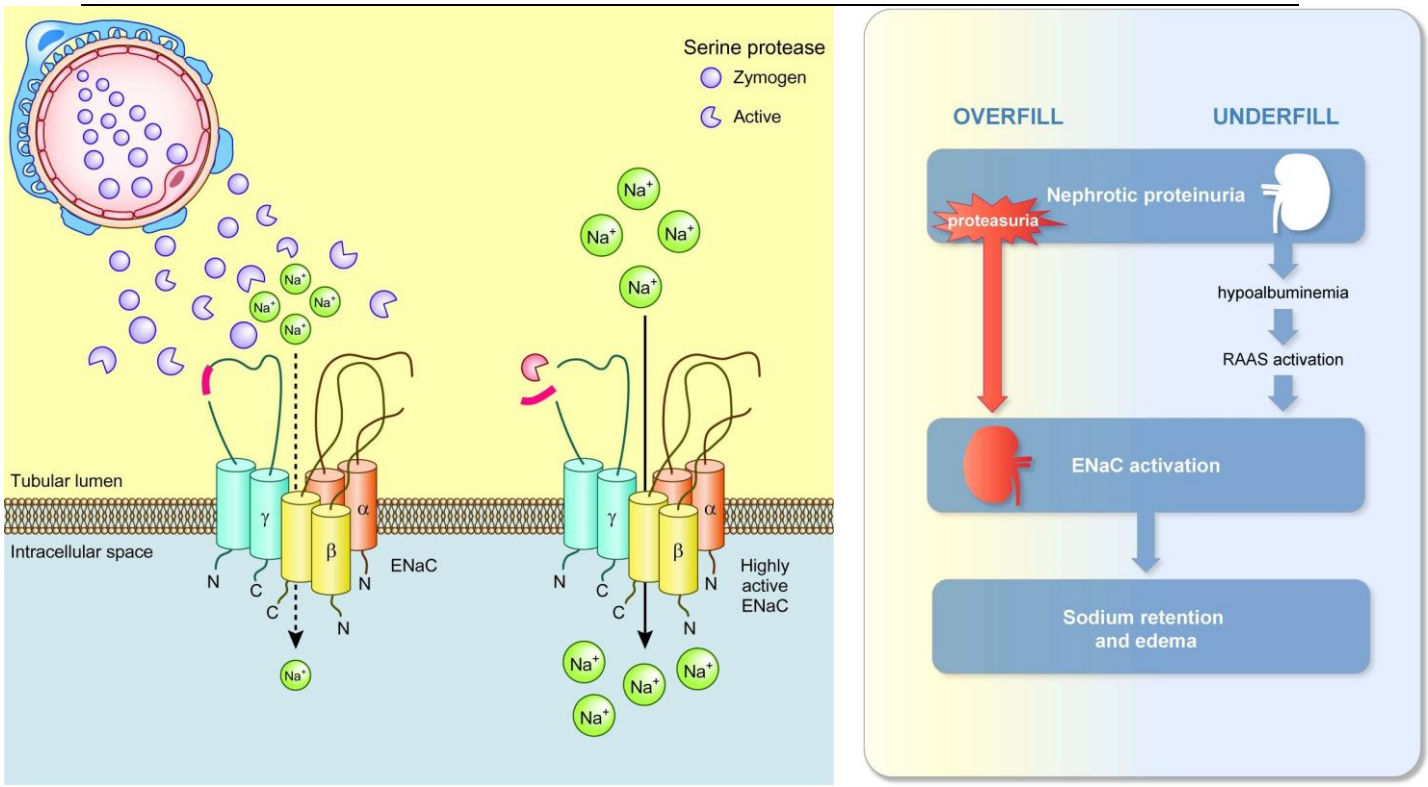
GCP-compliance	The present trial will be conducted in accordance with the valid versions of the trial protocol and the internationally recognised Good Clinical Practice Guidelines (ICH-GCP), including archiving of essential documents and the applicable regulatory requirements.
Financing	Intramural funding by the AKF (“Angewandte Klinische Forschung”) program has been approved.

1. Introduction

The nephrotic syndrome is characterized by a high ("nephrotic") proteinuria> 3.5 g / day with resulting hypoalbuminemia, as well as by edema and hyperlipidemia. Affected patients suffer from massive generalized edema involving the face and eyelids, effusions into the body cavities pleura, peritoneum and rarely pericardium as well as increased body weight. [1]

Mechanisms of edema formation are still not fully understood, but two models have been established [2]: The underfill hypothesis describes the development of edema as a consequence of decreased oncotic pressure due to hypoproteinemia that is caused by renal protein loss. As a result, intravascular volume deficiency developes, that activates the renin-angiotensin-aldosterone-system (RAAS) leading to a secondary renal sodium retention. In contrast, the overfill theory postulates a primary stimulation of renal sodium and water retention by the diseased kidney, resulting in volume overload with increased hydrostatic pressure and volume shift into the interstitium. [3] In this situation, RAAS components are typically suppressed [4].

Recent animal studies have demonstrated that primary renal sodium and water retention could be caused by activation of the epithelial sodium channel (ENaC) in the distal tubule system of the kidney by aberrantly filtered serine proteases (Figure 1) [5-7]. ENaC activation in the nephrotic mouse model can be prevented by the serine protease inhibitor aprotinin [8]. The pathophysiologically relevant serine proteases originate from the plasma and their identification is the subject of current investigations of our group [9]. So far, urokinase, plasminogen or plasma kallikrein have been identified as serin proteases that can activate the ENaC in vitro and are also aprotinin-sensitive [3, 7, 8, 10-12]. Data from urine samples from nephrotic humans and mice are identical with regard to the content of various serine proteases [9]. We have reported an aprotinin-sensitive proteasuria also in human proteinuric urine [8] and plasminuria was associated with overhydration in chronic kidney disease [13]. Therefore, it can be assumed that the pathophysiological processes described in the mouse model also apply to humans.



a b

Figure 1: Scheme illustrating the pathophysiology of ENaC-mediated sodium retention (a) and model of sodium retention in nephrotic syndrome (b) [3]

Aprotinin-sensitive serine proteases enter the urine in glomerular disease and can activate ENaC endoluminally by cleavage at the γ subunit (a). Proteasuria, as part of nephrotic proteinuria, results in sodium retention according to the overflow theory through this direct ENaC activation; if the proteinuria is sufficient to induce hypoalbuminemia and an "underfill", the ENaC might be activated additionally by RAAS (b).

In addition to the disease specific treatment commonly involving immunosupression, supportive therapy of patients with acute nephrotic syndrome includes reduction of proteinuria by RAAS blockade, blood pressure control, lipid lowering, thromboembolic prophylaxis and the correction of volume overload [1], which is achieved by a dietary salt restriction and diuretic therapy. Currently, loop diuretics are preferred as they have the highest diuretic potency [14]. However, the diuretic effect of loop diuretics is lower in nephrotic syndrome than in other forms of edema or in healthy volunteers [1, 15]. A possible cause of this resistance to loop diuretics could be activation of ENaC [16] that is located distally to the sodium-potassium-chloride cotransporter (NKCC2), the target of loop diuretics. Sodium retention via ENaC may therefore counteract the inhibition of NKCC2. To overcome refractory edema, either higher doses of loop diuretics or combination with thiazide diuretics (e.g., hydrochlorothiazide, HCT) for sequential nephron blockade or with distally acting diuretics such as the EnaC inhibitors amiloride and triamterene blockers are conceivable [1].

Considering the recent evidence of increased EnaC activity after activation by aberrantly filtered serine proteases, EnaC inhibition appears to be a promising approach to treat nephrotic edema syndrome [3, 16]. In nephrotic mice with overactivated ENaC, there is a stronger natriuresis after administration of the same dose of the EnaC inhibitor amiloride compared to the healthy state, and a daily treatment with amiloride prevents edema formation completely [8, 11]. There are a few smaller systematic studies on the use of amiloride, in particular with regard to the treatment of arterial hypertension in patients with proteinuria without nephrotic syndrome [17-19]. In nephrotic syndrome, the use of the ENaC inhibitors amiloride or triamterene has been reported in case reports, but there have been no controlled clinical trials yet. In an impressive case report by Hinrichs et al., a 38-year-old patient with type 1 diabetes and severe nephrotic syndrome, who underwent intensive combined antihypertensive and diuretic therapy without achieving blood pressure control and amelioration of edema, was treated with additional amiloride 5 mg daily, which lead to a marked decrease of systolic blood pressure, weight and edema, and increase of sodium excretion [20]. ENaC inhibitor triamterene has been tested with positive results for its application in nephrotic syndrome longer time ago already [21, 22] and was also used in a recently reported case [16]. Our own clinical experience of treating nephrotic patients with amiloride (in combination with hydrochlorothiazide) also confirms the very good efficacy of amiloride-based diuretic therapy.

To give a résumé, activation of ENaC in nephrotic syndrome has been characterized in different mouse models by our group and others [3, 7, 8, 12, 23]; our investigations in patients with proteinuric kidney disease and nephrotic syndrome show that activation of ENaC by serine proteases also appears to be of central importance as a mechanism of edema formation in humans [12, 13]. These findings are now to be translated into an improved treatment of sodium retention and overhydration in patients with nephrotic syndrome. This randomized interventional trial investigates the superiority of amiloride to reduce edema in the nephrotic syndrome in comparison to the loop diuretic furosemide.

1.1. Trial Rationale / Justification

Treatment of resistant edema and overhydration in nephrotic syndrome can be challenging and we successfully use ENaC inhibitors (in combination with hydrochlorothiazide) in these patients. However, most clinicians treat nephrotic edema with higher doses of loop diuretics, or combination therapy with thiazide diuretics. Worldwide, ENaC inhibitors are rarely used in

nephrotic syndrome. This is due to lack of evidence of the role of activated ENaC and the effectiveness of ENaC inhibitors in human nephrotic syndrome. In this clinical trial, we aim to investigate the therapeutic effect of amiloride on the reduction of edema and overhydration in the nephrotic syndrome. A recent review from January 2019 on this topic highlights the urgent need for such a study and concludes with the phrase "it is time to undertake new clinical trials to direct clinical practice [in nephrotic syndrome]" [24].

