Hydrochlorothiazide for Kidney Stone Recurrence Prevention (NOSTONE trial; NCT03057431)

Study Protocol (last version 28. November 2019)
Statistical Analysis Plan (last version 11. November 2021)

For details see next page.

I. Study protocols
    1. First/original trial protocol (version 1.0; Aug 30th, 2016)
    2. Last/final protocol (version 1.7; Nov 28th, 2019)
    3. Revision history

II. Statistical Analysis Plans (SAP)
    1. First/original SAP (version 1.0; Nov 6th, 2018)
    2. Last/final SAP (version 4.0; Nov 11th, 2021)
    3. Revision history

Note: The protocol version control employed a two-level system (#.#) whereas the version control of the Statistical Analysis Plan actually only used a one-level system, although versions where formally named with two levels.

First/original trial protocol

Version 1.0

August 30th, 2016

Clinical Study Protocol

Randomized double-blind placebo-controlled trial assessing the efficacy of standard and low dose hydrochlorothiazide treatment in the prevention of recurrent nephrolithiasis

NOSTONE TRIAL

Study Type:	Clinical trial with Investigational Medicinal Product (IMP)
Study Categorisation:	Risk category B
Study Registration:	ClinicalTrials.gov, Swiss National Clinical Trials Portal (SNCTP)
Study Identifier:	Swiss National Science Foundation # 33IC30_166785/ 1
Sponsor-Investigator:	Prof. Dr. med. Daniel Fuster Leitender Arzt Division of Nephrology, Hypertension and Clinical Pharmacology Bern University Hospital, Bern, Switzerland Phone: +41 (0)31 632 31 44 Email: daniel.fuster@insel.ch
Investigational Product:	Hydrochlorothiazide
Protocol Version and Date:	Version 1, 30.08.2016

Signature Page(s)

Study identifier: Swiss National Science Foundation # 33IC30_166785/ 1
Study title: Randomized double-blind placebo-controlled trial assessing the efficacy of standard and low dose hydrochlorothiazide treatment in the prevention of recurrent nephrolithiasis

The Sponsor-Investigator and Trial Statistician/ Methodologist have approved this trial protocol and confirm hereby to conduct the study according to the protocol, current version of the World Medical Association Declaration of Helsinki, ICH-GCP guidelines and the local legally applicable requirements.

Sponsor-Investigator: Prof. Dr. med. Daniel Fuster

Bern, 30.8.16 [redacted]

Place/ Date Signature

Trial Statistician and Methodologist: PD Dr. med. Sven Trelle

Bern, 20.8.2016 [redacted]

Place/ Date Signature

Local Principal Investigator at study site:

I have read and understood this trial protocol and agree to conduct the trial as set out in this study protocol, the current version of the World Medical Association Declaration of Helsinki, ICH-GCP guidelines and the local legally applicable requirements.

Site:

Principal Investigator:

Place/ Date	Signature

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STUDY SYNOPSIS

Sponsor/ Sponsor-Investigator:	Inselspital Bern (Sponsor) represented by Prof. Dr. med. Daniel Fuster (Sponsor-Investigator)
Study Title:	Randomized double-blind placebo-controlled trial assessing the efficacy of standard and low dose hydrochlorothiazide treatment in the prevention of recurrent nephrolithiasis
Short Title/ Study ID:	NOSTONE Trial
Protocol Version and Date:	Version 1, 30.08.2016
Trial registration:	ClinicalTrials.gov, Swiss national clinical trials portal (SNCTP)
Study category and Rationale:	Risk category B Clinical trial with an IMP authorized in Switzerland that is not used in accordance with its prescribing information.
Clinical Phase:	Therapeutic use
Background and Rationale:	Nephrolithiasis is a global healthcare problem with a current lifetime risk of 18.8% in men and 9.4% in women. Without specific treatment, 5- and 20-year recurrence rates are 40% and 75%, respectively. Given the high cost of medical treatments and surgical interventions as well as the morbidity related to symptomatic stone disease, medical prophylaxis for stone recurrence is an attractive approach. About 80–90% of stones are composed of calcium oxalate with various admixtures of calcium phosphate. Increased excretion of calcium in the urine, hypercalciuria, is the most common metabolic abnormality encountered in patients with recurrent nephrolithiasis. Thiazide diuretics have been the cornerstone of pharmacologic metaphylaxis for more than 40 years. The effect of thiazides to reduce the risk of stone recurrence has been attributed to their ability to decrease urinary calcium excretion. However, other factors, such as reduction of urinary pH and urinary oxalate excretion, probably contribute to this effect. Efficacy of thiazides on recurrence prevention of calcareous nephrolithiasis was tested in 11 randomized controlled trials (RCTs). With the exception of two trials, thiazides significantly reduced stone recurrence. Most of these trials are from the 1980's and 90's and the cumulative number of patients studied is remarkably low for such a prevalent disease. Our systematic review of these RCTs revealed major methodological deficiencies in all trials, including: lack of double-blinding and intention-to-treat analysis, unclear allocation concealment, lack of adverse event and drop out reporting and unknown baseline risk of disease severity. Furthermore, high doses of thiazides were employed in all trials, in the case of the best studied thiazide, hydrochlorothiazide (HCTZ), up to 100 mg daily. At such high doses, side effects occur frequently. Nowadays, thiazides are widely used in the treatment of recurrent nephrolithiasis and arterial hypertension, but at significantly lower doses. In the case of recurrent nephrolithiasis, however, this practice is not supported by randomized evidence and consequently, we do not know whether the currently employed low dose thiazide regimens are effective in reducing the risk for stone recurrence. Thus, evidence for benefits and harms of thiazide diuretics in the prevention of calcium-containing kidney stones in general remains unclear. In addition, the efficacy of the currently employed low dose thiazide regimens to prevent stone recurrence is not known.
Objective(s):	We plan to assess the efficacy of standard and low dose HCTZ treatment in the recurrence prevention of calcium-containing kidney stones. More specifically, we aim to assess the dose-response relationship for three different dosages of HCTZ.

Outcome(s):	Primary: relationship of the incidence of stone recurrences (a composite of symptomatic or radiologic recurrence) during study treatment and dose group. Secondary: individual components of the composite primary outcome, changes in urinary biochemistry elicited by HCTZ treatment and impact of baseline disease severity, biochemical abnormalities and stone composition on treatment response.
Study design:	Multicenter, randomized, placebo-controlled, double-blind, parallel-group trial.
Inclusion/ Exclusion criteria:	Main inclusion criteria: • Informed Consent as documented by signature • Age 18 years or older • Recurrent kidney stone disease (≥ 2 stone events within the last 10 years prior to randomization) • Any past kidney stone containing 50% or more of calcium oxalate, calcium phosphate or a mixture of both Main exclusion criteria: • Subjects with secondary causes of recurrent calcareous nephrolithiasis • Subjects with the following medications: Thiazide or loop diuretics, carbonic anhydrase inhibitors, xanthine oxidase inhibitors, alkali, active Vitamin D, calcium supplementation, bisphosphonates, denusomab, teriparatide, glucocorticoids • Known allergy to the study drug
Measurements and procedures:	An overview on the study measurements and procedures is provided in the study schedules. The main measurements and procedures performed are the following: Screening: medical history, vital signs, clinical laboratory tests Efficacy: symptomatic stone recurrence, low-dose renal-limited computed tomography for radiologic stone recurrence. Safety: vital signs, complete physical examination, SAEs and selected AEs, clinical laboratory tests.
Study Product/ Intervention:	HCTZ 12.5 mg, 25 mg or 50 mg once daily per os for 24 or 36 months. In addition, all patients in HCTZ treatment arms will receive state-of-the-art non-pharmacologic recommendations for stone prevention according to current guidelines.
Control Intervention (if applicable):	Placebo once daily per os for 24 to 36 months. In addition, all patients in the placebo arm will receive state-of-the-art non-pharmacologic recommendations for stone prevention according to current guidelines.
Number of Participants with Rationale:	416 patients in total, 104 patients per study arm.
Study Duration:	01/2017 – 02/2021
Study Schedule:	Month/Year of First-Participant-In: 01/2017 Month/Year of Last-Participant-Out: 02/2021
Investigator(s):	Principal Study Investigator: Prof. Dr. med. Daniel Fuster, Leitender Arzt Division of Nephrology, Hypertension and Clinical Pharmacology, Bern University Hospital Freiburgstrasse 11, 3010 Bern, Switzerland Email: daniel.fuster@insel.ch Phone: +41 (0)31 632 31 44
Study Centre(s):	8 sites in Switzerland including University Hospitals of Basel, Bern, Geneva, Lausanne and Zürich as well as Cantonal Hospitals of Aarau, Lugano and St. Gallen.

Statistical Considerations:	Sample size: Calculation was based on the primary objective i.e. to assess the dose-response relationship and the primary outcome i.e. recurrence with the following assumptions: • Uniform recruitment over 24 months with allocation ratio fixed at 1 across all arms. • A maximum and minimum follow-up time of 36 and 24 months, respectively. • Cumulative drop-out rate of 10% at 24 months after study start. • Risk of recurrence in the placebo group of 0.20 and 0.45 at 12 and 36 months after study start, respectively. • Hazard ratios for the 12.5, 25 and 50 mg HCTZ doses of 0.90, 0.65 and 0.50, respectively. • Power was set to be at least 80% and alpha was fixed at a two-sided level of 0.05. • An unweighted log-rank test for linear trend with local alternatives. Primary efficacy analysis: On the intention-to-treat analysis set by a stratified log-rank test for linear trend with the hazard ratio as primary effect measure. The test addresses the primary objective of the trial i.e. test for any dose-response relationship. Comparisons between placebo and the three active trial arms will be considered exploratory as the trial is not powered to detect differences there. Missing data will be handled by multiple imputation. All statistical analyses will be presented as effect measure plus 95% confidence interval. Secondary efficacy analysis: Analysis will follow principles as described for primary efficacy analysis. Continuous outcomes (laboratory values) will be analyzed using linear regression.
GCP Statement:	This study will be conducted in compliance with the protocol, the current version of the Declaration of Helsinki, the ICH-GCP as well as all national legal and regulatory requirements.

STUDY SUMMARY IN LOCAL LANGUAGE

Titel des Forschungsprojektes:

Randomisierte plazebokontrollierte Doppelbindstudie über die Wirksamkeit von normal- und tiefdosiertem Hydrochlorothiazid zur Vorbeugung von Rückfällen kalziumhaltiger Nierensteine: die NOSTONE-Studie

Lead:

In dieser klinischen Studie wird die Wirksamkeit des Medikaments Hydrochlorothiazid zur Vorbeugung von Rückfällen kalziumhaltiger Nierensteine getestet.

Inhalt und Ziele des Forschungsprojektes:

Nierensteine sind ein weltweites Gesundheitsproblem, etwa 10% aller Menschen sind mindestens einmal im Leben davon betroffen. Nierensteine sind äusserst schmerzhaft, treten wiederholt auf und verursachen hohe direkte und indirekte Kosten. Eine effektive und kostengünstige Vorbeugung gegen Rückfälle von Nierensteinen ist deshalb äusserst wünschenswert.
Etwa 80-90% aller Nierensteine enthalten Kalzium, eine vermehrte Ausscheidung von Kalzium im Urin ist die häufigste Stoffwechselstörung bei Patienten mit Nierensteinen. Thiazide reduzieren die Kalziumausscheidung im Urin und werden daher seit Jahrzehnten zur Vorbeugung von Rückfällen kalziumhaltiger Nierensteine eingesetzt. Deren Wirksamkeit wurde in den letzten 30 Jahren in mehreren Studien getestet. Leider war die Durchführung dieser Studien mangelhaft, die Gesamtzahl der untersuchten Patienten klein, und es wurden hohe Medikamentendosen getestet, welche häufig Nebenwirkungen verursachen. Zur Zeit ist daher der Stellenwert der Thiazide in der Behandlung von Patienten mit Nierensteinen unklar. Ziel dieser Studie ist es, die Wirksamkeit von normal- und tiefdosiertem Hydrochlorothiazid zur Vorbeugung von Rückfällen kalziumhaltiger Nierensteine zu testen.

Wissenschaftlicher und gesellschaftlicher Kontext des Forschungsprojekts:

Das Nierensteinleiden gehört zu den häufigsten menschlichen Erkrankungen. Mit dieser klinischen Studie soll eine grosse Wissenslücke in der Nierensteinbehandlung geschlossen werden. Die Resultate dieser Studie werden die Behandlung von Nierensteinpatienten weltweit beeinflussen.

ABBREVIATIONS

AE	Adverse Event
BMI	Body Mass Index
CA	Competent Authority (Swissmedic)
CEC	Competent Ethics Committee
CI	Confidence Interval
CRF	Case Report Form
eCRF	Electronic Case Report Form
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
EOS	End of Study
EOT	End of Treament
GCP	Good Clinical Practice
HCTZ	Hydrochlorothiazide
IMP	Investigational Medicinal Product
IVU	Intravenous Urography
NIH	National Institute of Health
RCT	Randomized Controlled Trial
SAE	Serious Adverse Event
SNCTP	Swiss National Clinical Trials Portal
SUSAR	Suspected Unexpected Serious Adverse Reaction

STUDY SCHEDULE 1 – PARTICIPANTS RECRUITED IN THE FIRST 12 MONTHS OF THE RECRUITMENT PERIOD

Study Period	Screening[1]		Treatment period								Safety follow-up
Visit	1	2	3	4 or ✆[2]	✆	5	✆	6	✆	7	✆ EOS
Visit time points NOSTONE trial	-14 to -1 day(s)	0	2 weeks	3 months	6, 9 months	12 months	15, 18, 21 months	24 months	27, 30, 33 months	36 months/ EOT	30 days after EOT
Allowed visit window		0	± 2 weeks	± 2 weeks	± 4 weeks	± 4 weeks	± 4 weeks	± 4 weeks	± 4 weeks	± 4 weeks	± 2 weeks
Patient Information & Informed Consent	x										
In-/ Exclusion Criteria	x		x								
Demographics	x										
Medical History	x										
Stone composition	x[3]		x [composition of any recurrent stone(s) if data available per routine]								
Physical Examination	x			x		x		x		x	
Vital Signs[4]	x			x		x		x		x	
Blood analysis[5]		x[6]		x		x		x		x	
Urine analysis[5]		x[7]		x[8]		x[7]		x[7]		x[7]	
Pregnancy test (blood or urine)[10]		x									
Randomization			x								
Low-dose renal CT for radiologic recurrence[5]			x[9]	x (in case of a suspicion of symptomatic recurrence)						x[9]	
Assessment of symptomatic stone recurrence				x	x	x	x	x	x	x	
Adherence reminder				x	x	x	x	x	x		
Screening criteria for interrupting or discontinuing IMP				x	x	x	x	x	x		
AEs of special interest				x	x	x	x	x	x	x	
SAEs		x	x	x	x	x	x	x	x	x	x
Concomitant medication	x	x	x	x	x	x	x	x	x	x	
Hand out of (new) IMP			x	x	x	x	x	x	x		

Collection of used/ unused IMP packs				x		x		x		x	
Daily intake of IMP			x (start on day after visit 3)								
Non-pharmacological preventive measures			x								

EOT end of treatment
EOS end of study

1 For participants newly referred to the study site and in metabolic work-up for recurrent stone disease, screening assessments are performed at visits 1 and 2. For participants who are in regular follow-up at the site for recurrent stone disease, visit 1 is not performed and all screening assessments are done at visit 2.
2 For participants recruited via new referrals: visit at the study site; for participants who are recruited from patients in routine follow-up at the study site: phone call assessing all parameters as required at the three-monthly phone calls with all study participants (see e.g. phone call at 6 months).
3 Information on the composition of all stones analyzed prior to randomisation as available.
4 Heart rate, systolic and diastolic blood pressure at the right arm in sitting position after at least 5 minutes at rest.
5 Analyzed centrally.
6 If at visit 2, blood sodium was in the range from 125 to 130 mmol/L and/or blood potassium in the range from 3 to 3.5 mmol/L (or if deemed necessary by the responsible principal investigator for any other reason), blood sodium and/or potassium level(s) must be re-checked within 1 month after visit 3.
7 Two 24 hour urine collections by the patient starting 48 hours and 24 hours respectively prior to the visit.
8 One 24 hour urine collection by the patient starting 24 hours prior to the visit.
9 If a routine non-iv contrast renal CT was performed within 2 months prior to visit 3 or visit 7 respectively, this CT can be used for the trial and no additional CT has to be performed at the respective visit.
10 For women of child-bearing potential, defined as women who are not surgically sterilized/ hysterectomized, and/ or who are postmenonpausal for less than 12 months), analyzed locally

STUDY SCHEDULE 2 – PARTICIPANTS RECRUITED ≥ 12 MONTHS AFTER RECRUITMENT START

Study Period	Screening[1]		Treatment period						Safety follow-up
Visit	1	2	3	4 or ☽[2]	☽	5	☽	6	☽ EOS
Visit time points NOSTONE trial	-14 to -1 day(s)	0	2 weeks	3 months	6, 9 months	12 months	15, 18, 21 months	24 months/ EOT	30 days after EOT
Allowed visit window		0	± 2 weeks	± 2 weeks	± 4 weeks	± 4 weeks	± 4 weeks	± 4 weeks	± 2 weeks
Patient Information & Informed Consent	x								
In-/ Exclusion Criteria	x		x						
Demographics	x								
Medical History	x								
Stone composition	x[3]		x [composition of any recurrent stone(s) if data available per routine]						
Physical Examination	x			x		x		x	
Vital Signs[4]	x			x		x		x	
Blood analysis[5]		x[6]		x		x		x	
Urine analysis[5]		x[7]		x[8]		x[7]		x[7]	
Pregnancy test (blood or urine)[10]		x							
Randomization			x						
Low-dose renal CT for radiologic recurrence[5]			x[9]	x (in case of a suspicion of symptomatic recurrence)				x[9]	
Assessment of symptomatic stone recurrence				x	x	x	x	x	
Adherence reminder				x	x	x	x		
Screening criteria for interrupting or discontinuing IMP				x	x	x	x		
AEs of special interest				x	x	x	x	x	
SAEs		x	x	x	x	x	x	x	x
Concomitant medication	x	x	x	x	x	x	x	x	

Hand out of (new) IMP			x	x	x	x	x		
Collection of used/ unused IMP packs				x		x		x	
Daily intake of IMP			x (start on day after visit 3)						
Non-pharmacological preventive measures			x						

EOT end of treatment
EOS end of study

1 For participants newly referred to the study site and in metabolic work-up for recurrent stone disease, screening assessments are performed at the two SKSC visits 1 and 2 as applicable. For participants who are in regular follow-up at the site for recurrent stone disease, visit 1 is not performed and all screening assessments are done at visit 2.
2 For participants recruited via new referrals: visit at the study site; for participants who are recruited from patients in routine follow-up at the study site: phone call assessing all parameters as required at the three-monthly phone calls with all study participants (see e.g. phone call at 6 months).
3 Information on the composition of all stones analyzed prior to randomisation as available.
4 Heart rate, systolic and diastolic blood pressure at the right arm in sitting position after at least 5 minutes at rest.
5 Analyzed centrally.
6 If at visit 2, blood sodium was in the range from 125 to 130 mmol/L and/or potassium in the range from 3 to 3.5 mmol/L (or if deemed necessary by the responsible principal investigator for any other reason), blood sodium and/or potassium level(s) must be re-checked within 1 month after visit 3.
7 Two 24 hour urine collections by the patient starting 48 hours and 24 hours respectively prior to the visit.
8 One 24 hour urine collection by the patient starting 24 hours prior to the visit.
9 If a routine non-iv contrast renal CT was performed within 2 months prior to visit 3 or visit 7 respectively, this CT can be used for the trial and no additional CT has to be performed at the respective visit.
10 For women of child-bearing potential, defined as women who are not surgically sterilized/ hysterectomized, and/ or who are postmenonpausal for less than 12 months), analyzed locally

1. STUDY ADMINISTRATIVE STRUCTURE

1.1 Sponsor-Investigator

Prof. Dr. med. Daniel Fuster, Leitender Arzt
Division of Nephrology, Hypertension and Clinical Pharmacology, Bern University Hospital
Freiburgstrasse 11, 3010 Bern, Switzerland
Email: daniel.fuster@insel.ch
Phone: +41 (0)31 632 31 44

1.2 Principal Study Investigator

Prof. Dr. med. Daniel Fuster, Leitender Arzt
Division of Nephrology, Hypertension and Clinical Pharmacology, Bern University Hospital
Freiburgstrasse 11, 3010 Bern, Switzerland
Email: daniel.fuster@insel.ch
Phone: +41 (0)31 632 31 44

1.3 Statistician ("Biostatistician")

PD Dr. med. Sven Trelle
CTU Bern, Department of Clinical Research, University of Bern
Finkelhubelweg 11, 3012 Bern, Switzerland
Email: sven.trelle@ctu.unibe.ch
Phone: +41 (0)31 631 35 04

1.4 Laboratory

The address of the central laboratory is documented elsewhere.

1.5 Monitoring institution

CTU Bern, Department of Clinical Research, University of Bern
Finkelhubelweg 11, 3012 Bern, Switzerland
Phone: +41 (0)31 631 33 72

1.6 Data Safety Monitoring Committee

Not applicable.

1.7 Any other relevant Committee, Person, Organisation, Institution

1.7.1 Study Steering Committee

The study steering committee will be responsible for the overall supervision of the trial and will regularly meet to discuss scientific and logistic aspects of the trial. Information on the composition of the committee is available in a separate document.

2. ETHICAL AND REGULATORY ASPECTS

Before the study will be conducted, the protocol, the proposed patient information and consent form, as well as other study-specific documents shall be submitted to a properly constituted Competent Ethics Committee (CEC) and competent authority (CA, Swissmedic) in agreement with local legal requirements, for formal approval. Any amendment to the protocol must as well be approved (if legally required) by these institutions.
The decision of the CEC and CA concerning the conduct of the study will be made in writing to the Sponsor-Investigator before commencement of this study. The clinical study can only begin once approval from all required authorities has been received. Any additional requirements imposed by the authorities shall be implemented.

2.1 Study registration

The study will be registered in the Clinical Trials Registry Platform of the National Institute of Health (NIH) – ClinicalTrials.gov. In addition, the trial will be registered in the Swiss National Clinical Trials Portal (SNCTP).

2.2 Categorisation of study

Risk category B. Clinical trial with an investigational medicinal product (IMP) authorized in Switzerland that is not used in accordance with its prescribing information.

2.3 Competent Ethics Committee (CEC)

Responsible investigators at each study site will ensure that approval from the appropriate constituted CEC is sought for the clinical study. Yearly intermediary reports (annual safety reports) will be forwarded to the CEC. Changes in the research activity and all unanticipated problems involving risks to humans will be reported to the CEC within 15 days. No changes will be made to the protocol without prior Sponsor and CEC approval, except where necessary to eliminate apparent immediate hazards to study participants.
Premature study end or interruption of the study will be reported to the CEC within 15 days. The regular end of the study will be reported to the CEC within 90 days, the final study report shall be submitted within one year after study end. Amendments will be reported according to section 2.10.

2.4 Competent Authorities (CA)

The Sponsor-Investigator will obtain approval from the CA (Swissmedic) before the start of the clinical trial. Yearly intermediary reports (annual safety reports) will be forwarded to the CA. Premature study end or interruption of the study will be reported to the CA within 15 days. The regular end of the study will be reported to the CA within 90 days, the final study report shall be submitted within one year after study end. Amendments are reported according to section 2.10. Non-substantial amendments shall be reported as soon as possible.

2.5 Ethical Conduct of the Study

The study will be carried out in accordance to the protocol and with principles enunciated in the current version of the Declaration of Helsinki, the guidelines of Good Clinical Practice (GCP) issued by ICH, the Swiss Law and Swiss regulatory authority's requirements. The CEC and regulatory authorities will receive annual safety and interim reports and be informed about study stop/ end in agreement with local requirements.

2.6 Declaration of interest

There are no conflicts of interest.

2.7 Patient Information and Informed Consent

All participants included in the trial are adults with recurrent kidney stone disease. No vulnerable individuals will be included in the trial. The participation in the trial is voluntary. The investigators will explain to each participant the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits and any discomfort it may entail. Each participant will be informed that the participation in the study is voluntary and that he/she may withdraw from the study at

any time and that withdrawal of consent will not affect his/her subsequent medical assistance and treatment. The participant will be informed that his/her medical records may be examined by authorised individuals other than their treating physician. All study participants will be provided with a participant information sheet and a consent form describing the study and providing sufficient information for the participant to make an informed decision about their participation in the study. Participants willing to participate will sign and date the informed consent form. The consent form will also be signed and dated by the investigator (or his designee) and it will be retained as part of the study records. A copy of the signed document will be given to the participants. Only after obtaining formal consent with the approved consent form, participants will be submitted to any study procedure. No compensation is foreseen and informed consent for general and further use of data and/ or biological samples will not be sought.

2.8 Participant privacy and confidentiality

The investigator affirms and upholds the principle of the participant's right to privacy and that they shall comply with applicable privacy laws. Especially, anonymity of the participants shall be guaranteed when presenting the data at scientific meetings or publishing them in scientific journals. Individual subject medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited. Subject confidentiality will be further ensured by utilising subject identification code numbers to correspond to treatment data in the computer files. For data verification purposes, authorised representatives of the Sponsor (-Investigator), the CA, or CEC may require direct access to parts of the medical records relevant to the study, including participants' medical history.

2.9 Early termination of the study

The Steering Committee may terminate the study prematurely according to certain circumstances, for example:

- ethical concerns,
- financial issues,
- insufficient participant recruitment,
- when the safety of the participants is doubtful or at risk, respectively,
- alterations in accepted clinical practice that make the continuation of a clinical trial unwise,
- early evidence of benefit or harm of the experimental intervention,
- or any other reason that would prevent the project execution according to the research plan.

Premature study end or interruption of the study will be reported to the CEC and CA within 15 days.

2.10 Protocol amendments

The Steering Committee will decide on protocol amendments. Any investigator, CEC or CA will be able to provide suggestions for a protocol amendment. Important protocol modifications will be communicated by the Sponsor-Investigator to relevant parties (CEC, CA, trial participants, trial registries and the Swiss National Science Foundation).
Substantial amendments will only be implemented after approval of the CEC and CA, respectively. Under emergency circumstances, deviations from the protocol to protect the rights, safety and well-being of human subjects may proceed without prior approval of the sponsor and the CEC/ CA. Such deviations shall be documented and reported to the sponsor and the CEC/ CA as soon as possible. All non-substantial amendments are communicated to the CA as soon as possible if applicable and to the CEC within the Annual Safety Report (ASR).

3. BACKGROUND AND RATIONALE

3.1 Background and Rationale

Introduction:
Nephrolithiasis is a worldwide healthcare problem with a current lifetime risk of ~18.8% in men and ~9.4% in women in Western civilizations [1]. Incidence and prevalence of renal stone disease are

increasing globally, irrespective of age, sex and race [1, 2]. Without a specific treatment, 5- and 20-year recurrence rates are ~40% and ~75%, respectively [3, 4]. In the United States, hospitalizations, surgery and lost work time associated with kidney stones cost more than 5 billion US Dollars annually [5]. Thus, given the high cost and the morbidity related to recurrent kidney stone disease, medical prophylaxis seems to be an attractive approach [6, 7]. Indeed, apart from its benefits to patients in terms of reduced morbidity and risk from procedures, medical prevention of nephrolithiasis is clearly cost effective [8].

Although various inherited and systemic diseases are associated with kidney stones, most stones are idiopathic [3]. Despite this, nephrolithiasis needs to be considered as a systemic disease. Chronic kidney disease (CKD), arterial hypertension, obesity, diabetes mellitus and low bone mass are much more prevalent in stone formers than in non-stone formers and contribute significantly to stone-related morbidity and mortality [9-15]. It is, however, currently unclear if stone disease is a cause of this co-morbidity or if it is a consequence of the same underlying conditions that lead to these disorders and renal stone disease.

80-90% of stones are composed of calcium oxalate, calcium phosphate or a mixture of both [16, 17]. Increased excretion of calcium in the urine, hypercalciuria, is the most frequent metabolic abnormality encountered in patients with recurrent idiopathic nephrolithiasis [17, 18]. Depending on the stone subtype, hypercalciuria is present in up to 87% of patients with calcium-containing kidney stones [17]. Other urinary factors frequently encountered in recurrent calcareous stone formers that contribute to the development of stones are hypocitraturia, hyperoxaluria, hyperuricosuria and low urinary volume [19]. Supersaturation of urinary calcium oxalate and calcium phosphate, expressed as the ratio of concentration to its solubility, is the driving force for stone formation. At a supersaturation < 1, crystals dissolve, at a supersaturation > 1, crystals form. Hypercalciuria increases supersaturation both for calcium oxalate and calcium phosphate and thus increases the risk for both calcium oxalate and calcium phosphate stones [3]. Urinary supersaturations calculated from ambulatory 24 hour urine collections are highly correlated with the type of kidney stones encountered in the individual stone former, with notable rare exceptions in patients with very high or very low urinary volumes [20]. The hypercalciuria encountered in recurrent stone formers is often familial and strongly influenced by diet but in most cases of unknown origin and hence designated “idiopathic” [3]. Gut absorption of calcium is enhanced in idiopathic hypercalciuria, but serum calcium remains typically normal because intestinally absorbed calcium is promptly excreted by the kidneys [21]. Despite intestinal calcium hyperabsorption, patients with idiopathic hypercalciuria are often in negative calcium balance because of excessive renal calcium losses, especially under a low calcium diet [19, 22]. The source of calcium in these circumstances is the skeleton, the largest repository of calcium in the body [19]. Not surprising therefore, low bone mass is a frequent finding in normo- and especially hypercalciuric stone formers [9]. At the moment, thiazide diuretics are the only drugs known to reduce urinary calcium excretion. This peculiar property is employed in the prevention of recurrent calcareous nephrolithiasis but also in the prevention of bone loss in patients with recurrent nephrolithiasis and/ or arterial hypertension [9, 23]. Observational data and evidence from prospective trials indicate that chronic thiazide therapy preserves bone mineral density and reduces fracture risk [24-27].

Clinical work-up of patients with recurrent nephrolithiasis includes a detailed history and physical examination as well as blood and urine analysis to detect potential causes of stones but also possible associated conditions [23]. Spot urine analysis is performed to search for crystalluria and rule out infection [23]. Paramount for the metabolic work-up are 24 h urine collections, which are typically performed twice, since mineral excretions have considerable day-to-day variations [23, 28, 29]. Metabolic work-up for stone disease is done at least 3 months after a symptomatic stone event in patients which have resumed their usual diet and activity [30]. All passed stones need to be analyzed for classification purposes but also to detect possible treatment related conversions of one stone type to another (e.g. calcium oxalate to calcium phosphate by citrate-induced urinary alkalinization) [3, 31]. Computed tomography (CT) without the use of contrast material is the most sensitive method to detect renal and ureteral stones and should, according to current guidelines, be preferred to plain abdominal X-ray imaging (KUB radiography) or intravenous urography (IVU) in the case of a symptomatic stone event [23, 31-33]. Modern low-dose CT protocols cause greatly reduced radiation exposure while preserving a high sensitivity of 96.6% and specificity of 94.9% [34]. Current radiation exposure of low dose CT protocols for the detection of urolithiasis is in the range of 0.97-1.9 mSV and thus similar to KUB radiography (0.5 – 1 mSv) but significantly lower than IVU (1.3 -3.5 mSv) or regular dose CT (4.5 – 5 mSv) [23, 35, 36].

Follow-up studies are necessary in patients taking preventive measures for stone recurrence [23, 37]. American and European guidelines recommend a first follow-up 24 hour urine collection 8-12 weeks after initiation of therapy to adjust drug dosage [23, 31]. Also, periodic blood testing in patients on

pharmacological treatment to assess for adverse events (AEs) are recommended [31]. In the case of thiazide diuretics, a repeat blood draw with serum electrolytes is typically done within the first few weeks of treatment initiation to rule out electrolyte abnormalities, especially hypokalemia. In the case of the latter, oral potassium supplementation or a potassium-sparing diuretic like spironolactone or amiloride can be additionally prescribed [31]. Once urinary parameters have been normalized and blood parameters are stable, yearly blood and urine analysis is recommended [23, 31]. In addition to metabolic studies, periodic follow-up imaging studies, preferably with low dose CT, are equally recommended to assess for stone growth or new stone formation [23, 31]. A one-year imaging interval is currently recommended for stable patients on medical stone prevention [23, 31].

Current knowledge on the efficacy of thiazides in recurrent nephrolithiasis:

The first thiazide diuretic chlorothiazide was synthesized in 1957 by Sprague and Novello [38]. In 1959, Lamberg and Kuhlbäck reported the observation that the thiazides chlorothiazide and hydrochlorothiazide (HCTZ) reduce urinary calcium excretion [39]. Soon thereafter, Lichtwitz and colleagues suggested that this peculiar property might be exploited to prevent recurrence of calcium-containing kidney stones [40]. In 1970, Yendt et al. reported initial observations of an uncontrolled series of 72 recurrent calcium stone formers treated with HCTZ in doses from 100 mg to 200 mg daily [41]. The treatment seemed highly effective: compared to pretreatment, HCTZ at such doses reduced stone episodes from 0.57 to 0.03 per patient year in patients without radiologic evidence of stone disease at treatment start and from 1.1 to 0.53 per patient year in patients with residual stone load at treatment initiation. Similar findings were reported by the same group in a follow up publication several years later with a total of 346 patients [42]. Based on these publications, despite the absence of randomized controlled trials (RCTs), thiazide rapidly became a cornerstone of medical stone prevention.