In our study, patients with nephrotic syndrome will be randomized to medication with the investigational drug amiloride (study arm 1) or the comparator furosemide (study arm 2) for a study period of 16 days. In case of treatment resistant edema, hydrochlorothiazid (HCT) is added at day 8 in both study arms. To prevent bias, patients included in the study are randomized to start treatment with amiloride or furosemide. We have opted against blinding, as laboratory results required for safety reasons and for definition of dose adjustments will inevitably reveal to which study group each patient was randomized (hypokalaemia – furosemide, hyperkaliemia – amiloride). Duration of follow up period was determined based on clinical experience on the duration of the reversion of edema and overhydration. During follow up, the parameters body weight, extracellular water and overhydration (measured by bioimpedance spectroscopy), 24-hour natriuresis, blood pressure, plasma creatinine concentration, plasma renin activity, serum aldosterone concentration and serum sodium and potassium concentration will be measured. Overhydration as primary endpoint can be measured with bioimpedance spectroscopy as a validated, investigator-independent, reproducible method [25] and directly reflects changes of volume status that are to be evaluated. The number of n = 36 patients (n = 18 per group) was chosen on the basis of statistical considerations in order to be able to statistically significantly evaluate the primary endpoint of the study. The potential for recruiting the required number of patients has been assessed by counting patients with nephrotic syndrome presenting in the outpatient clinic of the department of nephrology of the University Hospital of Tuebingen during the last years. Additionally, agreement from Nephrologists running offices nearby to send in suitable patients was obtained.

1.2. Benefit / Risk Assessment

Overhydration and edema are serious symptoms that affect patients with nephrotic syndrome. Establishment of a more effective treatment of edema with adding ENaC inhibitors to standard treatment in human nephrotic syndrome has the potential to improve the clinical outcome of patients suffering from sodium retention. Known side effects of

amiloride, furosemide and HCT are listed in the respective Summary of Product Characteristics (SmPC). All drugs have been used for a long time, and their effects and side effects are well known and can be well estimated. Study specific relevant side effects are increase of plasma potassium level by amiloride, decrease of plasma potassium level by furosemide, and decrease of sodium level, increase of urine volume and decrease of body fluid by both diuretic regimens. To minimize risk of these potential side effects, the following precautions have been taken: to avoid occurrence of potential side effects, additional risk factors, such as decreased glomerular filtration rate or pre-existing electrolyte disorders are considered in the exclusion criteria; to recognize potential side effects early, volume status and electrolytes are monitored closely during study intervention phase; to rule out negative consequences of potential side effects, dose of study medication is adjusted according to changes of fluid status and electrolyte levels as well as renal function as specified in this protocol. All patients participating in the study receive adequate diuretic therapy of their overhydration due to nephrotic syndrome. Study participants benefit from gaining access to an intense treatment at our University hospital and its resources.

All measurements performed in the study are without health risks and all devices used in the study are used according to their intended purpose. Venipuncture can be uncomfortable and slightly painful for the patient. The total volume of blood drawings during the entire study period does not exceed 100 mL within 8 days and does not pose participants at health risk.

1.3. Advisory Committes

A Data and Safety Monitoring Board and a Scientific Advisory Board are not forseen for this small scale exploratory study.

2. Study Objectives

2.1. Primary Objective and Endpoint

In the light of our current results on ENaC activation by serine proteases aberrantly filtered into urine in nephrotic syndrome, we hypothesize that targeting ENaC with amiloride will lead to a more effective reduction of overhydration in nephrotic syndrome than standard treatment with the loop diuretic furosemide.

Primary objective of the study is therefore to prove the efficacy and superiority of amiloride for reduction of edema and overhydration (OH) in human nephrotic syndrome in comparison to standard medication with furosemide. Primary endpoint of the study is the decrease of overhydration after 8 days, compared to baseline. OH is measured using bioimpedance spectroscopy with the device Body Composition Monitor, Fresenius Medical Care AG & Co; BCM) and expressed as percent of ectracellular water (% ECW).

2.2. Secondary Objectives and Endpoints

Secondary endpoints represent further parameters to evaluate course of overhydration and regulation of body volume status after initiation of study medication with amiloride or furosemide. Secondary endpoints include

1. decrease of OH after 16 days, measured using bioimpedance spectroscopy and expressed as % ECW
2. decrease of body weight after 8 and 16 days
3. decrease of edema circumference after 8 and 16 days, measured as the largest extent on the lower leg
4. decrease of systolic and diastolic blood pressure after 8 and 16 days
5. increase of urine volume and natriuresis after 8 and 16 days, determined from urine collection over 24 hours
6. course of plasma renin activity and serum aldosterone concentration after 8 and 16 days
7. number of required changes of dose of study medication
8. need for co-medication with HCT after 8 days
9. occurrence of adverse events

3. Study Design

The study design is a phase IIIb, monocenter, interventional, two-arm, randomized, open-label controlled clinical trial. We decided for a study type with randomization as method against bias and have omitted blinding, as laboratory results required for safety reasons and dose adjustments will inevitably reveal to which study group patients have been randomized. Overall study design is shown in figure 2. Participants are randomized to start medication with amiloride 5 mg (trial arm 1) or furosemide 40 mg (trial arm 2) at day 0. Dose adjustments depending on course of overhydration, sodium and potassium level in blood gas analysis and plasma creatinine are performed on day 2, 5, 8 and 12 as defined in section 6.6.1. In case of therapy-refractory overhydration, co-medication with HCT 12,5 mg is started at day 8. Last clinical study visit is at day 16. Follow up ends at day 23.

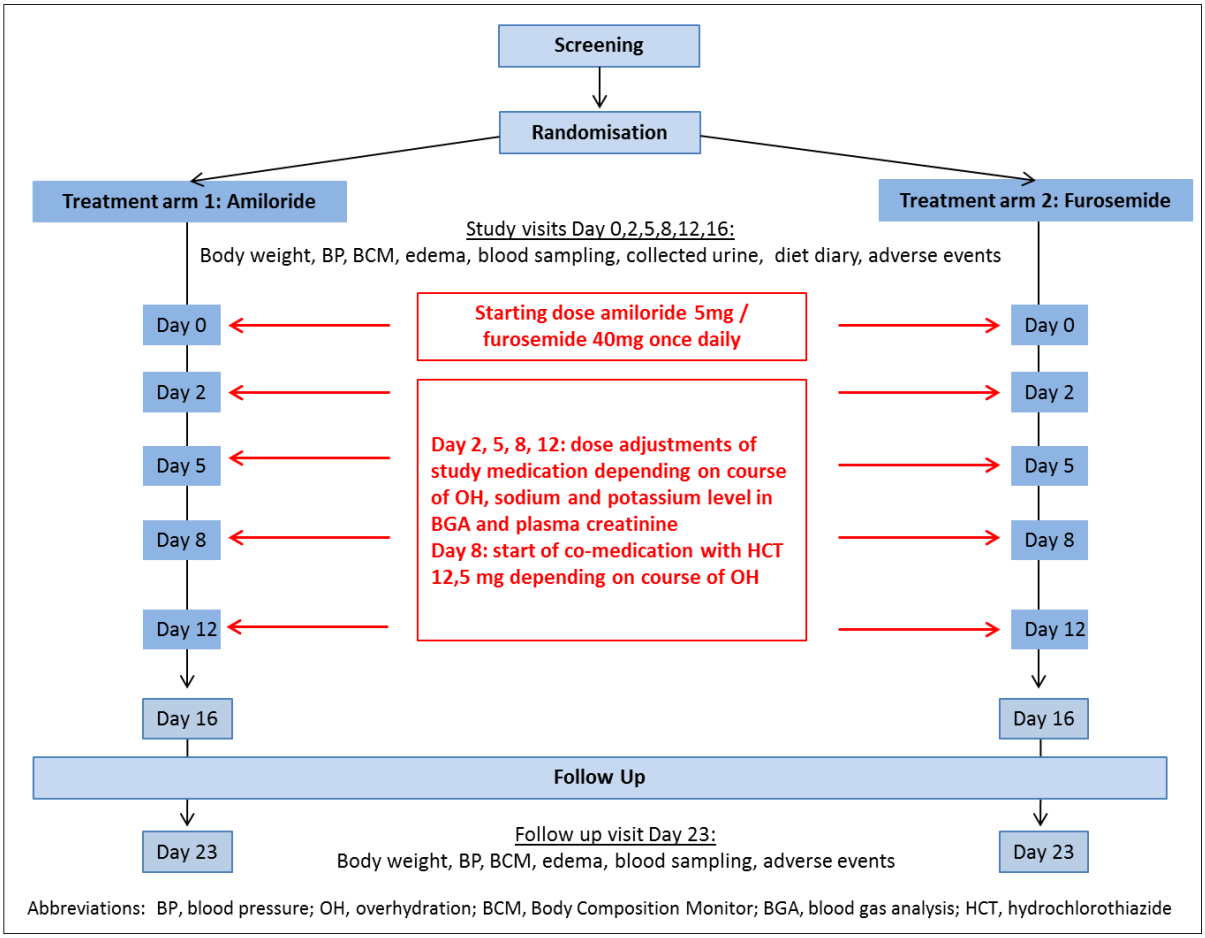


Figure 2: Overall study design

3.1. Study Duration and Schedule

The duration of the trial for each subject is 16 days of study treatment and 7 days of follow up.

The overall duration of the trial is expected to be approximately 3.0 years including preparatory and analysis / report phase. Recruitment of subjects is planned to start in 06/2020. The actual overall duration or recruitment may vary. The Study timelines are described in Table 1.

Table 1: Study Timelines

Total trial duration	36 months
Duration of clinical part (FSI – LSO)	24 months
Duration for in individual patient	Treatment: 16 days Follow up: 7 days
FSI (First Subject In)	06/2020
LSI (Last Subject In)	05/2022
LSO (Last Subject Out)	06/2022
DBL (Data Base Lock)	09/2022
Statistical Analyses Completed	12/2022
Trial Report Completed	06/2023

3.2. End of Study

The end of the study is defined as the date of the last study visit of the last patient in the trial. Last patient last visit (LPLV) is either the date of the last visit of the last patient to complete the study, or the date at which the last data point from the last patient, which was required for statistical analysis (i.e. key safety and efficacy results for decision making), was received, whichever is the later date.

4. Study Population

This study will include patients with acute nephrotic syndrome (definition see below) due to different underlying diseases (e.g. diabetic nephropathy, membranous nephropathy, minimal change disease, focal segmental glomerulosclerosis). The patients will be recruited from the nephrological outpatient department of the University Hospital Tuebingen or after referral from local nephrological practices.

4.1. General Criteria for Subject Selection

Adult male and female patients with acute nephrotic syndrome and fulfilling the below outlined inclusion criteria will be enrolled into the study.
Trial population will consist of both genders. Gender distribution in the trial is supposed to reflect the distribution in the real patient's population, i.e. there will be no prior defined quantitative ratio between females and males. No statistical analysis based on genders or differences between genders will be performed.

4.1.1. Inclusion Criteria

Subjects meeting all of the following criteria will be considered for admission to the trial:

1. Acute nephrotic syndrome with proteinuria > 3 g/day and formation of edema.
2. Age ≥ 18 years at the time of signing the informed consent.
3. Understand and voluntarily sign an informed consent document prior to any study related assessments/procedures.
4. Ability to adhere to the study visit schedule and other protocol requirements.
5. Use of adequate thrombosis prophylaxis due to the increased risk of thrombosis in nephrotic syndrome and the expected fluctuations in volume balance during study participation
6. Subject (male or female) is willing to use highly effective methods of contraception according to the "Clinical trial fertility group" recommendations (http://www.hma.eu/fileadmin/dateien/Human_Medicines/01About_HMA/Working_Groups/CTFG/2014_09_HMA_CTFG_Contraception.pdf) during treatment and for 28 days (male or female) after the end of treatment (adequate: intrauterine device, intrauterine hormone-releasoing system, bilateral tubal occlusion, vasectomized

partner[1], sexual abstinence[2]; due to the increased risk of thrombosis in nephrotic syndrome, no hormonal contraceptives are recommended).

7. Female Patients of childbearing potential (WOCBP)[3] must agree to pregnancy testing before inclusion in the study.
8. Female Patients must agree to abstain from breastfeeding during study participation and 28 days after study drug discontinuation.
9. All subjects must agree not to share medication.

[1] Vasectomized partner is a highly effective birth control method provided that partner is the sole sexual partner of the WOCBP trial participant and that the vasectomized partner has received medical assessment of the surgical success

[2] In the context of this guidance sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject

[3] For the purpose of this document, a woman is considered of childbearing potential (WOCBP), i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.

4.1.2. Exclusion Criteria

Subjects presenting with any of the following criteria will not be included in the trial:

1. Severe reduction of kidney function: Creatinine clearance or calculated GFR < 30 mL/min/1.73m² or acute kidney injury KDIGO stage 2 or 3 or anuria.
2. Hypovolemia or dehydration.
3. Uncontrolled diabetes mellitus.
4. Hypotension, systolic blood pressure < 90 mmHg.
5. Hyperkalemia, plasma potassium concentration > 4.8 mmol/l.
6. Hypokalemia, plasma potassium concentration < 3.3 mmol/l.
7. Hyponatremia, plasma sodium concentration < 128 mmol/l.
8. Hypercalcemia, ionized calcium > 2.0 mmol/l or total albumin corrected calcium > 3.0 mmol/.

9. Signs of cardiac decompensation (orthopnoe, dyspnoe NYHA IV).
10. Hepatic coma or precoma.
11. Symptoms of gout.
12. Current therapy with potassium-sparing diuretics (e.g. spironolactone) or potassium supplements.
13. Women during pregnancy and lactation.
14. History of hypersensitivity to the investigational medicinal product, comparator or co-medication or to any drug with similar chemical structure or to any excipient present in the pharmaceutical form of the investigational medicinal product, comparator or co-medication.
15. Any other clinical condition that would jeopardize the patient's safety while participating in this clinical trial.
16. Active participation in other clinical trials or observation period of competing trials.

5. Requirements for Trial Site and Investigator

The necessary equipment, including Body Composition Monitor, is permanent available at the trial site.

There are no special training measures required to use the investigational medical product or comparator.

6. General Information on the Study Medication

Investigational medicinal product: Amiloride 5 mg

Registered trade name: Modamide®

International Non-proprietary Name (INN): Amiloride

ATC code, if officially registered: C03DB01

Pharmaceutical formulation: tablet

Route of administration: oral

Storage conditions: No specific storage conditions

Stabilitiy: 3 years

Manufacturer: Laboratoires GERDA, 24 rue Erlanger, 75016 Paris / France

Source: Pharmacy of the University Hospital Tuebingen

Components: Quinoline Yellow, Red Iron Oxide, Corn Starch, Calcium Hydrogen Phosphate, Magnesium Stearate, Lactose

Indications: Cardiac edema; edema and ascites in cirrhosis; arterial hypertension

Contraindications: Hyperkalemia (above 5.5 mmol/l); therapy with other potassium-sparing diruetics; therapy with potassium supplements; impaired renal function (anuria, acute renal failure, severe progressive kidney disease and diabetic nephropathy); hypersensitivity to any component of the drug

Comparator: Furosemide 40 mg

Administration method: oral

Registered trade names: Furosemid-ratiopharm®

International Non-proprietary Name (INN): Furosemide

ATC code, if officially registered: C03CA01

Pharmaceutical formulation: tablet

Route of administration: oral

Storage conditions: No specific storage conditions

Stabilitiy: 5 years

Manufacturer: ratiopharm GmbH, Graf-Arco-Straße 3, 89079 Ulm, Germany

Source: Pharmacy of the University Hospital Tuebingen

Components: Lactose monohydrate, microcrystalline cellulose, Carboxymethyl starch sodium (type A), povidone K25, fumed silica, magnesium stearate

Indications: Cardiac or hepatic edema; renal edema; edema due to burns; arterial hypertension

Contraindications: Kidney failure with anuria; hepatic coma and praecoma; severe hypokalemia; severe hyponatremia, hypovolemia or dehydration; lactating women; hypersensitivity to any component of the drug

Co-medication: Hydrochlorothiazide (HCT) 12.5 mg

Administration method: oral

Registered trade names: HCT-Hexal®

International Non-proprietary Name (INN): Hydrochlorothiazide (HCT)

ATC code, if officially registered: C03AA03

Pharmaceutical formulation: tablet

Route of administration: oral

Storage conditions: No specific storage conditions

Stabilitiy: 3 years

Manufacturer: Hexal AG, Industriestraße 25, 83607 Holzkirchen / Germany

Source: Pharmacy of the University Hospital Tuebingen

Components: Corn starch, lactose monohydrate, microcrystalline cellulose, fumed silica, talc, poly (O-carboxymethyl) starch sodium salt, magnesium stearate

Indications: Arterial hypertension; cardiac, hepatic or renal edema; adjuvant symptomatic therapy of chronic heart failure in addition to ACE inhibitos

Contraindications: severe renal impairment (renal insufficiency with oliguria or anuria, creatinine clearance <30 ml/min and / or serum creatinine >1.8 mg/100 ml); acute glomerulonephritis; hepatic coma and praecoma; hypokalemia; hyponatremia; hypovolemia or dehydration; hypercalcaemia; gout; hypersensitivity to any component of the drug

6.1. Manufacturing of the Study Medication

Study medication (investigational medical product amiloride, comparator furosemide and co-medication hydrochlorothiazide) is commercially available and will be obtained via the Pharmacy of the University Hosptial Tuebingen directly from the respective manufacturer and

used for the study without further manufacturing processes or repackaging. The investigational medical product amiloride is not available as individual substance in Germany and will be imported from Laboratoires GERDA, 24 rue Erlanger, 75016 Paris / France. The comparator furosemide and co-medication hydrochlorothiazide are available in Germany from German manufactorers.

6.2. Labeling of the Study Medication

The trial medication furosemide and HCT will be provided by the pharmacy of the University Hospital Tuebingen as part of standard therapy medication from German manufacturers. As the trial is not blinded, no additional labelling has to be performed for furosemide and HCT.

The trial medication amiloride will be imported and labelled by the Pharmacy of the University Hospital Tuebingen according to § 5 (1) of GCP-V. The label will contain information in German language to ensure identification of the medical product and the clinical trial and to ensure a proper use of the medical product.
The label of the Investigational Medical Product Amilorid will contain the following details:

- Name, address and phone number of the representative of the trial sponsor
- Name and address of the Pharmacy
- Name and potency of investigational medicinal product
- Batch number or code number of the trial
- Administration form
- Content specified by weight, volume or quantity
- Application form
- Dosing instructions or a reference to instructions by the clinical investigators
- Expiry date using note “usable until”
- Discharge date from the pharmacy
- Code of Trial Protocol
- EudraCT-Number
- Note: “For use in clinical trial”
- Note: “Keep inaccessible for children”
- Note: “Bring this package including blister to every study visit”
- Note: “Return unused tablets at the end of the trial”
- Note: “Store below 25 °C”

The template of the label is shown in appendix 15.3. As accompanying document an instruction sheet, which can be seen in appendix 15.2, will be issued to every subject with study enrollment.

6.3. Storage of the Medical Product

All trial medication must be kept in a locked area with access restricted to designated trial staff. Amiloride and Furosemide must be stored below 25 °C. Otherwise, no trial medication requires any specific storage conditions according to the manufacturers' instructions.