In August 2015, we conducted a systematic database search for RCTs that tested the efficacy of thiazides in the prevention of recurrent nephrolithiasis. Our search covered MEDLINE, the Cochrane Database, Google Scholar and the Web of Science for English language trials that had clinical endpoints (symptomatic or radiologic stone recurrence) and at least 1 year of follow-up. We also searched for unpublished trials at the World Health Organization's International Clinical Trials Registry Platform (www.who.int/trialsearch), the NIH funded site ClinicalTrials.gov (https//:clinicaltrials.gov) and on internet databases provided by the US Food and Drug Administration and by the European Medicines Agency [42]. Our search revealed 11 published RCTs [43-53] and no unpublished trials. All 11 RCTs identified are depicted in Table 3.1-1. The first RCT for stone prevention with thiazide diuretics was published in 1981. At total of seven trials were conducted in the 1980's, three in the 1990's and only one trial was conducted after the year 2000. As shown in Table 1, with the exception of two trials [51, 53], which were the first two RCTs conducted in the field, thiazides significantly reduced stone recurrence compared to placebo or control, in average by about 50% (Table 1). The thiazides employed in the two negative trials were bendroflumethiazide at the dosage of 2.5 mg three times daily and HCTZ at the dosage of 25 mg twice daily. Follow-up duration of the two negative trials was very short (1 and 1.6 years, respectively) compared to 2 – 5 years in the positive trials, which may explain the lack of benefit observed. All trials were small and only one trial included > 100 patients [47]. The 11 trials included 693 patients in total (Table 1). In three trials, the outcome was symptomatic stone recurrence, in the other eight trials the outcome was a composite of symptomatic and radiologic recurrence (Table 3.1-1). In all trials, radiologic recurrence was studied with the low sensitivity and specificity imaging modalities, KUB or IVU. A consistent finding in these thiazide RCTs was that stone-formation rate between treated and control groups did not begin to diverge until after at least one year of therapy [54]. As detailed in Table 3.1-1, our review indicates that all thiazide RCTs thus far conducted have significant methodological deficiencies including unclear allocation concealment, lack of double-blinding, no intention-to-treat analysis and lack of AE and drop out reporting.

Interestingly, in only three of 11 RCTs hypercalciuria was an inclusion criterion and thiazides seem to be effective in reducing stone recurrence regardless of the presence or absence of hypercalciuria (Table 3.1-1). There are at least two possible explanations for this observation: first, Curhan et al. demonstrated in a large epidemiological study that calciuria is a gradual risk factor for the development of kidney stones [55]. An increase in the relative risk for kidney stone formation was observed when urinary calcium excretion exceeded 2.5 mmol/d, which is far below the current definition of hypercalciuria on a random outpatient diet (> 7.5 mmol/d). Second, thiazides, in addition to reducing calciuria, have favorable effects on other urinary constituents that may reduce stone risk. The latter include reduction of urinary pH and oxalate excretion and an increase of urinary magnesium and zinc excretion [56, 57].

HCTZ was used in five of the 11 thiazide trials and thus is currently the best studied thiazide in the

prevention of stone recurrence [45, 48, 50, 52, 53]. However, bendroflumethazide, chlorthalidone, trichlormethiazide and indapamide also reduced stone recurrence in one or more trial and seem to be effective as well (Table 3.1-1). In all trials, high thiazide doses were employed, in the case of HCTZ, 50 - 100 mg daily. In four of the five trials where HCTZ was employed, the diuretic was given twice daily, whereas in the treatment of arterial hypertension, HCTZ is given once daily [54]. Apart from reducing blood pressure, once daily HCTZ at 50 mg, 25 mg or 12.5 mg daily also reduced calciuria, a surrogate marker for stone prevention [58]. Twice daily HCTZ increases the frequency of side effects and augments diuresis at night and thereby likely affects compliance [54, 58].

A recent study showed that thiazide diuretics are often not used in an evidence-based fashion for the prevention of stone recurrence [59]. In that study, 107 patients with a filled prescription for thiazide diuretics that underwent a 24-hour urine stone risk factor analysis and had medical record documentation that the thiazide was prescribed for calcium-containing kidney stone recurrence were analyzed. Only 35% of HCTZ-treated patients received ≥50 mg/ d, a dose previously shown to reduce stone recurrence in RCTs. 52% of patients were prescribed 25 mg daily and 13% 12.5 mg daily, doses which were not studied in RCTs. The tendency to prescribe lower doses of thiazides in patients with recurrent nephrolithiasis was likely triggered by a paradigm shift in prescribing practices for thiazides used for the treatment of arterial hypertension. Starting in the 1980's, lower doses of HCTZ (12.5–25 mg daily) were increasingly employed [60]. While clinical and biochemical side effects were noted to be dose dependent, the antihypertensive effects remained robust, even at lower doses [60, 61]. If this is also true for recurrent calcareous nephrolithiasis is currently unknown.

Fink et al. recently conducted a systematic review of RCTs for the medical management to prevent recurrent nephrolithiasis [62]. RCTs involving dietary or pharmacologic treatments to prevent recurrent kidney stones in adults that reported clinical outcomes or harms were included in the meta-analysis. The search of the authors covered MEDLINE, the Cochrane Database, Google Scholar and the Web of Science for English-language studies published from 1948 through 2011. Using criteria developed by the Cochrane Collaboration, individual study quality was rated good, fair or poor on the basis of adequacy of allocation concealment, blinding, reporting reasons for attrition and how analyses accounted for incomplete data. Following methods developed by the Agency for Healthcare Research and Quality (AHRQ)'s Effective Health Care Program [63], strength of evidence (SOE) for the efficacy of each treatment was graded on the basis of risk of bias, consistency, directness and precision. The review was nominated to the AHRQ by the American Urological Association but was funded by the AHRQ. A total of 28 trials (8 dietary and 20 pharmacologic) were included in the meta-analysis. Six of the 11 thiazide trials we identified in our own database search were included in the meta-analysis (Table 3.1-1) [43-45, 50, 52, 53]. Patients enrolled in the selected thiazide trials had two or more stone events prior to study inclusion, mean treatment duration was 35 months with a cumulative number of 365 patients. Patients were assigned to thiazide and either placebo [44, 45, 53] or control [43, 50, 52]. The authors concluded that AEs were inconsistently reported but that compared with participants in the placebo and control groups, those randomly assigned to receive thiazides were statistically significantly more likely to withdraw for any reason or because of AEs [62]. Only one of the selected trials assessed symptomatic stone recurrence (treatment versus placebo RR 1.04, 95% CI 0.39 – 2.8) [53] and no trial reported radiologic outcomes separately. The other five trials with a cumulative number of 300 patients reported a composite endpoint with radiologic and symptomatic stone recurrence. Treatment with thiazides decreased the risk for this composite outcome (26 vs. 55%; RR 0.52, 95% CI 0.39 – 0.69; NNT 3.4). Fink et al. stated in their review that the meta-analysis was greatly limited by the available data and that additional trials are warranted [62]. Critical points raised were i) the low amount of trials available and the small sample sizes, ii) lack of data on treatment harms, iii) the fact that all trials included only adults with idiopathic calcium stones, iv) lack of symptomatic stone recurrence reporting as an isolated outcome, v) the fact that only one trial recruited patients from a primary care setting and vi) inconsistent reporting and categorization of baseline biochemistry measures.

Overall, the authors concluded that all six thiazide trials included in their meta-analysis were of fair quality and that there was moderate SOE for the composite end point of symptomatic and radiologic stone recurrence but that there was insufficient SOE for the individual outcomes symptomatic or radiologic stone recurrence [62].

Table 3.1-1: Randomized controlled trials of thiazides in the prevention of recurrent nephrolithiasis

Author, Year	Treatment, Dose	Allocation Concealment	Blinding	Intention-to-treat Analysis	Withdrawals described	Selection for Hypercalciuria	Follow-Up (Years)	Treated/ Placebo n/N	Events/ Total, n/N Thiazide	Events/ Total, n/N Placebo	RR [c]	Recurrence Outcome
Brocks, 1981 [51]	Bendroflumethiazide, 2.5 mg TID [a]	Unclear	Double-blind	No	No	No	1.6	33/29	5/33	5/29	NS	Composite
Scholz, 1982 [53]*	HCTZ, 25 mg BID [b]	Unclear	Double-blind	No	No	No	1	25/26	6/25	6/26	NS	Symptomatic
Laerum, 1984 [45]*	HCTZ, 25 mg BID	Unclear	Double-blind	Yes	Yes	No	3	23/25	5/23	12/25	0.45	Composite
Wilson, 1984 [48]	HCTZ, 100 mg daily	Unclear	Open-label	No	No	No	2.8	23/21	0.15 stones/ year	0.32 stones/ year	0.48	Symptomatic
Robertson, 1985 [49]	Bendroflumethazide, 2.5 mg TID	Unclear	Open-label	No	No	No	3-5	13/9	0.22 stones/ year	0.58 stones/ year	0.38	Symptomatic
Mortensen, 1986 [46]	Bendroflumethazide, 2.5 mg	Unclear	Double-blind	No	No	No	2	12/10	0/12	4/10	-	Composite
Ettinger, 1988 [44]*	Chlorthalidone, 25 mg / 50 mg	Adequate	Double-blind	No	Yes	No	3	19/23/31 (25 mg/ 50 mg/ placebo)	6/42	14/31	0.32	Composite
Ohkawa, 1992 [47]	Trichlormethiazide, 4 mg	Unclear	Open-label	No	No	Yes	2.14-2.21	82/93	24/82	57/93	0.42	Composite
Borghi, 1993 [43]*	Indapamide, 2.5 mg daily	Unclear	Open-label	No	Yes	Yes	3	43/21	6/43	9/21	0.37	Composite
Ahlstrand, 1996 [52]*	HCTZ, 25 mg BID	Unclear	Open-label	Yes	Yes	Yes	3.6-4.3	17/22	9/17	19/22	0.61	Composite
Fernandez-Rodriguez, 2006 [50]*	HCTZ, 50 mg daily	Unclear	None stated	Yes	No withdrawals	No	3	50/50	16/50	28/50	0.57	Composite

[a] TID, three times daily
[b] BID, two times daily
[c] RR, relative risk
* trials included in the meta-analysis by Fink et al. [62]

Rationale:
We and others [62] identified major issues in design, conduct, analysis and reporting in thiazide RCTs conducted thus far for the prevention of stone recurrence.

Major issues encountered include:
- Lack of double-blinding
- Lack of intention-to-treat analysis
- Unclear allocation concealment
- Lack of AE and drop out reporting
- Unknown baseline risk of disease severity or biochemical abnormalities
- Lack of patients stratification based on stone composition
- Lack of outcome uniformity
- Absence of data on the impact of pharmacological interventions on disease risk factors
- Low overall number of patients studied
- Use of high dose thiazide treatments
- Inadequate dietary measures in control and treatment groups
- Use of low sensitivity and low specificity imaging for radiologic outcomes
- Use of outdated dietary recommendations in placebo or control groups

The methodological deficiencies are of great concern. Review of the trials reveals that none of the 11 trials fulfills the quality standards expected of a randomized, placebo-controlled, double-blind trial. While the methodological deficiencies of the trials are obvious for anyone concerned with clinical research, several points peculiar to kidney stone disease deserve further explanation:

Drug dosing: High doses of thiazides were employed in these trials, in the case of the best studied thiazide, HCTZ, 50 - 100 mg daily. At such high doses, side effects occur in 30 – 40% of patients and include electrolyte disturbances, muscle cramps, orthostatic hypotension, gastrointestinal symptoms, hypocitraturia, gout, impaired glucose tolerance, skin rashes and erectile dysfunction [52, 56]. Since AE and drop out reporting in all trials was very poor, tolerability and harms of high dose thiazide treatments remain largely unknown. Nowadays, thiazides are widely used in the treatment of arterial hypertension and recurrent nephrolithiasis, but at significantly lower doses [54, 58, 59]. However, this practice is not supported by randomized evidence in the case of recurrent nephrolithiasis and consequently, we do not know whether the currently employed low dose thiazide regimens are effective in reducing the risk for stone recurrence.
Dietary interventions: Non-pharmacologic interventions for recurrence prevention are very effective [62]. Several landmark trials investigating the effect of dietary measures to prevent stone recurrence were published after the thiazide trials had been completed [64, 65]. In idiopathic recurrent calcareous stone formers, high fluid intake or a diet restricted in animal protein and salt combined with a normal calcium intake each reduce stone recurrence by ~50% [64, 65]. In fact, the thiazide trials were conducted at a time when recurrent calcareous stone formers were given the advice to limit dietary calcium intake, a long-held opinion that has since been refuted both by observational data and a prospective RCT [65, 66]. Thus, the added benefit of thiazides to current state-of-the non-pharmacologic measures in stone prevention remains unknown.
Imaging modality for radiologic outcomes: Some patients with “recurrent” stones in past thiazide RCTs may have already harbored the stones at baseline and experienced stone passage rather than progression of disease. The latter is also the most likely explanation for the repeated observation made in past RCTs (beyond thiazides) concerned with recurrence prevention of renal stones that stone incidence in treatment and control groups are diverting only after one year of treatment or later, although most patients were considered “stone free” at study initiation, based on KUB or IVU imaging. Although CT is by far the most sensitive and most specific imaging modality for renal stone disease, no thiazide trial (or any other RCT concerned with kidney stone prevention) thus far used this imaging modality to monitor progression of stone disease. As outlined earlier, modern low-dose CT protocols cause greatly reduced radiation exposure while preserving a high sensitivity and specificity, making low-dose CT the kidney stone imaging modality of choice to monitor disease progression.
Thus, in summary, benefits and harms of thiazide treatments in general for the prevention of stone recurrence are still unclear. In addition, the efficacy of the currently employed low dose thiazide regimens to prevent stone recurrence is not known.

3.2 Investigational Product (treatment, device) and Indication

HCTZ belongs to the group of thiazide diuretics. Thiazide diuretics inhibit the sodium/ chloride co-

transporter (NCC or SLC12A3) in the distal tubule of the kidney. Inhibition of NCC causes an increased excretion of sodium, chloride and water in the urine and thereby lowering of blood pressure. At the same time, thiazide diuretics reduce renal calcium excretion by a still ill-defined intrarenal mechanism. In Switzerland, HCTZ as monosubstance is marketed exclusively as Esidrex® by Novartis Pharma Schweiz AG in divisible tablets of 25 mg. The approved indications include: arterial hypertension, edemas, heart failure and recurrence prevention of calcium-containing kidney stones. For the treatment of arterial hypertension, Esidrex® is recommended in doses of 12.5 - 50 mg, once or twice daily. For the recurrence prevention of calcareous nephrolithiasis, Esidrex® is recommended in doses of 25 or 50 mg twice daily (Esidrex® summary of product characteristics, November 2014). In addition to the monosubstance, HCTZ is currently available in 78 different galenic formulations in Switzerland as combination with ACE-inhibitors, angiotensin II receptor blockers or non-thiazide diuretics (www.swissmedicinfo.ch, last accessed on 11.08.2016).
Detailed information to pharmacokinetics, side effects and interaction profile of HCTZ can be found at www.swissmedicinfo.ch.

3.3 Preclinical Evidence

There is no animal model, which recapitulates the pathophysiology of calcareous nephrolithiasis in humans. Hence, there is no supportive preclinical evidence for this trial. However, there is ample evidence that thiazide diuretics reduce calciuria, a surrogate marker for calcareous nephrolithiasis, in a wide variety of mammals.

3.4 Clinical Evidence to Date

The clinical evidence on the efficiency of HCTZ in recurrence prevention of calcareous nephrolithiasis has been reviewed in detail in section 3.1.

3.5 Dose Rationale: Rationale for the intended purpose in study

Our systematic review of thiazide trials in the prevention of recurrent nephrolithiasis in section 3.1 reveals that the type of thiazide used and the dose regimens employed varied widely. Although the evidence is currently very limited, no thiazide seems to be superior for stone prevention. As outlined earlier, a common theme of all trials was that very high doses of thiazides were employed. Only in one trial more than one dose of thiazide was tested [45]. In that trial, chlorthalidone 25 mg and 50 mg daily were equally effective in preventing the primary outcome stone recurrence compared to placebo. While the reduction in calciuria compared to placebo was also not different between the two dose regimens, 25 mg chlorthalidone daily reduced oxaluria significantly whereas 50 mg chlorthalidone daily did not. In contrast, however, 50 mg chlorthalidone daily caused more frequent and more pronounced hypokalemia than 25 mg chlorthalidone daily. HCTZ is by far the best studied thiazide diuretic for both currently recognized indications, arterial hypertension and recurrent calcareous nephrolithiasis [55]. In addition, HCTZ (once daily) is the thiazide most commonly found in combination drugs prescribed for the treatment of arterial hypertension. Based on these facts, we decided to use HCTZ for the NOSTONE trial. To improve compliance and reduce side effects, we plan to compare 12.5 mg or 25 mg or 50 mg HCTZ once daily to placebo. As is the case for all thiazides, HCTZ has an excellent bioavailability and thus will be given per os.

3.6 Explanation for choice of comparator (or placebo)

Dietary and lifestyle interventions are highly effective in preventing recurrence in calcareous nephrolithiasis and are thus always the basis of stone prevention [63]. As such, any trial assessing pharmacologic interventions for the prevention of stone recurrence must compare the efficacy of the drug treatment to the state-of-the-art non-pharmacologic interventions. On the basis of RCT evidence, current American [31] and European [23] nephrolithiasis guidelines recommend the following non-pharmacologic measures in adult patients with recurrent calcareous nephrolithiasis: increased fluid intake with circadian drinking to ensure daily urinary volumes of at least 2 – 2.5 L, a balanced diet rich in vegetables and fibers with normal calcium content (1-1.2 g/day) but limited NaCl (4-5 g/day) and animal protein (0.8-1 g/kg/day) content. Furthermore, patients must be advised to retain a normal body mass index (BMI), have adequate physical activity and balance excessive fluid loss. All patients in the placebo and treatment arms of our planned study will receive these current non-pharmacologic recommendations for stone prevention. Since the added benefit of thiazides to current state-of-the non-pharmacologic measures in stone prevention remains unknown, as outlined in detail in section 3.1, a placebo arm needs to be part of this trial.

3.7 Risks/ Benefits

Calcareous nephrolithiasis is a disease with high recurrence rates that causes high morbidity in affected individuals with repeated exposures to urological procedures. The risk a patient is willing to accept is a personal decision based on his/ her individual and familial experience with the disease. HCTZ potentially lowers the recurrence risk of the disease, although in the dosages applied in the study it has not yet been approved for this indication. HCTZ has been used for the treatment of arterial hypertension in the dosages tested in this study for over 40 years. Thus, the treatment risks by HCTZ are well characterized, manageable and must be weighed against the consequences of no treatment. The most common AEs of HCTZ therapy include those arising from saluresis including reduction of blood pressure, polyuria, dehydration and electrolyte abnormalities. Therefore, appropriate patient monitoring and management will be implemented to mitigate this potential risk in the kidney stone population. These AEs should be considered in light of the benefits of a reduced risk of stone recurrence, including pain, urinary tract infection and need for urological interventions. With sufficient knowledge of the benefits and risks and risk mitigation strategies, patients and their physicians may make informed decisions about HCTZ treatment. In the final assessment, the overall benefit-risk profile of HCTZ for the recurrence prevention of calcareous nephrolithiasis appears favourable, offering a real opportunity to fill a longstanding unmet medical need.
The results of the study, will yield important clinical information for the care of patients with recurrent calcareous nephrolithiasis, which is one of the most frequent human diseases (~10% of worldwide population affected). Study participants who finished the trial or stopped the IMP during the trial will be seen in the different ambulatory stone clinics of the participating centers at least annually, if medical condition warrants, more frequently.
Because the detection rate of plain abdominal X-ray imaging (KUB radiography) for urinary stones is far inferior to the detection rate of CT or IVU, and because often several KUBs in different angles have to be performed for a better detection of kidney stones, the international guidelines recommend low-dose CT for the detection and follow-up of urinary stone patients [23, 31-33]. Modern low-dose CT protocols cause greatly reduced radiation exposure while preserving a high sensitivity of up to 98% [69] and specificity of up to 97% [34, 35, 69]. Current radiation exposure of low dose CT protocols for the detection of urolithiasis is in the range of 0.5-1.9 mSV, and thus similar to (single) KUB radiography (0.5 – 1.1 mSv) but lower than IVU (1.3 -3.93 mSv) [20, 23, 35, 36, 70]. The international guidelines recommend imaging of recurrent stone formers at least once a year [31]. Thus, yearly imaging with low dose CT is in line with the international guidelines [31] and with our daily practice (usually 6-monthly to yearly imaging). It is a rather conservative imaging strategy compared to other centers, and leads to a relatively low radiation exposure which does not exceed the standard radiation dose for recurrent stone formers during regular follow-up. Even more, the radiation exposure of our patients is far lower than the 50 mSv throughout a single year or the 20 mSv per year over a 5-year period as accepted as the threshold levels for “safe” exposure by the International Commission on Radiological Protection (ICRP) [71].

3.8 Justification of choice of study population

For the trial, thiazide naïve adult individuals with recurrent calcareous nephrolithiasis will be recruited. 80 – 90% of patients with recurrent nephrolithiasis belong into this group. Hence, the study population will be representative of the far most common type of renal stone former encountered in clinical routine. Recurrent is defined as two or more stone episodes in the last 10 years, calcareous is defined as 50% or more calcium oxalate, calcium phosphate or a mixture of both in a previous stone analysis. No vulnerable individuals will be included in the study.

4. STUDY OBJECTIVES

4.1 Overall Objective

The study aims to describe an efficacy and safety profile of HCTZ for the recurrence prevention of calcareous nephrolithiasis.

4.2 Primary Objective

Dose-response relationship for three different dosages of HCTZ using incidence of stone recurrence (a composite of symptomatic or radiologic recurrence) as the primary outcome.

4.3 Secondary Objectives

Efficacy of the different dosages of HCTZ in terms of the primary outcome as well as the individual components of the composite primary outcome, i.e. incidence of symptomatic stone recurrence and incidence of radiologic stone recurrence. Effects of different dosages of HCTZ on urinary biochemistry (efficacy and safety aspects) and the impact of different baseline characteristics on the effects of the different dosages (effect modification).

4.4 Safety Objectives

Long-term safety and tolerability of HCTZ compared to placebo.

5. STUDY OUTCOMES

5.1 Primary Outcome

5.1.1 Primary outcome at patient level

The primary outcome at the patient level is the incidence of stone recurrences during study treatment. Stone recurrence is the composite of symptomatic or radiological recurrences.

Symptomatic stone recurrence is defined as visible passage of a stone with accompanying typical symptoms such as flank/ loin pain and hematuria or a symptomatic or asymptomatic stone requiring urological intervention for stone removal.

Radiological stone recurrence as assessed by low-dose non-intravenous (iv) contrast CT imaging is defined as the appearance of new caliculi or enlargement of preexisting caliculi with reference to the baseline CT performed at visit 3. Renal CTs for the assessment of stone recurrences are performed whenever there is a suspicion of symptomatic recurrence (standard procedure in symptomatic patients irrespective of the study) and at the end of study treatment.

5.1.2 Primary outcome at study level

Relationship of the incidence of stone recurrences during study treatment and dose group.

5.2 Secondary Outcomes

Secondary outcomes are the following:

- The individual components of the composite primary outcome, i.e. number of symptomatic stone recurrences and number of radiologic stone recurrences
- Changes in urinary biochemistry elicited by HCTZ or placebo
- Impact of baseline disease severity (incidence of stone recurrence during life time prior to randomisation) and biochemical abnormalities on stone recurrence
- Impact of stone composition on stone recurrence

Given the high prevalence of the disease [1, 2], the morbidity, pain and costs associated with symptomatic stone disease [5] as well as the high likelihood of asymptomatic stones to become symptomatic [67], our chosen outcomes are clinically highly relevant.

5.3 Other Outcomes of Interest

None.

5.4 Safety Outcomes

Safety endpoints to be analyzed include a descriptive summary of the following parameters:

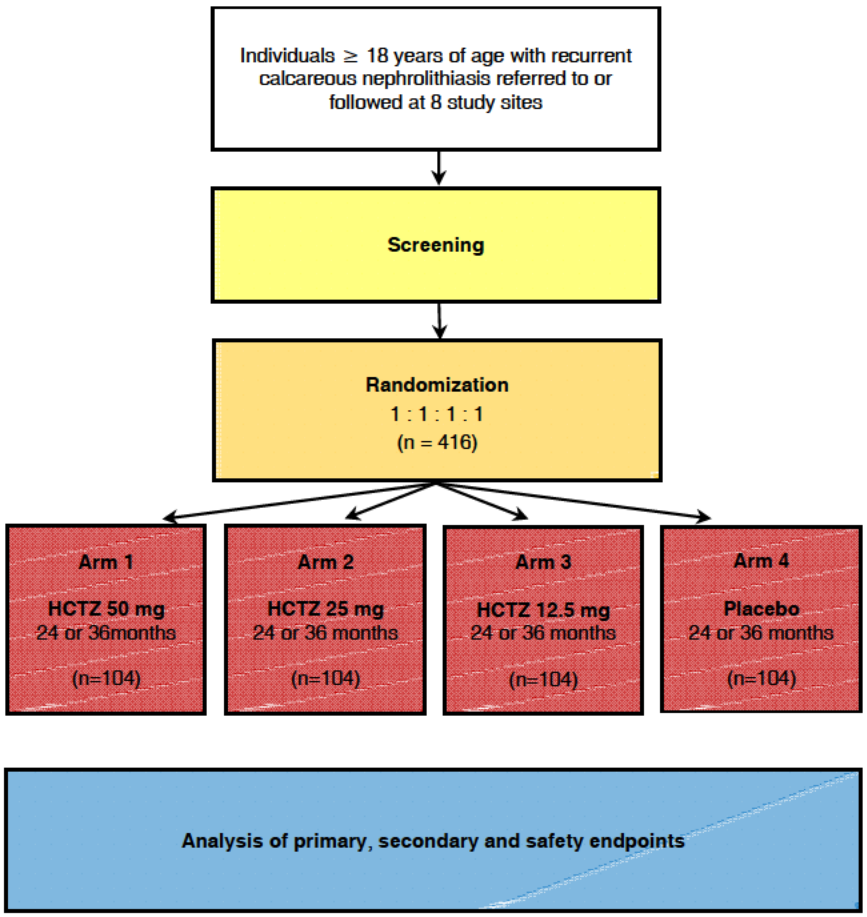
- SAEs
- Pre-specified AEs of special interest (defined in section 9.2.4.2)
- Vital signs

6. STUDY DESIGN

6.1 General study design and justification of design

This is a multicenter, randomized, placebo-controlled, observer-, clinician- and patient-blinded, parallel-group study to compare the efficacy and safety of HCTZ in the recurrence prevention of calcareous nephrolithiasis.
Eligible patients will be randomized in equal proportions between 12.5 mg, 25 mg or 50 mg HCTZ or placebo. All subjects will be given the IMP (HCTZ or placebo) once daily in the morning. Placebo will be administered to subjects randomized to that treatment in a form identical to the HCTZ capsules. The first dose of the IMP will be administered the day after the randomization.
A total of 416 participants, 104 in each group, will be included in the study. Recruitment of participants is planned to occur over a period of 24 months at all 8 study sites. Study treatment will be 36 months for subjects enrolled in the first 12 months of the recruitment period and 24 months for subjects recruited in the remainder of the recruitment period.

Figure 6.1-1: NOSTONE trial design schematic



6.2 Methods of minimizing bias

6.2.1 Randomization

We will use stratified randomization to assign participants to the different trial arms with the number of previous stone events as stratification factors: stratification group 1: 2 or 3 stone events within 10 years prior to randomization; stratification group 2: ≥ 4 stone events within 10 years prior to randomisation. Allocation will be concealed using sequentially coded drug packs that are otherwise identical. Preparation and handling of the unblinded drug packs will be done at a facility otherwise not involved in the trial. Randomization lists at CTU Bern will be stored electronically with no access for persons directly involved in the trial. Allocation to the trial arms will be done using numbered drug packs. Randomization lists will be generated by a statistician at CTU Bern otherwise not involved in the trial following dedicated standard operating procedures. The statistician will communicate directly with the facility, which prepares the drug packs (Grosse Apotheke Dr. G. Bichsel AG, Interlaken, Switzerland). Investigators at each site will assign the drug pack with the next sequential number to the next patient (consecutive). Content of the drug packs is based on randomization lists as described above.

6.2.2 Blinding procedures

All trial personnel but the statistician generating the list and the personnel at the facility preparing the drug packs (Grosse Apotheke Dr. G. Bichsel AG, Interlaken, Switzerland) will be blinded to the assigned treatment. Blinding will be upheld until all analyses will have been completed. HCTZ and placebo will be provided in identically looking blistered capsules in drug packs. Besides the consecutive number, packs and pack content will look identical. Therefore, all trial personnel that is involved in recruitment and care of patients, trial assessment, monitoring and analyses will be blinded to the assigned trial arm.

6.2.3 Other methods of minimising bias

To minimize referral bias, we plan to collaborate with external Nephrologists, Urologists, Internists and Family practitioners.
To minimize bias of primary outcome assessment for radiological recurrence, all renal CTs will be assessed centrally at Inselspital Bern.

6.3 Unblinding Procedures (Code break)

HCTZ is an established drug with a well-known safety profile. Although extremely rare, there are reports of allergic reactions either directly or because of sulfonamide cross-sensitivity. Unblinding will only be allowed in situations where knowledge of the allocation is needed for the care of a patient. Because there is no antidote and the allergic reactions as described in the literature as well as other serious adverse reactions such as aplastic anemia or angioedema are treated independently from any knowledge of treatment assignment, we do not expect any emergency unblinding. Nevertheless, all trial sites will get tamper-proof, sealed, opaque envelopes to allow for emergency unblinding. The integrity of the envelopes will be checked at monitoring visits. Opening of an envelope will need to be notified to the Sponsor-Investigator within 24 hours.
Note that a break of the randomization code per se is not a reason to stop study treatment or to withdraw the participant from the study.

7. STUDY POPULATION

This trial will include adult individuals with recurrent calcareous nephrolithiasis. Outpatients newly referred to stone clinics for metabolic stone work-up will be recruited for the trial at 3 Cantonal Hospitals (Aarau, Lugano, St. Gallen) and 5 University Hospitals (Basel, Bern, Geneva, Lausanne, Zürich) in Switzerland. If enrolment goals are not met, we will 1) try to enhance referrals to trial sites by contacting local Urologists and Nephrologists 2) extend recruitment to outpatients with recurrent calcareous nephrolithiasis that have already undergone metabolic work-up for stone disease but are

seen for regular follow-up visits at the trial sites.
In order to maximize power and minimize the possibility of a Type II error, trial enrollment will continue until at least 416 individuals are randomized.

7.1 Eligibility criteria

Individuals fulfilling all of the following inclusion criteria are eligible for the study:

1. Informed Consent as documented by signature
2. Age 18 years or older
3. Recurrent kidney stone disease ($\geq$ 2 stone events within the last 10 years prior to randomization)
4. Any past kidney stone containing 50% or more of calcium oxalate, calcium phosphate or a mixture of both

The presence of any one of the following exclusion criteria will lead to exclusion of the individual:

1. Pharmacologic prevention for stone recurrence less than 3 months prior to randomization
2. Patients with secondary causes of recurrent calcareous nephrolithiasis including:
   - Severe eating disorders (anorexia or bulimia)
   - Chronic bowel disease, intestinal or bariatric surgery
   - Sarcoidosis
   - Primary hyperparathyroidism
   - Hyperthyroidism
   - Complete distal tubular acidosis
   - Active malignancy
3. Patients with the following medications:
   - Thiazide or loop diuretics
   - Carbonic anhydrase inhibitors (including topiramate)
   - Xanthine oxidase inhibitors (febuxostat or allopurinol)
   - Alkali, including potassium citrate or sodium bicarbonate
   - Treatment with 1,25-OH Vitamin D (calcitriol)
   - Calcium supplementation
   - Bisphosphonates
   - Denusomab
   - Teriparatide
   - Glucocorticoids
4. Obstructive uropathy, if not treated successfully
5. Urinary tract infection, if not treated successfully
6. Chronic kidney disease (defined as CKD-EPI eGFR < 60 mL/min per 1,73 m^2 body surface area
7. Patients with a kidney transplant
8. > 3 gout arthritis episodes within one year prior to randomisation or gout arthritis requiring uric acid lowering therapy
9. Cystinuria at screening
10. Hypokalemia (blood potassium level < 3 mmol/L) at screening
11. Hyponatremia (blood sodium level < 125 mmol/L) at screening
12. Pregnant and lactating women [pregnancy test to be performed for women of child-bearing potential (defined as women who are not surgically sterilized/ hysterectomized, and/ or who are postmenonpausal for less than 12 months)]
13. Previous (within 3 months prior to randomization) or concomitant participation in another interventional clinical trial
14. Inability to understand and follow the protocol
15. Known allergy to the study drug

7.2 Recruitment and screening

Target population of the study are individuals who suffer from recurrent calcareous nephrolithiasis. Two different groups of individuals will be screened and recruited in the study by local investigators: 1) individuals newly referred for metabolic work-up for recurrent stone disease to study sites and 2) individuals who already underwent work-up for recurrent stone disease and are currently undergoing regular follow-up for recurrent stone disease at the study sites. After approval by the CEC and CA, the study will be announced to the Nephrology and Urology staff at the 8 study sites. We will also inform

external Nephrologists, Urologists, Internists and Family practitioners by emails and kick-off meetings upon approval by the CEC and CA.
If available medical history indicates that an individual may be eligible for study participation, the individual will be informed in detail about the study by local investigators. Inclusion in the study will take place only upon receipt of written informed consent and if all eligibility criteria are fulfilled.
No payment or compensation will be given to study participants.

7.3 Assignment to study groups

Stratified randomization will be used to assign participants to the different trial arms with the number of previous stone events as stratification factors (see section 6.2.1). Allocation will be concealed using sequentially coded drug packs that are otherwise identical. Randomization lists will be generated by a statistician at CTU Bern otherwise not involved in the trial following dedicated standard operating procedures. The statistician will communicate directly with the facility, which prepares the drug packs (Grosse Apotheke Dr. G. Bichsel AG, Interlaken, Switzerland). Investigators at each site will assign the drug pack with the next sequential number to the next patient (consecutive). Content of the drug packs is based on randomization lists as described above. Preparation and handling of the unblinded drug packs will be done at a facility otherwise not involved in the trial. Randomization lists at CTU Bern will be stored electronically with no access for persons directly involved in the trial.

7.4 Criteria for withdrawal/ discontinuation of participants

7.4.1 Discontinuation of study IMP

Study IMP must be permanently discontinued if any of the following occurs:
- If any exclusion criterion applies during the trial
- If the responsible study investigator feels that treatment with the study regimen is harmful to the participant's well-being
- If patient is non-compliant with the study intervention as judged by the investigator and/ or the sponsor
- Pregnancy in a study participant
- Hypokalemia (blood potassium level < 3 mmol/L) not responsive to supplementation therapy
- Profound hyponatremia (blood sodium level < 125 mmol/L) recurring after normalization upon temporary suspension of IMP
- Blood creatinine > 150% of creatinine value at screening (visit 2)
- Gouty arthritis recurring > 3 times per year or requiring uric acid lowering therapy.
- Allergic reaction of skin as judged by the investigator
- > 2 recurrences of symptomatic stone events during the trial

Participants who permanently discontinue the IMP are expected to continue in the follow-up period and to attend all protocol-specified study visits. If study visits are not possible, a telephone consultation will be performed to determine if relevant health events/ endpoints have occurred.

7.4.2 Discontinuation of study

Study participants must be withdrawn from the study if the following occurs:
- At the participants own request
- If, in the investigator's opinion, continuation of the study would be harmful to the subject's well-being

A study participant who discontinues study participation prematurely for any reason is defined as dropout if the participant has already been randomized. A study participant who terminates the study before randomization is regarded as a screening failure.

No replacement of participants discontinuing study treatment or study participation is foreseen.

8. STUDY INTERVENTION

8.1 Identity of Investigational Products

8.1.1 Experimental Intervention

Identical looking gelatin capsules containing 12.5 mg or 25 mg or 50 mg HCTZ will be supplied by Grosse Apotheke Dr. G. Bichsel AG, Interlaken, Switzerland according to applicable regulations.

8.1.2 Control Intervention

Placebo gelatin capsules indistinguishable from HCTZ capsules will be supplied by Grosse Apotheke Dr. G. Bichsel AG, Interlaken, Switzerland according to applicable regulations.

8.1.3 Packaging, Labelling and Supply (re-supply)

IMP capsules will be provided as bulk supply in bottles labelled with trial-specific labels according to Annex 13, "Manufacturing of investigational medicinal products" to Volume IV of the EU guideline to Good Manufacturing Practice:

- Name, address and telephone number of the sponsor-investigator
- Pharmaceutical dosage form (capsules), route of administration (per os), number of capsules
- Batch number, Lot number
- Trial acronym (Nostone-trial)
- Trial subject identification number
- Storage conditions
- Expiry date
- Safety warning “Keep out of reach of children”

8.1.4 Storage Conditions

All IMPs will be stored in a securely locked cabinet or enclosure. Access will be limited to investigators and their designees. Neither investigators nor any designees may provide IMP to any subject not participating in this protocol. The IMP will be stored according to the conditions specified in the IMP label. The clinical site staff will maintain a temperature log in the drug storage area recording the temperature using (at minimum) a Min/ Max thermometer at least weekly.

8.2 Administration of experimental and control interventions

8.2.1 Experimental Intervention

HCTZ capsules will be administered once daily per os in the morning. HCTZ has excellent bioavailability and thus per os administration is chosen for this trial (www.swissmedicinfo.ch). The rationale for AM dosing is the fact that HCTZ induces a diuresis that starts 1-2 hours after ingestion, peaks 4-6 hours after ingestion and lasts until 10-12 hours after ingestion (www.swissmedicinfo.ch). Thus, HCTZ-induced diuresis potentially disturbs sleep of participants when PM dosing is chosen. Minimal effective dose for HCTZ-induced diuresis and blood pressure reduction in humans is 12.5 mg per os once daily, maximal effective dose 50 mg per os once daily (www.swissmedicinfo.ch). Hence, 12,5 mg, 25 mg and 50 mg per os once daily were chosen as HCTZ doses for this trial.

8.2.2 Control Intervention

Placebo capsules will be administered once daily per os in the morning, identical to HCTZ capsules.

8.3 Dose modifications

8.3.1 Electrolyte Disturbances

Hypokalemia, hyponatremia and hypomagnesemia can occur in patients with HCTZ treatment. In the case of hypokalemia (< 3 mmol/L), supplementation with oral potassium chloride or co-administration of the potassium-sparing diuretic is recommended. In the case of hypomagnesemia (< 0.5 mmol/L), supplementation with oral magnesium aspartate is recommended. Potassium citrate or potassium

bicarbonate may affect stone recurrence and are not allowed during the trial. If hypokalemia or hypomagnesemia are not responsive to the above recommendations, the IMP will be withdrawn from the patient. In the case of profound hyponatremia (< 125 mmol/L), temporary suspension of the IMP until normalization of the blood sodium concentration is recommended. The IMP can then be restarted. If profound hyponatremia recurs, the IMP will be withdrawn from the patient.

8.3.2 Renal function impairment

Because of blood pressure lowering and volume depletion, therapy with HCTZ may worsen renal function. If blood creatinine equals or exceeds 150% of the baseline creatinine, the IMP will be withdrawn from the patient.

8.3.3 Gout arthritis

HCTZ may increase blood uric acid levels which can cause gout arthritis. An acute gout flare should be treated symptomatically with analgesics. Xanthine oxidase inhibitors to lower blood and urine uric acid levels may affect stone recurrence and are not allowed during the trial. If gouty arthritis recurs > 3 times/ year or a uric acid lowering therapy is needed, the IMP will be withdrawn from the patient.

8.3.4 Diabetes

HCTZ may worsen glucose tolerance or induce diabetes mellitus. If overt diabetes mellitus develops during the trial (fasting glucose > 7 mmol/L, random glucose > 11 mmol/L or hemoglobin A1c > 7%), the patient will be referred to a diabetologist.

8.3.5 Allergic reactions

Occasionally allergic reactions of the skin have been observed in patients with HCTZ treatment. If this is suspected, the IMP will be withdrawn from the patient.

8.3.6 Symptomatic stone recurrence during the trial

Patients will remain on study treatment with up to two symptomatic stone events. If a third symptomatic stone event occurs during the trial, the IMP will be withdrawn from the patient.

For additional criteria for discontinuing study IMP treatment or study discontinuation see section 7.4.

8.4 Compliance with study intervention

At initial product dispensing and the follow-up visits, including the three-monthly telephone contacts, the participants will be reminded about the following:

- The importance of following the study guidelines, including the non-pharmaceutical preventive measures and taking the study product once daily
- Instructions about IMP administration including dose, importance of taking the IMP as a whole, timing, and noting done any missed dose, and instructions about proper IMP storage
- Notification that there will be IMP counts performed during the trial
- To keep all medication containers, including any unused capsules and to return them to the study site at the next scheduled on-site visit
- That IMP may be HCTZ or placebo
- Importance of calling the clinic if experiencing problems possibly related to the study product such as symptoms or lost IMP

At the follow-up visits and three-monthly telephone calls, the participants will be asked about any problems with regard to taking the IMP using a structured and open-ended questionnaire. There will be a discussion of reasons for missed doses and simple strategies for enhancing adherence, e.g. linking IMP taking to meals or other daily activities. Participants will have an opportunity to ask questions and key messages from the initial session will be reviewed as needed.

Participants will return used, partially used and unused IMP containers at each follow-up visit at the study sites and IMP counts will be performed to assess medication adherence and thus enhance data validity.
Depending on the circumstances leading to non-compliance, the subject may be discontinued from IMP administration by the investigator and/ or sponsor. A subject who proactively wishes to discontinue IMP administration, or has IMP discontinued by the investigator, will be encouraged to continue limited participation in the trial, as described in section 7.4.

8.5 Data Collection and Follow-up for withdrawn participants

Participants who permanently discontinue the study treatment are expected to continue in the follow-up period and to attend all protocol-specified study visits. If study visits are not possible, a telephone consultation will be performed to determine if relevant health events/ endpoints have occurred.
Participants who are withdrawn from further study participation are invited to attend a final visit at which the assessments of the End of Treatment visit are performed.

8.6 Trial-specific preventive measures

Dietary and lifestyle interventions are highly effective in preventing recurrence in calcareous nephrolithiasis and are the basis of stone prevention [63]. Any trial assessing pharmacologic interventions for the prevention of stone recurrence must compare the efficacy of the drug treatment to the state-of-the-art non-pharmacologic interventions. Thus, all patients will receive state-of-the-art non-pharmacologic recommendations for stone prevention according to current American [31] and European [23] nephrolithiasis guidelines including: increased fluid intake with circadian drinking to ensure daily urinary volumes of at least 2 – 2.5 L, a balanced diet rich in vegetables and fibers with normal calcium content (1-1.2 g/day) but limited NaCl (4-6 g/day) and animal protein (0.8-1 g/kg/day) content. Furthermore, patients must be advised to retain a normal BMI, have adequate physical activity and balance excessive fluid loss.

The following medications will be prohibited during the trial (reason given in brackets):
- Thiazide diuretics (pharmacodynamic interference with IMP)
- Loop diuretics (pharmacodynamics interference with IMP)
- Carbonic anhydrase inhibitors (increase risk for stone recurrence)
- Xanthine oxidase inhibitors (potentially decrease risk of stone recurrence)
- Alkali, including potassium citrate or sodium bicarbonate (potentially decrease risk of stone recurrence)
- Treatment with 1,25-OH Vitamin D (increases risk of stone recurrence)
- Calcium supplementation (increases risk of stone recurrence)
- Bisphosphonates (potentially decreases risk of stone recurrence)
- Denusomab (potentially decreases risk of stone recurrence)
- Teriparatide (increases risk of stone recurrence)
- Glucocorticoids (increases risk of stone recurrence)

8.7 Concomitant Interventions (treatments)

Active recurrence prevention for calcareous nephrolithiasis with thiazide diuretics, alkali or xanthine oxidase inhibitors will have to be stopped at least 3 months before an individual can be enrolled (randomized) in the study. All medications not listed in section 8.6 will be allowed before and during the study as concomitant treatments. The use of concomitant medications will be recorded in the eCRF. There are no restrictions to medications or treatments after the study.
All urological interventions related to symptomatic and asymptomatic stone disease are permitted during the trial. Generally, it is recommended to follow patients with asymptomatic stones and wait until stones become symptomatic. However, the likelihood for the eventual need of an urological intervention depends on the stone diameter. Ureteral stones with a diameter > 7 mm have a likelihood of 99% to require a urological intervention in the future [68]. Thus, kidney stones with a diameter > 7 mm are often removed, even if asymptomatic. In patients with a high risk of complications, including those with a solitary kidney or in patients with certain professions (e.g. pilots), even elective removal of asymptomatic stones with a diameter $\leq$ 7 mm may be indicated. Interventional treatment of asymptomatic stones of patients enrolled in the trial will be left to the discretion of the treating urologists. Removal of an asymptomatic stone will be considered as symptomatic stone event in this trial.

8.8 Study Drug Accountability

The investigator or designee must maintain an inventory record of IMP at the site level (received from the sponsor, returned to the sponsor) and at the patient level (dispensed to the patient, returned by the patient).

8.9 Return or Destruction of Study Drug/ Medical Device

Upon completion or termination of the trial, all unused IMP must be destructed on site following the

site's standard procedures for medication destruction and the destruction documented. Documentation on IMP destruction must be provided to the sponsor-investigator for filing in the trial master file.

9. STUDY ASSESSMENTS

9.1 Study flow chart(s)/ table of study procedures and assessments

For work-up and follow-up of participants, the NOSTONE protocol strictly adheres to recommendations of the American and European guidelines on nephrolithiasis [23, 31] with regard to scheduling of patient visits, lab analyses and imaging.
For the schedule of assessments refer to "Study Schedule" table at the beginning of this document.

9.2 Assessments of outcomes

In accordance with recommendations of the American and European guidelines on nephrolithiasis [23, 31], only study participants undergoing first-time work-up for stone disease will be scheduled for Visit 4 (V4). Participants with past work-up for stone disease that are currently undergoing regular follow-up will omit V4, all other Visits (including numbering of Visits) are identical to participants with first-time work-up for stone disease.

9.2.1 Assessment of primary outcome

Symptomatic recurrence (for definition see section 5.1.1) will be assessed at the follow-up visits 3, 4, 5 and 6 as well as at the three-monthly phone calls in between the visits.
Radiologic recurrence (for definition see section 5.1.1) will be assessed by low-dose, renal-limited non-iv contrast CT imaging at visit 2 (defined as reference for stone recurrence during the study), if a stone recurrence is suspected during the treatment phase (standard procedure in symptomatic patients irrespective of the study), and at treatment end (visit 7 for participants recruited in the first 12 months of the recruitment period and visit 6 for participants recruited in the remainder of the recruitment period). At treatment end, a renal CT is performed only if no other non-iv contrast renal CT was performed within 3 months prior to the visit.

9.2.2 Assessment of secondary outcomes

Individual components of the composite primary outcome, i.e. incidence of symptomatic stone recurrence and incidence of radiologic stone recurrence will be assessed as described for the primary outcome. Changes in urinary biochemistry elicited by the IMP compared to baseline (visit 2) will be assessed by chemical analysis of urine samples collected immediately prior to visits 4, 5, 6 and 7 (one 24 hour collection immediately prior to visit 4; two 24 hours collections within 48 hours immediately prior to visits 5, 6 and 7). Impact of baseline disease severity and biochemical abnormalities on stone recurrence will be assessed by baseline blood and urine analysis and baseline medical history and assessment of the primary outcome as described. Impact of stone composition on stone recurrence will be assessed by baseline medical history and assessment of the primary outcome as described.

9.2.3 Assessment of other outcomes of interest

No other outcome assessments are planned.

9.2.4 Assessment of safety outcomes

9.2.4.1 Serious adverse events (SAEs)
SAEs will be collected, fully investigated and documented in the source documents and the eCRF for all participants from the date of ICF signature until the last protocol-specific procedure has been completed, including a safety follow-up period of 30 days.

9.2.4.2 Adverse events (AEs) of special interest
For this trial, (non-serious) AEs will not be documented in the eCRF with exception of the following events "of special interest" occurring from the date of randomizatzion (visit 2) until visit 7:
- Hypokalemia, defined as blood potassium level < 3 mmol/L
- Hyponatremia, defined as blood sodium level < 125 mmol/L

- Hypomagnesemia, defined as blood magnesium level < 0.5 mmol/L
- Blood creatinine > 150% of baseline creatinine
- Gouty arthritis if recurrence > 3 times per year or requiring Uric acid lowering therapy
- Newly developed overt diabetes mellitus (defined as fasting glucose level > 7 mmol/L or random Glucose > 11 mmol/L or hemoglobin A1c > 7%)
- Allergic reaction of skin if considered by the local investigator to be potentially related to the study medication

9.2.4.3 Vital signs

Heart rate, systolic and diastolic blood pressure at the right arm in sitting position after at least 5 minutes rest will be recorded at visits 1, 4, 5, 6 and 7.

9.2.5 Assessments in participants who prematurely stop the study

Participants who permanently discontinue the study treatment will undergo all protocol-specified study visits and assessments. If study visits are not possible, a telephone consultation will be performed to determine if relevant health events/ endpoints have occurred.

9.3 Procedures at each visit

9.3.1 Visit 1 (screening day 1*; -14 days to day -1)

- Patient information and delivery of written study information to subject
- Pre-assessment of eligibility (based on data available per routine)
- Demographics
- Medical history
- Stone composition (all stones analyzed prior to randomization as available)
- General physical examination, including body weight, height
- Vital signs (heart rate, systolic and diastolic blood pressure at the right arm after at least 5 minutes at rest)
- Concomitant medication

* For participants with first time work-up for kidney stone disease (new referrals), screening assessments are performed at visits 1 and 2. For participants in current follow-up for past stone disease, all assessments of visit 1 are performed at visit 2.

9.3.2 Visit 2 (screening day 2; day 0)

- Written informed consent (if not obtained earlier). Note: In order to give subjects in current follow-up for past stone disease sufficient time for the decision on study participation, initial patient information may be done via phone and the written study information provided to the subject via normal post prior to the visit
- Assessment of eligibility
- Blood analysis (analyzed centrally): sodium, potassium, chloride, calcium, magnesium, inorganic phosphate, creatinine, urea, uric acid, bicarbonate, PTH, 25-OH Vitamin D, 1,25-OH Vitamin D, glucose (fasting), hemoglobin A1c, cholesterol, alkaline phosphatase)
- Urine analysis (two 24 hour collections in the 48 hours immediately prior to the visit; analyzed centrally): sodium, potassium, chloride, calcium, magnesium, inorganic phosphate, creatinine, urea, uric acid, protein, albumin, oxalate, citrate, ammonium, pH, volume
- Pregnancy test (performed locally, from blood or urine) for women of child-bearing potential (defined as women who are not surgically sterilized/ hysterectomized, and/ or who are postmenonpausal for less than 12 months)
- Assessment of SAEs
- Concomitant medication

9.3.3 Visit 3 (+2 weeks, visit window ±2 weeks)

- Re-assessment of eligibility
- Randomisation
- Baseline low-dose non-iv contrast renal CT (analyzed centrally; if a routine non-iv contrast renal CT was performed within 2 months prior to this visit, the CT does not have to be repeated)
- Collection of information on composition of any new kidney stone since last visit
- Instruction of participant on non-pharmacologic recommendations for stone prevention
- Dispense of IMP (first intake of IMP on the day after visit 3)

- Assessment of SAEs
- Concomitant medication
- If at visit 2, blood sodium was in the range from 125 to 130 mmol/L and/or potassium in the range from 3 to 3.5 mmol/L (or if deemed necessary by the responsible principal investigator for any other reason), blood sodium and/or potassium level(s) must be re-checked within 1 month after visit 3.

9.3.4 Visit 4 (+3 months, visit window ±2 weeks)

Participants with first-time work-up of stone disease:
- General physical examination, including body weight
- Vital signs (heart rate, systolic and diastolic blood pressure at the right arm after at least 5 minutes at rest)
- Collection of information on composition of any new kidney stone since last visit
- Blood analysis (analyzed centrally): sodium, potassium, chloride, calcium, magnesium, inorganic phosphate, creatinine, urea, uric acid, bicarbonate, PTH, 25-OH Vitamin D, 1,25-OH Vitamin D, glucose (fasting), hemoglobin A1c, cholesterol, alkaline phosphatase)
- Urine analysis (one 24 hour collections in the 48 hours immediately prior to the visit; analyzed centrally): sodium, potassium, chloride, calcium, magnesium, inorganic phosphate, creatinine, urea, uric acid, protein, albumin, oxalate, citrate, ammonium, pH, volume
- Assessment of symptomatic stone recurrence
- Compliance reminder (intake of IMP and adherence to non-pharmacologic recommendations for stone prevention)
- Screening for criteria for interrupting or discontinuing study intervention
- Assessment of SAEs and AEs of special interest
- Concomitant medication
- Dispense of IMP and collection of used/ unused IMP packs from participant

Participants in current follow-up for past kidney stone disease:
- Telephone call to ask participant about possible trial-related problems (including any SAEs since last visit and regarding the potential occurrence of events of special interest as far as known to the participant) and answer any questions from participant
- Assessment of symptomatic stone recurrence
- Compliance reminder (intake of IMP and adherence to non-pharmacologic recommendations for stone prevention)
- Dispense of IMP via normal post

9.3.5 In between Visits 4 and 5 (+6 and +9 months, visit window ±4 weeks)
- Telephone call to ask participant about possible trial-related problems (including any SAEs since last contact and regarding the potential occurrence of AEs of special interest as far as known to the participant) and answer any questions from participant
- Assessment of symptomatic stone recurrence
- Compliance reminder (intake of IMP and adherence to non-pharmacologic recommendations for stone prevention)
- Dispense of IMP via normal post

9.3.6 Visit 5 (+12 months, visit window ±4 weeks)
- General physical examination, including body weight
- Vital signs (heart rate, systolic and diastolic blood pressure at the right arm after at least 5 minutes at rest)
- Blood analysis (analyzed centrally): sodium, potassium, chloride, calcium, magnesium, inorganic phosphate, creatinine, urea, uric acid, bicarbonate, PTH, 25-OH Vitamin D, 1,25-OH Vitamin D, glucose (fasting), hemoglobin A1c, cholesterol, alkaline phosphatase)
- Urine analysis (two 24 hour collections in the 48 hours immediately prior to the visit; analyzed centrally): sodium, potassium, chloride, calcium, magnesium, inorganic phosphate, creatinine, urea, uric acid, protein, albumin, oxalate, citrate, ammonium, pH, volume
- Assessment of symptomatic stone recurrence
- Compliance reminder (intake of IMP and adherence to non-pharmacologic recommendations for stone prevention)
- Screening for criteria for interrupting or discontinuing study intervention

- Assessment of SAEs and AEs of special interest
- Concomitant medication
- Dispense of IMP and collection of used/ unused IMP packs from participant

9.3.7 In between Visits 5 and 6 (+15, +18 and +21 months, visit window ±4 weeks)

- Telephone call to ask participant about possible trial-related problems (including any SAEs since last contact and regarding the potential occurrence of AEs of special interest as far as known to the participant) and answer any questions from participant
- Assessment of symptomatic stone recurrence
- Compliance reminder (intake of IMP and adherence to non-pharmacologic recommendations for stone prevention)
- Dispense of IMP via normal post

9.3.8 Visit 6 (+24 months, visit window ±4 weeks)

Participants recruited during the first 12 months of the recruitment period:

- Vital signs (heart rate, systolic and diastolic blood pressure at the right arm after at least 5 minutes at rest)
- Blood analysis (analyzed centrally): sodium, potassium, chloride, calcium, magnesium, inorganic phosphate, creatinine, urea, uric acid, bicarbonate, PTH, 25-OH Vitamin D, 1,25-OH Vitamin D, glucose (fasting), hemoglobin A1c, cholesterol, alkaline phosphatase)
- Urine analysis (two 24 hour collections in the 48 hours immediately prior to the visit; analyzed centrally): sodium, potassium, chloride, calcium, magnesium, inorganic phosphate, creatinine, urea, uric acid, protein, albumin, oxalate, citrate, ammonium, pH, volume
- Assessment of symptomatic stone recurrence
- Compliance reminder (intake of IMP and adherence to non-pharmacologic recommendations for stone prevention)
- Screening for criteria for interrupting or discontinuing study intervention
- Assessment of SAEs and AEs of special interest
- Concomitant medication
- Dispense of IMP and collection of used/ unused IMP packs from participant

Participants recruited ≥ 12 months after start of the recruitment period:

This visit corresponds to the end of treatment visits for participants who were recruited ≥ 12 months after start of the recruitment period. This visit is followed by the safety follow-up telephone call (see section 9.3.11)

- General physical examination, including body weight
- Vital signs (heart rate, systolic and diastolic blood pressure at the right arm after at least 5 minutes at rest)
- Blood analysis (analyzed centrally): sodium, potassium, chloride, calcium, magnesium, inorganic phosphate, creatinine, urea, uric acid, bicarbonate, PTH, 25-OH Vitamin D, 1,25-OH Vitamin D, glucose (fasting), hemoglobin A1c, cholesterol, alkaline phosphatase)
- Urine analysis (two 24 hour collections in the 48 hours immediately prior to the visit; analyzed centrally): sodium, potassium, chloride, calcium, magnesium, inorganic phosphate, creatinine, urea, uric acid, protein, albumin, oxalate, citrate, ammonium, pH, volume
- Low-dose non-iv contrast renal CT (analyzed centrally; if a routine non-iv contrast renal CT was performed within 3 months prior to this visit, the CT does not have to be repeated)
- Assessment of symptomatic stone recurrence
- Assessment of SAEs and AEs of special interest
- Concomitant medication
- Collection of used/ unused IMP packs from participant

9.3.9 In between Visits 6 and 7 (+27, +30 and +33 months, visit window ±4 weeks)

Only for participants recruited during the first 12 months of the recruitment period:

- Telephone call to ask participant about possible trial-related problems (including any SAEs since last contact and regarding the potential occurrence of AEs of special interest as far as known to the participant) and answer any questions from participant
- Assessment of symptomatic stone recurrence
- Compliance reminder (intake of IMP and adherence to non-pharmacologic recommendations for

stone prevention)
- Dispense of IMP via normal post

9.3.10 Visit 7 (+36 months, visit window ±4 weeks)

Only for participants recruited during the first 12 months of the recruitment period:

- General physical examination, including body weight
- Vital signs (heart rate, systolic and diastolic blood pressure at the right arm after at least 5 minutes at rest)
- Blood analysis (analyzed centrally): sodium, potassium, chloride, calcium, magnesium, inorganic phosphate, creatinine, urea, uric acid, bicarbonate, PTH, 25-OH Vitamin D, 1,25-OH Vitamin D, glucose (fasting), hemoglobin A1c, cholesterol, alkaline phosphatase)
- Urine analysis (two 24 hour collections in the 48 hours immediately prior to the visit; analyzed centrally): sodium, potassium, chloride, calcium, magnesium, inorganic phosphate, creatinine, urea, uric acid, protein, albumin, oxalate, citrate, ammonium, pH, volume
- Low-dose non-iv contrast renal CT (analyzed centrally; if a routine non-iv contrast renal CT was performed within 2 months prior to this visit, the CT does not have to be repeated)
- Assessment of symptomatic stone recurrence
- Assessment of SAEs and AEs of special interest
- Concomitant medication
- Collection of used/ unused IMP packs from participant

9.3.11 Safety follow-up (30 days after end of treatment, visit window ±2 weeks)

- Assessment of SAEs (telephone call)

9.3.12 Study-specific procedures

The following procedures are study-specific:
- Pregnancy test for women of child-bearing potential at visit 2
- Baseline low-dose non-iv contrast renal CT at visit 3
- Re-assessment of blood sodium and potassium within one month after visit 3 if required (see section 9.3.3)
- Telephone call at visit 4 for patients in routine follow-up for past kidney stone disease
- 3-monthly telephone calls in between on-site visits and telephone call at 30 days after end of treatment
- Administration of IMP

All other procedures are part of the routine assessments in kidney stone patients.

10. SAFETY

During the entire duration of the study, all SAEs are collected, fully investigated and documented in source documents and eCRF. Study duration encompassed the time from when the participant signs the informed consent until the last protocol-specific procedure has been completed, including a safety follow-up period of 30 days.
Non-serious AEs are not collected with the exception of AEs of special interest (see section 9.2.4.2) occurring from the time point of randomization until the end of treatment visit.

10.1 Definition and assessment of (serious) adverse events and other safety related events

An **adverse event (AE)** is any untoward medical occurrence in a patient or a clinical investigation participant who administered a pharmaceutical product and which does not necessarily have a causal relationship with the study procedure. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product. [ICH E6 1.2]

A **serious adverse event (SAE)** is classified as any untoward medical occurrence that:

- results in death,
- is life-threatening,
- requires in-patient hospitalization or prolongation of existing hospitalisation,
- results in persistent or significant disability/ incapacity, or
- is a congenital anomaly/ birth defect.

In addition, important medical events that may not be immediately life-threatening or result in death, or require hospitalisation, but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed above should also usually be considered serious. [ICH E2A]

SAEs should be followed until resolution or stabilisation. Participants with ongoing SAEs at study termination (including safety visit) will be further followed-up until recovery or until stabilisation of the event or until the participant is lost to follow-up.

10.1.1 Assessment of Causality

Both Investigator and Sponsor-investigator make a causality assessment of the event to the study drug, based on the criteria listed in the ICH E2A guidelines:

Relationship	Description
Definitely	Temporal relationship Improvement after dechallenge* Recurrence after rechallenge (or other proof of drug cause)
Probably	Temporal relationship Improvement after dechallenge No other cause evident
Possibly	Temporal relationship Other cause possible
Unlikely	Any assessable reaction that does not fulfil the above conditions
Not related	Causal relationship can be ruled out
*Improvement after dechallenge only taken into consideration, if applicable to reaction	

10.1.2 Unexpected Adverse Drug Reaction

An "unexpected" adverse drug reaction is an adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g. Investigator's Brochure for drugs that are not yet approved and Product Information for approved drugs, respectively). [ICH E2A]

10.1.3 Suspected unexpected serious adverse reactions (SUSARs)

The Sponsor-Investigator evaluates any SAE that has been reported regarding seriousness, causality and expectedness. If the event is related to the investigational product and is both serious and unexpected, it is classified as a SUSAR. Unblinding will be needed in order to determine a SUSAR.

10.1.4 Assessment of Severity

Classification and severity grading scale of SAEs and AEs of special interest (see section 9.2.4.2) in this study will be performed in accordance with "Common Terminology Criteria for Adverse Events CTCAE Version 4.03" terminology.

10.2 Reporting of serious adverse events (SAE) and other safety-related events

Reporting of SAEs

All SAEs must be reported immediately and within a maximum of 24 hours of learning of its occurrence to the Sponsor-Investigator of the study via email or fax:

Fax: +41 (0)31 632 97 34
e-mail: daniel.fuster@insel.ch

The Sponsor-Investigator will re-evaluate the SAE and return the form to the site.
SAEs resulting in death will be reported to the coordinating and, if applicable, to the local Ethics Committee within 7 days.

Non-serious Events that require discontinuation of IMP
Non-serious events that require discontinuation of IMP (including laboratory abnormalities – see section 7.4.1) should be reported to the Sponsor-Investigator within 3 days.

Reporting of SUSARs
A SUSAR needs to be reported to the local Ethics Committee (local event via local Investigator) and to Swissmedic (via Sponsor-Investigator) within 7 days, if the event is fatal, or within 15 days (all other events).
The Sponsor-Investigator must inform all Investigators participating in the clinical study of the occurrence of a SUSAR. All in the trial involved Ethics Committees will be informed about SUSARs in Switzerland via Sponsor-Investigator according to the same timelines.

Reporting of Safety Signals
All suspected new risks and relevant new aspects of known adverse reactions that require safety-related measures, i.e. so called safety signals, must be reported to the Sponsor-Investigator within 24 hours. The Sponsor-Investigator must report the safety signals within 7 days to the local Ethics Committee and to Swissmedic. The Sponsor-Investigator must immediately inform all participating Investigators about all safety signals. The other in the trial involved Ethics Committees will be informed about safety signals in Switzerland via the Sponsor-Investigator.

Reporting and Handling of Pregnancies
Pregnant participants must immediately discontinue IMP. Any pregnancy during the treatment phase of the study and within 30 days after discontinuation of IMP must be reported to the Sponsor-Investigator within 24 hours. The course and outcome of the pregnancy will be followed up carefully and any abnormal outcome regarding the mother or the child should be documented and reported.

Periodic reporting of safety
An annual safety report will be prepared and submitted once a year to the local Ethics Committees and to Swissmedic by the Sponsor-Investigator. The annual safety report will contain information from all study sites.

10.3 Follow up of (serious) adverse events

Participants with any reported ongoing SAE at the last scheduled study contact will be followed until resolution of the event or a stabilized condition of the subject has been achieved or until the subject is lost to follow-up.
Any new SAEs the Investigator gets aware of that occur after the last scheduled study contact and are determined by the Investigator to be reasonably associated with the use of the IMP, should be reported to the Sponsor-Investigator. The Investigator should follow potentially IMP-related SAEs identified after the last scheduled contact (and report any significant follow-up information to the Sponsor-Investigator) until the events are resolved or stabilized, or the subject is lost to follow-up.

11. STATISTICAL METHODS

11.1 Hypothesis

The null hypothesis that there is no linear trend between dose and the survival function for the primary outcome (i.e. recurrence) will be tested against the alternative that there is a linear trend.

11.2 Determination of Sample Size

The sample size calculation was based on the primary objective i.e. to assess the dose-response relationship and the primary outcome i.e. recurrence with the following assumptions:

- Uniform recruitment over 24 months with allocation ratio fixed at 1 across all arms.
- A maximum and minimum follow-up time of 36 and 24 months, respectively.
- A cumulative drop-out rate of 10% at 24 months after study start.
- Based on the available literature we assumed a risk of recurrence in the placebo group of 0.20 and 0.45 at 12 and 36 months after study start, respectively.
- Hazard ratios for the 12.5, 25 and 50 mg HCTZ doses of 0.90, 0.65 and 0.50, respectively. These effects were based on the literature review presented in Table 3.1-1.
- Power was set to be at least 80% and alpha was fixed at a two-sided level of 0.05.
- An unweighted log-rank test for linear trend with local alternatives.

Using the command artsurv in Stata we calculated that we will need to recruit 416 patients i.e. 104 in each arm.

11.3 Statistical criteria of termination of trial

Not applicable as no formal interim analysis is planned.

11.4 Planned Analyses

The statistical analysis of the trial will be done at CTU Bern by a statistician blinded to the allocation. This process is defined in standard operating procedures. After start of the trial but before recruitment ends, a statistical analysis plan will be written. The plan will determine all necessary data preparation steps (e.g. additional validations, generation of new variables), definitions (e.g. analysis sets), and statistical analyses (e.g. models, outputs such as tables and graphs).
All statistical analyses will be presented as effect measure plus 95% confidence interval. A significance level of 5% will be used.

11.4.1 Datasets to be analysed, analysis populations

All analyses will be done on the intention-to-treat analysis set whereby all randomized patients will be analyzed in the allocated group regardless of any protocol violations such as cross-overs (which can only happen accidently in this trial) or early treatment discontinuations.

11.4.2 Primary Analysis

The primary outcome will be analyzed using a stratified log-rank test for linear trend with the hazard ratio as primary effect measure. The test addresses the primary objective of the trial i.e. test for any dose-response relationship. We will start with testing for a linear relationship but will also consider more complex relationships by using fractional polynomials. Kaplan-Meier curves will be used to present results on the primary outcome graphically. In case of a relevant number of multiple events within patients we will consider a model for recurrent events.
Components of the primary outcome measure (clinical and radiological recurrence) will be analyzed similar to the primary analysis
Continuous secondary outcomes (changes in urinary biochemistry) will be analyzed using linear regression. Comparisons between placebo and the three active trial arms will be considered exploratory as the trial is not powered to detect differences there.