6.4. Drug Accountability, Therapy Compliance and Disposal

The site investigator will keep an account of the trial medication and acknowledge the receipt of all shipments of the trial medication.
Trial medication will be dispensed to the subjects by the investigator at day 0 of study participation. The investigator will document the date of dispensary, subject identification, batch/ serial numbers or other identification of trial medication. The investigator will also keep accurate records of the quantities of trial medication dispensed, used, and returned by each subject. Subjects will be instructed to intake the IMP / comparator daily and to bring all remaining trial medication and empty blisters to the trial site at every study visit. Compliance will be assessed by count of tablets. Details will be recorded in the Case Report Form (CRF). The University Hospital Tübingen is responsible to discard the unused trial medication at the end of the trial.

6.5. Method of Treatment Assignment

See Section 7.1.4 (Randomisation).

6.6. Dose Schedule

Participating patients will take the study medication orally once daily in the morning in fasting state, unchewed with adequate amount of fluid. Initial dose will be amiloride 5 mg or furosemide 40 mg. Initial dose of amilorde was chosen by virtue of clinical expertise and former study results from studies using amiloride in related groups of patients [17-19], with reflection of clinical effectiveness and prevention of adverse events. Initial dose of furosemide was chosen as a typically used initial dose. The duration of treatment is 16 days,

since the major loss of overhydration is expected to be reached in this time period. Depending on course of overhydration, co-medication with HCT 12,5 mg is started at ay 8.

6.6.1. Dose Modification

The following considerations were taken into account when determining the dose adjustments:

- The initial dosages determined on the basis of clinical experience and common clinical practice are amiloride 5 mg and furosemide 40 mg. In the treatment of overhydration in nephrotic syndrome with furosemide, treatment resistance must be expected [26, 27]. The dose should therefore be increased depending on the clinical effect. To ensure the comparability of the results of the two study arms, the dose should be increased by steps of one whole tablet of each, amiloride or furosemide. Based on available clinical data [17-20], maximum dose of amiloride is 15 mg and of furosemide should be 120 mg.
- Based on data from trial measurements of baseline overhydration in nephrotic patients prior to treatment (n = 14), an overhydration of 1 L corresponds to 4.25 %ECW. The daily weight loss of water should not exceed 1 L (corresponding to 4.25 %ECW) according to usual recommendations (safety criterion).
- With a weight loss of less than 0.4 L (corresponding to 1.7 %ECW) per day, the response to the therapy is too low and the diuretic therapy should be increased (efficacy criterion).
- If weight loss under medication with amilorid or furosemide is treatment refractory, co-medication with HCT should be initiated after 8 days, with maximum dose 25 mg.
- Electrolyte disorders should lead to dose adjustments in case of plasma potassium < 3.3 mmol/l or > 5.3 mmol/l and plasma sodium < 125 mmol/l (safety criteria).
- Indications of acute kidney injury (increase of serum creatinine by ≥ 0.4 mg/dl) should lead to a dose reduction.
- Low blood pressure (BP) should lead to a dose reduction.
- When overhydration is no longer present, the dose should not be increased further, but a maintenance dose based on previous needed dose should be continued. Overhydration is no longer present, if OH is within the normal range of -1 to +1 L, corresponding to -4.25 to +4.25 %ECW.

The dose after dose adjustment is valid until the next study visit. If a safety criterion and an efficacy criterion are both met, the dose adjustment shall be performed on the basis of the safety criterion.

Dose adjustments are performed as follows:

Dose adjustment at day 2:

Reasons for incease (efficacy criteria)	Reasons for decrease (safety criteria)	Reasons for pause of study medication (safety criteria)
- Decrease of OH since day 0 is less than 3.4 %ECW	- Decrease of OH since day 0 is more than 8.5 %ECW - Potassium in BGA >5.3 or <3.3 mmol/l - Systolic BP < 90 mmHg or diastolic BP < 60 mmHg	- Increase of creatinine by >0.4 mg/dl since day 0 - K in BGA >5.6 or <3.0 mmol/l - Na in BGA <125 mmol/l

Dose adjustment at day 5:

Reasons for incease (efficacy criteria)	Reasons for decrease (safety criteria)	Reasons for pause of study medication (safety criteria)
- Decrease of OH since day 2 is less than 5.1 %ECW	- Decrease of OH since day 2 is more than 12.8 %ECW - Potassium in BGA >5.3 or <3.3 mmol/l - Systolic BP < 90 mmHg or diastolic BP < 60 mmHg	- Increase of creatinine by >0.4 mg/dl since day 0 - K in BGA >5.6 or <3.0 mmol/l - Na in BGA <125 mmol/l

Dose adjustment at day 8:

Reasons for increase (efficacy criteria)	Reasons for decrease (safety criteria)	Reasons for pause of study medication (safety criteria)
- Decrease of OH since day 5 is less than 5.1 %ECW	- Decrease of OH since day 5 is more than 12.8 %ECW - Potassium in BGA >5.3 or <3.3 mmol/l - Systolic BP < 90 mmHg or diastolic BP < 60 mmHg	- Increase of creatinine by >0.4 mg/dl since day 0 - K in BGA >5.6 or <3.0 mmol/l - Na in BGA <125 mmol/l

Dose adjustment at day 12:

Reasons for increase (efficacy criteria)	Reasons for decrease(safety criteria)	Reasons for pause of study medication (safety criteria)
Decrease of OH since day 5 is less than 6.8 %ECW	- Decrease of OH since day 8 is more than 17.0 %ECW - Potassium in BGA >5.3 or <3.3 mmol/l - Systolic BP < 90 mmHg or diastolic BP < 60 mmHg	- Increase of creatinine by >0.4 mg/dl since day 0 - K in BGA >5.6 or <3.0 mmol/l - Na in BGA <125 mmol/l

Increase is defined as

- Increase of study medication by amiloride 5 mg or furosemide 40 mg if current dose is amiloride 5 or 10 mg or furosemide 40 or 80 mg
- Addition of HCT 12,5 mg or 25 mg at day 8 or 12

Decrease is defined as

- pause of study medication for 1 day if current dose is amiloride 5 mg or furosemide 40 mg and then continue with amiloride 5 mg or furosemide 40mg
- reduction by amiloride 5 mg or furosemide 40 mg if current dose is amilorid 10 or 15 mg or furosemide 80 or 120 mg
- reduction of HCT dose at day 12

Pause of study medication is defined as pause of study medication until the next visit.

7. Study Procedures and Examination Method

This Study will consist of the following consecutive phases: Study entry, Treatment and Follow-up. Time-points and trial procedures are listed in Table 2.

Table 2 Table of Events

	Study entry	**Treatment**						**Follow-up**
Visit number	1	2	3	4	5	6	7	8
Day	-3 to -1	0	2	5	8	12	16	23
Study Entry								
Informed Consent	X							
Demographics, including sex, body weight, high, age	X							
Inclusion/Exclusion criteria: anamnesis, clinical examination, blood pressure, pre-existing medication, proteinuria in 24 hours collected urine, general laboratory control (including blood count, creatinine, urea, liver enzymes, lactat dehydrogenase, c-reactive protein, electrolytes (potassium, sodium, calcium), glucose, HbA1c, pregnancy test in females)	X							
Safety Assessments								
Adverse events		X	X	X	X	X	X	X
Concomitant medication	X	X	X	X	X	X	X	X
Check of the study medication		X	X	X	X	X	X	
Return of any remaining study medication							X	
Plasma creatinine	X	X	X	X	X	X	X	X
Potassium and sodium level in blood gas analysis	X	X	X	X	X	X	X	X
Efficacy Assessment								
Body weight	X	X	X	X	X	X	X	X
Bioimpedance spectroscopy with BCM	X	X	X	X	X	X	X	X

Edema circumference	X	X	X	X	X	X	X	X
Blood pressure	X	X	X	X	X	X	X	X
Plasma renin activity, serum aldosterone concentration	X	X	X	X	X	X	X	X
24 hours urine volume and natriuresis	X		X	X	X	X	X	
Diet diary during urine collection	X		X	X	X	X	X	
Check for dose adjustment		X	X	X	X	X		

7.1. Study Entry

7.1.1. Patient's Informed Consent (see also 11.2.)

The patient is to be informed both in writing and verbally by the investigator before any study-specific procedure is performed. Each patient will be informed about the modalities of the clinical study in accordance with the provided patient information. The patient is given sufficient time (24h) to consider participation in the clinical trial and ask for additional advise if needed. Informed consent from the patient will be obtained using a form approved by the ethical committee of the University Hospital Tuebingen. The patient and informing physician/ investigator must each personally date and sign the informed consent form and declaration on data privacy protection (section 11.4) on the same day. The original signed documents will be part of the investigator's site file and retained with it and a copy included the insurance policy of the trial will be handed to the patient.

7.1.2. Enrollment

After the patient's informed consent, a unique subject number for identification purposes will be assigned to the patient in order to maintain his/her pseudonymity. The subject number will be used for the patient throughout the study (= ID number). Screening failures will also be assigned to a subject number and all collected data will be recorded in the CRF. The PI or a nominated investigator will decide on enrolment after screening.

7.1.3. Screening

Screening will be performed within 3 days prior to first administration of study medication. After having signed the informed consent, patients will undergo all assessments listed below:

- Anamnesis including medical history and pre-existing medication
- Questions about participation in other clinical trials or research projects
- Demographics and clinical examination
- Blood pressure

- Assessment of edema, measurement of highest edema circumference at the lower leg
- Blood sampling for measurement of laboratory parameters and blood gas analysis; laboratory parameters include blood count, creatinine, urea, liver enzymes, lactat dehydrogenase, C-reactive protein, electrolytes (potassium, sodium, calcium), glucose, HbA1c, pregnancy test in females
- Proteinuria in 24 hours collected urine
- Natriuresis in 24 hours collected urine
- Diet diary during urine collection
- Bioimpedance spectroscopy (BCM)

Patients who fulfill all the inclusion criteria and none of the exclusion criteria will be eligible to participate in the trial. Screening failures, i.e. screened patients not in compliance with all criteria; are to be excluded and the reason will be recorded in the patient notes.
Information of patient's trial participation can be provided to the patient's general practitioner upon request.

7.1.4. Randomisation

The randomization will follow a randomization scheme of a 1:1 (amiloride and furosemide). Subjects will be assigned to their respective treatment by randomization. A randomisation number will be assigned (= Random. number). Subjects will then receive their study medication during their personal visit at day 0 of study participation.

7.1.5. Concomitant Medication and Treatments

Relevant additional medications and treatments administered to the subjects on entry to the trial or at any time during the trial are regarded as concomitant medications and treatments and must be documented on the appropriate pages of the CRF.

7.1.6. Permitted Prior and Concomitant Medications and Treatments

The following concomitant medications and treatments are permitted during the trial and for 3 days prior to enrolment into the clinical trial:

- Proteinuria lowering medication (ACE inhibitors and Angiotensin II receptor) in a low dose (maximally 8 mg Candesartan or 2,5 mg Ramipril per day or equivalent)
- Corticosteroids and other immunosuppressive therapy as therapy of underlying disease starting from day 8
- Antihypertensive therapy with calcium antagonists.

7.1.7. Prohibited Prior and Concomitant Medications and Treatments

Medication that are contraindicated in association with amilorid, furosemide or HCT as defined in the respective SmPCs have been considered in the Exclusion criteria, this includes in particular potassium sparing diuretics and potassium salts.

Further drug interactions are reflected in the following.

The SmPC of Amiloride lists potassium salts, potassium diuretics, angiotensin-converting enzyme inhibitors, angiotensin II antagonists, nonsteroidal anti-inflammatory drugs, heparins (low molecular weight or unfractionated), immunosuppressants such as ciclosporin or tacrolimus, trimethoprim as medications that can promote the occurrence of hyperkaliemia under treatment with amiloride. The SmPC points out that this risk is particularly important with potassium-sparing diuretics and potassium salts and these drug classes have therefore been considered as prohibited prior and concomitant medication and in the exclusion criteria. Other listed potential interactions include increased risk of toxicity of HCT (baclofen, antidepressants, neuroleptics) or other drugs (lithium, metformin), increased risk of hypotension or acute kidey injury especially in case ot hypovolemia (nonsteroidal anti-inflammatory drugs, ACE inhibitors, iodinated contrast media, other diuretics, alpha blocker antihypertensives, amifostine), decreased effect of HCT (corticosteroids).

The SmPC of furosemide lists interactions with other pharmaceuticals, like increased risk of hypokalemia and therefore increased risk of arrhythmia (glucocorticoids, carbenoxolone or laxatives), increased risk of acute kidney injury especially if hypovolemia is caused (non-steroidal anti-inflammatory drugs, antibiotics like aminoglycosides, cephalosporins or polymyxins, cisplatin, iodinated contrast medium), weakening of the effect of furosemide (probenecid, methotrexate, phenytoin, sucralfate) or of the other drug (antidiabetics, sympathomimetics), increased risk of ototoxicity (aminoglycosides, cisplatin, increased risk of hypotonia (ACE inhibitors, angiotensin II receprot antagonists), decrease of renal elimination (of lithium, probenecid, methotrexate), increased risk of hyperuricemia (cycosporin A).

The SmPC of HCT lists interactions like increased risk of hypotonia (guanethidin, methyldopa, calcium antagonists, ACE inhibitors, angiotensin II receptor blockers, dopamine reuptake inhibitor, beta receptor blocker, nitrates, barbiturates, phenothiazine, thicyclic antidepressants, vasodilatatirs, ethanol), acute kidney injury (ACE inibitors, non steroidal antiinflammatoric drugs), increased risk of hyperglycemia (beta receptor blockers), reduced effect of HCT (non steroidal antiinflammatoric drugs), increased risk of hypokalemia and therefore increased risk of arrhythmia (other diuretics, glucocorticoids, ACTH, cabenoxolon, penicillin G, salicylates, amphotericin B, antiarrhythmics, glycosides, laxans), increased risk of hyponatremia (diuretics, antidepressants, neuroleptics, antiepileptics), increased toxicity of

other drugs (salicylate, allopurinol, amantadine), decreased effect of HCT (colestyramin, colestipol) or other drugs (insulin, anti-hyperurikemic drugs, noradrenalin, adrenalin), increased toxicity of other drugs (caclophosphamid, fluorouracil, methotrexate, lithium, muscle relaxans, vitamine D, calcium, ciclosporin, diazoxid, methyldopa), increased effect of HCT (atropine, biperiden).

Most of the listed co-medication with potential interactions is not expected to be prescribed or indicated in the study cohort of patients with nephrotic syndrome. However, serum creatinine levels and serum electrolyte concentrations are monitored closely during the study as safety parameters (section 7.4). If any other co-medication with potential interaction is found during check of co-medication at screening visit, any condition that could jeopardize the patient's safety is defined as exclusion criteria. Patients are informed in the patient information sheet that they may only undergo other medical treatment, except in emergencies, after prior consultation with the study investigator, they have to notify the investigator immediately of any emergency treatment received and they have to inform other treating physicians about the study participation to prevent pharmaceutical interactions.

7.1.8. Required Prior and Concomitant Medications and Treatments

As co-medication according to study protocol, hydrochlorothiazide 12.5 mg to 25 mg in addition to the initial medication with amiloride or furosemide is added at day 8 in case of treatment refractory edema as defined in section 6.6.1. Otherwise, there are no concomitant medications and treatments required during the trial.

7.2. Treatment Phase

During the treatment phase (day 0 – 15) of this trial, participants will receive either amiloride with an initial dose of 5 mg once daily or furosemide with an initial dose of 40 mg once daily. Dose adjustments will be performed as defined in 6.6.1. Beginning at day 8, participants will receive hydrochlorothiazide (initial dose 12.5 mg) as co-medication in addition to the initial medication with amiloride or furosemide in case of treatment refractory edema as defined in section 6.6.1.

7.2.1. Description of Patients' Visits

Visit 1 is the screening visit and described in 7.1.3. The patient will be randomized if all inclusion criteria and no exclusion criteria are fulfilled.

The patient wil be instructed to take study medication in the morning on all study days during treatment phase (at day 0 – 15)

Visit 2 – 7 take place in the morning and all have the same schedule. Examinations performed on visit 2 – 7 include:

- interview for adverse events and concomitant medication
- body weight, bioimpedance spectroscopy (BCM) and edema circumference
- blood pressure
- blood sampling: blood gas analysis (1 mL) is carried out immediately; Li-Hep-plasma (3 mL) is taken for measurement of serum creatinine directly after blood collection; further blood samples including EDTA-plasma (8 mL), Li-Hep-plasma (5 mL) and serum (8 mL) are centrifuged and frozen at -20 °C for later measurements)
- 24 hours collected urine volume and natriuresis and diet diary during urine collection (the patient will be instructed to collect urine for 24 hours and write a diet diary prior to the study visit, see instruction sheet, appendix 15.2).
- handing out / check / receive back (visit 7) the patient's study medication
- check for dose adjustments as defined in section 6.6.1 and completing the medication and dose for the next study days on the patient's instruction sheet (see appendix 15.2).

After the last examination at study visit 7, treatment phase ends for the respective patient. Treatment after the study will be operated as described in section 7.8.1

At day 23, a follow up (**visit 8**) will be performed. The follow-up visit invludes:

- interview for adverse events and concomitant medication
- body weight, bioimpedance spectroscopy (BCM) and edema circumference
- blood pressure
- blood sampling: blood gas analysis (1 mL) is carried out immediately; Li-Hep-plasma (3 mL) is taken for measurement of serum creatinine directly after blood collection; further blood samples including EDTA-plasma (8 mL), Li-Hep-plasma (5 mL) and serum (8 mL) are centrifuged and frozen at -20 °C for later measurements)

7.3. Assessment of Efficacy

7.3.1. Efficacy Parameters

Aim of the study is to prove the efficacy of amiloride for reduction of edema and overhydration in human nephrotic syndrome in comparison to standard medication with

furosemide. Overhydration as the primary endpoint variable is determined directly using bioimpedance spectrosycopy with the Body Composition Monitor in an investigator independent and reproducible way. As further clinical parameters to assess overhydration, body weight, edema circumference and systolic and diastolic blood pressure are measured in a clinical examination.

Laboratory parameters performed for assessment of efficacy include plasma renin activity, serum aldosterone concentration and urine volume and natriuresis in 24 hours collected urine. Plasma renin activity and serum aldosterone concentration are relevant parameters of the body's fluid regulation and suitable laboratory parameters to assess efficacy of diuretic medication. Increase of natriuresis represents the mechanism of acting of diuretic medication and leads to higher urine volume output with the consequence of reduction of overhydration. Therefore, urine volume and natriuresis are necessary parameters to assess the efficacy of diuretic medication. To consider that natriuresis is affected by sodium intake in further analysis, patients are instructed to write a diet diary to enable estimation of sodium intake.

Additional efficacy parameters are the number of required changes of the dose of the study medication and the need for co-medication with hydrochlorothiazide as defined in this protocol.

7.3.2. Methods and Timing for Assessing, Recording, and Analysing of Efficacy Parameters

A) Measurement of body fluid status with the Body Composition Monitor:

Body fluid status will be determined using bioimpedance spectroscopy with the device Body Composition Monitor (BCM, Fresenius Medical Care AG & Co). Bioimpedance spectroscopy measures the electrical resistance of the body tissue at 50 different frequencies in the range from 5 to 1000 kHz. Due to the principle that high-frequency measuring currents flow unhindered through the whole body water, while the low-frequency measuring currents can not penetrate the cell membrane and flow only through the extracellular space, intra- and extracellular water can be determined separately. Based on two physiological models, the volume model and the body composition model, the relevant parameters for assessing the hydration status are calculated from the measured resistances at the different measurement frequencies. The volume model is used to calculate total body water (TBW), ECW and intracellular water (ICW). The Body Composition model calculates the so-called overhydration (OH) as excess fluid volume out of total body mass, normal hydrated fatty tissue mass and normal hydrated lean tissue mass. [25]

Measurement with the BCM is performed as follows: The patient is already in a supine position for two minutes before taking the measurement. The patient should not be in skin contact with metal objects. Two measuring electrodes with at least 3 cm distance are attached to the back of the right hand and foot and connected to the BCM. After entering the patient data necessary for calculating the results, namely gender, age, daily updated size and daily updated weight, the measurement can be started. During the few seconds of measurement, the patient must lie still and not speak. After a short calculation time, the device displays the results.