11.4.3 Secondary Analyses

The following baseline characteristics were identified to potentially modify the expected treatment effect:
- Baseline disease severity (Highest vs. lowest tertile in stone frequency prior to study entry)
- Biochemical abnormalities (Hypercalciuria vs. no hypercalciuria)
- Stone composition (pure CaOx vs. pure CaP vs. mixed CaOx/ CaP vs. mixed CaOx/ UA)

These characteristics will be used for stratified analyses. All stratified analyses will be accompanied by tests for interaction.

11.4.4 Interim analyses

No formal interim analysis is planned.

11.4.5 Safety analysis

Safety endpoints to be analyzed include a descriptive summary of pre-specified AEs of special interest and vital signs. No formal statistical testing will be applied.

11.4.6 Deviation(s) from the original statistical plan

Deviations from the statistical analysis plan will be stated and justified in the final analysis report..

11.5 Handling of missing data and drop-outs

For the primary outcome, drop-outs will be censored at the last available visit. Missing data for continuous outcomes will be handled by multiple imputation.

12. QUALITY ASSURANCE AND CONTROL

12.1 Data handling and record keeping/ archiving

The Investigators will maintain appropriate medical and research records for this trial, in compliance with ICH-GCP and regulatory and institutional requirements for the protection of confidentiality of subjects. The Principal Investigator, Sub-investigator, and Clinical Research Nurses or Coordinators will have access to the records. The Principal Investigators will permit authorized representatives of the Sponsor and regulatory agencies to examine clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress.

12.1.1 Case Report Forms

The CRF will be electronic. All data requested on the eCRF must be recorded and the recorded data should be consistent with the source documents or the discrepancies should be explained. The Investigator should ensure the accuracy, completeness, and timeliness of the data reported in the eCRF and all other required reports. Generally, the eCRF should be completed within one week of completion of a participant's visit/ follow-up phone call.

12.1.2 Specification of source documents

Source documents must be available at the site to document the existence of the study participants and must include the original documents relating to the study, as well as the medical treatment and medical history of the participant.
For all data captured in the eCRF, the location of the source should be documented on a list of source documents (source data location list), which will be stored in the investigator site file at each study site. If certain data are directly entered into the eCRF (and are thus considered as source data) this must be specified on the source data location list accordingly.
Any change or correction to source data should be dated, initialed, and explained (if necessary) and should not obscure the original entry.

12.1.3 Record keeping/ archiving

All study data (written and electronic), including any images, must be retained for a period of at least 10 years from the completion or premature termination of the trial. The Investigators should take measures to prevent accidental or premature destruction of these documents.

12.2 Data management

12.2.1 Data Management System

The CRFs in this trial are implemented electronically using a dedicated electronic data capturing (EDC) system (secuTrial®). The EDC system is activated for the trial only after successfully passing a formal test procedure. All data entered in the eCRF are stored on a Linux server in a dedicated Oracle database.
Responsibility for hosting the EDC system and the database lies with Inselspital Bern.

12.2.2 Data security, access and back-up

The server hosting the EDC system and the database is kept in a locked server-room. Only the system administrators have direct access to the server. A role concept with personal passwords (site investigator, statistician, monitor, administrator etc.) regulates permission for each user to use the system and database as he/ she requires.

All data entered into the eCRF are transferred to the database using Secure Sockets Layer (SSL) encryption. Each data point has attributes attached to it identifying the user who entered it with the exact time and date. Retrospective alterations of data in the database are recorded in an audit table. Time, table, data field and altered value, and the person are recorded (audit trail). A multi-level back-up system is implemented.

12.2.3 Analysis and archiving

At interim (if applicable) and final analyses, data files will be extracted from the database into statistical packages to be analyzed. The status of the database at this time is recorded in special archive tables.
The study database with all archive tables will be securely stored by Inselspital Bern. The sponsor also keeps the Trial Master File and interim and final reports both in electronic and in hard copy form for at least 10 years.

12.2.4 Electronic and central data validation

Data is checked by the EDC system for completeness and plausibility. Furthermore, selected data points are cross-checked for plausibility with previously entered data for that participant. In addition (if applicable), central data reviews will be performed on a regular basis to ensure completeness of the data collected and accuracy of the primary outcome data.

12.3 Monitoring

For quality control of the study conduct and data retrieval, all study sites will be visited on-site by appropriately trained and qualified Monitors. Any findings and comments will be documented in site visit reports and communicated to the local Investigator and to the Sponsor as applicable. Investigators at the participating study sites will support the Monitor in his/ her activities. Prior to study start (first participant enrolled) a plan detailing all monitoring-related procedures will be developed.
All source data and relevant documents will be accessible to Monitors and questions of Monitors are answered during site visits.

12.4 Audits and Inspections

Source data/ documents must be available to audits by the Sponsor or designee or to inspections by health authorities. The CA (Swissmedic) or CEC may wish to conduct an inspection (during the study or after its completion). If an inspection is requested, the Investigator must inform the Sponsor immediately that this request has been made. The Investigators at the participating sites will support the inspectors in their activities and will answer questions from inspectors as needed. All involved parties must keep the participant data strictly confidential.

12.5 Confidentiality, Data Protection

The Investigator ensures anonymity of the patients; patients will not be identified by names in any documents. Signed informed consent forms and patient enrollment log will be kept strictly confidential to enable patient identification at the site.

12.6 Storage of biological material and related health data

All baseline and follow-up CTs for central analysis will be coded and uploaded into the picture archiving and communication system (PACS) of the Inselspital. A secure exchange platform will be used for file exchange.

13. PUBLICATION AND DISSEMINATION POLICY

The trial protocol will be published in an open access journal. Study results will be presented at national and international meetings and will be submitted for publication to high impact, peer reviewed journals. Upon completion of the analysis, trial results will be communicated to all participants. Public reporting will be done through patient organizations and via the communication Departments at the individual Universities and Hospitals.

Once results have been published, trial data will be accessible to external researchers and anonymized datasets corresponding to each publication will be made available. Investigators wishing to replicate the analyses or to do an individual patient meta-analysis may request the data from the steering committee. Access to data will be granted in an unbureaucratic way.

14. FUNDING AND SUPPORT

14.1 Funding

This trial trial is financed by the Swiss National Science Foundation via the “Investigator-initiated clinical trials – IICT” grant # 33IC30_166785.

14.2 Other Support

The trial will also receive intramural support of the Bern University Hospital.

15. INSURANCE

Insurance will be provided by the Sponsor. A copy of the certificate is filed in each investigator site file and the trial master file.

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17. APPENDICES

Not applicable.

Last/final trial protocol

Version 1.7

November 28th, 2019

Clinical Study Protocol

Randomized double-blind placebo-controlled trial assessing the efficacy of standard and low dose hydrochlorothiazide treatment in the prevention of recurrent nephrolithiasis

NOSTONE TRIAL

Study Type:	Clinical trial with Investigational Medicinal Product (IMP)
Study Categorisation:	Risk category B
Study Registration:	ClinicalTrials.gov, Swiss National Clinical Trials Portal (SNCTP)
Study Identifier:	Swiss National Science Foundation # 33IC30_166785/ 1
Sponsor-Investigator:	Prof. Dr. med. Daniel Fuster Leitender Arzt Division of Nephrology and Hypertension Bern University Hospital, Bern, Switzerland Phone: +41 (0)31 632 31 44 Email: daniel.fuster@insel.ch
Investigational Product:	Hydrochlorothiazide
Protocol Version and Date:	Version 1.7, 28.11.2019

Signature Page(s)

Study identifier: Swiss National Science Foundation # 33IC30_166785/ 1
Study title: Randomized double-blind placebo-controlled trial assessing the efficacy of standard and low dose hydrochlorothiazide treatment in the prevention of recurrent nephrolithiasis

The Sponsor-Investigator and Trial Statistician/ Methodologist have approved this trial protocol and confirm hereby to conduct the study according to the protocol, current version of the World Medical Association Declaration of Helsinki, ICH-GCP guidelines and the local legally applicable requirements.

Sponsor-Investigator: Prof. Dr. med. Daniel Fuster

Bern, 21/2/2020 [redacted]

Place/ Date Signature

Trial Statistician and Methodologist: PD Dr. med. Sven Trelle

Bern 21.2.2020 [redacted]

Place/ Date Signature

Local Principal Investigator at study site:

I have read and understood this trial protocol and agree to conduct the trial as set out in this study protocol, the current version of the World Medical Association Declaration of Helsinki, ICH-GCP guidelines and the local legally applicable requirements.

Site:

Principal Investigator:

Place/ Date	Signature

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STUDY SYNOPSIS

Sponsor/ Sponsor-Investigator:	Inselspital Bern (Sponsor) represented by Prof. Dr. med. Daniel Fuster (Sponsor-Investigator)
Study Title:	Randomized double-blind placebo-controlled trial assessing the efficacy of standard and low dose hydrochlorothiazide treatment in the prevention of recurrent nephrolithiasis
Short Title/ Study ID:	NOSTONE Trial
Protocol Version and Date:	Version 1.7, 28.11.2019
Trial registration:	ClinicalTrials.gov, Swiss national clinical trials portal (SNCTP)
Study category and Rationale:	Risk category B Clinical trial with an IMP authorized in Switzerland that is not used in accordance with its prescribing information.
Clinical Phase:	Therapeutic use
Background and Rationale:	Nephrolithiasis is a global healthcare problem with a current lifetime risk of 18.8% in men and 9.4% in women. Without specific treatment, 5- and 20-year recurrence rates are 40% and 75%, respectively. Given the high cost of medical treatments and surgical interventions as well as the morbidity related to symptomatic stone disease, medical prophylaxis for stone recurrence is an attractive approach. About 80–90% of stones are composed of calcium oxalate with various admixtures of calcium phosphate. Increased excretion of calcium in the urine, hypercalciuria, is the most common metabolic abnormality encountered in patients with recurrent nephrolithiasis. Thiazide diuretics have been the cornerstone of pharmacologic metaphylaxis for more than 40 years. The effect of thiazides to reduce the risk of stone recurrence has been attributed to their ability to decrease urinary calcium excretion. However, other factors, such as reduction of urinary pH and urinary oxalate excretion, probably contribute to this effect. Efficacy of thiazides on recurrence prevention of calcareous nephrolithiasis was tested in 11 randomized controlled trials (RCTs). With the exception of two trials, thiazides significantly reduced stone recurrence. Most of these trials are from the 1980's and 90's and the cumulative number of patients studied is remarkably low for such a prevalent disease. Our systematic review of these RCTs revealed major methodological deficiencies in all trials, including: lack of double-blinding and intention-to-treat analysis, unclear allocation concealment, lack of adverse event and drop out reporting and unknown baseline risk of disease severity. Furthermore, high doses of thiazides were employed in all trials, in the case of the best studied thiazide, hydrochlorothiazide (HCTZ), up to 100 mg daily. At such high doses, side effects occur frequently. Nowadays, thiazides are widely used in the treatment of recurrent nephrolithiasis and arterial hypertension, but at significantly lower doses. In the case of recurrent nephrolithiasis, however, this practice is not supported by randomized evidence and consequently, we do not know whether the currently employed low dose thiazide regimens are effective in reducing the risk for stone recurrence. Thus, evidence for benefits and harms of thiazide diuretics in the prevention of calcium-containing kidney stones in general remains unclear. In addition, the efficacy of the currently employed low dose thiazide regimens to prevent stone recurrence is not known.
Objective(s):	We plan to assess the efficacy of standard and low dose HCTZ treatment in the recurrence prevention of calcium-containing kidney stones. More specifically, we aim to assess the dose-response relationship for three different dosages of HCTZ.

Outcome(s):	Primary: relationship of the incidence of stone recurrences (a composite of symptomatic or radiologic recurrence) during study treatment and dose group. Secondary: individual components of the composite primary outcome, changes in urinary biochemistry elicited by HCTZ treatment and impact of baseline disease severity, biochemical abnormalities and stone composition on treatment response.
Study design:	Multicenter, randomized, placebo-controlled, double-blind, parallel-group trial.
Inclusion/ Exclusion criteria:	Main inclusion criteria: • Informed Consent as documented by signature • Age 18 years or older • Recurrent kidney stone disease (≥ 2 stone events within the last 10 calendar years prior to randomization) • Any past kidney stone containing 50% or more of calcium oxalate, calcium phosphate or a mixture of both Main exclusion criteria: • Subjects with secondary causes of recurrent calcareous nephrolithiasis • Subjects with the following medications: Thiazide or loop diuretics, carbonic anhydrase inhibitors, xanthine oxidase inhibitors, alkali, active Vitamin D, calcium supplementation, bisphosphonates, denusomab, teriparatide, glucocorticoids • Known allergy to the study drug
Measurements and procedures:	An overview on the study measurements and procedures is provided in the study schedules. The main measurements and procedures performed are the following: Screening: medical history, vital signs, clinical laboratory tests Efficacy: symptomatic stone recurrence, low-dose renal-limited computed tomography for radiologic stone recurrence. Safety: vital signs, complete physical examination, SAEs and selected AEs, clinical laboratory tests.
Study Product/ Intervention:	HCTZ 12.5 mg, 25 mg or 50 mg once daily per os for 24 or 36 months. In addition, all patients in HCTZ treatment arms will receive state-of-the-art non-pharmacologic recommendations for stone prevention according to current guidelines.
Control Intervention (if applicable):	Placebo once daily per os for 24 to 36 months. In addition, all patients in the placebo arm will receive state-of-the-art non-pharmacologic recommendations for stone prevention according to current guidelines.
Number of Participants with Rationale:	416 patients in total, 104 patients per study arm.
Study Duration:	03/2017 – 08/2021
Study Schedule:	Month/Year of First-Participant-In: 03/2017 Month/Year of Last-Participant-Out: 08/2021
Investigator(s):	Principal Study Investigator: Prof. Dr. med. Daniel Fuster, Leitender Arzt Division of Nephrology, Hypertension and Clinical Pharmacology, Bern University Hospital Freiburgstrasse 11, 3010 Bern, Switzerland Email: daniel.fuster@insel.ch Phone: +41 (0)31 632 31 44
Study Centre(s):	12 sites in Switzerland including University Hospitals of Basel, Bern, Geneva, Lausanne and Zurich as well as Cantonal Hospitals of Aarau, Bellinzona, Chur, Lugano, Sion, Lucerne and St. Gallen.

Statistical Considerations:	Sample size: Calculation was based on the primary objective i.e. to assess the dose-response relationship and the primary outcome i.e. recurrence with the following assumptions: • Uniform recruitment over 24 months with allocation ratio fixed at 1 across all arms. • A maximum and minimum follow-up time of 36 and 24 months, respectively. • Cumulative drop-out rate of 10% at 24 months after study start. • Risk of recurrence in the placebo group of 0.20 and 0.45 at 12 and 36 months after study start, respectively. • Hazard ratios for the 12.5, 25 and 50 mg HCTZ doses of 0.90, 0.65 and 0.50, respectively. • Power was set to be at least 80% and alpha was fixed at a two-sided level of 0.05. • An unweighted log-rank test for linear trend with local alternatives. Primary efficacy analysis: On the intention-to-treat analysis set by a stratified log-rank test for linear trend with the hazard ratio as primary effect measure. The test addresses the primary objective of the trial i.e. test for any dose-response relationship. Comparisons between placebo and the three active trial arms will be considered exploratory as the trial is not powered to detect differences there. Missing data will be handled by multiple imputation. All statistical analyses will be presented as effect measure plus 95% confidence interval. Secondary efficacy analysis: Analysis will follow principles as described for primary efficacy analysis. Continuous outcomes (laboratory values) will be analyzed using linear regression.
GCP Statement:	This study will be conducted in compliance with the protocol, the current version of the Declaration of Helsinki, the ICH-GCP as well as all national legal and regulatory requirements.

STUDY SUMMARY IN LOCAL LANGUAGE

Titel des Forschungsprojektes:

Randomisierte plazebokontrollierte Doppelblindstudie über die Wirksamkeit von normal- und tiefdosiertem Hydrochlorothiazid zur Vorbeugung von Rückfällen kalziumhaltiger Nierensteine: die NOSTONE-Studie

Lead:

In dieser klinischen Studie wird die Wirksamkeit des Medikaments Hydrochlorothiazid zur Vorbeugung von Rückfällen kalziumhaltiger Nierensteine getestet.

Inhalt und Ziele des Forschungsprojektes:

Nierensteine sind ein weltweites Gesundheitsproblem, etwa 10% aller Menschen sind mindestens einmal im Leben davon betroffen. Nierensteine sind äusserst schmerzhaft, treten wiederholt auf und verursachen hohe direkte und indirekte Kosten. Eine effektive und kostengünstige Vorbeugung gegen Rückfälle von Nierensteinen ist deshalb äusserst wünschenswert.
Etwa 80-90% aller Nierensteine enthalten Kalzium und eine vermehrte Ausscheidung von Kalzium im Urin ist die häufigste Stoffwechselstörung bei Patienten mit Nierensteinen. Thiazide reduzieren die Kalziumausscheidung im Urin und werden daher seit Jahrzehnten zur Vorbeugung von Rückfällen kalziumhaltiger Nierensteine eingesetzt. Deren Wirksamkeit wurde in den letzten 30 Jahren in mehreren Studien getestet. Leider war die Durchführung dieser Studien mangelhaft, die Gesamtzahl der untersuchten Patienten klein, und es wurden hohe Medikamentendosen getestet, welche häufig Nebenwirkungen verursachen. Zur Zeit ist daher der Stellenwert der Thiazide in der Behandlung von Patienten mit Nierensteinen unklar. Ziel dieser Studie ist es, die Wirksamkeit von normal- und tiefdosiertem Hydrochlorothiazid zur Vorbeugung von Rückfällen kalziumhaltiger Nierensteine zu testen.

Wissenschaftlicher und gesellschaftlicher Kontext des Forschungsprojekts:

Das Nierensteinleiden gehört zu den häufigsten menschlichen Erkrankungen. Mit dieser klinischen Studie soll eine grosse Wissenslücke in der Nierensteinbehandlung geschlossen werden. Die Resultate dieser Studie werden die Behandlung von Nierensteinpatienten weltweit beeinflussen.

ABBREVIATIONS

AE	Adverse Event
BMI	Body Mass Index
CA	Competent Authority (Swissmedic)
CEC	Competent Ethics Committee
CI	Confidence Interval
CRF	Case Report Form
eCRF	Electronic Case Report Form
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
EOS	End of Study
EOT	End of Treament
GCP	Good Clinical Practice
HCTZ	Hydrochlorothiazide
IMP	Investigational Medicinal Product
IVU	Intravenous Urography
NIH	National Institute of Health
RCT	Randomized Controlled Trial
SAE	Serious Adverse Event
SNCTP	Swiss National Clinical Trials Portal
SUSAR	Suspected Unexpected Serious Adverse Reaction

STUDY SCHEDULE 1 – PARTICIPANTS RECRUITED IN THE FIRST 12 MONTHS OF THE RECRUITMENT PERIOD

Study Period	Screening[1]		Treatment period								Safety follow-up
Visit	1 or ☽	2	3	4 or ☽[2]	☽	5 or ☽	☽	6 or ☽	☽	7 or ☽[10]	☽ EOS
Visit time points NOSTONE trial	-1 months to -14 day(s)	-2 weeks	0	3 months	6, 9 months	12 months	15, 18, 21 months	24 months	27, 30, 33 months	36 months/ EOT	30 days after EOT
Allowed visit window		± 2 weeks	0	± 2 weeks	± 4 Weeks	± 4 weeks	± 4 weeks	± 4 weeks	± 4 weeks	± 4 weeks	± 2 weeks
Patient Information & Informed Consent	x										
In-/ Exclusion Criteria	x		x								
Demographics	x										
Medical History	x										
Stone composition	x[3]		x [composition of any recurrent stone(s) if data available per routine]								
Physical Examination	x			x		x		x		x	
Vital Signs[4]	x			x		x		x		x	
Blood analysis[5]		x		x		x		x		x	
Urine analysis[5]		x[6]		x[7]		x[6]		x[6]		x[6]	
Pregnancy test (blood or urine)[9]		x									
Randomization			x								
Low-dose renal CT for radiologic recurrence[5]			x[8]	x (in case of a suspicion of symptomatic recurrence)						x[8]	
Assessment of symptomatic stone recurrence				x	x	x	x	x	x	x	
Adherence reminder				x	x	x	x	x	x		
Screening criteria for interrupting or discontinuing IMP				x	x	x	x	x	x		
AEs of special interest				x	x	x	x	x	x	x	
SAEs		x	x	x	x	x	x	x	x	x	x
Concomitant medication	x	x	x	x	x	x	x	x	x	x	

Hand out of (new) IMP			x	x	x	x	x	x	x		
Collection of used/ unused IMP packs				x		x		x		x	
Daily intake of IMP			x (start on day after visit 3)								
Non-pharmacological preventive measures			x								

EOT end of treatment
EOS end of study

1 For participants who are in regular follow-up at the site for recurrent stone disease, visit 1 is not performed and all screening assessments are done at visit 2. For participants newly referred to the study site and in metabolic work-up for recurrent stone disease, screening assessments should be performed at visits 1 and 2, but if this is impracticable, the same procedure as for participants in regular follow-up applies.
2 For participants who are recruited from patients in routine follow-up at the study site: phone call assessing all parameters as required at the three-monthly phone calls with all study participants (see e.g. phone call at 6 months). For participants recruited via new referrals: either visit at the study site (recommended) or phone call if visit impracticable.
3 Information on the composition of all stones analyzed prior to randomization as available.
4 Heart rate, systolic and diastolic blood pressure at the right arm in sitting position after at least 5 minutes at rest.
5 Analyzed centrally; laboratory analyses performed only with patient's approval for visits 4,5, 6 and 7. Nonetheless, safety lab values must be checked at V4 (can be done also at the GP).
6 Two 24 hour urine collections by the patient starting 48 hours and 24 hours respectively prior to the visit or one 24 hour urine collection (with added paraffin/thymol) by the patient in the 48 hours prior to the visit (if two 24 hour collections by the patient are impracticable).
7 One 24 hour urine collection (with added paraffin/ thymol) by the patient starting 24 hours prior to the visit.
8 If a routine non-iv contrast renal CT was performed within 2 months prior to visit 3 or visit 7 respectively, this CT can be used for the trial and no additional CT has to be performed at the respective visit.
9 For women of child-bearing potential, defined as women who are not surgically sterilized/ hysterectomized, and/ or who are postmenopausal for less than 12 months), analyzed locally
10 This visit can be replaced by a phone call in case the patient is unable to come for the visit in person, but the final CT scan and a detailed report of stone events during the treatment period from the last visit must be performed.

STUDY SCHEDULE 2 – PARTICIPANTS RECRUITED ≥ 12 MONTHS AFTER RECRUITMENT START (***)

Study Period	Screening[1]		Treatment period						Safety follow-up
Visit	1 or ✆	2	3	4 or ✆[2]	✆	5 or ✆	✆	6 or ✆[10]	✆ EOS
Visit time points NOSTONE trial	-1month to -14 day(s)	-2 weeks	0	3 months	6, 9 months	12 months	15, 18, 21 months	24 months/ EOT	30 days after EOT
Allowed visit window		± 2 weeks	0	± 2 weeks	± 4 weeks	± 4 weeks	± 4 weeks	± 4 weeks	± 2 weeks
Patient Information & Informed Consent	x								
In-/ Exclusion Criteria	x		x						
Demographics	x								
Medical History	x								
Stone composition	x[3]		x [composition of any recurrent stone(s) if data available per routine]						
Physical Examination	x			x		x		x	
Vital Signs[4]	x			x		x		x	
Blood analysis[5]		x		x		x		x	
Urine analysis[5]		x[6]		x[7]		x[6]		x[6]	
Pregnancy test (blood or urine)[9]		x							
Randomization			x						
Low-dose renal CT for radiologic recurrence[5]			x[8]	x (in case of a suspicion of symptomatic recurrence)				x[8]	
Assessment of symptomatic stone recurrence and asymptomatic stone removal				x	x	x	x	x	
Adherence reminder				x	x	x	x		
Screening criteria for interrupting or discontinuing IMP				x	x	x	x		

AEs of special interest				x	x	x	x	x	
SAEs		x	x	x	x	x	x	x	x
Concomitant medication	x	x	x	x	x	x	x	x	
Hand out of (new) IMP			x	x	x	x	x		
Collection of used/ unused IMP packs				x		x		x	
Daily intake of IMP			x (start on day after visit 3)						
Non-pharmacological preventive measures			x						

EOT end of treatment
EOS end of study

1 For participants who are in regular follow-up at the site for recurrent stone disease, visit 1 is not performed and all screening assessments are done at visit 2. For participants newly referred to the study site and in metabolic work-up for recurrent stone disease, screening assessments should be performed at visits 1 and 2, but if this is impracticable, the same procedure as for participants in regular follow-up applies.
2 For participants who are recruited from patients in routine follow-up at the study site: phone call assessing all parameters as required at the three-monthly phone calls with all study participants (see e.g. phone call at 6 months); for participants recruited via new referrals: either visit at the study site (recommended) or phone call) or phone call if visit impracticable.
3 Information on the composition of all stones analyzed prior to randomization as available.
4 Heart rate, systolic and diastolic blood pressure at the right arm in sitting position after at least 5 minutes at rest.
5 Analyzed centrally; laboratory analyses performed only with patient's approval for visits 4, 5 and 6. Nonetheless, safety lab values must be checked at V4 (can be done also at the GP).
6 Two 24 hour urine collections by the patient starting 48 hours and 24 hours respectively prior to the visit or one 24 hour urine collection (with added paraffin/thymol) by the patient in the 48 hours prior to the visit (if two 24 hour collections by the patient are impracticable).
7 One 24 hour urine collection (with added paraffin/ thymol) by the patient starting 24 hours prior to the visit.
8 If a routine non-iv contrast renal CT was performed within 2 months prior to visit 3 or visit 7 respectively, this CT can be used for the trial and no additional CT has to be performed at the respective visit.
9 For women of child-bearing potential, defined as women who are not surgically sterilized/ hysterectomized, and/ or who are postmenopausal for less than 12 months), analyzed locally
10 This visit can be replaced by a phone call in case the patient is unable to come for the visit in person, but the final CT scan and a detailed report of stone events during the treatment period from the last visit must be performed.

(***) Patients can be followed up for a minimum of 24 months up to max 36 months, same as for the patients enrolled during the first 12 months

1. STUDY ADMINISTRATIVE STRUCTURE

1.1 Sponsor-Investigator

Prof. Dr. med. Daniel Fuster, Leitender Arzt
Division of Nephrology and Hypertension, Bern University Hospital
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Email: daniel.fuster@insel.ch
Phone: +41 (0)31 632 31 44

1.2 Principal Study Investigator

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Division of Nephrology and Hypertension, Bern University Hospital
Freiburgstrasse 11, 3010 Bern, Switzerland
Email: daniel.fuster@insel.ch
Phone: +41 (0)31 632 31 44

1.3 Statistician ("Biostatistician")

PD Dr. med. Sven Trelle
CTU Bern, Department of Clinical Research, University of Bern
Mittelstrasse 43, 3012 Bern, Switzerland
Email: sven.trelle@ctu.unibe.ch
Phone: +41 (0)31 631 35 04

1.4 Laboratory

The address of the central laboratory is documented elsewhere.

1.5 Monitoring institution

CTU Bern, Department of Clinical Research, University of Bern
Mittelstrasse 43, 3012 Bern, Switzerland
Phone: +41 (0)31 631 33 72

1.6 Data Safety Monitoring Committee

Not applicable.

1.7 Any other relevant Committee, Person, Organisation, Institution

1.7.1 Study Steering Committee

The study steering committee will be responsible for the overall supervision of the trial and will regularly meet to discuss scientific and logistic aspects of the trial. Information on the composition of the committee is available in a separate document.

2. ETHICAL AND REGULATORY ASPECTS

Before the study will be conducted, the protocol, the proposed patient information and consent form, as well as other study-specific documents shall be submitted to a properly constituted Competent Ethics Committee (CEC) and competent authority (CA, Swissmedic) in agreement with local legal requirements, for formal approval. Any amendment to the protocol must as well be approved (if legally required) by these institutions.
The decision of the CEC and CA concerning the conduct of the study will be made in writing to the Sponsor-Investigator before commencement of this study. The clinical study can only begin once approval from all required authorities has been received. Any additional requirements imposed by the authorities shall be implemented.

2.1 Study registration

The study will be registered in the Clinical Trials Registry Platform of the National Institute of Health (NIH) – ClinicalTrials.gov. In addition, the trial will be registered in the Swiss National Clinical Trials Portal (SNCTP).

2.2 Categorisation of study

Risk category B. Clinical trial with an investigational medicinal product (IMP) authorized in Switzerland that is not used in accordance with its prescribing information.

2.3 Competent Ethics Committee (CEC)

Responsible investigators at each study site will ensure that approval from the appropriate constituted CEC is sought for the clinical study. Yearly intermediary reports (annual safety reports) will be forwarded to the CEC. All unanticipated problems involving risks to humans will be reported to the CEC within 7 days. No changes will be made to the protocol without prior Sponsor and, in case of substantial amendments, CEC approval, except where necessary to eliminate apparent immediate hazards to study participants. Amendments will be reported according to section 2.10.
Premature study end or interruption of the study will be reported to the CEC within 15 days. The regular end of the study will be reported to the CEC within 90 days, the final study report shall be submitted within one year after study end.

2.4 Competent Authorities (CA)

The Sponsor-Investigator will obtain approval from the CA (Swissmedic) before the start of the clinical trial. Yearly intermediary reports (annual safety reports) will be forwarded to the CA. Premature study end or interruption of the study will be reported to the CA within 15 days. The regular end of the study will be reported to the CA within 90 days, the final study report shall be submitted within one year after study end. Amendments are reported according to section 2.10. Non-substantial amendments shall be reported as soon as possible.

2.5 Ethical Conduct of the Study

The study will be carried out in accordance to the protocol and with principles enunciated in the current version of the Declaration of Helsinki, the guidelines of Good Clinical Practice (GCP) issued by ICH, the Swiss Law and Swiss regulatory authority's requirements. The CEC and regulatory authorities will receive annual safety and interim reports and be informed about study stop/ end in agreement with local requirements.

2.6 Declaration of interest

There are no conflicts of interest.

2.7 Patient Information and Informed Consent

All participants included in the trial are adults with recurrent kidney stone disease. No vulnerable individuals will be included in the trial. The participation in the trial is voluntary. The investigators will explain to each participant the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits and any discomfort it may entail. Each participant will be informed that the participation in the study is voluntary and that he/she may withdraw from the study at

any time and that withdrawal of consent will not affect his/her subsequent medical assistance and treatment. The participant will be informed that his/her medical records may be examined by authorised individuals other than their treating physician. All study participants will be provided with a participant information sheet and a consent form describing the study and providing sufficient information for the participant to make an informed decision about their participation in the study. Participants willing to participate will sign and date the informed consent form. The consent form will also be signed and dated by the investigator (or his designee) and it will be retained as part of the study records. A copy of the signed document will be given to the participants. Only after obtaining formal consent with the approved consent form, participants will be submitted to any study procedure. No compensation is foreseen and informed consent for general and further use of data and/ or biological samples will not be sought.
Patients must sign an additional patient informed consent for the NOSTONE biobank.

2.8 Participant privacy and confidentiality

The investigator affirms and upholds the principle of the participant's right to privacy and that they shall comply with applicable privacy laws. Especially, anonymity of the participants shall be guaranteed when presenting the data at scientific meetings or publishing them in scientific journals. Individual subject medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited. Subject confidentiality will be further ensured by utilising subject identification code numbers to correspond to treatment data in the computer files. For data verification purposes, authorised representatives of the Sponsor (-Investigator), the CA, or CEC may require direct access to parts of the medical records relevant to the study, including participants' medical history.

2.9 Early termination of the study

The Steering Committee may terminate the study prematurely according to certain circumstances, for example:

- ethical concerns,
- financial issues,
- insufficient participant recruitment,
- when the safety of the participants is doubtful or at risk, respectively,
- alterations in accepted clinical practice that make the continuation of a clinical trial unwise,
- early evidence of benefit or harm of the experimental intervention,
- or any other reason that would prevent the project execution according to the research plan.

Premature study end or interruption of the study will be reported to the CEC and CA within 15 days.

2.10 Protocol amendments

The Steering Committee will decide on protocol amendments. Any investigator, CEC or CA will be able to provide suggestions for a protocol amendment. Important protocol modifications will be communicated by the Sponsor-Investigator to relevant parties (CEC, CA, trial participants, trial registries and the Swiss National Science Foundation).
Substantial amendments will only be implemented after approval of the CEC and CA, respectively. Under emergency circumstances, deviations from the protocol to protect the rights, safety and well-being of human subjects may proceed without prior approval of the sponsor and the CEC/ CA. Such deviations shall be documented and reported to the sponsor and the CEC/ CA as soon as possible. All non-substantial amendments are communicated to the CA as soon as possible if applicable and to the CEC within the Annual Safety Report (ASR).

3. BACKGROUND AND RATIONALE

3.1 Background and Rationale

Introduction:
Nephrolithiasis is a worldwide healthcare problem with a current lifetime risk of ~18.8% in men and ~9.4% in women in Western civilizations [1]. Incidence and prevalence of renal stone disease are increasing globally, irrespective of age, sex and race [1, 2]. Without a specific treatment, 5- and 20-year

recurrence rates are ~40% and ~75%, respectively [3, 4]. In the United States, hospitalizations, surgery and lost work time associated with kidney stones cost more than 5 billion US Dollars annually [5]. Thus, given the high cost and the morbidity related to recurrent kidney stone disease, medical prophylaxis seems to be an attractive approach [6, 7]. Indeed, apart from its benefits to patients in terms of reduced morbidity and risk from procedures, medical prevention of nephrolithiasis is clearly cost effective [8].