The BCM device carries a CE certificate (see appendix 15.4) and is used during the study exclusively for its intended purpose and by trained personnel.

For the study, overhydration is expressed as percent of ectracellular water (% ECW). Measurement with the Body Composition Monitor is performed at every study visit (study entry, treatment phase and end of follow up) and documented in the case report form.

B) Measurement of body weight, edema circumference, systolic and diastolic blood pressure:

The body weight is measured with a calibrated scale. For each measurement of the patient's body weight during participation in the study the same scale will be used. Body weight will be recorded with the patient wearing street clothes without shoes, outerwear or accessories.

Edema circumference is measured using a calibrated measuring tape. Maximal circumference of the lower leg is measured on both sides.

For blood pressure measurements, a calibrated electric blood pressure monitor with upper arm cuff is used. Blood pressure will be measured as office blood pressure in a sitting position after at least 5 minutes of rest at the patient’s dominant side (right side if right-handed, left side if left-handed) twice. The average of the measured values is documented.

Measurement of body weight, body fluid status, edema circumference, systolic and diastolic blood pressure is performed at every study visit and documented immediately in the case report form.

C) Measurement of laboratory parameters:

Blood and urine samples for measurement of the laboratory parameters are taken at every study visit during treatment phase. This includes EDTA-plasma for measurement of plasma renin activity, serum for serum-aldosterone concentration and 24 hours collected urine for natriuresis. Urine volume of 24 hours collected urine is documented immediately. Test tubes

with blood samples are centrifuged and blood and urine samples are frozen at -20 °C. Measurement of plasma renin activity, serum aldosterone concentration and urine sodium concentration are performed at the end of the patient's follow up in the nephrological laboratory and in the central laboratory of the Univerity Hospital of Tuebingen. To enable consideration of salt content of the food in later analysis and interpretation of study results, study participants are instructed to write food diaries on the days of the urine collection.

7.4. Assessment of Safety

7.4.1. Safety Parameters

Safety assessment includes adverse events, concomitant medication and check of study medication. As safety parameters for specific risks of the study, serum potasium and sodium levels and plasma creatinine concentration are monitored during study participation.

(Serious) Adverse Events (see section 10)

7.4.2. Methods and Timing for Assessing, Recording, and Analysing Safety Parameters

Check for adverse events is performed continuously from beginning of study medication until 7 days after end of study treatment. Check of concomitant medication is performed at screening visit and continuously until end of follow up. Check of study medication (integrity, correct number of remaining tablets) is checked at every study visit during treatment phase. Any remaining study medication is to be returned at the end of treatment phase.

Specific risks from the study medication are shifts in the electrolyte balance and volume status. Parameters of volume status are assessed as efficacy parameters as described above (section 7.3). Sodium and potassium levels are measured in blood gas analysis at every study visit. Therefore, blood gas analysis is performed directly after blood collection using the blood gas analysis device at the dialysis department. If this blood gas analysis device is not available at the respective moment, blood gas analysis devices at the emergency department or intensive care unit are used alternatively. All BGA devices used are subject to continuous quality assurance and inspection. Possible changes in body fluid status and electrolyte levels are counterregulated by dose adjustment requirements specified in this protocol (section 6.6.1).

As changes in body fluid status could lead to acute (prerenal) kidney failure, serum creatinine levels are measured at every study visit. The blood sample is sent to the central laboratory of

the University Hospital Tuebingen directly after blood collection and plasma creatine levels are measured immediately. If plasma ceratinine increases by >0.4 mg/dl, study medication is discontinued as specified in section 6.6.1.

7.5. Premature termination of clinical trial for a trial subject

Reasons for premature termination of trial for an individual trial subject are:

1. Death
2. Withdrawal of consent
3. Patient lost to follow-up
4. Major protocol violation
5. At their own request or at request of the legal representative
6. If, in the investigator's opinion, continuation of the trial would be detrimental to the subject's well-being
7. Occurrence of serious adverse event caused by the investigational medicinal product
8. For women, if it becomes known that the subject is pregnant

The PI decides about withdrawal of subjects from trial treatment in case of occurrence of criteria mentioned above. In all cases, the reason for withdrawal must be recorded in the CRF and in the subject's medical records. In case of withdrawal of a subject at his/ her own request, the reason should be determined and documented. All examinations scheduled for the last trial day will be performed and documented as far as possible, subject to the consent of the patient. These subjects will enter the regular follow up of the trial, unless the subject has withdrawn his/her consent to any further study-related procedure. All ongoing Adverse Events (AEs)/ Serious Adverse Events (SAEs) of withdrawn subjects have to be followed up until no more signs and symptoms are verifiable or the subject is on stable condition.

7.6. Premature closure of a trial site

Not applicable since monocentric study.

7.7. Premature termination of the trial

The trial may be prematurely terminated, if in the opinion of the sponsor and coordinating investigator there is sufficient reasonable cause. Written notification documenting the reason for study termination will be provided to the investigators.

In case of the following situations, a premature termination of the trial has to be considered:

- Serious adverse drug reactions / not justifiable toxicity
- Substantial changes in risk-benefit considerations
- New insights from other trials
- Insufficient recruitment rate

7.8. End of Study of Subjects

The End of Study for a patient enrolled in this trial is defined as the date of the last visit of the respective patient in the trial.

7.8.1. Plan for Treatment or Care after End of Study

At the end of study tretmant, further medication for nephrotic syndrome will be determined by the nephrologist of the patient's medical practice or the attending physician in the nephrologic outpatient clinic of the University Hospital Tübingen. The study medication may be continued or adjusted at the discretion of the responsible nephrologist. The responsible nephrologist will therefore be informed of the patient's medication during study participation. There are no special requirements regarding the termination of the medication taken during the study. If diuretic therapy with amiloride, furosemide or hydrochlorothiazide is continued, further controls of the electrolytes and renal function should be performed as they would be without any study participation.

8. Quality Control and Quality insurance

8.1. Monitoring

Monitoring for this study is provided by the Zentrum für Klinische Studien Tübingen (ZKS Tübingen). The monitoring will be conducted according to ZKS Tübingen's internal SOPs and a dedicated monitoring manual for the study. The monitoring timelines include, for all centres, an initiation visit, regular monitor visits during the course of the trial as well a close out visit. Monitoring will end with the last visit after full documentation of the last patient enrolled (close out visit). All investigators agree that the monitors regularly visit the trial site, assure that the monitors will receive appropriate support in their activities and will have access to all trial-related documents.

The aims and scope of activities of the monitoring visits will be specified in a monitoring manual. Briefly, visits will include:

- To check the informed consent documents
- To monitor trial subject safety (occurrence and documentation/reporting of Adverse Events (AEs) and Serious AEs).
- To check the completeness and accuracy of entries on the CRFs.
- To validate the entries on the CRFs against those in the source documents (source data verification (SDV) (section 8.3.2).
- To evaluate the progress of the trial.
- To evaluate compliance with the trial protocol.
- To assess whether the trial is being performed according to GCP at the trial site.
- To discuss with the investigator aspects of trial conduct and any deficiencies found.
- A monitoring visit report is prepared for each visit describing the progress of the clinical trial and any problems.

8.2. Audits/ Inspections

In addition to the monitoring activities, a comprehensive quality control will be conducted by CI in the form of audits. These may include checking the whole course of the study, the documentation, statistical analysis, the trial centre, the investigators, and the monitor. The competent regulatory authorities may also conduct audits or inspections.

With his participation in the study, the investigator agrees to support the activities of the auditor/inspector, provide her/him with direct access to the source documents and give her/him the opportunity to inspect the laboratory facilities, storage of the investigational product, etc.

8.3. Documentation: Collection, Handling, Storage and Archiving of Data

8.3.1. Case Report Form

The trial case report form (CRF) is the primary data collection instrument for the trial. All data requested on the CRF must be recorded. All missing data must be explained.

For this project, paper Case Report Forms (CRFs) will be used. The investigator is responsible for ensuring that all sections of the CRF are completed correctly and that entries can be verified against source data. Any errors should have a single line drawn through them so that the original entry remains legible and the correct data should be entered at the side with the investigator's signature, date and reason for change. Self-explanatory corrections do not need to be justified.

The correctness of entries in CRFs will be confirmed by dated signature of the responsible investigator.

8.3.2. Source Data

Source data is all information, original records of clinical findings, observations or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, patients' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, CTs, MRIs, ultrasound reports, patient files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

8.3.3. Data Handling

After first check for plausibility by eye, all data will be entered in a database as recorded in the CRF. To ensure data quality a double data entry will be performed.

The trial master file, the CRFs, and other material supplied for the conduct of the study will be retained by Sponsor/CRO according to applicable regulations and laws.

8.3.4. Storage and Archiving of Data

According to the §13 of the German GCP-Ordinance all important trial documents (e.g. CRF) will be archived for at least 10 years after the trial termination.

The investigator(s) will archive all trial data (source data and Investigator Site File (ISF) including subject identification list and relevant correspondence) according to the section 4.9 of the ICH Consolidated Guideline on GCP (E6) and to local law or regulations.

9. Statistical Analyses

9.1. Study Population Definition

9.1.1. Sample Size and Power Consideration

Primary endpoint of the study is decrease of overhydration (measured using bioimpedance spectroscopy) after 8 days, compared to baseline. Based on data from a baseline sample (n = 14) and clinical experience on reduction of overhydration baseline overhydration is expected to be 26.5 % of extracellular water (standard deviation 12.3; normal distribution is assumed) and it is expected that the overhydration will be reduced by 90% in the amiloride group and by 50% in the furosemide group. For the final t-test for two groups, a one-tailed significance level of 0.05 and a power of 80% should be ensured. From these conditions results a sample size of n = 18 patients per group (total n = 36; calculated for a t-test with the nQuery 7.0 program). In order to take into account dropouts, the sample size is defined as n = 22 per group (total n = 44).

9.1.2. Intention-to-Treat Population

All statistical analyses will be based on the Intention-to-Treat Population (ITT). The ITT includes all randomised patients with exception of patients who withdraw their informed consent for the analysis of their data during the study.

9.2. Analysis of Primary Variable

The primary endpoint variable is change of overhydration (OH) after 8 days compared to baseline, measured by bioimpedance spectroscopy using the Body Composition Monitor and expressed in percent of ectracellular water (% ECW). The primary endpoint variable will be compared between the two study arms, which are patients with amilorid treatment and patients with furosemide treatment.

The null-hypothesis is that there is equal or greater decrease of OH after 8 days in the group of patients with furosemide treatment compared to the group of patients with amiloride treatment. The alternative hypothesis is that there is a greater decrease of OH after 8 days in the group of patients with amiloride treatment compared to the group of patients with furosemide treatment. For analysis of the primary endpoint variable a one-sided t-test for two groups will be performed to test the null hypothesis against the alternative hypothesis. Effect size and 95%-confidence interval for effect size will be estimated. Only the rejection of the null hypothesis will be interpreted as statistical evidence for the efficacy of the amiloride treatment.

9.3. Analysis of Secondary Variables

Secondary endpoint variables are listed in section 2.2. All secondary endpoints will be compared and statistically assessed for descriptive purposes and not in a confirmatory sense. The aim of the analysis is explorative data analysis, not hypothesis testing or generation of evidence for efficacy and no attempt will be made to adjust the p-values of statistical tests of the secondary endpoints for multiple testing. If adequate, secondary endpoints will be compared and statistically assessed using t-tests for two groups or covariance techniques with baseline values as covariates in case of quantitative variables. Depending on distribution, non-parametric tests may be indicated. Dichtomous data will be compared and statistically assessed using Mantel-Haenszel chi-squared tests including relative risks and 95%-confidene intervals for relative risks.

9.4. Safety Analysis

Safety will be assessed by frequency tabulations and line listings.

9.5. Descriptive Analysis

Descriptive analyses will include absolute and percentage frequencies for categorical variables, means, medians, standard deviations, quartiles and ranges for quantitative variables and medians, quartiles and ranges for ordinal variables.

9.6. Handling of Missing Data

All variables included in the CRF are mandatory. The monitoring will assure quality of the assessments. Thus, missing values are to be expected only due to refusal by patients. In the analysis of the primary endpoint missing values will be imputed using multiple imputation approaches, complete case and last observation carried forward analyses will be performed as sensitivity analyses. All other missing data will be given in the descriptive tables but excluded from the statistical test.

9.7. Subgroup Analysis

No subgroup analysis is planned.

9.8. Biometric Report

The statistical analysis will be conducted by the IKEAB, once the database is declared closed. In this study protocol the directions of the planned analyses are given. Before starting the final analysis, a detailed statistical analysis plan (SAP) will be written and signed by the responsible statistician and the coordinating investigator. If any major deviations in the SAP from the original study protocol are necessary, the reason for that will be given in detail. A protocol amendment will be adequate for major deviations (e.g. change of primary endpoint, sample size). The final SAP will be documented in the final biometrical report. The biometrical report will be written by the responsible statistician and should include the complete statistical analysis.

10. Safety

10.1. Definition of Adverse Events and Side Effects

10.1.1. Adverse Events

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

An AE may be:

- New symptoms/ medical conditions.
- New diagnosis.
- Changes of laboratory parameters.
- Diseases and accidents.
- Worsening of medical conditions/ diseases existing before clinical trial start.
- Recurrence of disease.
- Increase of frequency or intensity of episodical diseases.

A pre-existing disease or symptom will not be considered an adverse event unless there will be an untoward change in its intensity, frequency or quality. This change will be documented by the investigator.

Surgical procedures themselves are not AEs; they are therapeutic measures for conditions that require surgery. The condition for which the surgery is required may be an AE. Planned surgical measures permitted by the clinical trial protocol and the condition(s) leading to these measures are not AEs, if the condition leading to the measure was present prior to inclusion into the trial.

AEs are classified as "non-serious" or "serious".

10.1.2. Adverse Drug Reaction

Adverse reaction means all untoward and unintended responses to an investigational medicinal product unrelated to the dose administered.

10.1.3. Unexpected Adverse Drug Reaction

An unexpected Adverse Drug Reaction (ADR) is a reaction which nature or severity is not consistent with the applicable product information available for the IMP. Expected ADRs are

listed in the appropriate reference documents, e.g. Investigator's Brochure; Summary of Product Characteristics (SmPC [Fachinformation in Germany]).

10.1.4. Serious Adverse Event and Serous Adverse Reaction

AEs are classified as "non-serious" or "serious".

A serious adverse event (SAE) is one that at any dose:

- Results in death.
- Is life-threatening (the term life-threatening refers to an event in which the subject was at risk of death at the time of event and not to an event which hypothetically might have cause death if it was more severe).
- Requires subject hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/ incapacity.
- A congenital anomaly / birth defect.

10.2. Period of Observation

For the purpose of this trial, the period of observation for collection of adverse events extends from the time the patient starts study medication until 7 days after the last dose administrated.

All adverse events that occur in the course of a clinical trial regardless of the causal relationship must be monitored and followed up until the outcome is known.

10.3. Documentation and Reporting of Adverse Events

10.3.1. Documentation and Reporting of Adverse Events by the Investigator

The investigator must document all adverse events that occur during the observation period set in this protocol (see Section 10.2) on the pages provided in the case report form. Additional instructions may be provided in the investigator file and in the case report form itself. The following approach will be taken for documentation:

All adverse events (whether serious or non-serious) must be documented on the "adverse event" page of the case report form.

If the adverse event is serious (see Section 10.1.4), the investigator must complete, in addition to the "adverse event" page in the case report form, a "serious adverse event report form" at the time when the serious adverse event is detected. The investigator will document the date when he/she or any employee was first aware of the report and fax all SAE reports (initial and follow-up reports), even if they are incomplete, immediately (without culpable

delay), at the latest 24 hours after obtaining knowledge to the safety department/representative of the Sponsor:

Zentrum für Klinische Studien Tuebingen (ZKS Tuebingen)
Otfried-Mueller-Str. 45
72076 Tuebingen, Germany
Phone.: +49 7071 29-85635
SAE-Fax: +49 7071 29-25205
e-mail: zks-pv@med.uni-tuebingen.de

The investigator should also assess the severity and the causal relationship between the event and the trial medication (section 10.3.2).

10.3.2. Assessment of Severity and Causality

The investigator will also provide an assessment of the severity of the event according to CTCAE criteria (Version 5.0) and causal relationship between the event and each of the investigational products or trial procedures.

AEs and SAEs should be evaluated for severity according to the following scale:

- Grade 1 - mild event: Causing no limitations of usual activities; the patient may experience slight discomfort.
- Grade 2 - moderate event: Causing some limitation of usual activities; the patient may experience annoying discomfort.
- Grade 3 - severe event: Causing inability to carry out usual activities; the patient may experience intolerable discomfort or pain.
- Grade 4 - life threatening or disabling event
- Grade 5 - death related to event

The investigator must determine also the relationship between the administration of IMP and the occurrence of an AE/SAE as defined below:

Related: There is a reasonable possibility that the SAE may be related to the IMP (e.g. favourable temporal relationship, positive dechallenge: symptoms are receding when IMP is withdrawn or the dose reduced, positive rechallenge: symptoms are reappearing when the IMP is reintroduced or the full dose is re-administered)

Not Related: There is no reasonable possibility that the SAE is related to the IMP (e.g. there is a plausible alternative cause for the SAE that better explains the occurrence of the SAE)

10.3.3. Sponsors Assessment of the SAEs

All SAE will be subject to a second assessment by the trial Sponsor or authorized second assessors.

The second assessor will fill out a 'Second Assessment Form' for each SAE containing.

- Event serious yes/no
- Relationship between SAE and IMP
- Expectedness of SAE according to the reference document: SmPCs
- Benefit / risk assessment for the trial regarding change as a result of SAE.

10.3.4. Follow-up of Initial Report

Information not available at the time of the initial report (e.g. an end date for the adverse event or laboratory values received after the report) must be documented on a "Serious Adverse Event" form with the box "Follow-up" checked under "Report type".

All patients who have adverse events, whether considered associated with the use of the investigational products or not, must be monitored to determine the outcome. The clinical course of the adverse event will be followed up according to accepted standards of medical practice even after the end of the period of observation, until a satisfactory explanation is found or the investigator considers it medically justifiable to terminate follow-up. Should the adverse event result in death, a full pathologist's report should be supplied, if possible.

The sponsor will identify missing information for each SAE report and will require follow up information in regular intervals from the investigators until all queries are resolved or no further information can be reasonably expected. All responses to queries and supply of additional information by the investigator should follow the same reporting route and timelines as the initial report.

10.3.5. Suspected Unexpected Serious Adverse Reaction (SUSAR)

SAEs have to be assessed by the second assessor wether they are both suspected, i.e. possibly related to IMP and 'unexpected', i.e. the nature and / or severity of which is not consistent with the applicable product information. They are then to be classified as Suspected Unexpected Serious Adverse Reactions (SUSARs).

In case, either the investigator or the second assessor classify the SAE as 'suspected' i.e. either as related or probable, or possible, or unlikely-related to IMP (section 9.3.2) and the SAE is unexpected as assessed by the Sponsor / CI it will be categorized as a SUSAR.

All SUSARs are subject to an expedited reporting (section 9.3.6) to the responsible ethics committee(s), the competent higher federal authority (i.e. either BfArM or PEI) and to all participating investigators.