Although various inherited and systemic diseases are associated with kidney stones, most stones are idiopathic [3]. Despite this, nephrolithiasis needs to be considered as a systemic disease. Chronic kidney disease (CKD), arterial hypertension, obesity, diabetes mellitus and low bone mass are much more prevalent in stone formers than in non-stone formers and contribute significantly to stone-related morbidity and mortality [9-15]. It is, however, currently unclear if stone disease is a cause of this co-morbidity or if it is a consequence of the same underlying conditions that lead to these disorders and renal stone disease.

80-90% of stones are composed of calcium oxalate, calcium phosphate or a mixture of both [16, 17]. Increased excretion of calcium in the urine, hypercalciuria, is the most frequent metabolic abnormality encountered in patients with recurrent idiopathic nephrolithiasis [17, 18]. Depending on the stone subtype, hypercalciuria is present in up to 87% of patients with calcium-containing kidney stones [17]. Other urinary factors frequently encountered in recurrent calcareous stone formers that contribute to the development of stones are hypocitraturia, hyperoxaluria, hyperuricosuria and low urinary volume [19]. Supersaturation of urinary calcium oxalate and calcium phosphate, expressed as the ratio of concentration to its solubility, is the driving force for stone formation. At a supersaturation < 1, crystals dissolve, at a supersaturation > 1, crystals form. Hypercalciuria increases supersaturation both for calcium oxalate and calcium phosphate and thus increases the risk for both calcium oxalate and calcium phosphate stones [3]. Urinary supersaturations calculated from ambulatory 24 hour urine collections are highly correlated with the type of kidney stones encountered in the individual stone former, with notable rare exceptions in patients with very high or very low urinary volumes [20]. The hypercalciuria encountered in recurrent stone formers is often familial and strongly influenced by diet but in most cases of unknown origin and hence designated “idiopathic” [3]. Gut absorption of calcium is enhanced in idiopathic hypercalciuria, but serum calcium remains typically normal because intestinally absorbed calcium is promptly excreted by the kidneys [21]. Despite intestinal calcium hyperabsorption, patients with idiopathic hypercalciuria are often in negative calcium balance because of excessive renal calcium losses, especially under a low calcium diet [19, 22]. The source of calcium in these circumstances is the skeleton, the largest repository of calcium in the body [19]. Not surprising therefore, low bone mass is a frequent finding in normo- and especially hypercalciuric stone formers [9]. At the moment, thiazide diuretics are the only drugs known to reduce urinary calcium excretion. This peculiar property is employed in the prevention of recurrent calcareous nephrolithiasis but also in the prevention of bone loss in patients with recurrent nephrolithiasis and/ or arterial hypertension [9, 23]. Observational data and evidence from prospective trials indicate that chronic thiazide therapy preserves bone mineral density and reduces fracture risk [24-27].

Clinical work-up of patients with recurrent nephrolithiasis includes a detailed history and physical examination as well as blood and urine analysis to detect potential causes of stones but also possible associated conditions [23]. Spot urine analysis is performed to search for crystalluria and rule out infection [23]. Paramount for the metabolic work-up are 24 h urine collections, which are typically performed twice, since mineral excretions have considerable day-to-day variations [23, 28, 29]. Metabolic work-up for stone disease is done at least 3 months after a symptomatic stone event in patients which have resumed their usual diet and activity [30]. All passed stones need to be analyzed for classification purposes but also to detect possible treatment related conversions of one stone type to another (e.g. calcium oxalate to calcium phosphate by citrate-induced urinary alkalinization) [3, 31]. Computed tomography (CT) without the use of contrast material is the most sensitive method to detect renal and ureteral stones and should, according to current guidelines, be preferred to plain abdominal X-ray imaging (KUB radiography) or intravenous urography (IVU) in the case of a symptomatic stone event [23, 31-33]. Modern low-dose CT protocols cause greatly reduced radiation exposure while preserving a high sensitivity of 96.6% and specificity of 94.9% [34]. Current radiation exposure of low dose CT protocols for the detection of urolithiasis is in the range of 0.97-1.9 mSV and thus similar to KUB radiography (0.5-1 mSv) but significantly lower than IVU (1.3 -3.5 mSv) or regular dose CT (4.5-5 mSv) [23, 35, 36].

Follow-up studies are necessary in patients taking preventive measures for stone recurrence [23, 37]. American and European guidelines recommend a first follow-up 24 hour urine collection 8-12 weeks after initiation of therapy to adjust drug dosage [23, 31]. Also, periodic blood testing in patients on pharmacological treatment to assess for adverse events (AEs) are recommended [31]. In the case of thiazide diuretics, a repeat blood draw with serum electrolytes is typically done within the first few weeks

of treatment initiation to rule out electrolyte abnormalities, especially hypokalemia. In the case of the latter, oral potassium supplementation or a potassium-sparing diuretic like spironolactone or amiloride can be additionally prescribed [31]. Once urinary parameters have been normalized and blood parameters are stable, yearly blood and urine analysis is recommended [23, 31]. In addition to metabolic studies, periodic follow-up imaging studies, preferably with low dose CT, are equally recommended to assess for stone growth or new stone formation [23, 31]. A one-year imaging interval is currently recommended for stable patients on medical stone prevention [23, 31].

Current knowledge on the efficacy of thiazides in recurrent nephrolithiasis:

The first thiazide diuretic chlorothiazide was synthesized in 1957 by Sprague and Novello [38]. In 1959, Lamberg and Kuhlbäck reported the observation that the thiazides chlorothiazide and hydrochlorothiazide (HCTZ) reduce urinary calcium excretion [39]. Soon thereafter, Lichtwitz and colleagues suggested that this peculiar property might be exploited to prevent recurrence of calcium-containing kidney stones [40]. In 1970, Yendt et al. reported initial observations of an uncontrolled series of 72 recurrent calcium stone formers treated with HCTZ in doses from 100 mg to 200 mg daily [41]. The treatment seemed highly effective: compared to pretreatment, HCTZ at such doses reduced stone episodes from 0.57 to 0.03 per patient year in patients without radiologic evidence of stone disease at treatment start and from 1.1 to 0.53 per patient year in patients with residual stone load at treatment initiation. Similar findings were reported by the same group in a follow up publication several years later with a total of 346 patients [42]. Based on these publications, despite the absence of randomized controlled trials (RCTs), thiazide rapidly became a cornerstone of medical stone prevention.

In August 2015, we conducted a systematic database search for RCTs that tested the efficacy of thiazides in the prevention of recurrent nephrolithiasis. Our search covered MEDLINE, the Cochrane Database, Google Scholar and the Web of Science for English language trials that had clinical endpoints (symptomatic or radiologic stone recurrence) and at least 1 year of follow-up. We also searched for unpublished trials at the World Health Organization's International Clinical Trials Registry Platform (www.who.int/trialsearch), the NIH funded site ClinicalTrials.gov (https//:clinicaltrials.gov) and on internet databases provided by the US Food and Drug Administration and by the European Medicines Agency [42]. Our search revealed 11 published RCTs [43-53] and no unpublished trials. All 11 RCTs identified are depicted in Table 3.1-1. The first RCT for stone prevention with thiazide diuretics was published in 1981. At total of seven trials were conducted in the 1980's, three in the 1990's and only one trial was conducted after the year 2000. As shown in Table 1, with the exception of two trials [51, 53], which were the first two RCTs conducted in the field, thiazides significantly reduced stone recurrence compared to placebo or control, in average by about 50% (Table 1). The thiazides employed in the two negative trials were bendroflumethiazide at the dosage of 2.5 mg three times daily and HCTZ at the dosage of 25 mg twice daily. Follow-up duration of the two negative trials was very short (1 and 1.6 years, respectively) compared to 2 – 5 years in the positive trials, which may explain the lack of benefit observed. All trials were small and only one trial included > 100 patients [47]. The 11 trials included 693 patients in total (Table 1). In three trials, the outcome was symptomatic stone recurrence, in the other eight trials the outcome was a composite of symptomatic and radiologic recurrence (Table 3.1-1). In all trials, radiologic recurrence was studied with the low sensitivity and specificity imaging modalities, KUB or IVU. A consistent finding in these thiazide RCTs was that stone-formation rate between treated and control groups did not begin to diverge until after at least one year of therapy [54]. As detailed in Table 3.1-1, our review indicates that all thiazide RCTs thus far conducted have significant methodological deficiencies including unclear allocation concealment, lack of double-blinding, no intention-to-treat analysis and lack of AE and drop out reporting.

Interestingly, in only three of 11 RCTs hypercalciuria was an inclusion criterion and thiazides seem to be effective in reducing stone recurrence regardless of the presence or absence of hypercalciuria (Table 3.1-1). There are at least two possible explanations for this observation: first, Curhan et al. demonstrated in a large epidemiological study that calciuria is a gradual risk factor for the development of kidney stones [55]. An increase in the relative risk for kidney stone formation was observed when urinary calcium excretion exceeded 2.5 mmol/d, which is far below the current definition of hypercalciuria on a random outpatient diet (> 7.5 mmol/d). Second, thiazides, in addition to reducing calciuria, have favorable effects on other urinary constituents that may reduce stone risk. The latter include reduction of urinary pH and oxalate excretion and an increase of urinary magnesium and zinc excretion [56, 57].

HCTZ was used in five of the 11 thiazide trials and thus is currently the best studied thiazide in the prevention of stone recurrence [45, 48, 50, 52, 53]. However, bendroflumethazide, chlorthalidone, trichlormethiazide and indapamide also reduced stone recurrence in one or more trial and seem to be effective as well (Table 3.1-1). In all trials, high thiazide doses were employed, in the case of HCTZ, 50 - 100 mg daily. In four of the five trials where HCTZ was employed, the diuretic was given twice daily,

whereas in the treatment of arterial hypertension, HCTZ is given once daily [54]. Apart from reducing blood pressure, once daily HCTZ at 50 mg, 25 mg or 12.5 mg daily also reduced calciuria, a surrogate marker for stone prevention [58]. Twice daily HCTZ increases the frequency of side effects and augments diuresis at night and thereby likely affects compliance [54, 58].

A recent study showed that thiazide diuretics are often not used in an evidence-based fashion for the prevention of stone recurrence [59]. In that study, 107 patients with a filled prescription for thiazide diuretics that underwent a 24-hour urine stone risk factor analysis and had medical record documentation that the thiazide was prescribed for calcium-containing kidney stone recurrence were analyzed. Only 35% of HCTZ-treated patients received ≥50 mg/ d, a dose previously shown to reduce stone recurrence in RCTs. 52% of patients were prescribed 25 mg daily and 13% 12.5 mg daily, doses which were not studied in RCTs. The tendency to prescribe lower doses of thiazides in patients with recurrent nephrolithiasis was likely triggered by a paradigm shift in prescribing practices for thiazides used for the treatment of arterial hypertension. Starting in the 1980's, lower doses of HCTZ (12.5–25 mg daily) were increasingly employed [60]. While clinical and biochemical side effects were noted to be dose dependent, the antihypertensive effects remained robust, even at lower doses [60, 61]. If this is also true for recurrent calcareous nephrolithiasis is currently unknown.

Fink et al. recently conducted a systematic review of RCTs for the medical management to prevent recurrent nephrolithiasis [62]. RCTs involving dietary or pharmacologic treatments to prevent recurrent kidney stones in adults that reported clinical outcomes or harms were included in the meta-analysis. The search of the authors covered MEDLINE, the Cochrane Database, Google Scholar and the Web of Science for English-language studies published from 1948 through 2011. Using criteria developed by the Cochrane Collaboration, individual study quality was rated good, fair or poor on the basis of adequacy of allocation concealment, blinding, reporting reasons for attrition and how analyses accounted for incomplete data. Following methods developed by the Agency for Healthcare Research and Quality (AHRQ)'s Effective Health Care Program [63], strength of evidence (SOE) for the efficacy of each treatment was graded on the basis of risk of bias, consistency, directness and precision. The review was nominated to the AHRQ by the American Urological Association but was funded by the AHRQ. A total of 28 trials (8 dietary and 20 pharmacologic) were included in the meta-analysis. Six of the 11 thiazide trials we identified in our own database search were included in the meta-analysis (Table 3.1-1) [43-45, 50, 52, 53]. Patients enrolled in the selected thiazide trials had two or more stone events prior to study inclusion, mean treatment duration was 35 months with a cumulative number of 365 patients. Patients were assigned to thiazide and either placebo [44, 45, 53] or control [43, 50, 52]. The authors concluded that AEs were inconsistently reported but that compared with participants in the placebo and control groups, those randomly assigned to receive thiazides were statistically significantly more likely to withdraw for any reason or because of AEs [62]. Only one of the selected trials assessed symptomatic stone recurrence (treatment versus placebo RR 1.04, 95% CI 0.39 – 2.8) [53] and no trial reported radiologic outcomes separately. The other five trials with a cumulative number of 300 patients reported a composite endpoint with radiologic and symptomatic stone recurrence. Treatment with thiazides decreased the risk for this composite outcome (26 vs. 55%; RR 0.52, 95% CI 0.39 – 0.69; NNT 3.4). Fink et al. stated in their review that the meta-analysis was greatly limited by the available data and that additional trials are warranted [62]. Critical points raised were i) the low amount of trials available and the small sample sizes, ii) lack of data on treatment harms, iii) the fact that all trials included only adults with idiopathic calcium stones, iv) lack of symptomatic stone recurrence reporting as an isolated outcome, v) the fact that only one trial recruited patients from a primary care setting and vi) inconsistent reporting and categorization of baseline biochemistry measures.

Overall, the authors concluded that all six thiazide trials included in their meta-analysis were of fair quality and that there was moderate SOE for the composite end point of symptomatic and radiologic stone recurrence but that there was insufficient SOE for the individual outcomes symptomatic or radiologic stone recurrence [62].

Table 3.1-1: Randomized controlled trials of thiazides in the prevention of recurrent nephrolithiasis

Author, Year	Treatment, Dose	Allocation Concealment	Blinding	Intention-to-treat Analysis	Withdrawals described	Selection for Hypercalciuria	Follow-Up (Years)	Treated/ Placebo n/N	Events/ Total, n/N Thiazide	Events/ Total, n/N Placebo	RR [c]	Recurrence Outcome
Brocks, 1981 [51]	Bendroflumethiazide, 2.5 mg TID [a]	Unclear	Double-blind	No	No	No	1.6	33/29	5/33	5/29	NS	Composite
Scholz, 1982 [53]*	HCTZ, 25 mg BID [b]	Unclear	Double-blind	No	No	No	1	25/26	6/25	6/26	NS	Symptomatic
Laerum, 1984 [45]*	HCTZ, 25 mg BID	Unclear	Double-blind	Yes	Yes	No	3	23/25	5/23	12/25	0.45	Composite
Wilson, 1984 [48]	HCTZ, 100 mg daily	Unclear	Open-label	No	No	No	2.8	23/21	0.15 stones/ year	0.32 stones/ year	0.48	Symptomatic
Robertson, 1985 [49]	Bendroflumethazide, 2.5 mg TID	Unclear	Open-label	No	No	No	3-5	13/9	0.22 stones/ year	0.58 stones/ year	0.38	Symptomatic
Mortensen, 1986 [46]	Bendroflumethazide, 2.5 mg	Unclear	Double-blind	No	No	No	2	12/10	0/12	4/10	-	Composite
Ettinger, 1988 [44]*	Chlorthalidone, 25 mg / 50 mg	Adequate	Double-blind	No	Yes	No	3	19/23/31 (25 mg/ 50 mg/ placebo)	6/42	14/31	0.32	Composite
Ohkawa, 1992 [47]	Trichlormethiazide, 4 mg	Unclear	Open-label	No	No	Yes	2.14-2.21	82/93	24/82	57/93	0.42	Composite
Borghi, 1993 [43]*	Indapamide, 2.5 mg daily	Unclear	Open-label	No	Yes	Yes	3	43/21	6/43	9/21	0.37	Composite
Ahlstrand, 1996 [52]*	HCTZ, 25 mg BID	Unclear	Open-label	Yes	Yes	Yes	3.6-4.3	17/22	9/17	19/22	0.61	Composite
Fernandez-Rodriguez, 2006 [50]*	HCTZ, 50 mg daily	Unclear	None stated	Yes	No withdrawals	No	3	50/50	16/50	28/50	0.57	Composite

[a] TID, three times daily
[b] BID, two times daily
[c] RR, relative risk
* trials included in the meta-analysis by Fink et al. [62]

Rationale:
We and others [62] identified major issues in design, conduct, analysis and reporting in thiazide RCTs conducted thus far for the prevention of stone recurrence.

Major issues encountered include:
- Lack of double-blinding
- Lack of intention-to-treat analysis
- Unclear allocation concealment
- Lack of AE and drop out reporting
- Unknown baseline risk of disease severity or biochemical abnormalities
- Lack of patients stratification based on stone composition
- Lack of outcome uniformity
- Absence of data on the impact of pharmacological interventions on disease risk factors
- Low overall number of patients studied
- Use of high dose thiazide treatments
- Inadequate dietary measures in control and treatment groups
- Use of low sensitivity and low specificity imaging for radiologic outcomes
- Use of outdated dietary recommendations in placebo or control groups

The methodological deficiencies are of great concern. Review of the trials reveals that none of the 11 trials fulfills the quality standards expected of a randomized, placebo-controlled, double-blind trial. While the methodological deficiencies of the trials are obvious for anyone concerned with clinical research, several points peculiar to kidney stone disease deserve further explanation:

Drug dosing: High doses of thiazides were employed in these trials, in the case of the best studied thiazide, HCTZ, 50 - 100 mg daily. At such high doses, side effects occur in 30 – 40% of patients and include electrolyte disturbances, muscle cramps, orthostatic hypotension, gastrointestinal symptoms, hypocitraturia, gout, impaired glucose tolerance, skin rashes and erectile dysfunction [52, 56]. Since AE and drop out reporting in all trials was very poor, tolerability and harms of high dose thiazide treatments remain largely unknown. Nowadays, thiazides are widely used in the treatment of arterial hypertension and recurrent nephrolithiasis, but at significantly lower doses [54, 58, 59]. However, this practice is not supported by randomized evidence in the case of recurrent nephrolithiasis and consequently, we do not know whether the currently employed low dose thiazide regimens are effective in reducing the risk for stone recurrence.
Dietary interventions: Non-pharmacologic interventions for recurrence prevention are very effective [62]. Several landmark trials investigating the effect of dietary measures to prevent stone recurrence were published after the thiazide trials had been completed [64, 65]. In idiopathic recurrent calcareous stone formers, high fluid intake or a diet restricted in animal protein and salt combined with a normal calcium intake each reduce stone recurrence by ~50% [64, 65]. In fact, the thiazide trials were conducted at a time when recurrent calcareous stone formers were given the advice to limit dietary calcium intake, a long-held opinion that has since been refuted both by observational data and a prospective RCT [65, 66]. Thus, the added benefit of thiazides to current state-of-the non-pharmacologic measures in stone prevention remains unknown.
Imaging modality for radiologic outcomes: Some patients with “recurrent” stones in past thiazide RCTs may have already harbored the stones at baseline and experienced stone passage rather than progression of disease. The latter is also the most likely explanation for the repeated observation made in past RCTs (beyond thiazides) concerned with recurrence prevention of renal stones that stone incidence in treatment and control groups are diverting only after one year of treatment or later, although most patients were considered “stone free” at study initiation, based on KUB or IVU imaging. Although CT is by far the most sensitive and most specific imaging modality for renal stone disease, no thiazide trial (or any other RCT concerned with kidney stone prevention) thus far used this imaging modality to monitor progression of stone disease. As outlined earlier, modern low-dose CT protocols cause greatly reduced radiation exposure while preserving a high sensitivity and specificity, making low-dose CT the kidney stone imaging modality of choice to monitor disease progression.
Thus, in summary, benefits and harms of thiazide treatments in general for the prevention of stone recurrence are still unclear. In addition, the efficacy of the currently employed low dose thiazide regimens to prevent stone recurrence is not known.

3.2 Investigational Product (treatment, device) and Indication

HCTZ belongs to the group of thiazide diuretics. Thiazide diuretics inhibit the sodium/ chloride co-

transporter (NCC or SLC12A3) in the distal tubule of the kidney. Inhibition of NCC causes an increased excretion of sodium, chloride and water in the urine and thereby lowering of blood pressure. At the same time, thiazide diuretics reduce renal calcium excretion by a still ill-defined intrarenal mechanism. In Switzerland, HCTZ as monosubstance is marketed exclusively as Esidrex® by Medius AG, 4132 Muttenz in divisible tablets of 25 mg. The approved indications include: arterial hypertension, edemas, heart failure and recurrence prevention of calcium-containing kidney stones. For the treatment of arterial hypertension, Esidrex® is recommended in doses of 12.5-50 mg, once or twice daily. For the recurrence prevention of calcareous nephrolithiasis, Esidrex® is recommended in doses of 25 or 50 mg twice daily (Esidrex® summary of product characteristics, November 2018). In addition to the monosubstance, HCTZ is currently available in 78 different galenic formulations in Switzerland as combination with ACE-inhibitors, angiotensin II receptor blockers or non-thiazide diuretics (www.swissmedicinfo.ch, last accessed on 11.08.2016).
Detailed information to pharmacokinetics, side effects and interaction profile of HCTZ can be found at www.swissmedicinfo.ch.

3.3 Preclinical Evidence

There is no animal model, which recapitulates the pathophysiology of calcareous nephrolithiasis in humans. Hence, there is no supportive preclinical evidence for this trial. However, there is ample evidence that thiazide diuretics reduce calciuria, a surrogate marker for calcareous nephrolithiasis, in a wide variety of mammals.

3.4 Clinical Evidence to Date

The clinical evidence on the efficiency of HCTZ in recurrence prevention of calcareous nephrolithiasis has been reviewed in detail in section 3.1.

3.5 Dose Rationale: Rationale for the intended purpose in study

Our systematic review of thiazide trials in the prevention of recurrent nephrolithiasis in section 3.1 reveals that the type of thiazide used and the dose regimens employed varied widely. Although the evidence is currently very limited, no thiazide seems to be superior for stone prevention. As outlined earlier, a common theme of all trials was that very high doses of thiazides were employed. Only in one trial more than one dose of thiazide was tested [45]. In that trial, chlorthalidone 25 mg and 50 mg daily were equally effective in preventing the primary outcome stone recurrence compared to placebo. While the reduction in calciuria compared to placebo was also not different between the two dose regimens, 25 mg chlorthalidone daily reduced oxaluria significantly whereas 50 mg chlorthalidone daily did not. In contrast, however, 50 mg chlorthalidone daily caused more frequent and more pronounced hypokalemia than 25 mg chlorthalidone daily. HCTZ is by far the best studied thiazide diuretic for both currently recognized indications, arterial hypertension and recurrent calcareous nephrolithiasis [55]. In addition, HCTZ (once daily) is the thiazide most commonly found in combination drugs prescribed for the treatment of arterial hypertension. Based on these facts, we decided to use HCTZ for the NOSTONE trial. To improve compliance and reduce side effects, we plan to compare 12.5 mg or 25 mg or 50 mg HCTZ once daily to placebo. As is the case for all thiazides, HCTZ has an excellent bioavailability and thus will be given per os.

3.6 Explanation for choice of comparator (or placebo)

Dietary and lifestyle interventions are highly effective in preventing recurrence in calcareous nephrolithiasis and are thus always the basis of stone prevention [63]. As such, any trial assessing pharmacologic interventions for the prevention of stone recurrence must compare the efficacy of the drug treatment to the state-of-the-art non-pharmacologic interventions. On the basis of RCT evidence, current American [31] and European [23] nephrolithiasis guidelines recommend the following non-pharmacologic measures in adult patients with recurrent calcareous nephrolithiasis: increased fluid intake with circadian drinking to ensure daily urinary volumes of at least 2 – 2.5 L, a balanced diet rich in vegetables and fibers with normal calcium content (1-1.2 g/day) but limited NaCl (4-5 g/day) and animal protein (0.8-1 g/kg/day) content. Furthermore, patients must be advised to retain a normal body mass index (BMI), have adequate physical activity and balance excessive fluid loss. All patients in the placebo and treatment arms of our planned study will receive these current non-pharmacologic recommendations for stone prevention. Since the added benefit of thiazides to current state-of-the non-pharmacologic measures in stone prevention remains unknown, as outlined in detail in section 3.1, a placebo arm needs to be part of this trial.

3.7 Risks/ Benefits

Calcareous nephrolithiasis is a disease with high recurrence rates that causes high morbidity in affected individuals with repeated exposures to urological procedures. The risk a patient is willing to accept is a personal decision based on his/ her individual and familial experience with the disease. HCTZ potentially lowers the recurrence risk of the disease, although in the dosages applied in the study it has not yet been approved for this indication. HCTZ has been used for the treatment of arterial hypertension in the dosages tested in this study for over 40 years. Thus, the treatment risks by HCTZ are well characterized, manageable and must be weighed against the consequences of no treatment. The most common AEs of HCTZ therapy include those arising from saluresis including reduction of blood pressure, polyuria, dehydration and electrolyte abnormalities. Therefore, appropriate patient monitoring and management will be implemented to mitigate this potential risk in the kidney stone population. These AEs should be considered in light of the benefits of a reduced risk of stone recurrence, including pain, urinary tract infection and need for urological interventions. With sufficient knowledge of the benefits and risks and risk mitigation strategies, patients and their physicians may make informed decisions about HCTZ treatment. In the final assessment, the overall benefit-risk profile of HCTZ for the recurrence prevention of calcareous nephrolithiasis appears favourable, offering a real opportunity to fill a longstanding unmet medical need.

The results of the study, will yield important clinical information for the care of patients with recurrent calcareous nephrolithiasis, which is one of the most frequent human diseases (~10% of worldwide population affected). Study participants who finished the trial or stopped the IMP during the trial will be seen in the different ambulatory stone clinics of the participating centers at least annually, if medical condition warrants, more frequently.

Because the detection rate of plain abdominal X-ray imaging (KUB radiography) for urinary stones is far inferior to the detection rate of CT or IVU, and because often several KUBs in different angles have to be performed for a better detection of kidney stones, the international guidelines recommend low-dose CT for the detection and follow-up of urinary stone patients [23, 31-33]. Modern low-dose CT protocols cause greatly reduced radiation exposure while preserving a high sensitivity of up to 98% [69] and specificity of up to 97% [34, 35, 69]. Current radiation exposure of low dose CT protocols for the detection of urolithiasis is in the range of 0.5-1.9 mSV, and thus similar to (single) KUB radiography (0.5 – 1.1 mSv) but lower than IVU (1.3 -3.93 mSv) [20, 23, 35, 36, 70]. The international guidelines recommend imaging of recurrent stone formers at least once a year [31]. Thus, yearly imaging with low dose CT is in line with the international guidelines [31] and with our daily practice (usually 6-monthly to yearly imaging). It is a rather conservative imaging strategy compared to other centers, and leads to a relatively low radiation exposure which does not exceed the standard radiation dose for recurrent stone formers during regular follow-up. Even more, the radiation exposure of our patients is far lower than the 50 mSv throughout a single year or the 20 mSv per year over a 5-year period as accepted as the threshold levels for “safe” exposure by the International Commission on Radiological Protection (ICRP) [71].

3.8 Justification of choice of study population

For the trial, thiazide naïve adult individuals with recurrent calcareous nephrolithiasis will be recruited. 80 – 90% of patients with recurrent nephrolithiasis belong into this group. Hence, the study population will be representative of the far most common type of renal stone former encountered in clinical routine. Recurrent is defined as two or more stone episodes in the last 10 calendar years, calcareous is defined as 50% or more calcium oxalate, calcium phosphate or a mixture of both in a previous stone analysis. No vulnerable individuals will be included in the study.

4. STUDY OBJECTIVES

4.1 Overall Objective

The study aims to describe an efficacy and safety profile of HCTZ for the recurrence prevention of calcareous nephrolithiasis.

4.2 Primary Objective

Dose-response relationship for three different dosages of HCTZ using incidence of stone recurrence (a composite of symptomatic or radiologic recurrence) as the primary outcome.

4.3 Secondary Objectives

Efficacy of the different dosages of HCTZ in terms of the primary outcome as well as the individual components of the composite primary outcome, i.e. incidence of symptomatic stone recurrence and incidence of radiologic stone recurrence. Effects of different dosages of HCTZ on urinary biochemistry (efficacy and safety aspects) and the impact of different baseline characteristics on the effects of the different dosages (effect modification).

4.4 Safety Objectives

Long-term safety and tolerability of HCTZ compared to placebo.

5. STUDY OUTCOMES

5.1 Primary Outcome

5.1.1 Primary outcome at patient level

The primary outcome at the patient level is the incidence of stone recurrences during study treatment. Stone recurrence is the composite of symptomatic or radiological recurrences.

Symptomatic stone recurrence is defined as visible passage of a stone with or without accompanying typical symptoms such as flank/ loin pain and hematuria or a symptomatic or asymptomatic stone requiring urological intervention for stone removal. In case of potential stone passages without visible stones, it will be up to the local investigators to evaluate patients' symptoms and determine whether a stone passage occurred or the symptoms were rather due to a different cause.

Baseline stone recurrence includes all stone events that occurred in the 10 calendar years preceding the year of randomization and until randomization. For the report of stone events, if the exact month a stone event occurred is not known, the first day of the month will be conventionally reported as date of the event, if only the calendar year is known, the first of January of the same year will be used as conventional date for the event. Several events occurring at unknown dates within a known period of time will be distributed throughout the period of interest at regular intervals for the purpose of reporting.

Radiological stone recurrence as assessed by low-dose non-intravenous (iv) contrast CT imaging is defined as the appearance of new calculi or enlargement of preexisting calculi with reference to the baseline CT performed at visit 3 to the end of study treatment. The processing and evaluation of the CT images is described in a specific SOP (SOP_CT_V1.3).

Stone events during the first six weeks after randomization will not be considered for analysis.

5.1.2 Primary outcome at study level

Relationship of the incidence of stone recurrences during study treatment and dose group.

5.2 Secondary Outcomes

Secondary outcomes are the following:

- The individual components of the composite primary outcome, i.e. number of symptomatic stone recurrences and number of radiologic stone recurrences
- Changes in urinary biochemistry elicited by HCTZ or placebo
- Impact of baseline disease severity (incidence of stone recurrence during the last 10 calendar years prior to and until randomization) and biochemical abnormalities on stone recurrence
- Impact of stone composition on stone recurrence

Given the high prevalence of the disease [1, 2], the morbidity, pain and costs associated with symptomatic stone disease [5] as well as the high likelihood of asymptomatic stones to become symptomatic [67], our chosen outcomes are clinically highly relevant.

5.3 Other Outcomes of Interest

During one of the scheduled follow up visits, the investigators will collect a DNA sample from the patients. DNA will be stored in the NOSTONE Biobank at the Bern University Hospital (Abteilung für Humangenetik), Switzerland. Patients must sign a separate patient informed consent for the NOSTONE Biobank. The rules and procedures of the NOSTONE biobank are described in a specific booklet (NOSTONE Biobank Reglement).

5.4 Safety Outcomes

Safety endpoints to be analyzed include a descriptive summary of the following parameters:

- SAEs
- Pre-specified AEs of special interest (defined in section 9.2.4.2)
- Vital signs

6. STUDY DESIGN

6.1 General study design and justification of design

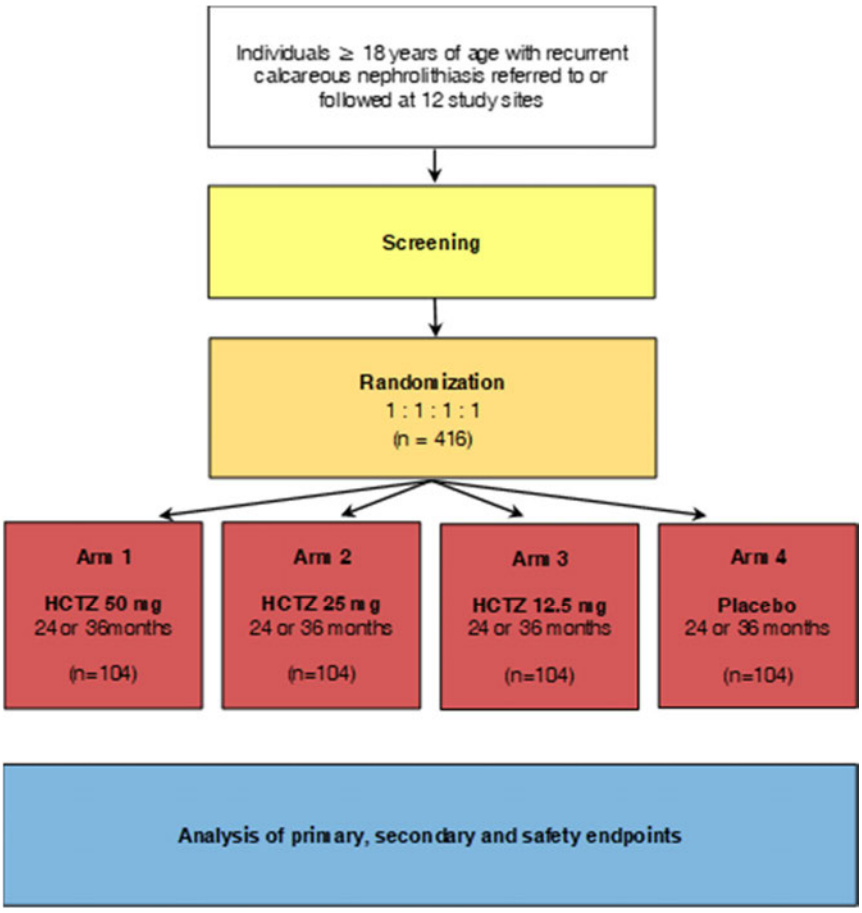

Figure 6.1-1: NOSTONE trial design schematic

This is a multicenter, randomized, placebo-controlled, observer-, clinician- and patient-blinded, parallel-group study to compare the efficacy and safety of HCTZ in the recurrence prevention of calcareous nephrolithiasis.
Eligible patients will be randomized in equal proportions between 12.5 mg, 25 mg or 50 mg HCTZ or placebo. All subjects will be given the IMP (HCTZ or placebo) once daily in the morning. Placebo will be administered to subjects randomized to that treatment in a form identical to the HCTZ capsules. The first dose of the IMP will be administered the day after the randomization.
A total of 416 participants, 104 in each group, will be included in the study. Recruitment of participants is planned to occur over a period of 30 months at all 12 study sites. Recruitment in Bern will be extended for another 2 months. Study treatment will be 36 months for subjects enrolled in the first 12 months of the recruitment period and for a minimum of 24 months and maximum of 36 months for subjects recruited in the remainder of the recruitment period.