10.3.6. Expedited Reporting to the Regulatory Authorities

Fatal and life-threatening SUSARs

The competent authority and the ethics committee responsible must be informed by the Sponsor/CI of all fatal or life-threatening SUSARs. This must be done immediately, at the latest seven calendar days after becoming aware of the minimum criteria for reporting. In all cases, attempts must be made to obtain further relevant information, which must be supplied to the competent authority and the ethics committee within a further eight days. Furthermore, if a trial subject dies, this information must be passed on to the ethics committee responsible for the region in which the death occurred.

SUSARs that are not fatal or life threatening

The authority and the ethics committee responsible will be informed without delay by the sponsor or CI of all SUSARs, at the latest within 15 calendar days of becoming aware of the minimum criteria for reporting. Further relevant details will be passed on as soon as possible. If the information at the time of reporting is incomplete, further information to enable adequate assessment of the case will be requested from the reporter or other available sources.

10.3.7. Examination and Report of Changes in the Risk to Benefit Ratio

Without delay, and at the latest within 15 days of the decision for the need to do so, the Sponsor / CI will inform the competent authority, the ethics committee responsible of any events or factors that could result in a review of the risk-benefit ratio of the IMP. These consist of especially:

- Individual reports of expected serious ADRs with an unexpected outcome.
- A clinically relevant increase in the rate of occurrence of expected ADRs.
- SUSARs in trial subjects who have already completed the follow-up period of the clinical trial (”end-of-trial visit”).
- Factors emerging in connection with trial conduct or the development of the IMP that may affect the safety of persons concerned.

10.3.8. Report to the Investigator

The Sponsor / CI will inform investigators of all SUSARs including all relevant further information within the periods set by the authority.

If new information becomes known that is different from the scientific information given to the investigator, all investigators will be informed of this by the sponsor.

10.4. Annual Safety Report

Once a year, the Sponsor / CI will supply a report on the safety of trial subjects with all available relevant information concerning patient safety during the reference period to the competent authorities. This report will also be supplied to the responsible ethics committee.
The annual safety report will be compiled according to the corresponding ICH guideline E2F „Development Safety Update Report – DSUR“

10.5. Deviations from the protocol

Any deviation from the protocol will be noted in the CRF. The PI or a nominated person will evaluate this deviation from the protocol and will decide on the further course of the trial for the respective subject.

10.6. Reporting of pregnancy

Pregnancies and suspected pregnancies (including a positive pregnancy test regardless of age or disease state) of a female patient or the female partner of a male patient occurring while the patient is on study drug or within 28 days of the patient's last dose of study drug are considered events to be reported immediately to the Sponsor. All study drugs have to be discontinued immediately and the patient instructed to return any unused portion of the study drug to the investigator(s). The pregnancy, suspected pregnancy, or positive pregnancy test must be immediately reported to Sponsor after investigator's knowledge using the "Pregnancy Reporting Form".

The female should be referred to an obstetrician.

The investigator(s) will follow the female patient until completion of the pregnancy, and must notify the Sponsor of the outcome of the pregnancy (including notification of false-positive tests) within 24 hours of having knowledge of the event as a follow-up to the initial report.

If the outcome of the pregnancy meets the SAE-criteria , the investigator(s) should follow the procedures for reporting SAEs (i.e., report the event within 24 hours of the investigator's knowledge of the event).

All neonatal deaths that occur within 28 days of birth should be reported, without regard to causality, as SAE.

If the female is found not to be pregnant, any determination regarding the patient's continued participation in the study will be determined by the investigator(s).

In case of study discontinuation due to pregnancy, protocol-required procedures for study discontinuation and follow-up must be performed on the patient unless contraindicated by pregnancy (e.g., x-ray studies). Other appropriate pregnancy follow-up procedures should be considered if indicated.

Information on pregnancy must be collected on the “Pregnancy Reporting Form”. In order for Sponsor or designee to collect any pregnancy surveillance information from the female partner, the female partner must sign an informed consent form for disclosure of this information.

11. Regulatory Consideration

11.1. Ethical Conduct of Clinical Study

11.1.1. Good Clinical Practice, Declaration of Helsinki and legal Provision

The procedures set out in this trial protocol, pertaining to the conduct, evaluation, and documentation of this trial, are designed to ensure that all persons involved in the trial act according to Good Clinical Practice (GCP) and the ethical principles described in the applicable version of the Declaration of Helsinki.
This is a scientific clinical study; the German Medicines Act (AMG) §40 is applicable without restrictions according to section §42.

11.2. Subject Information and Informed Consent

Each patient will be informed about the modalities of the clinical study in accordance with the provided patient informed consent (IC). The patient is to be informed both in writing and verbally by the investigator before any study-specific procedure is performed. The patient must be given sufficient time (i.e. >24 h) to decide whether to participate in this comparative study and to ask questions concerning this trial. It must also be made clear to the patient that he / she can withdraw from the study at any time without giving reasons and that he / she will not be in any way disadvantaged for this. The subject must give consent in writing. The patient and informing physician must each personally date and sign the informed consent form with an integrated declaration on data privacy protection, whereby the physician must not sign before the patient. Original signed documents will be part of the investigator's file and retained with it. A copy of the signed informed consent document and study insurance policy must be given to the subject. The documents must be in a language understandable to the subject and must specify who informed the subject. The subjects will be informed as soon as possible if new information may influence his/her decision to participate in the trial. The communication of this information should be documented in the patient chart.

11.3. Insurance

Each patient is insured against any health impairment occurring as a result of participation in the study in accordance with the laws and regulations of the Deutsches Arzneimittelgesetz. The insurance is covered by HDI-Gerling Industrie Versicherung AG, Am Schönenkamp 45, 40599 Düsseldorf, phone +49 211 177 69 0, fax +49 211 352 345; the Policy number is 5701031101013 and the insurance is valid throughout the conduct of the study including 16

days follow-up for each individual patient. A copy of the insurance policy and conditions are distributed to the patient upon enrolment into the study and the patient is advised to adhere to the conditions of the insurance policy to safeguard a valid patient insurance.

11.4. Confidentiality

The data obtained in the course of the trial will be treated according to the European General Data Protection Regulation (Datenschutz-Grundverordnung; DSGVO) and the applicable local data protection regulations as well as the AMG. The details on confidentiality according to EU-DSGVO and AMG are provided in the patient information using the text templates of the Medical Ethics Commissions in the Federal Republic of Germany (see appendix 15.1).

11.5. Responsibility of the the Investigator

The investigator should ensure that all persons assisting with the trial are adequately informed about the protocol, any amendments to the protocol, the trial treatments, and their trial-related duties and functions.
The investigator should maintain a list of subinvestigators and other appropriately qualified persons to whom he or she has delegated significant trial-related duties.

11.6. Registration of the Trial

Prior to the beginning of the clinical phase (First Patient In) the Sponsor / CI will register the trial in http://www.clinicaltrials.gov. The trial has be given a unique EudraCT number.

11.7. Continuous Information to Independent Ethic Committee

According to the German Drug Law (AMG) and the GCP Ordinance, the EC and the competent authority will be informed of all suspected serious unexpected adverse reactions (SUSARs). Both institutions will be informed in case the risk/ benefit assessment did change or any others new and significant hazards for subjects' safety or welfare did occur. Furthermore, a report on all observed SAEs will be submitted once a year – Annual Safety Report.
The EC and the regulatory authorities must be informed of the end of the trial. They will be provided with a summary of trial results within one year after the end of clinical phase.

11.8. Approval of Protocol and Subsequent Amendments

Before the start of the trial, the trial protocol, informed consent document, and any other appropriate documents will be submitted to the independent Ethics Committee (EC) as well as to the competent authority (BfArM or PEI). A written favourable vote of the EC and an (implicit) approval by the competent higher federal authority are a prerequisite for initiation of this clinical trial. Before the first subject is enrolled in the trial, all ethical and legal requirements must be met. All planned substantial changes (see §10, (1) of German GCP-Regulation) will be submitted for approval to EC and the competent authority in writing as protocol amendments.

12. Publications

12.1. Reports

Within one year of the completion of the trial, the competent authority and the ethics committee will be supplied with a summary of the final report on the clinical trial containing the principle results.

All reports to the sponsor will be written in English language. All clinical, analytical and statistical results will be presented in a final clinical trial report (CTR). The outline of this report will accord to the ICH Topic E3.

Section 10.4 describes the requirements for Annual Safety Reports on the safety of trial subjects.

12.2. Publication

The final results of this study will be presented at scientific meetings and published in a peer reviewed leading medical journal of general medicine or a leading nephrological journal. All publications in result of this study are the responsibility of the principal coordinating investigator and the authorship will reflect the contributions of each collaborating centre. Any publication, abstract or presentation based on patients included in this study must be approved by the coordinating investigator.

13. Financing

The study is financed with intramural funding by the AKF ("Angewandte Klinische Forschung") program of the University Tuebingen.

Table 3 lists the financial resources requested for funding by the intramural program. There are no further financial resources required to complete the study.

Table 3: Budget draft

	Details of requested funds	**Amount of requested funds**
Staff resources	Clinical Investigator: 10% / TV-Ä (88.200€/Year)	17.640,00 €
	Assistant scientist (16,79 €/h x 85h /Monat)	17.126,00 €
Material resources	Submission Ethics Committee / Federal authority (ZKS/PI)	maximum. 9.330,00 €
	Monitoring (ZKS)	13.150,00 €
	Pharmakovigilance (ZKS)	22.740,00 €
	Fees first submission	8.500,00 €
	Fees Amendment	1.250,00 €
	Fees DSUR	2.000,00 €
	Biometrics, data management (IKEAB)	13.900,00 €
	Subjects insurance	3.000,00 €
	Travel reimbursement	5.400,00 €
	Material costs (Elektrodes for BCM measurement, laboratory material)	8.400,00 €
	Study medication + Labelling	1.150,00 €
	Presentation of study results by participation in congresses	5.000,00 €
	Publication costs	2.500,00 €
Total amount		130.436,00 €

14. Literature

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15. Appendices

15.1. Patient Information and Informed Consent

15.2. Instruction sheet for participants

15.3. Label of the Investigational Medical Product Amiloride

15.4. CE certificate of the Body Composition Monitor