6.2 Methods of minimizing bias

6.2.1 Randomization

We will use stratified randomization to assign participants to the different trial arms with the number of previous stone events as stratification factors: stratification group 1: 2 or 3 stone events within 10 calendar years prior to randomization; stratification group 2: $\geq$ 4 stone events within 10 calendar years prior to randomization. Allocation will be concealed using sequentially coded drug packs that are otherwise identical. Preparation and handling of the unblinded drug packs will be done at a facility otherwise not involved in the trial. Randomization lists at CTU Bern will be stored electronically with no access for persons directly involved in the trial. Allocation to the trial arms will be done using numbered drug packs. Randomization lists will be generated by a statistician at CTU Bern otherwise not involved in the trial following dedicated standard operating procedures. The statistician will communicate directly with the facility, which prepares the drug packs (Grosse Apotheke Dr. G. Bichsel AG, Interlaken, Switzerland). Investigators at each site will assign the drug pack with the next sequential number to the next patient (consecutive). Content of the drug packs is based on randomization lists as described above.

6.2.2 Blinding procedures

All trial personnel but the statistician generating the list and the personnel at the facility preparing the drug packs (Grosse Apotheke Dr. G. Bichsel AG, Interlaken, Switzerland) will be blinded to the assigned treatment. Blinding will be upheld until all analyses will have been completed. HCTZ and placebo will be provided in identically looking blistered capsules in drug packs. Besides the consecutive number, packs and pack content will look identical. Therefore, all trial personnel that is involved in recruitment and care of patients, trial assessment, monitoring and analyses will be blinded to the assigned trial arm.
Blinding will remain in place until the statistician codes the primary analysis of the primary and secondary outcomes and produces a dummy report of the primary analysis using a randomly generated group variable. The true group variable becomes open after the completion of the dummy report and gives place to the final report of all the analysis as well as the quality control by the independent statistician.

6.2.3 Other methods of minimising bias

To minimize referral bias, we plan to collaborate with external Nephrologists, Urologists, Internists and Family practitioners.
To minimize bias of primary outcome assessment for radiological recurrence, all renal CTs will be assessed centrally at Inselspital Bern.

6.3 Unblinding Procedures (Code break)

HCTZ is an established drug with a well-known safety profile. Although extremely rare, there are reports of allergic reactions either directly or because of sulfonamide cross-sensitivity. Unblinding will only be allowed in situations where knowledge of the allocation is needed for the care of a patient. Because there is no antidote and the allergic reactions as described in the literature as well as other serious adverse reactions such as aplastic anemia or angioedema are treated independently from any knowledge of treatment assignment, we do not expect any emergency unblinding. Note that a break of the randomization code per se is not a reason to stop study treatment or to withdraw the participant from

the study.
Unblinding will be managed centrally for all the centers at the pharmacy of the Inselspital. Dedicated instructions will be provided to the centers.

7. STUDY POPULATION

This trial will include adult individuals with recurrent calcareous nephrolithiasis. Outpatients newly referred to stone clinics for metabolic stone work-up will be recruited for the trial at 7 Cantonal Hospitals (Aarau, Bellinzona, Chur, Lugano, Lucerne, Sion, St. Gallen) and 5 University Hospitals (Basel, Bern, Geneva, Lausanne, Zurich) in Switzerland. If enrolment goals are not met, we will 1) try to enhance referrals to trial sites by contacting local Urologists and Nephrologists 2) extend recruitment to outpatients with recurrent calcareous nephrolithiasis that have already undergone metabolic work-up for stone disease but are seen for regular follow-up visits at the trial sites.
In order to maximize power and minimize the possibility of a Type II error, trial enrollment will continue until at least 416 individuals are randomized.

7.1 Eligibility criteria

Individuals fulfilling all of the following inclusion criteria are eligible for the study:

1. Informed Consent as documented by signature
2. Age 18 years or older
3. Recurrent kidney stone disease ($\geq$ 2 stone events within the last 10 calendar years prior to randomization, i.e. during the 10 calendar years preceding the year of randomization and until randomization)
4. Any past kidney stone containing 50% or more of calcium oxalate, calcium phosphate or a mixture of both

The presence of any one of the following exclusion criteria will lead to exclusion of the individual:

1. Pharmacologic prevention for stone recurrence less than 3 months prior to randomization
2. Patients with secondary causes of recurrent calcareous nephrolithiasis including:
   - Severe eating disorders (anorexia or bulimia)
   - Chronic inflammatory bowel disease, bariatric surgery, intestinal surgery with malabsorbtion or chronic diarrheal status
   - Sarcoidosis
   - Primary hyperparathyroidism
   - Complete distal tubular acidosis
   - Active malignancy
3. Patients with the following medications:
   - Thiazide or loop diuretics
   - Carbonic anhydrase inhibitors (including topiramate)
   - Xanthine oxidase inhibitors (febuxostat or allopurinol)
   - Alkali, including potassium citrate or sodium bicarbonate
   - Treatment with 1,25-OH Vitamin D (calcitriol)
   - Calcium supplementation
   - Bisphosphonates
   - Denusomab
   - Teriparatide
   - Glucocorticoids
4. Obstructive uropathy, if not treated successfully
5. Urinary tract infection, if not treated successfully
6. Chronic kidney disease (defined as CKD-EPI eGFR < 30 mL/min per 1,73 m^2 body surface area for more than 3 months)
7. Patients with a kidney transplant
8. > 3 gout arthritis episodes within one year prior to randomization or gout arthritis requiring uric acid lowering therapy
9. Cystinuria at screening
10. Hypokalemia (blood potassium level < 3 mmol/L) at screening

11. Hyponatremia (blood sodium level < 125 mmol/L) at screening
12. Pregnant and lactating women [pregnancy test to be performed for women of child-bearing potential (defined as women who are not surgically sterilized/ hysterectomized, and/ or who are postmenopausal for less than 12 months)]
13. Previous (within 3 months prior to randomization) or concomitant participation in another interventional clinical trial
14. Inability to understand and follow the protocol
15. Known allergy to the study drug

7.2 Recruitment and screening

Target population of the study are individuals who suffer from recurrent calcareous nephrolithiasis. Two different groups of individuals will be screened and recruited in the study by local investigators: 1) individuals newly referred for metabolic work-up for recurrent stone disease to study sites and 2) individuals who already underwent work-up for recurrent stone disease and are currently undergoing regular follow-up for recurrent stone disease at the study sites. After approval by the CEC and CA, the study will be announced to the Nephrology and Urology staff at the 12 study sites. We will also inform external Nephrologists, Urologists, Internists and Family practitioners by emails and kick-off meetings upon approval by the CEC and CA.
If available medical history indicates that an individual may be eligible for study participation, the individual will be informed in detail about the study by local investigators. Inclusion in the study will take place only upon receipt of written informed consent and if all eligibility criteria are fulfilled.
No payment or compensation will be given to study participants.

7.3 Assignment to study groups

Stratified randomization will be used to assign participants to the different trial arms with the number of previous stone events as stratification factors (see section 6.2.1). Allocation will be concealed using sequentially coded drug packs that are otherwise identical. Randomization lists will be generated by a statistician at CTU Bern otherwise not involved in the trial following dedicated standard operating procedures. The statistician will communicate directly with the facility, which prepares the drug packs (Grosse Apotheke Dr. G. Bichsel AG, Interlaken, Switzerland). Investigators at each site will assign the drug pack with the next sequential number to the next patient (consecutive). Content of the drug packs is based on randomization lists as described above. Preparation and handling of the unblinded drug packs will be done at a facility otherwise not involved in the trial. Randomization lists at CTU Bern will be stored electronically with no access for persons directly involved in the trial.

7.4 Criteria for withdrawal/ discontinuation of participants

7.4.1 Discontinuation of study IMP

Study IMP must be permanently discontinued if any of the following occurs:
- If any exclusion criterion applies during the trial, except the incompatible medications. The IMP will be discontinued only if the patient took the medications listed in the exclusion criteria for more than 4 months*
- If the responsible study investigator feels that treatment with the study regimen is harmful to the participant's well-being
- If patient is non-compliant with the study intervention as judged by the investigator and/ or the sponsor
- Pregnancy in a study participant
- Hypokalemia (blood potassium level < 3 mmol/L) not responsive to supplementation therapy
- Profound hyponatremia (blood sodium level < 125 mmol/L) recurring after normalization upon temporary suspension of IMP
- Gouty arthritis recurring > 3 times per year or requiring uric acid lowering therapy.
- Allergic reaction of skin as judged by the investigator
- > 3 recurrences of symptomatic stone events during the trial

*The patients are either contacted via phone or visited every 3 months (+/- 1 month). If a patient is taking a medication from the prohibited list, the prescription will be discussed with the family practitioner (Hausarzt) and it will be evaluated if it is possible to stop the prohibited medication or if the IMP needs to be suspended.

Participants who permanently discontinue the IMP are expected to continue in the follow-up period and to attend all protocol-specified study visits. If study visits are not possible, a telephone consultation will be performed to determine if relevant health events/ endpoints have occurred.

7.4.2 Discontinuation of study

Study participants must be withdrawn from the study if the following occurs:
- At the participants own request
- If, in the investigator's opinion, continuation of the study would be harmful to the subject's well-being

A study participant who discontinues study participation prematurely for any reason is defined as dropout if the participant has already been randomized. A study participant who terminates the study before randomization is regarded as a screening failure.

No replacement of participants discontinuing study treatment or study participation is foreseen.

Any samples and data collected until study withdrawal will remain coded for the analysis. It is not possible to anonymize the data and samples upon withdrawal.

8. STUDY INTERVENTION

8.1 Identity of Investigational Products

8.1.1 Experimental Intervention

Identical looking gelatin capsules containing 12.5 mg or 25 mg or 50 mg HCTZ will be supplied by Grosse Apotheke Dr. G. Bichsel AG, Interlaken, Switzerland according to applicable regulations.

8.1.2 Control Intervention

Placebo gelatin capsules indistinguishable from HCTZ capsules will be supplied by Grosse Apotheke Dr. G. Bichsel AG, Interlaken, Switzerland according to applicable regulations.

8.1.3 Packaging, Labelling and Supply (re-supply)

IMP capsules will be provided as bulk supply in bottles labelled with trial-specific labels according to Annex 13, "Manufacturing of investigational medicinal products" to Volume IV of the EU guideline to Good Manufacturing Practice:

- Name, address and telephone number of the sponsor-investigator
- Pharmaceutical dosage form (capsules), route of administration (per os), number of capsules
- Batch number, Lot number
- Trial acronym (Nostone-trial)
- Trial subject identification number
- Storage conditions
- Expiry date
- Safety warning “Keep out of reach of children”

8.1.4 Storage Conditions

All IMPs will be stored in a securely locked cabinet or enclosure. Access will be limited to investigators and their designees. Neither investigators nor any designees may provide IMP to any subject not participating in this protocol. The IMP will be stored according to the conditions specified in the IMP label. The clinical site staff will maintain a temperature log in the drug storage area recording the temperature using (at minimum) a Min/ Max thermometer at least weekly.

8.2 Administration of experimental and control interventions

8.2.1 Experimental Intervention

HCTZ capsules will be administered once daily per os in the morning. HCTZ has excellent bioavailability and thus per os administration is chosen for this trial (www.swissmedicinfo.ch). The rationale for AM dosing is the fact that HCTZ induces a diuresis that starts 1-2 hours after ingestion, peaks 4-6 hours after ingestion and lasts until 10-12 hours after ingestion (www.swissmedicinfo.ch). Thus, HCTZ-induced diuresis potentially disturbs sleep of participants when PM dosing is chosen. Minimal effective dose for HCTZ-induced diuresis and blood pressure reduction in humans is 12.5 mg per os once daily, maximal effective dose 50 mg per os once daily (www.swissmedicinfo.ch). Hence, 12,5 mg, 25 mg and 50 mg per os once daily were chosen as HCTZ doses for this trial.

8.2.2 Control Intervention

Placebo capsules will be administered once daily per os in the morning, identical to HCTZ capsules.

8.3 Dose modifications

8.3.1 Electrolyte Disturbances

Hypokalemia, hyponatremia and hypomagnesemia can occur in patients with HCTZ treatment. In the case of hypokalemia (< 3 mmol/L), supplementation with oral potassium chloride or co-administration of the potassium-sparing diuretic is recommended. In the case of hypomagnesemia (< 0.5 mmol/L), supplementation with oral magnesium aspartate is recommended. Potassium or magnesium citrate or bicarbonate salts may affect stone recurrence and are not allowed during the trial. If hypokalemia or hypomagnesemia are not responsive to the above recommendations, the IMP will be withdrawn from the patient. In the case of profound hyponatremia (< 125 mmol/L), temporary suspension of the IMP until normalization of the blood sodium concentration is recommended. The IMP can then be restarted. If profound hyponatremia recurs, the IMP will be withdrawn from the patient.

8.3.2 Renal function impairment

Because of blood pressure lowering and volume depletion, therapy with HCTZ may worsen renal function. The IMP will be withdrawn if CKD-EPI eGFR drops below 30 mL/min per 1,73 m^2 body surface area for more than 3 months.

8.3.3 Gout arthritis

HCTZ may increase blood uric acid levels which can cause gout arthritis. An acute gout flare should be treated symptomatically with analgesics. Xanthine oxidase inhibitors to lower blood and urine uric acid levels may affect stone recurrence and are not allowed during the trial. If gouty arthritis recurs > 3 times/ year or a uric acid lowering therapy is needed, the IMP will be withdrawn from the patient.

8.3.4 Diabetes

HCTZ may worsen glucose tolerance or induce diabetes mellitus. If overt diabetes mellitus develops during the trial (fasting glucose ≥ 7 mmol/L, random glucose ≥ 11 mmol/L or hemoglobin A1c ≥ 6.5%), the patient will be asked to see his general practitioner to initiate appropriate treatment.

8.3.5 Allergic reactions

Occasionally allergic reactions of the skin have been observed in patients with HCTZ treatment. If this is suspected, the IMP will be withdrawn from the patient.

8.3.6 Symptomatic stone recurrence during the trial

Patients will remain on study treatment with up to three symptomatic stone events. If a fourth symptomatic stone event occurs during the trial, the IMP will be withdrawn from the patient.

For additional criteria for discontinuing study IMP treatment or study discontinuation see section 7.4.

8.4 Compliance with study intervention

At initial product dispensing and the follow-up visits, including the three-monthly telephone contacts, the participants will be reminded about the following:

- The importance of following the study guidelines, including the non-pharmaceutical preventive

measures and taking the study product once daily
- Instructions about IMP administration including dose, importance of taking the IMP as a whole, timing, and noting done any missed dose, and instructions about proper IMP storage
- Notification that there will be IMP counts performed during the trial
- To keep all medication containers, including any unused capsules and to return them to the study site at the next scheduled on-site visit
- That IMP may be HCTZ or placebo
- Importance of calling the clinic if experiencing problems possibly related to the study product such as symptoms or lost IMP

At the follow-up visits and three-monthly telephone calls, the participants will be asked about any problems with regard to taking the IMP using a structured and open-ended questionnaire. There will be a discussion of reasons for missed doses and simple strategies for enhancing adherence, e.g. linking IMP taking to meals or other daily activities. Participants will have an opportunity to ask questions and key messages from the initial session will be reviewed as needed.

Participants will be asked to return used, partially used and unused IMP containers at each follow-up visit at the study sites.
Depending on the circumstances leading to non-compliance, the subject may be discontinued from IMP administration by the investigator and/ or sponsor. A subject who proactively wishes to discontinue IMP administration, or has IMP discontinued by the investigator, will be encouraged to continue limited participation in the trial, as described in section 7.4.

8.5 Data Collection and Follow-up for withdrawn participants

Participants who permanently discontinue the study treatment are expected to continue in the follow-up period and to attend all protocol-specified study visits. If study visits are not possible, a telephone consultation will be performed to determine if relevant health events/ endpoints have occurred.
Participants who are withdrawn from further study participation are invited to attend a final visit at which the assessments of the End of Treatment visit are performed.

8.6 Trial-specific preventive measures

Dietary and lifestyle interventions are highly effective in preventing recurrence in calcareous nephrolithiasis and are the basis of stone prevention [63]. Any trial assessing pharmacologic interventions for the prevention of stone recurrence must compare the efficacy of the drug treatment to the state-of-the-art non-pharmacologic interventions. Thus, all patients will receive state-of-the-art non-pharmacologic recommendations for stone prevention according to current American [31] and European [23] nephrolithiasis guidelines including: increased fluid intake with circadian drinking to ensure daily urinary volumes of at least 2 – 2.5 L, a balanced diet rich in vegetables and fibers with normal calcium content (1-1.2 g/day) but limited NaCl (4-6 g/day) and animal protein (0.8-1 g/kg/day) content. Furthermore, patients must be advised to retain a normal BMI, have adequate physical activity and balance excessive fluid loss.

The following medications will be prohibited during the trial (reason given in brackets)*:
- Thiazide diuretics (pharmacodynamic interference with IMP)
- Loop diuretics (pharmacodynamics interference with IMP)
- Carbonic anhydrase inhibitors (increase risk for stone recurrence)
- Xanthine oxidase inhibitors (potentially decrease risk of stone recurrence)
- Alkali, including potassium citrate or sodium bicarbonate (potentially decrease risk of stone recurrence)
- Treatment with 1,25-OH Vitamin D (increases risk of stone recurrence)
- Calcium supplementation (increases risk of stone recurrence)
- Bisphosphonates (potentially decreases risk of stone recurrence)
- Denusomab (potentially decreases risk of stone recurrence)
- Teriparatide (increases risk of stone recurrence)
- Glucocorticoids (increases risk of stone recurrence)

*a grace period of maximal 4 months will be tolerated (see section 7.4.1)

8.7 Concomitant Interventions (treatments)

Active recurrence prevention for calcareous nephrolithiasis with thiazide diuretics, alkali or xanthine oxidase inhibitors will have to be stopped at least 3 months before an individual can be enrolled (randomized) in the study. All medications not listed in section 8.6 will be allowed before and during the study as concomitant treatments. The use of concomitant medications will be recorded in the eCRF. There are no restrictions to medications or treatments after the study.
All urological interventions related to symptomatic and asymptomatic stone disease are permitted during the trial. Generally, it is recommended to follow patients with asymptomatic stones and wait until stones become symptomatic. However, the likelihood for the eventual need of an urological intervention depends on the stone diameter. Ureteral stones with a diameter > 7 mm have a likelihood of 99% to require a urological intervention in the future [68]. Thus, kidney stones with a diameter > 7 mm are often removed, even if asymptomatic. In patients with a high risk of complications, including those with a solitary kidney or in patients with certain professions (e.g. pilots), even elective removal of asymptomatic stones with a diameter $\leq$ 7 mm may be indicated. Interventional treatment of asymptomatic stones of patients enrolled in the trial will be left to the discretion of the treating urologists. Removal of an asymptomatic stone will be considered as symptomatic stone event in this trial.

8.8 Study Drug Accountability

The investigator or designee must maintain an inventory record of IMP at the site level (received from the sponsor, returned to the sponsor) and at the patient level (dispensed to the patient, returned by the patient).

8.9 Return or Destruction of Study Drug/ Medical Device

Upon completion or termination of the trial, all unused IMP must be destructed on site following the site's standard procedures for medication destruction and the destruction documented. Documentation on IMP destruction must be provided to the sponsor-investigator for filing in the trial master file.

9. STUDY ASSESSMENTS

9.1 Study flow chart(s)/ table of study procedures and assessments

For work-up and follow-up of participants, the NOSTONE protocol strictly adheres to recommendations of the American and European guidelines on nephrolithiasis [23, 31] with regard to scheduling of patient visits, lab analyses and imaging.
For the schedule of assessments refer to "Study Schedule" table at the beginning of this document.

9.2 Assessments of outcomes

In accordance with recommendations of the American and European guidelines on nephrolithiasis [23, 31], study participants undergoing first-time work-up for stone disease will be scheduled for Visit 4 (V4), unless they are unable to attend the visit in person. Participants with past work-up for stone disease that are currently undergoing regular follow-up will omit V4, all other Visits (including numbering of Visits) are identical to participants with first-time work-up for stone disease.

9.2.1 Assessment of primary outcome

Symptomatic recurrence (for definition see section 5.1.1) will be assessed at the follow-up visits 3, 4, 5 and 6 as well as at the three-monthly phone calls in between the visits.
Radiologic recurrence (for definition see section 5.1.1) will be assessed by low-dose, renal-limited non-iv contrast CT imaging at visit 3 (defined as reference for stone recurrence during the study), if a stone recurrence is suspected during the treatment phase (standard procedure in symptomatic patients irrespective of the study), and at treatment end (visit 7 for participants recruited in the first 12 months of the recruitment period and visit 6 for participants recruited in the remainder of the recruitment period). Stone events occurring during the first 6 weeks after randomization will not be considered as events for the analysis. At treatment end, a renal CT is performed only if no other non-iv contrast renal CT was performed within 3 months prior to the visit.

9.2.2 Assessment of secondary outcomes

Individual components of the composite primary outcome, i.e. incidence of symptomatic stone recurrence and incidence of radiologic stone recurrence will be assessed as described for the primary outcome. Changes in urinary biochemistry elicited by the IMP compared to baseline (visit 2) will be assessed by chemical analysis of urine samples collected immediately prior to visits 4, 5, 6 and 7 (one 24 hour collection immediately prior to visit 4; two 24 hours collections within 48 hours immediately prior to visits 5, 6 and 7). Impact of baseline disease severity and biochemical abnormalities on stone recurrence will be assessed by baseline blood and urine analysis and baseline medical history and assessment of the primary outcome as described. If the collection of 48 hour urines is difficult for the patient, a single, 24 hour urine collection (with added paraffin/ thymol) can be taken into consideration. Chemical blood and urine analysis are only performed at visits 4, 5, 6 and 7 with the explicit (unwritten) approval of the patient. If these visits are replaced by a phone call, blood and urine analysis is not performed. Impact of stone composition on stone recurrence will be assessed by baseline medical history and assessment of the primary outcome as described.
Given the average stability over time of urine and blood composition in this patients population, the time windows outlined in “Study schedule 1” and “Study schedule 2” (pages 11-14) are meant to be indicative, not restrictive.

9.2.3 Assessment of other outcomes of interest

Genetic data generated from the DNA stored in the biobank will be used in different biomedical projects (e.g genetic markers vs urine/blood analysis; genetic markers vs stone incidence; genetic markers vs. treatment response).

9.2.4 Assessment of safety outcomes

9.2.4.1 Serious adverse events (SAEs)

SAEs will be collected, fully investigated and documented in the source documents and the eCRF for all participants from the date of ICF signature until the last protocol-specific procedure has been completed, including a safety follow-up period of 30 days.

9.2.4.2 Adverse events (AEs) of special interest

For this trial, (non-serious) AEs will not be documented in the eCRF with exception of the following events “of special interest” occurring from the date of randomizatzion (visit 3) until visit 7:

- Hypokalemia, defined as blood potassium level < 3 mmol/L
- Hyponatremia, defined as blood sodium level < 125 mmol/L
- Hypomagnesemia, defined as blood magnesium level < 0.5 mmol/L
- Gouty arthritis if recurrence > 3 times per year or requiring Uric acid lowering therapy
- Newly developed overt diabetes mellitus (defined as fasting glucose level $\geq$ 7 mmol/L or random Glucose $\geq$ 11 mmol/L or hemoglobin A1c $\geq$ 6.5%)
- Allergic reaction of skin if considered by the local investigator to be potentially related to the study medication

9.2.4.3 Vital signs

Heart rate, systolic and diastolic blood pressure at the right arm in sitting position after at least 5 minutes rest will be recorded at visits 1, 4, 5, 6 and 7.

9.2.5 Assessments in participants who prematurely stop the study

Participants who permanently discontinue the study treatment will undergo all protocol-specified study visits and assessments. If study visits are not possible, a telephone consultation will be performed to determine if relevant health events/ endpoints have occurred.

9.3 Procedures at each visit

9.3.1 Visit or phone call 1 (screening day 1*; -1month to day -14)

- Patient information and delivery of written study information to subject
- Pre-assessment of eligibility (based on data available per routine)
- Demographics
- Medical history
- Stone composition (all stones analyzed prior to randomization as available)
- General physical examination, including body weight, height

- Vital signs (heart rate, systolic and diastolic blood pressure at the right arm after at least 5 minutes at rest)
- Concomitant medication

* For participants in current follow-up for past stone disease, all assessments of visit 1 are performed at visit 2. For participants with first time work-up for kidney stone disease (new referrals), screening assessments should be performed at visits 1 and 2, unless the distribution over 2 visits is impracticable, in which case the same procedure as for participants in current follow-up applies.

9.3.2 Visit 2 (screening day 2; -14 days to -1 day; window ±2 weeks)

- Written informed consent (if not obtained earlier). Note: In order to give subjects in current follow-up for past stone disease sufficient time for the decision on study participation, initial patient information may be done via phone and the written study information provided to the subject via normal post prior to the visit; same for new referrals if they are unable to come extra for Visit 1
- Assessment of eligibility
- Blood analysis (analyzed centrally): sodium, potassium, chloride, calcium, magnesium, inorganic phosphate, creatinine, urea, uric acid, bicarbonate, PTH, 25-OH Vitamin D, 1,25-OH Vitamin D, glucose (fasting), hemoglobin A1c, cholesterol, alkaline phosphatase)
- Urine analysis (two 24 hour collections in the 48 hours immediately prior to the visit or one 24 hour collection in case of impediment; analyzed centrally): sodium, potassium, chloride, calcium, magnesium, inorganic phosphate, creatinine, urea, uric acid, protein, albumin, oxalate, citrate, ammonium, pH, volume
- Pregnancy test (performed locally, from blood or urine) for women of child-bearing potential (defined as women who are not surgically sterilized/ hysterectomized, and/ or who are postmenopausal for less than 12 months)
- Assessment of SAEs
- Concomitant medication

9.3.3 Visit 3 (day 0)

- Re-assessment of eligibility
- Randomization
- Baseline low-dose non-iv contrast renal CT (analyzed centrally; if a routine non-iv contrast renal CT was performed within 2 months prior to this visit, the CT does not have to be repeated)
- Collection of information on composition of any new kidney stone since last visit
- Instruction of participant on non-pharmacologic recommendations for stone prevention
- Dispense of IMP (first intake of IMP on the day after visit 3)
- Assessment of SAEs
- Concomitant medication
- Collection of an additional 2.0 ml of EDTA blood for the NOSTONE biobank.

9.3.4 Visit or phone call 4 (+3 months, visit window ±2 weeks)

Participants with first-time work-up of stone disease:

- General physical examination, including body weight
- Vital signs (heart rate, systolic and diastolic blood pressure at the right arm after at least 5 minutes at rest)
- Collection of information on composition of any new kidney stone since last visit
- Blood analysis (analyzed centrally): sodium, potassium, chloride, calcium, magnesium, inorganic phosphate, creatinine, urea, uric acid, bicarbonate, PTH, 25-OH Vitamin D, 1,25-OH Vitamin D, glucose (fasting), hemoglobin A1c, cholesterol, alkaline phosphatase)
- Urine analysis (one 24 hour collection (with added paraffin/ thymol) in the 48 hours immediately prior to the visit; analyzed centrally): sodium, potassium, chloride, calcium, magnesium, inorganic phosphate, creatinine, urea, uric acid, protein, albumin, oxalate, citrate, ammonium, pH, volume
- Assessment of symptomatic stone recurrence
- Compliance reminder (intake of IMP and adherence to non-pharmacologic recommendations for stone prevention)
- Screening for criteria for interrupting or discontinuing study intervention
- Assessment of SAEs and AEs of special interest
- Concomitant medication
- If not done before, collection of an additional 2.0 ml of EDTA blood for the NOSTONE biobank.

Participants in current follow-up for past kidney stone disease or participants with first-time work-up of stone disease that are unable to come to Visit 4:

- Telephone call to ask participant about possible trial-related problems (including any SAEs since last visit and regarding the potential occurrence of events of special interest as far as known to the participant) and answer any questions from participant
- Assessment of symptomatic stone recurrence
- Compliance reminder (intake of IMP and adherence to non-pharmacologic recommendations for stone prevention)

9.3.5 In between Visits 4 and 5 (+6 and +9 months, visit window ±4 weeks)

- Telephone call to ask participant about possible trial-related problems (including any SAEs since last contact and regarding the potential occurrence of AEs of special interest as far as known to the participant) and answer any questions from participant
- Assessment of symptomatic stone recurrence
- Compliance reminder (intake of IMP and adherence to non-pharmacologic recommendations for stone prevention)
- Dispense of IMP via normal post

9.3.6 Visit or phone call 5 (+12 months, visit window ±4 weeks)

- General physical examination, including body weight
- Vital signs (heart rate, systolic and diastolic blood pressure at the right arm after at least 5 minutes at rest)
- Blood analysis (analyzed centrally): sodium, potassium, chloride, calcium, magnesium, inorganic phosphate, creatinine, urea, uric acid, bicarbonate, PTH, 25-OH Vitamin D, 1,25-OH Vitamin D, glucose (fasting), hemoglobin A1c, cholesterol, alkaline phosphatase)
- Urine analysis (two 24 hour collections in the 48 hours immediately prior to the visit or one 24 hour collection (with added paraffin/ thymol) in case of impediment; analyzed centrally): sodium, potassium, chloride, calcium, magnesium, inorganic phosphate, creatinine, urea, uric acid, protein, albumin, oxalate, citrate, ammonium, pH, volume
- Assessment of symptomatic stone recurrence
- Compliance reminder (intake of IMP and adherence to non-pharmacologic recommendations for stone prevention)
- Screening for criteria for interrupting or discontinuing study intervention
- Assessment of SAEs and AEs of special interest
- Concomitant medication
- Dispense of IMP and collection of used/ unused IMP packs from participant

9.3.7 If not done before, collection of an additional 2.0 ml of EDTA blood for the NOSTONE biobank. In between Visits 5 and 6 (+15, +18 and +21 months, visit window ±4 weeks)

- Telephone call to ask participant about possible trial-related problems (including any SAEs since last contact and regarding the potential occurrence of AEs of special interest as far as known to the participant) and answer any questions from participant
- Assessment of symptomatic stone recurrence
- Compliance reminder (intake of IMP and adherence to non-pharmacologic recommendations for stone prevention)

9.3.8 Visit or phone call 6 (+24 months, visit window ±4 weeks)

Participants recruited ≥ 12 months after start of the recruitment period:
Patients recruited ≥ 12 months after start can stay in the trial up to 36 months same as for the patients enrolled during the first 12 months. They will follow the regular schedule as the participants enrolled during the first 12 months. However, the end of treatment visit (V7) is flexible and will take place between the months 24 and 36.

- Vital signs (heart rate, systolic and diastolic blood pressure at the right arm after at least 5 minutes at rest)
- Blood analysis (analyzed centrally): sodium, potassium, chloride, calcium, magnesium, inorganic phosphate, creatinine, urea, uric acid, bicarbonate, PTH, 25-OH Vitamin D, 1,25-OH Vitamin D, glucose (fasting), hemoglobin A1c, cholesterol, alkaline phosphatase)
- Urine analysis (two 24 hour collections in the 48 hours immediately prior to the visit or one 24 hour

collection (with added paraffin/ thymol) in case of impediment; analyzed centrally): sodium, potassium, chloride, calcium, magnesium, inorganic phosphate, creatinine, urea, uric acid, protein, albumin, oxalate, citrate, ammonium, pH, volume
- Assessment of symptomatic stone recurrence
- Compliance reminder (intake of IMP and adherence to non-pharmacologic recommendations for stone prevention)
- Screening for criteria for interrupting or discontinuing study intervention
- Assessment of SAEs and AEs of special interest
- Concomitant medication
- Dispense of IMP and collection of used/ unused IMP packs from participant
- If not done before, collection of an additional 2.0 ml of EDTA blood for the NOSTONE biobank. For participants that terminate their treatment period with visit 6 ("Study schedule 2", p.13-14): if this visit is replaced by a phone call, the patient must receive an appointment for the CT scan anyway (unless this has been performed within 2 months prior to this visit), and the stone assessment must be performed by phone.

Participants recruited ≥ 12 months of the recruitment period that stop treatment after 24 months:
- Low-dose non-iv contrast renal CT (analyzed centrally; if a routine non-iv contrast renal CT was performed within 2 months prior to this visit, the CT does not have to be repeated)

9.3.9 In between Visits 6 and 7 (+27, +30 and +33 months, visit window ±4 weeks)

- Telephone call to ask participant about possible trial-related problems (including any SAEs since last contact and regarding the potential occurrence of AEs of special interest as far as known to the participant) and answer any questions from participant
- Assessment of symptomatic stone recurrence
- Compliance reminder (intake of IMP and adherence to non-pharmacologic recommendations for stone prevention)

9.3.10 Visit or phone call 7 (+36 months, visit window ±4 weeks)

- General physical examination, including body weight
- Vital signs (heart rate, systolic and diastolic blood pressure at the right arm after at least 5 minutes at rest)
- Blood analysis (analyzed centrally): sodium, potassium, chloride, calcium, magnesium, inorganic phosphate, creatinine, urea, uric acid, bicarbonate, PTH, 25-OH Vitamin D, 1,25-OH Vitamin D, glucose (fasting), hemoglobin A1c, cholesterol, alkaline phosphatase)
- Urine analysis (two 24 hour collections in the 48 hours immediately prior to the visit or one 24 hour collection (with added paraffin/ thymol) in case of impediment; analyzed centrally): sodium, potassium, chloride, calcium, magnesium, inorganic phosphate, creatinine, urea, uric acid, protein, albumin, oxalate, citrate, ammonium, pH, volume
- Low-dose non-iv contrast renal CT (analyzed centrally; if a routine non-iv contrast renal CT was performed within 2 months prior to this visit, the CT does not have to be repeated)
- Assessment of symptomatic stone recurrence
- Assessment of SAEs and AEs of special interest
- Concomitant medication
- Collection of used/ unused IMP packs from participant
- If not done before, collection of an additional 2.0 ml of EDTA blood for the NOSTONE biobank.
- If this visit is replaced by a phone call, the patient must receive an appointment for the CT scan anyway (unless this has been performed within 2 months prior to this visit), and the stone assessment must be performed by phone.

9.3.11 Safety follow-up (30 days after end of treatment, visit window ±2 weeks)
- Assessment of SAEs (telephone call)

9.3.12 Study-specific procedures
The following procedures are study-specific:
- Pregnancy test for women of child-bearing potential at visit 2

- Baseline low-dose non-iv contrast renal CT at visit 3
-
- Telephone call at visit 4 for patients in routine follow-up for past kidney stone disease or phone call at visit 4 to newly referred patients that according to usual routine procedures should have come for a visit but were not able to
- 3-monthly telephone calls in between on-site visits and telephone call at 30 days after end of treatment
- Administration of IMP
- Collection of blood sample for the NOSTONE biobank

All other procedures are part of the routine assessments in kidney stone patients.

10. SAFETY

During the entire duration of the study, all SAEs are collected, fully investigated and documented in source documents and eCRF. Study duration encompassed the time from when the participant signs the informed consent until the last protocol-specific procedure has been completed, including a safety follow-up period of 30 days.
Non-serious AEs are not collected with the exception of AEs of special interest (see section 9.2.4.2) occurring from the time point of randomization until the end of treatment visit.

10.1 Definition and assessment of (serious) adverse events and other safety related events

An **adverse event (AE)** is any untoward medical occurrence in a patient or a clinical investigation participant who administered a pharmaceutical product and which does not necessarily have a causal relationship with the study procedure. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product. [ICH E6 1.2]

A **serious adverse event (SAE)** is classified as any untoward medical occurrence that:

- results in death,
- is life-threatening,
- requires in-patient hospitalization or prolongation of existing hospitalisation, Patients who are hospitalized because of a kidney stone related episode are not recorder as SAE.
- results in persistent or significant disability/ incapacity, or
- is a congenital anomaly/ birth defect.

In addition, important medical events that may not be immediately life-threatening or result in death, or require hospitalisation, but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed above should also usually be considered serious. [ICH E2A]

SAEs should be followed until resolution or stabilisation. Participants with ongoing SAEs at study termination (including safety visit) will be further followed-up until recovery or until stabilisation of the event or until the participant is lost to follow-up.

10.1.1 Assessment of Causality

Both Investigator and Sponsor-investigator make a causality assessment of the event to the study drug, based on the criteria listed in the ICH E2A guidelines:

Relationship	Description
Definitely	Temporal relationship Improvement after dechallenge* Recurrence after rechallenge (or other proof of drug cause)

Probably	Temporal relationship Improvement after dechallenge No other cause evident
Possibly	Temporal relationship Other cause possible
Unlikely	Any assessable reaction that does not fulfil the above conditions
Not related	Causal relationship can be ruled out
*Improvement after dechallenge only taken into consideration, if applicable to reaction	

10.1.2 Unexpected Adverse Drug Reaction

An "unexpected" adverse drug reaction is an adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g. Investigator's Brochure for drugs that are not yet approved and Product Information for approved drugs, respectively). [ICH E2A]

10.1.3 Suspected unexpected serious adverse reactions (SUSARs)

The Sponsor-Investigator evaluates any SAE that has been reported regarding seriousness, causality and expectedness. If the event is related to the investigational product and is both serious and unexpected, it is classified as a SUSAR. Unblinding will be needed in order to determine a SUSAR.

10.1.4 Assessment of Severity

Classification and severity grading scale of SAEs and AEs of special interest (see section 9.2.4.2) in this study will be performed in accordance with "Common Terminology Criteria for Adverse Events CTCAE Version 4.03" terminology.

10.2 Reporting of serious adverse events (SAE) and other safety-related events

Reporting of SAEs
All SAEs must be reported immediately and within a maximum of 24 hours of learning of its occurrence to the Sponsor-Investigator of the study via the SAEreport form on secuTrial.

The Sponsor-Investigator will re-evaluate the SAE on secuTrial.
SAEs resulting in death will be reported to the coordinating and, if applicable, to the local Ethics Committee within 7 days.

Non-serious Events that require discontinuation of IMP
Non-serious events that require discontinuation of IMP (including laboratory abnormalities – see section 7.4.1) should be reported to the Sponsor-Investigator within 3 days.

Reporting of SUSARs
A SUSAR needs to be reported to the local Ethics Committee (via Sponsor-Investigator) and to Swissmedic (via Sponsor-Investigator) within 7 days, if the event is fatal, or within 15 days (all other events).
The Sponsor-Investigator must inform all Investigators participating in the clinical study of the occurrence of a SUSAR. All in the trial involved Ethics Committees will be informed about SUSARs in Switzerland via Sponsor-Investigator according to the same timelines.

Reporting of Safety Signals
All suspected new risks and relevant new aspects of known adverse reactions that require safety-related measures, i.e. so called safety signals, must be reported to the Sponsor-Investigator within 24 hours. The Sponsor-Investigator must report the safety signals within 7 days to the local Ethics Committee and to Swissmedic. The Sponsor-Investigator must immediately inform all participating Investigators about all safety signals. The other in the trial involved Ethics Committees will be informed about safety signals in Switzerland via the Sponsor-Investigator.

Reporting and Handling of Pregnancies
Pregnant participants must immediately discontinue IMP. Any pregnancy during the treatment phase of the study and within 30 days after discontinuation of IMP must be reported to the Sponsor-Investigator within 24 hours. The course and outcome of the pregnancy will be followed up carefully and any abnormal outcome regarding the mother or the child should be documented and reported.

Periodic reporting of safety
An annual safety report will be prepared and submitted once a year to the local Ethics Committees and to Swissmedic by the Sponsor-Investigator. The annual safety report will contain information from all study sites.

10.3 Follow up of (serious) adverse events

Participants with any reported ongoing SAE at the last scheduled study contact will be followed until resolution of the event or a stabilized condition of the subject has been achieved or until the subject is lost to follow-up.
Any new SAEs the Investigator gets aware of that occur after the last scheduled study contact and are determined by the Investigator to be reasonably associated with the use of the IMP, should be reported to the Sponsor-Investigator. The Investigator should follow potentially IMP-related SAEs identified after the last scheduled contact (and report any significant follow-up information to the Sponsor-Investigator) until the events are resolved or stabilized, or the subject is lost to follow-up.

11. STATISTICAL METHODS

11.1 Hypothesis

The null hypothesis that there is no linear trend between dose and the survival function for the primary outcome (i.e. recurrence) will be tested against the alternative that there is a linear trend.

11.2 Determination of Sample Size

The sample size calculation was based on the primary objective i.e. to assess the dose-response relationship and the primary outcome i.e. recurrence with the following assumptions:

- Uniform recruitment over 24 months with allocation ratio fixed at 1 across all arms.
- A maximum and minimum follow-up time of 36 and 24 months, respectively.
- A cumulative drop-out rate of 10% at 24 months after study start.
- Based on the available literature we assumed a risk of recurrence in the placebo group of 0.20 and 0.45 at 12 and 36 months after study start, respectively.
- Hazard ratios for the 12.5, 25 and 50 mg HCTZ doses of 0.90, 0.65 and 0.50, respectively. These effects were based on the literature review presented in Table 3.1-1.
- Power was set to be at least 80% and alpha was fixed at a two-sided level of 0.05.
- An unweighted log-rank test for linear trend with local alternatives.

Using the command artsurv in Stata we calculated that we will need to recruit 416 patients i.e. 104 in each arm.

11.3 Statistical criteria of termination of trial

Not applicable as no formal interim analysis is planned.

11.4 Planned Analyses

The statistical analysis of the trial will be done at CTU Bern by a statistician blinded to the allocation. This process is defined in standard operating procedures. After start of the trial but before recruitment ends, a statistical analysis plan will be written. The plan will determine all necessary data preparation steps (e.g. additional validations, generation of new variables), definitions (e.g. analysis sets), and statistical analyses (e.g. models, outputs such as tables and graphs).
All statistical analyses will be presented as effect measure plus 95% confidence interval. A significance level of 5% will be used.

11.4.1 Datasets to be analysed, analysis populations

All analyses will be done on the intention-to-treat analysis set whereby all randomized patients will be analyzed in the allocated group regardless of any protocol violations such as cross-overs (which can

only happen accidently in this trial) or early treatment discontinuations.

11.4.2 Primary Analysis

The primary outcome (time to stone event) will be analyzed using a stratified log-rank test for linear trend with the hazard ratio as primary effect measure. The test addresses the primary objective of the trial i.e. test for any dose-response relationship. We will start with testing for a linear relationship but will also consider more complex relationships by using fractional polynomials. Kaplan-Meier curves will be used to present results on the primary outcome graphically. In case of a relevant number of multiple events within patients we will consider a model for recurrent events.
Components of the primary outcome measure (clinical and radiological recurrences represented by the number of stones during the trial or time to exclusion from study due to too many stones) will be analyzed similar to the primary analysis (for counts using frailty (count) or marginal count model).
Continuous secondary outcomes (changes in urinary biochemistry) will be analyzed using mixed effects model. Comparisons between placebo and the three active trial arms will be considered exploratory as the trial is not powered to detect differences there.

11.4.3 Secondary Analyses

The following baseline characteristics were identified to potentially modify the expected treatment effect:

- Baseline disease severity (Highest vs. lowest tertile in stone frequency prior to study entry)
- Biochemical abnormalities (Hypercalciuria vs. no hypercalciuria)
- Stone composition (pure CaOx vs. pure CaP vs. mixed CaOx/ CaP vs. mixed CaOx/ UA)

These characteristics will be used for stratified analyses. All stratified analyses will be accompanied by tests for interaction.

11.4.4 Interim analyses

No formal interim analysis is planned.

11.4.5 Safety analysis

Safety endpoints to be analyzed include a descriptive summary of pre-specified AEs of special interest and vital signs. No formal statistical testing will be applied.

11.4.6 Deviation(s) from the original statistical plan

Deviations from the statistical analysis plan will be stated and justified in the final analysis report.

11.5 Handling of missing data and drop-outs

For the primary outcome, drop-outs will be censored at the last available visit. Missing data for continuous outcomes will be handled by multiple imputation.

12. QUALITY ASSURANCE AND CONTROL

12.1 Data handling and record keeping/ archiving

The Investigators will maintain appropriate medical and research records for this trial, in compliance with ICH-GCP and regulatory and institutional requirements for the protection of confidentiality of subjects. The Principal Investigator, Sub-investigator, and Clinical Research Nurses or Coordinators will have access to the records. The Principal Investigators will permit authorized representatives of the Sponsor and regulatory agencies to examine clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress.

12.1.1 Case Report Forms

The CRF will be electronic. All data requested on the eCRF must be recorded and the recorded data should be consistent with the source documents or the discrepancies should be explained. The Investigator should ensure the accuracy, completeness, and timeliness of the data reported in the eCRF and all other required reports. Generally, the eCRF should be completed within two weeks of completion

of a participant's visit/ follow-up phone call.

12.1.2 Specification of source documents

Source documents must be available at the site to document the existence of the study participants and must include the original documents relating to the study, as well as the medical treatment and medical history of the participant.
For all data captured in the eCRF, the location of the source should be documented on a list of source documents (source data location list), which will be stored in the investigator site file at each study site. If certain data are directly entered into the eCRF (and are thus considered as source data) this must be specified on the source data location list accordingly.
Any change or correction to source data should be dated, initialed, and explained (if necessary) and should not obscure the original entry.

12.1.3 Record keeping/ archiving

All study data (written and electronic), including any images, must be retained for a period of at least 10 years from the completion or premature termination of the trial. The Investigators should take measures to prevent accidental or premature destruction of these documents.

12.2 Data management

12.2.1 Data Management System

The CRFs in this trial are implemented electronically using a dedicated electronic data capturing (EDC) system (secuTrial®). The EDC system is activated for the trial only after successfully passing a formal test procedure. All data entered in the eCRF are stored on a Linux server in a dedicated Oracle database. Responsibility for hosting the EDC system and the database lies with Inselspital Bern.

12.2.2 Data security, access and back-up

The server hosting the EDC system and the database is kept in a locked server-room. Only the system administrators have direct access to the server. A role concept with personal passwords (site investigator, statistician, monitor, administrator etc.) regulates permission for each user to use the system and database as he/ she requires.
All data entered into the eCRF are transferred to the database using Secure Sockets Layer (SSL) encryption. Each data point has attributes attached to it identifying the user who entered it with the exact time and date. Retrospective alterations of data in the database are recorded in an audit table. Time, table, data field and altered value, and the person are recorded (audit trail). A multi-level back-up system is implemented.

12.2.3 Analysis and archiving

At interim (if applicable) and final analyses, data files will be extracted from the database into statistical packages to be analyzed. The status of the database at this time is recorded in special archive tables.
The study database with all archive tables will be securely stored by Inselspital Bern. The sponsor also keeps the Trial Master File and interim and final reports both in electronic and in hard copy form for at least 10 years.

12.2.4 Electronic and central data validation

Data is checked by the EDC system for completeness and plausibility. Furthermore, selected data points are cross-checked for plausibility with previously entered data for that participant. In addition (if applicable), central data reviews will be performed on a regular basis to ensure completeness of the data collected and accuracy of the primary outcome data.

12.3 Monitoring

For quality control of the study conduct and data retrieval, all study sites will be visited on-site by appropriately trained and qualified Monitors. Any findings and comments will be documented in site visit reports and communicated to the local Investigator and to the Sponsor as applicable. Investigators at the participating study sites will support the Monitor in his/ her activities. Prior to study start (first participant enrolled) a plan detailing all monitoring-related procedures will be developed.
All source data and relevant documents will be accessible to Monitors and questions of Monitors are answered during site visits.

12.4 Audits and Inspections

Source data/ documents must be available to audits by the Sponsor or designee or to inspections by health authorities. The CA (Swissmedic) or CEC may wish to conduct an inspection (during the study or after its completion). If an inspection is requested, the Investigator must inform the Sponsor immediately that this request has been made. The Investigators at the participating sites will support the inspectors in their activities and will answer questions from inspectors as needed. All involved parties must keep the participant data strictly confidential.

12.5 Confidentiality, Data Protection

The Investigator ensures anonymity of the patients; patients will not be identified by names in any study documents leaving the study site. Subject confidentiality will be ensured by utilizing subject identification codes consisting of three random letters and three random numbers, such as toj838. Signed informed consent forms and patient enrollment log will be kept strictly confidential to enable patient identification at the site.

12.6 Storage of biological material and related health data

All baseline and follow-up CTs for central analysis will be coded and uploaded into the picture archiving and communication system (PACS) of the Inselspital. A secure exchange platform will be used for file exchange.
DNA collected from patients will be stored indefinitely in the NOSTONE Biobank at the Bern University Hospital. The samples will not be destroyed at the end of the study. Patient's samples and genetic data will be stored only after a separate informed consent is signed.
The procedures and rules of the NOSTONE biobank are described in a specific booklet (NOSTONE Biobank Reglement).

13. PUBLICATION AND DISSEMINATION POLICY

The trial protocol will be published in an open access journal. Study results will be presented at national and international meetings and will be submitted for publication to high impact, peer reviewed journals. Upon completion of the analysis, trial results will be communicated to all participants. Public reporting will be done through patient organizations and via the communication Departments at the individual Universities and Hospitals.
Once results have been published, trial data will be accessible to external researchers and anonymized datasets corresponding to each publication will be made available. Investigators wishing to replicate the analyses or to do an individual patient meta-analysis may request the data from the steering committee. Access to data will be granted in an unbureaucratic way.

14. FUNDING AND SUPPORT

14.1 Funding

This trial trial is financed by the Swiss National Science Foundation via the "Investigator-initiated clinical trials – IICT" grant # 33IC30_166785.

14.2 Other Support

The trial will also receive intramural support of the Bern University Hospital.

15. INSURANCE

Insurance will be provided by the Sponsor. A copy of the certificate is filed in each investigator site file and the trial master file.

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17. APPENDICES

Not applicable.

Revision history

NOSTONE Protocol Amendments

Revision history

Protocol version	Justification	Timing
1.0	First (original) version	30.08.2016
1.1	– Revision of the handling of collected data and samples upon participant withdrawal §7.4.2 – Revision of confidentiality and data protection §12.5	26.09.2016
1.2	– Addition of 4 new clinical sites (Synopsis) – Revision of unblinding procedures §6.3 – Revision of exclusion criteria (inflammatory bowel disease and intestinal surgery) §7.1 – Harmonization of number of recurrences for IMP stop (= 3) for consistency within the text §7.4.1 – Adaptation of the blood parameters for diabetes mellitus diagnosis §8.3.4 & 9.2.4.2 – Investigational Medicinal Product (IMP) count eliminated §8.4 – IMP dispensing specifications eliminated §9.3 – Revision of kidney stone hospitalization within the SAE definition §10.1 – SAE reporting changed from email/fax to electronic §10.2 – Margin for eCRF completion extended from 1 to 2 weeks §12.1.1	20.2.2017
1.3	– Hyperthyroidism eliminated from exclusion criteria §7.1 – CKD-EPI eGFR definition for CKD changed to "< 30 mL/min per 1,73 m2 body surface area for more than 3 months" §7.1 – Revision of conditions for IMP discontinuation upon concomitant forbidden medication intake §7.4.1	12.5.2017
1.4	– Revision of conditions for discontinuation of study IMP/ discontinuation of forbidden medications §7.4.1 – Grace period of maximal 4 months for forbidden medications introduced §8.6	14.08.2017
1.5	– Study duration and study schedule adapted (Synopsis)	18.04.2018

	– Revision of follow-up duration (Study schedule, §6.1, §§9.3.8 - 9.3.10,) – Revision of unblinding procedure (no unblinding envelopes at study sites) §6.1	
1.6	– CTU Bern, address change §1.3, §1.5 – Introduction of sampling for DNA biobank: • Informed consent DNA biobank §2.7 • Other outcomes of interest §5.3 • Assessment of other outcomes of interest §9.2.3 • Visits procedures §9.3 – Unblinding procedures clarified §6.2.2 – Condition "Blood creatinine > 150% of creatinine value at screening (visit 2) for IMP discontinuation eliminated §7.4.1 – Condition for IMP withdraw "The IMP will be withdrawn if CKD-EPI eGFR drops below 30 mL/min per 1,73 m^2 body surface area for more than 3 months " introduced in §8.3.2 – Condition "Stone events occurring during the first 6 weeks after randomization will not be considered as events for the analysis" introduced in §9.2.1 – "Blood creatinine > 150% of baseline creatinine" eliminated from AE of special interest §9.2.4.2	02.06.2018
1.7	• Revision of inclusion criteria "Recurrent kidney stone disease (≥ 2 stone events within the last 10 *calendar* years prior to randomization)" (Synopsis, §3.8, §6.1.1, §7.1) – Extension of study duration to 08/2021 (Synopsis) – Revision of study schedule • Possibility to substitute Visit 1 with a phone call (Synopsis, §9.2, §9.3); • Possibility to merge Visit 1 and Visit 2 also for new referrals (Synopsis, §9.2, §9.3); • Possibility to skip lab analysis at Visit 4, Visit 5, Visit 6 and Visit 7 on patient's request (Synopsis, §9.2, §9.3); • Possibility to replace Visits 4-7 with a phone call, provided the final CT scan is done on site (Synopsis, §9.2, §9.3) – Adaptation Esidrex® patent owner address §3.2 – Revision of the definition of the primary and secondary outcome §3.1 & §3.2	28.11.2019

	– Revision of recruitment timeline §6.1 – "Magnesium" implemented in forbidden medications for electrolyte disturbances §8.3.1 – Revision of action to take in case of new onset diabetes §8.3.4 – Harmonization of rules for "Symptomatic stone recurrence during the trial" §8.3.6	

First/original SAP

Version 1.0

November 6th, 2018

Statistical Analysis Plan

Randomized double-blind placebo-controlled trial assessing the efficacy of standard and low dose hydrochlorothiazide treatment in the prevention of recurrent nephrolithiasis

Acronym of study: NOSTONE

Administrative Information

Project number:	640
Study title:	Randomized double-blind placebo-controlled trial assessing the efficacy of standard and low dose hydrochlorothiazide treatment in the prevention of recurrent nephrolithiasis
Trial registration number:	Swiss National Clinical Trials Portal: SNCTP000002101; ClinicalTrials.gov: NCT03057431
SAP version:	1.0 of 06.11.2018
Protocol version:	1.5 (18.04.2018)

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Revision history

Revision	Justification	Timing

Approved by

Name	Affiliation	Study Role	Date and Signature (wet ink)
Marie Roumet, PhD	CTU Bern	Trial Statistician	[redacted] 2.11.18
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As a minimum requirement, the main statistician, a supervising senior statistician and the sponsor/sponsor-investigator have to sign the SAP.

Contents

1. Introduction

1.1 Background and rationale

Background: Nephrolithiasis is a global healthcare problem with a current lifetime risk of 18.8% in men and 9.4% in women. Without specific treatment, 5- and 20-year recurrence rates are 40% and 75%, respectively. About 80–90% of stones are composed of calcium oxalate with various admixtures of calcium phosphate. Increased excretion of calcium in the urine, hypercalciuria, is the most common metabolic abnormality encountered in patients with recurrent nephrolithiasis. Thiazide diuretics have been the cornerstone of pharmacologic metaphylaxis for more than 40 years. The effect of thiazides to reduce the risk of stone recurrence has been attributed to their ability to decrease urinary calcium excretion. However, other factors, such as reduction of urinary pH and urinary oxalate excretion, probably contribute to this effect. Efficacy of thiazides on recurrence prevention of calcareous nephrolithiasis was tested in 11 randomized controlled trials (RCTs). With the exception of two trials, thiazides significantly reduced stone recurrence. Most of these trials are from the 1980's and 90's and the cumulative number of patients studied is remarkably low for such a prevalent disease. Our systematic review of these RCTs revealed major methodological deficiencies in all trials, including: lack of double-blinding and intention-to-treat analysis, unclear allocation concealment, lack of adverse event and drop out reporting and unknown baseline risk of disease severity. Furthermore, high doses of thiazides were employed in all trials, in the case of the best studied thiazide, hydrochlorothiazide (HCTZ), up to 100 mg daily. At such high doses, side effects occur frequently. Nowadays, thiazides are widely used in the treatment of recurrent nephrolithiasis and arterial hypertension, but at significantly lower doses. In the case of recurrent nephrolithiasis, however, this practice is not supported by randomized evidence and consequently, we do not know whether the currently employed low dose thiazide regimens are effective in reducing the risk for

stone recurrence. Thus, evidence for benefits and harms of thiazide diuretics in the prevention of calcium-containing kidney stones in general remains unclear. In addition, the efficacy of the currently employed low dose thiazide regimens to prevent stone recurrence is not known.

Aim of the study: The aim of the study is to compare the efficacy and safety of standard and low dose HCTZ treatment in the recurrence prevention of kidney stones. More specifically, the study aims to describe the dose-response relationship for placebo and three different dosages of HCTZ.

Hypothesis: HCTZ significantly reduces stone recurrence compared to placebo. There is a significant dose-response relationship.

Design of the study: This is a multicenter, randomized, placebo-controlled, double blind, parallel-group trial.

Outcomes: The **primary outcome** as measured on the individual patient-level is stone recurrence during study treatment.

The primary outcome of the trial is the relationship between treatment group and stone recurrence during study treatment.

Secondary outcomes include:

- The individual components of the composite primary outcome:
    - symptomatic stone recurrence
    - radiologic stone recurrence
- Changes in urinary biochemistry from baseline to visits 4 (3 months), 5 (12 months), 6 (24 months) and 7 (36 months):
    - calcium,
    - citrate,
    - oxalate,
    - sodium,
    - total urine volume per 24 hours,
    - pH.

1.2 Objectives

1.2.1 Primary objective

The primary objective is to assess the dose-response relationship between placebo and the three different dosages of HCTZ and stone recurrence.

1.2.2 Secondary objectives

Secondary objectives are to assess the efficacy of the different dosages of HCTZ in terms of the primary outcome as well as the individual components of the composite primary outcome, i.e. symptomatic stone recurrence and radiologic stone recurrence. Further objectives are to study effects of different dosages of HCTZ on urinary biochemistry (efficacy and safety aspects) and the impact of different baseline characteristics on stone recurrence and on the effects of the different dosages on stone recurrence (effect modification).

1.2.3 Safety objectives

The safety objective is to assess the long-term safety and tolerability of HCTZ compared to placebo

2. Study methods

2.1 Trial design

NOSTONE is a randomized, placebo-controlled, observer-, clinician- and patient-blinded, parallel-group study.

Eligible patients will be randomized in equal proportions between 12.5 mg, 25 mg or 50 mg HCTZ or placebo. All subjects will be given the IMP (HCTZ or placebo) once daily in the morning. Placebo will be administered to subjects randomized to that treatment in a form identical to the HCTZ capsules. The first dose of the IMP will be administered the day after the randomization.

A total of 416 participants, 104 in each group, will be included in the study. Recruitment of participants is planned to occur over a period of 24 months at all 12 study sites. Study treatment will be 36 months for subjects enrolled in the first 12 months of the recruitment period and 24 months for subjects recruited in the remainder of the recruitment period.

2.2 Randomization

Stratified randomization is used to assign participants to the different trial arms with the number of previous stone events as stratification factors:

- Stratification group 1: 2 or 3 stone events within 10 years prior to randomization
- Stratification group 2: ≥4 stone events within 10 years prior to randomization.

Allocation is concealed using sequentially coded drug packs that are otherwise identical. Randomization lists were generated by a statistician at CTU Bern following dedicated standard operating procedures. The statistician communicated directly with the facility, which prepared the drug packs (Grosse Apotheke Dr. G. Bichsel AG, Interlaken, Switzerland). Investigators at each site assign the drug pack with the next sequential number to the next patient (consecutive). Content of the drug packs is based on randomization lists as described above.

To ensure allocation concealment, the statistician preparing the randomization list as well as the facility handling of the un-blinded drug packs are otherwise not involved in the trial; randomization lists are stored electronically at CTU Bern with no access for persons directly involved in the trial.

2.3 Sample size

A total of 416 patients, i.e. 104 in each arm, will be included in this study.

The sample size was calculated using the artsurv command in Stata and was based on the primary objective i.e. to assess the dose-response relationship between treatment group and the primary outcome, based on the following assumptions:

- Uniform recruitment over 24 months with allocation ratio fixed at 1 across all arms
- A maximum and minimum follow-up time of 36 and 24 months, respectively
- A cumulative drop-out rate of 10% at 24 months after study start
- Based on the available literature we assumed a risk of recurrence in the placebo group of 0.20 and 0.45 at 12 and 36 months after study start, respectively.

- Hazard ratios for the 12.5, 25 and 50 mg HCTZ doses of 0.90, 0.65 and 0.50, respectively. These effects were based on the literature review presented in Table 3.1-1 in the study protocol.
- Power was set to be at least 80% and alpha was fixed at a two-sided level of 0.05
- An unweighted log-rank test for linear trend with local alternatives.

2.4 Framework

The null hypothesis that there is no linear trend between dose and the survival function for the primary outcome (i.e. recurrence) will be tested against the alternative that there is a linear trend.

2.5 Statistical interim analyses and stopping guidance

There is no interim analysis planned.

2.6 Timing of final analysis

Analysis will start after completion of data export, preparation and validation as described in the paragraphs 3.1, 3.2 and 3.3 of the present document. Data validation starts at the beginning of the trial and goes on continuously until the validation of the last follow-up of the last patient.

The analysis will use all outcomes except for the dosage group variable as explained further in the paragraph 2.8.

2.7 Timing of outcome assessments

2.7.1 Participants recruited in the first 12 months of the recruitment period

Study Period	**Screening**		**Treatment period**								**Safety follow-up**
Visit	**1**	**2**	**3**	**4 or tel-ephone call**	**Tele-phone calls**	**5**	**Tele-phone calls**	**6**	**Tele-phone calls**	**7**	**Tele-phone call, EOS**
Visit time points	-14 to -1 day(s)	0	2 weeks	3 Months	6, 9 months	12 months	15, 18, 21 months	24 months/ EOT	27, 30, 33 months	36 months/ EOT	30 days after EOT
Allowed visit window		0	± 2 weeks	± 2 Weeks	± 4 weeks	± 4 weeks	± 4 weeks	± 4 weeks	± 4 weeks	± 2 weeks	± 2 weeks

2.7.2 Participants recruited ≥12 months after recruitment start

Study Period	**Screening**		**Treatment period**						**Safety follow-up**
Visit	**1**	**2**	**3**	**4 or tel-ephone call**	**Tele-phone call**	**5**	**Tele-phone call**	**6**	**Telephone call, EOS**
Visit time points	-14 to -1 day(s)	0	2 weeks	3 months	6, 9 months	12 months	15, 18, 21 months	24 months/ EOT	30 days after EOT
Allowed visit win-dow		0	± 2 weeks	± 2 weeks	± 4 weeks	± 4 weeks	± 4 weeks	± 4 weeks	± 2 weeks

2.8 Blinding

All trial personnel is blinded to the assigned trial arm through all the stages of the study: recruitment, care of patients, trial assessment, monitoring - the only un-blinded people are 1) the independent statistician generating the randomization list; and 2) the personnel at the facility preparing the drug packs (Grosse Apotheke Dr. G. Bichsel AG, Interlaken, Switzerland). HCTZ and placebo are provided in identically looking capsules in plastic bottles with unique consecutive numbers.

Blinding will remain in place until the statistician codes the primary analysis of the primary and secondary outcomes and produces a dummy report of the primary analysis using a randomly generated group variable. The true group variable becomes open after the completion of the dummy report and gives place to the final report of all the analysis as well as the quality control by the independent statistician described in the paragraph 6.7.

3. Data management

3.1 Data export

Clinical study data are provided by the CTU Bern (University of Bern) in a database format (secuTrial®) and will be imported into R by the trial statistician for data preparation, validation and analysis.

3.2 Data validation

First line data validation is performed by the online eCRF system at real-time as defined in the data dictionary. Second line data validation and cleaning will be performed according to the SOP for data validation [3] with trimestrial Data Quality Review meetings during the data collection; and additionally after the completion of overall data collection, before database export for the final analysis.

3.3 Data preparation

We will derive the primary and the secondary outcomes from the data. Time will be calculated as days since randomization.

When recorded, calcium, citrate, oxalate, sodium and pH are measured in one (pH) or two (calcium, citrate, oxalate and sodium) 24-hour urine samples. Measures of calcium, citrate, oxalate and sodium will be averaged between the two samples and reported as absolute excretion per 24h.

When recorded, heart rate, systolic and diastolic blood pressures are measured three times; the exact value will be approximated by the mean of these three measurements.

3.3.1 Categorization of data

Treatment groups:

Four treatment groups are considered, 12.5 mg, 25 mg or 50 mg HCTZ, or placebo.

Treatment centers:

There are twelve treatment centers in Switzerland:

- 7 Cantonal Hospitals: Aarau, Bellinzona, Chur, Lugano, Luzern, Sion and St. Gallen
- and 5 University Hospitals: Basel, Bern, Geneva, Lausanne, Zürich

Other

- Stratification group 1: 2 or 3 stone events within 10 years prior to randomization; stratification group 2: ≥4 stone events within 10 years prior to randomization.
- Baseline disease severity approximated by the number of stones within the last 10 years prior to randomization. The variable will be categorized into three groups using the first and the second tertiles as cut-offs. Patients with values below or equal to the first, second and third tertiles will be defined as patients with low, moderate disease and severe disease severity, respectively.
- Biochemical abnormality is defined as the occurrence of Hypercalciuria.
- Hypercalciuria is defined as above 6.25 mmol/d (=250 mg/d) in women and above 7.5 mmol/d (=300 mg/d) in men.

- Biochemical abnormalities at baseline - hypercalciuria vs. no hypercalciuria in the baseline urine analysis.
- Hypertension is considered occurring when the systolic blood pressure exceed 89 mmHg or when the diastolic blood pressure exceed 139 mmHg or when the subject is treated by antihypertensive medication.
- Diabetes is defined as the occurrence of blood glucose level (fasting) of 7.0 mmol/l or more; or random (non-fasting) glucose 11 mmol/l or more; or HbA1c 6.5 or higher; or treated (oral antidiabetic or insulin).
- Stone composition is categorized at baseline as
  (i) calcium oxalate (i.e. > 50% ca-ox)
  (ii) calcium phosphate (i.e. > 50% ca-p)
  (iii) other.

3.4 Outcome derivation

Table: Derivation of primary, secondary outcomes and some of the variables important for the primary and secondary analysis.

Outcome	Visit	eCRF sheet	Variable	Variable type	Derivation	Outcome type
Primary						
Stone event	Any visit or call	stone	stone_event	Binary: yes or no	none	Part of the primary outcome
Count of stone event per subject	Any visit or call	stone	sts_count	date	none	Part of the primary outcome
Date of the stone event (start date)	Any visit or call	stone	Sts_inc_date	date	none	Part of the primary outcome
HCTZ Dose group allocation[1]	Randomization	Separate blinded database		Group number	none	Part of the primary outcome
Secondary						
Total urine volume (ml), first collection	2,4,5,6,7	Urine Analysis	lab_ur1_tvol	Continuous, greater than 0: ml	none	Not an outcome without derivation
Total urine volume (ml), second collection	2,4,5,6,7	Urine Analysis	lab_ur2_tvol	Continuous, greater than 0: ml	none	Not an outcome without derivation
Duration (hrs), first collection	2,4,5,6,7	Urine Analysis	lab_ur1_dur3	Continuous, greater than 0: hrs	none	Not an outcome without derivation
Duration (hrs), second collection	2,4,5,6,7	Urine Analysis	lab_ur1_dur6	Continuous, greater than 0: hrs	none	Not an outcome without derivation
Urine Calcium, absolute excretion per 24hrs, first collection	2,4,5,6,7	Urine Analysis	lab_ur1_ca	Number continuous, greater than 0, detection limits are unknown	Absolute excretion per 24 hrs for calcium= lab_ur1_ca (mmol/l)* 1000ml*lab_ur1_tvol (ml)*24hrs/ lab_ur1_dur3	Secondary Continuous: mmol/24hrs

[1] This variable will be un-blinded after the dummy report on primary and secondary analysis as described in paragraph 2.8

Outcome	Visit	eCRF sheet	Variable	Variable type	Derivation	Outcome type
Urine Calcium, absolute excretion per 24hrs, second collection	2,4,5,6,7	Urine Analysis	lab_ur2_ca	Number continuous, greater than 0, detection limits are unknown	Absolute excretion per 24 hrs for calcium= lab_ur2_ca (mmol/l)* 1000ml*lab_ur2_tvol (ml)*24hrs/ lab_ur1_dur6	Secondary Continuous: mmol/24hrs
Urine Oxalate, absolute excretion per 24hrs, first collection	2,4,5,6,7	Urine Analysis	lab_ur1_oxa	Number continuous, greater than 0, detection limits are unknown µmol/l	Absolute excretion per 24 hrs for oxalate = lab_ur1_oxa (µmol/l)* 1000ml*lab_ur1_tvol (ml)*24hrs/ lab_ur1_dur3	Secondary Continuous: µmol/24hrs
Urine Oxalate, absolute excretion per 24hrs, second collection	2,4,5,6,7	Urine Analysis	lab_ur2_oxa	Number continuous, greater than 0, detection limits are unknown µmol/l	Absolute excretion per 24 hrs for oxalate = lab_ur2_oxa (µmol/l)* 1000ml*lab_ur2_tvol (ml)*24hrs/ lab_ur1_dur6	Secondary Continuous: µmol/24hrs
Urine Citrate, absolute excretion per 24hrs, first collection	2,4,5,6,7	Urine Analysis	lab_ur1_citr	Number continuous, greater than 0, detection limits are unknown mmol/l	Absolute excretion per 24 hrs for citrate = lab_ur1_citr (mmol/l)* 1000ml*lab_ur1_tvol (ml)*24hrs/ lab_ur1_dur3	Secondary Continuous: mmol/24hrs
Urine Citrate, absolute excretion per 24hrs, second collection	2,4,5,6,7	Urine Analysis	lab_ur2_citr	Number continuous, greater than 0, detection limits are unknown mmol/l	Absolute excretion per 24 hrs for citrate = lab_ur2_citr (mmol/l)* 1000ml*lab_ur2_tvol (ml)*24hrs/ lab_ur1_dur6	Secondary Continuous: mmol/24hrs
Urine Sodium, absolute excretion per 24hrs, first collection	2,4,5,6,7	Urine Analysis	lab_ur1_na	Number continuous, greater than 0, detection limits are unknown: mmol/l	Absolute excretion per 24 hrs for sodium = lab_ur1_na (mmol/l)* 1000ml*lab_ur1_tvol (ml)*24hrs/ lab_ur1_dur3	Secondary Continuous: mmol/24hrs
Urine Sodium, absolute excretion per 24hrs, second collection	2,4,5,6,7	Urine Analysis	lab_ur2_na	Number continuous, greater than 0, detection limits are unknown: mmol/l	Absolute excretion per 24 hrs for sodium = lab_ur2_na (mmol/l) * 1000ml * lab_ur2_tvol (ml) * 24hrs/ lab_ur1_dur6	Secondary Continuous: mmol/24hrs

3.5 Data sharing (if applicable)

Data will be shared as described in the Protocol: "Once results have been published, trial data will be accessible to external researchers and anonymized datasets corresponding to each publication will be made available. Investigators wishing to replicate the analyses or to do an individual patient meta-analysis may request the data from the steering committee. Access to data will be granted in an unbureaucratic way."

4. Statistical principles

4.1 Confidence intervals and *P* values

All confidence intervals will be two-sided and relate to the 95% level and the two-sided significance level will be set at $\alpha = 0.05$.

4.2 Analysis populations

4.2.1 Full analysis set (FAS)

The full analysis set consists of all randomized patients regardless of whether they actually received the allocated intervention or not or any other protocol deviations. Patients will be analysed in the group they were randomized regardless of any cross-overs or loss to follow-up (intention-to-treat principle).

4.2.2 Per-protocol (PP)

Per-protocol population consists of all subjects in the FAS who did not have any protocol deviations that could confound the interpretation of analyses conducted on the FAS. The following are common major protocol deviations:

- Not receiving the allocated treatment.
- Missing treatment doses. We will consider as non-compliant participants who missed more than 20% of IMP during their study period (period when they were followed in the study).
- Failure to perform primary endpoint assessment, i.e. assessment not done. Not fulfilling the eligibility criteria.

4.2.3 Safety population

In the safety analysis data set, patients will be included in the treatment group they actually received (at least one dose).

4.3 Estimands

The ICH E9 (R1) addendum on estimands and sensitivity analyses [1] defines different treatment estimators that are of interest in clinical trials. An estimand is the target of estimation to address the scientific question of interest posed by the trial objective. Attributes of an estimand include the population of interest, the outcome of interest, the specification of how intercurrent events such as cross-overs and non-

compliance are reflected in the scientific question of interest, and the population-level summary for the outcome.

4.3.1 Treatment policy estimand

The estimand that addresses the main objective of this trial is based on the treatment policy strategy. The occurrence of an intercurrent event such as a cross-over, IMP missed doses or discontinuation is irrelevant; the value for the outcome of interest is used regardless of whether or not the intercurrent event occurs. The primary intention-to-treat analysis of the primary and all secondary outcomes will be based on this estimand using the FAS.

4.3.2 While on treatment estimand

Moreover, we will assess the estimand based on the 'while on treatment' strategy. Subjects who

- missed more than 20% treatment doses.

will be excluded from this analysis. This estimand will be addressed in a secondary analysis using the PP set.

5. Trial Population

5.1 Screening data

No screening data were collected and the representativeness of trial sample cannot be described.

5.2 Eligibility

Individuals fulfilling all of the following inclusion criteria are eligible for the study:

- Informed Consent as documented by signature
- Age 18 years or older
- Recurrent kidney stone disease (≥2 stone events within the last 10 years prior to randomization)
- Any past kidney stone containing 50% or more of calcium oxalate, calcium phosphate or a mixture of both

The presence of any one of the following exclusion criteria will lead to exclusion of the individual:

1. Pharmacologic prevention for stone recurrence less than 3 months prior to randomization.
2. Patients with secondary causes of recurrent calcareous nephrolithiasis including:
   - Severe eating disorders (anorexia or bulimia);
   - Chronic inflammatory bowel disease, bariatric surgery, intestinal surgery with malabsorbtion or chronic diarrheal status;
   - Sarcoidosis;
   - Primary hyperparathyroidism;
   - Hyperthyroidism;
   - Complete distal tubular acidosis;
   - Active malignancy.

3. Patients with the following medications. According to the Protocol' criteria for withdrawal/ discontinuation of participants "The IMP will be discontinued only if the patient took the medications listed in the exclusion criteria for more than 4 months":
   - Thiazide or loop diuretics;
   - Carbonic anhydrase inhibitors (including topiramate) ;
   - Xanthine oxidase inhibitors (febuxostat or allopurinol);
   - Alkali, including potassium citrate or sodium bicarbonate;
   - Treatment with 1,25-OH Vitamin D (calcitriol);
   - Calcium supplementation;
   - Bisphosphonates;
   - Denusomab;
   - Teriparatide;
   - Glucocorticoids.
4. Obstructive uropathy, if not treated successfully.
5. Urinary tract infection, if not treated successfully.
6. Chronic kidney disease (defined as CKD-EPI eGFR < 30 mL/min per 1,73 m2 body surface area for more than 3 months).
7. Patients with a kidney transplant.
8. > 3 gout arthritis episodes within one year prior to randomisation or gout arthritis requiring uric acid lowering therapy.
9. Cystinuria at screening.
10. Hypokalemia (blood potassium level < 3 mmol/L) at screening.
11. Hyponatremia (blood sodium level < 125 mmol/L) at screening.
12. Pregnant and lactating women [pregnancy test to be performed for women of child-bearing potential (defined as women who are not surgically sterilized/ hysterectomized, and/ or who are postmenonpausal for less than 12 months)].
13. Previous (within 3 months prior to randomization) or concomitant participation in another interventional clinical trial.
14. Inability to understand and follow the protocol.
15. Known allergy to the study drug.

5.3 Recruitment

A CONSORT patient flow diagram will be completed following the CONSORT 2010 standards (http://www.consort-statement.org/consort-2010).

The flow chart will consider specifically:

- N assessed for eligibility
- N eligible and not included into the trial
- N randomized
- Ns allocated to intervention
- Ns receiving allocated intervention
- Ns not receiving allocated intervention (with reasons)
- Ns discontinued intervention (with reasons)
- Ns lost to follow up (with reasons)

- Ns analyzed
- Ns excluded from primary analysis

5.4 Baseline patient characteristics

The patient characteristics of the FAS at baseline will be presented in a table, stratified by treatment arm, as number and percentage or mean and standard deviation for categorical and normally distributed continuous variables, respectively. For data severely deviating from a normal distribution, we will present median and interquartile ranges. No statistical comparisons of patient characteristics at baseline will be performed.

Total	Placebo n=	12.5mg HCTZ n=	25mg HCTZ n=	50 mg HCTZ n=
DEMOGRAPHICS				
Age, years (mean ± SD)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Sex (Female, n [%])	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
DISEASE SEVERITY AND STONE COMPOSITION				
Number of previous stone events within the 10 years prior to randomization	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
– [2,3] stones	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
– ≥4 stones	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
Number of previous stone events within the 10 years prior to randomization	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
– First tertile	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
– Second tertile	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
– Third tertile	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
Stone composition:				
– calcium oxalate (i.e. > 50% ca-ox)	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
– calcium phosphate (i.e. > 50% ca-p)	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
– other.	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
PHYSICAL EXAMINATION & VITAL SIGNS				
BMI	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Systolic blood pressure [2]	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Diastolic blood pressure[2]	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Heart rate[2]	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x

[2] Values averaged over 3 measurements

HABITS & CARDIOVASCULAR RISK FACTORS				
Smoking				
– Never	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
– Current	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
– Former	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
Alcohol consumption (unit/day)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Hypertension	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
Treated for hypertension (yes, n [%])	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
Diabetes (yes, n [%])	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
Treated for diabetes (yes, n [%])	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
Gout arthritis (yes, n [%])	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
RENAL SYSTEM				
Lower urinary tract infection(s) in the past (yes, n [%])	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
Upper urinary tract infection(s) in the past (yes, n [%])	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
Kidney malformations/Urinary tract malformations (yes, n [%])	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
BLOOD ANALYSIS (SERUM)				
Potassium (K) (mmol/l)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Sodium (Na) (mmol/l)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Calcium (mmol/l)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Magnesium (mmol/l)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Bicarbonate (mmol/l)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Glucose (fasting) (mmol/l)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Haemoglobin A1c (%)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Creatinine (umol/l)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Uric acid (µmol/l)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Total Cholesterol (mmol/l)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
HDL Cholesterol (mmol/l)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
LDL Cholesterol (mmol/l)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Triglycerides (mmol/l)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
URINE ANALYSIS (FIRST 24 HR COLLECTION[3])				
Urine volume (l /24 h)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Calcium (mmol/24 h)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Sodium (mmol/24 h)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Citrate (mmol/24 h)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Oxalate (mmol/24 h)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
pH	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x

[3] Values averaged over 2 measurements (OIL and NATIVE)

BIOCHEMICAL ABNORMALITIES (urine based) Hypercalciuria (Calcium urine > 7.5 mmol/d in men or > 6.25 mmol/d in women) (yes, n [%])	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)

5.5 Adherence and protocol deviations

At each follow-up visit, participants are asked whether they were able to take their study medication every day. For each visit, we will present the number and percentage of patients who were or were not able to take their study medication every day. Among the patients who were not able to take their study medication every day, we will present the percentages of the following categories: missed 1-5 pills, 6-10 pills, 11-20 pills, more than 20 pills of the 180 expected in the last 3 months.

The common major protocol deviations are listed in the paragraph 4.2.2 above. The number of patients presenting each of these deviations will be summarized in a table.

5.6 Withdrawal/follow-up

The number of withdrawal and loss to follow-up at each visit in the control and in the treatment arm will be presented in a CONSORT patient flow diagram (for details see 5.3 above).

6. Analysis

6.1 Outcome definitions

6.1.1 Primary outcome

The primary outcome of the trial is the relationship between dose group and stone recurrence during study treatment (up to 36 months).

The primary outcome at the patient level is stone recurrence during study treatment. Stone recurrence is the composite of symptomatic and radiological recurrence.

Symptomatic stone recurrence is defined as visible passage of a stone with accompanying typical symptoms such as flank/ loin pain and hematuria or a symptomatic or asymptomatic stone requiring urological intervention for stone removal. It will be assessed at the follow-up visits 3 (2 weeks), 4 (3 months), 5 (12 months) and 6 (24 months) as well as at the three-monthly phone calls in between the visits.

Radiological stone recurrence as assessed by low-dose non-intravenous (iv) contrast CT imaging is defined as the appearance of new caliculi or enlargement of preexisting caliculi with reference to the baseline CT performed at visit 3 (2 weeks) or within 2 months prior this visit. To minimize bias of primary outcome assessment for radiological recurrence, all renal CTs will be assessed by two blinded observers independently and centrally at Inselspital Bern.

Renal CTs for the assessment of stone recurrences are performed whenever there is a suspicion of symptomatic recurrence, and at the end of study treatment. The end of study treatment corresponds to visit 7 (36 months) for participants recruited in the first 12 months of the recruitment period and to visit 6 (24 months) for participants recruited in the remainder of the recruitment period. At treatment end, a renal CT is performed only if no other non-iv contrast renal CT was performed within 3 months prior to the visit.

6.1.2 Secondary outcomes

Secondary outcomes include:

- The individual components of the composite primary outcome:
  - symptomatic stone recurrence (assessed as described for the primary outcome).
  - radiologic stone recurrence (assessed as described for the primary outcome).
- Urinary biochemistry: urinary volume[4], calcium, citrate, oxalate, sodium and pH at visits 4 (3 months), 5 (12 months), 6 (24 months) and 7 (36 months).

[4] Urinary volume per say is not biochemistry, however it is an absolute prerequisite for properly evaluating the eventual biochemistry changes as for equal microelement excretions per 24 hours its urinary concentrations might differ depending on volume..

6.1.3 Assessment of safety outcomes

Serious adverse events (SAEs)

SAEs will be collected, fully investigated and documented in the source documents and the eCRF for all participants from the date of ICF signature until the last protocol-specific procedure has been completed, including a safety follow-up period of 30 days.

Adverse events (AEs) of special interest

For this trial, (non-serious) AEs will not be documented in the eCRF with exception of the following events "of special interest" occurring from the date of randomization (visit 2) until visit 7 (36 months):

- Hypokalemia, defined as blood potassium level < 3 mmol/L;
- Hyponatremia, defined as blood sodium level < 125 mmol/L;
- Hypomagnesemia, defined as blood magnesium level < 0.5 mmol/L;
- Blood creatinine > 150% of baseline creatinine;
- Newly developed overt diabetes;
- Allergic reaction of skin if considered by the local investigator to be potentially related to the study medication;
- Gouty arthritis if recurrence > 3 times per year or requiring Uric acid lowering therapy.

To assess the AEs of special interest the following blood biochemistry parameters are collected as safety outcome: potassium, sodium, magnesium, creatinine, glucose (fasting), HbA1c at visits 4 (3 months), 5 (12 months), 6 (24 months) and 7 (36 months). These parameters will be assessed according to the protocol. Moreover, we will perform the additional analysis as below in the paragraph 6.2.5.

6.1.4 Assessments in participants who prematurely stop the study

Participants who permanently discontinue the study treatment will undergo all protocol-specified study visits and assessments. If study visits are not possible, a telephone consultation will be performed to determine if relevant health events/ endpoints have occurred.

6.2 Analysis methods

6.2.1 Primary analysis

The CTU Bern statistician will perform the analysis of the study. The statistician will be first blinded to the group allocation, then un-blinded as described in the paragraph 2.8. In the primary analysis, all patients will be analysed using the FAS according to the intention-to-treat principle. All effect measures will be accompanied by 95% confidence intervals and all p-values will be two-sided without adjustment for multiple testing.

Primary outcome

Using two below approaches we will address the primary objective of the trial, i.e. test for any dose-response relationship between dosage group and stone recurrence.

First, we will test for a linear trend using a log-rank test stratified for the number of stones (<4 vs. ≥4 within 10 years prior to randomization) and present Kaplan-Meier curves by treatment dose.

Second, we will calculate hazard ratios between dosage groups using the Cox proportional hazards model stratified for number of stones at baseline (<4 vs. ≥4), with treatment dose as categorical variable and the placebo group as reference. We will consider exploratory the comparisons between placebo and each of the three active trial arms, because the trial size does not provide enough statistical power to detect differences with placebo.

Secondary outcomes

Components of the primary outcome measure (clinical and radiological recurrence) will be analyzed as the primary outcome.

Changes in urinary biochemistry from baseline to visits 4 (3 months), 5 (12 months), 6 (24 months) and 7 (36 months):

- urine volume,
- calcium,
- citrate,
- oxalate,
- sodium,
- pH,

will be analysed using a repeated-measures random-effects linear regression model in order to assess differences between treatment groups over time. Models will include fixed effects for the intervention group (continuous), the baseline value (continuous), the time point (categorical), an interaction term between group and time point, and an indicator for number of stones (<4 vs. ≥4), and a random intercept for the patient.

$$OUTCOME_{ij} \sim OUTCOME_{0j} + ARM_j + TIME_i + ARM_j * TIME_i + STRAT_j + (1|j),$$

where j is for subject and i is for visit number, i=0 being screening visit.

6.2.2 Secondary analyses

In a secondary per-protocol analysis, we will evaluate primary and secondary outcomes based on the PP analysis set.

We will use a multivariable Cox-model to assess the impact of baseline disease severity (<4 vs. ≥4 within 10 years prior to randomization) on stone recurrence; the impact of biochemical abnormalities on stone recurrence; and the impact of stone composition on stone recurrence using the categorization of exploratory variables from the paragraph 3.3.1.

6.2.3 Sensitivity analyses

We will compare the FAS and PP analysis of continuous outcomes based on multiple imputations with the analysis of all available cases.

We will assess the sensitivity of time-to-first-event approach (log-rank test, Kaplan-Meier curves and the Cox proportional hazards model) comparing it with multiple event models or frailty (count) models or marginal count models.

6.2.4 Subgroup analyses

The following baseline characteristics may modify the expected treatment effect:

- Baseline disease severity (defined by cut-offs at highest, middle and lowest tertiles in stone frequencies within the last 10 years prior to randomization.)

- Biochemical abnormalities (hypercalciuria vs. no hypercalciuria)

- Stone composition (calcium oxalate, calcium phosphate and other).

Although power may be too low for the definitive results, we will perform subgroup analyses for all outcomes according to the subpopulations above defined. All subgroup analyses will be accompanied by tests for interactions.

6.2.5 Additional analyses

- In the secondary analysis of the paragraph 6.2.2 in case of a relevant number of patients with multiple events (i.e. more than 5% of patients with more than one event), we will consider a shared-frailty Cox model for multiple recurrent events.
- We will additionally address the primary objective of the trial, i.e. the dose-response relationship for dosage groups and the stone recurrence hazard during the study by considering treatment dose as continuous variable and fitting a Cox proportional hazards model stratified for the number of stones (<4 vs. ≥4). In this model, we will assess the linear versus non-linear dependence of the dosage by introducing fractional polynomial dosage terms and evaluating their significance.
- In order to assess differences between treatment groups and occurrence of adverse events of special interest over time we will additionally analyze the changes in continuous safety outcomes from baseline to discharge (visits 4, 5, 6 and 7) using a repeated-measures linear random-effects model. Models will include fixed effects for the intervention group (continuous), the baseline value (continuous), the time point (categorical or continuous), an interaction term between group and time point, and baseline stratification for the number of stones (<4 vs. ≥4), and a random intercept for the patient:

$$OUTCOME_{ij} \sim OUTCOME_{0j} + ARM_j + TIME_i + ARM_j * TIME_i + STRAT_j + (1|j),$$

where j is for subject and i is for visit number, $i = 0$ being screening visit. The following variables from blood serum will be subject to this analysis: sodium, potassium, magnesium, creatinine, glucose (fasting), HbA1c, total cholesterol, LDL and HDL cholesterol, and triglycerides - In repeated-measures random-effects models of continuous secondary outcomes, we will also consider to use the treatment group categorical and the time point continuous, and will assess different relationships between the continuous variables and the outcome of interest using fractional polynomial functions.

6.2.6 Assessment of statistical assumptions

For all models, we will perform model validation i.e. check for the goodness-of-fit. In case of deviations from the applied models, to achieve a good fit to the data we will apply 1) common transformations and 2) non-parametric models.

6.3 Interim analyses

No formal interim analysis is planned.

6.4 Missing data

For the primary outcome, drop-outs will be censored at the last available visit.

Missing data for continuous outcomes will be handled by multiple imputation including the primary outcome in the multiple imputation model besides other baseline and follow-up characteristics.

We will assume missingness at random. Each outcome will be imputed separately. We will use relevant baseline variables, the respective outcome measure and relevant variables at all time-points as well as the treatment indicator as predictors in the imputation models. Age, BMI, systolic and diastolic blood pressure, heart rate, alcohol consumption, blood analysis values (i.e. potassium, magnesium, glucose, creatinine, uric acid and cholesterol) and urine analysis values (i.e. calcium, creatinine, citrate, oxalate and PH) will be used as continuous, the other variables will be treated as categorical. Binary variables with a frequency of less than 5% will be omitted. We will use multiple imputation by chained equation. In total, fifty imputed data sets will be generated, which will be analyzed as described by van Buuren [2].

6.5 Harms

Safety analysis will include a descriptive summary of pre-specified AEs of special interest, all SAEs, and vital signs. All safety endpoints will be reported separately for each treatment group based on the safety analysis data set.

No formal statistical testing will be applied.

6.6 Statistical software

We will use the R statistical software to calculate outcome and co-variate values, and to perform all analyses. We will list the R version and all used packages in the statistical report.

6.7 Quality control

A second statistician will double code the primary analysis from the raw data.

6.8 Changes from the protocol

The SAP is consistent with principle features of the statistical methods described in the protocol. Any deviation from the protocol is detailed hereunder with reason.

Header	Change	Reason

6.9 References

1. European Medicines Agency, C.f.H.M.P., *Draft ICH E9 (R1) addendum on estimands and sensitivity analysis in clinical trials to the guideline on statistical principles for clinical trials, step 2b*. 2017.
2. Rubin, D.B., *Multiple imputation for nonresponse in surveys*. Wiley series in probability and mathematical statistics Applied probability and statistics. 1987, New York etc.: John Wiley.
3. SOP Statistical data validation, CS_STA_SOP_02, version 03, 11.09.2018

Last/final SAP

Version 4.0

November 11th, 2021

Statistical Analysis Plan

Randomized double-blind placebo-controlled trial assessing the efficacy of standard and low dose hydrochlorothiazide treatment in the prevention of recurrent nephrolithiasis

Acronym of study: NOSTONE

Administrative Information

Project number:	640
Study title:	Randomized double-blind placebo-controlled trial assessing the efficacy of standard and low dose hydrochlorothiazide treatment in the prevention of recurrent nephrolithiasis
Trial registration number:	Swiss National Clinical Trials Portal: SNCTP000002101; ClinicalTrials.gov: NCT03057431
SAP version:	4.0 of 11.11.21
Protocol version:	1.7 (28.11.2019)

Authored by

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Revision history

Revision	Justification	Timing
1.0	First version	06.11.2018
2.0	**Definition**: Definitions of symptomatic and radiologic stone recurrence updated according to the last version of the protocol (i.e. 1.7). **Derivation & analysis of the primary outcome** 1. According to protocol (v1.7), stones occurring during the first 6 weeks after randomization will not be considered as events for the analysis. 2. Time to event analysis: In case of radiological recurrence, we will assume the date of the event to be equal to the date of the end of study CT. In case of multiple radiological stones, we will consider them as multiple events separated by 1h each. In case of a violation of the proportional hazard assumption, we will analyze the outcome as a binary outcome (i.e. occurrence of any symptomatic or radiologic stone recurrence yes/no). **Secondary outcomes** – Stone recurrence will be analyzed as a count outcome. The total number of stone recurrence will be assessed as: N(symptomatic stones) + N(radiologic stones) and analyzed using a generalized linear model. – Symptomatic recurrence will be analyzed as time to event as well as count outcome.	27.04.2020 Data still blinded; change implemented without inspection of data

	– Radiologic recurrence will be analyzed both as a binary outcome (i.e. yes/no) as well as a count outcome, but not as a time to event outcome. – Changes in urinary biochemistry : If the timing of urinary analysis is highly variable among patients, we will not consider time as a discrete but as a continuous variable.	
3.0	**Definition of the PP population** The following issues are not considered anymore as major protocol deviations: – Failure to perform primary endpoint assessment, i.e. assessment not done. We will only remove patients who didn't perform any visit/call: patients for whom neither the occurrence of symptomatic events nor the occurrence of radiological stone events have been assessed. – Missing treatment doses: we will use inverse probability censoring weighting methods to deal with non-compliance.	3.5.2021 Data still blinded; change implemented without inspection of data
3.0 updated in 4.0	**Derivation & analysis of the primary outcome** 1. Derivation Symptomatic events occurring more than 3 months after the end-of-study CT, will not be considered for the analysis Radiological results will only be used for analysis when the baseline CT was performed in a window of +/- 3 months around the randomization date, and the end-of-study CT less than 3 months after the last visit. In case of radiological recurrence, we will assume the date of the event to be equal to the date of the last performed visit or, if earlier, to the date of the end-of-study CT+3 months. In cases where no symptomatic stone events are reported and the radiological stone recurrence is not assessed (e.g. because of no CT performed at the end of study) we will use different strategies. - For the main analysis we will assume that, at the time of censoring no radiological stone occurred - In a first sensitivity analysis, we will assume the occurrence of a radiological stone 1h before censoring (i.e., worst case scenario)	3.5.2021 updated on 9.11.21 Data still blinded; change implemented without inspection of data

	- In a second sensitivity analysis we will remove all the patients without a radiological stone assessment.	
3.0	2. Analysis Instead of reporting the hazard ratios between dosage groups using the Cox proportional hazards model, we will calculate rate ratios with the Mantel–Cox method.	3.5.2021 Data still blinded; change implemented without inspection of data
4.0	**Derivation of the secondary outcomes** Radiological results will only be used for analysis when the baseline CT was performed in a window of +/- 3 months around the randomization date, and the end-of-study CT less than 3 months after the last visit. Symptomatic events occurring more than 3 months after the end-of-study CT, will not be considered for the analysis The number of radiologic stone recurrence will be approximated by the number of new radiological stones. For the biochemical outcomes, we will use the results of the 24 h urines collected with thymol and paraffin oil (i.e., oil measurements).	9.11.2021 Data still blinded; change implemented without inspection of data
3.0	**Sensitivity analysis** The primary outcome (i.e., occurrence of any symptomatic or radiologic stone recurrence yes/no) will be analyzed as a binary outcome in a sensitivity analysis.	3.5.2021 Data still blinded; change implemented without inspection of data
4.0	For the primary outcome as well as for the number of symptomatic stone events we will present a Kaplan-Meier curve including the events occurring in the first 6 weeks of the study and perform a landmark analysis at the 6 weeks landmark point.	9.11.2021 Data still blinded; change implemented without inspection of data
4.0	**Definition** Diabetes is defined as the occurrence of HbA1c 6.5% or higher; or treated (oral or sc antidiabetic or insulin).	9.11.2021 Data still blinded; change implemented without inspection of data

Approved by

Name	Affiliation	Study Role	Date and Signature (wet ink)
Marie Roumet, PhD	CTU Bern	Trial Statistician	[redacted]
Andreas Limacher, PhD	CTU Bern	Senior Statistician	
Sven Trelle, MD	CTU Bern	Senior Statistician	
Prof. Dr. med. Daniel Fuster	University Hospital Bern	Sponsor-Investigator	

As a minimum requirement, the main statistician, a supervising senior statistician and the sponsor/sponsor-investigator have to sign the SAP.

Contents

1 Introduction

1.1 Background and rationale

Background: Nephrolithiasis is a global healthcare problem with a current lifetime risk of 18.8% in men and 9.4% in women. Without specific treatment, 5- and 20-year recurrence rates are 40% and 75%, respectively. About 80–90% of stones are composed of calcium oxalate with various admixtures of calcium phosphate. Increased excretion of calcium in the urine, hypercalciuria, is the most common metabolic abnormality encountered in patients with recurrent nephrolithiasis. Thiazide diuretics have been the cornerstone of pharmacologic metaphylaxis for more than 40 years. The effect of thiazides to reduce the risk of stone recurrence has been attributed to their ability to decrease urinary calcium excretion. However, other factors, such as reduction of urinary pH and urinary oxalate excretion, probably contribute to this effect. Efficacy of thiazides on recurrence prevention of calcareous nephrolithiasis was tested in 11 randomized controlled trials (RCTs). With the exception of two trials, thiazides significantly reduced stone recurrence. Most of these trials are from the 1980's and 90's and the cumulative number of patients studied is remarkably low for such a prevalent disease. Our systematic review of these RCTs revealed major methodological deficiencies in all trials, including: lack of double-blinding and intention-to-treat analysis, unclear allocation concealment, lack of adverse event and drop out reporting and unknown baseline risk of disease severity. Furthermore, high doses of thiazides were employed in all trials, in the case of the best studied thiazide, hydrochlorothiazide (HCTZ), up to 100 mg daily. At such high doses, side effects occur frequently. Nowadays, thiazides are widely used in the treatment of recurrent nephrolithiasis and arterial hypertension, but at significantly lower doses. In the case of recurrent nephrolithiasis, however, this practice is not supported by randomized evidence and consequently, we do not know whether the currently employed low dose thiazide regimens are effective in reducing the risk for stone recurrence. Thus, evidence for benefits and harms of thiazide diuretics in the prevention of calcium-containing kidney stones in general remains unclear. In addition, the efficacy of the currently employed low dose thiazide regimens to prevent stone recurrence is not known.

Aim of the study: The aim of the study is to compare the efficacy and safety of standard and low dose HCTZ treatment in the recurrence prevention of kidney stones. More specifically, the study aims to describe the dose-response relationship for placebo and three different dosages of HCTZ.

Hypothesis: HCTZ significantly reduces stone recurrence compared to placebo. There is a significant dose-response relationship.

Design of the study: This is a multicenter, randomized, placebo-controlled, double blind, parallel-group trial.

Outcomes: The **primary outcome** as measured on the individual patient-level is stone recurrence during study treatment.

The primary outcome of the trial is the relationship between treatment group and stone recurrence during study treatment.

Secondary outcomes include:

- The individual components of the composite primary outcome:
    - symptomatic stone recurrence
    - radiologic stone recurrence
- Changes in urinary biochemistry from baseline to visits 4 (3 months), 5 (12 months), 6 (24 months) and 7 (36 months):
    - calcium,
    - citrate,
    - oxalate,

- sodium,
- total urine volume per 24 hours,
- pH.

1.2 Objectives

1.2.1 Primary objective

The primary objective is to assess the dose-response relationship between placebo and the three different dosages of HCTZ and stone recurrence.

1.2.2 Secondary objectives

Secondary objectives are to assess the efficacy of the different dosages of HCTZ in terms of the primary outcome as well as the individual components of the composite primary outcome, i.e. symptomatic stone recurrence and radiologic stone recurrence. Further objectives are to study effects of different dosages of HCTZ on urinary biochemistry (efficacy and safety aspects) and the impact of different baseline characteristics on stone recurrence and on the effects of the different dosages on stone recurrence (effect modification).

1.2.3 Safety objectives

The safety objective is to assess the long-term safety and tolerability of HCTZ compared to placebo.

2 Study methods

2.1 Trial design

NOSTONE is a randomized, placebo-controlled, observer-, clinician- and patient-blinded, parallel-group study.

Eligible patients will be randomized in equal proportions between 12.5 mg, 25 mg or 50 mg HCTZ or placebo. All subjects will be given the IMP (HCTZ or placebo) once daily in the morning. Placebo will be administered to subjects randomized to that treatment in a form identical to the HCTZ capsules. The first dose of the IMP will be administered the day after the randomization.

A total of 416 participants, 104 in each group, will be included in the study. Recruitment of participants is planned to occur over a period of 24 months at all 12 study sites. Study treatment will be 36 months for subjects enrolled in the first 12 months of the recruitment period and 24 months for subjects recruited in the remainder of the recruitment period.

2.2 Randomization

Stratified randomization is used to assign participants to the different trial arms with the number of previous stone events as stratification factors:

- Stratification group 1: 2 or 3 stone events within 10 years prior to randomization
- Stratification group 2: ≥ 4 stone events within 10 years prior to randomization.

Allocation is concealed using sequentially coded drug packs that are otherwise identical. Randomization lists were generated by a statistician at CTU Bern following dedicated standard operating procedures. The statistician communicated directly with the facility, which prepared the drug packs (Grosse Apotheke Dr. G. Bichsel AG, Interlaken, Switzerland). Investigators at each site assign the drug pack with the next sequential number to the next patient (consecutive). Content of the drug packs is based on randomization lists as described above.

To ensure allocation concealment, the statistician preparing the randomization list as well as the facility handling of the un-blinded drug packs are otherwise not involved in the trial; randomization lists are stored electronically at CTU Bern with no access for persons directly involved in the trial.

2.3 Sample size

A total of 416 patients, i.e. 104 in each arm, will be included in this study.

The sample size was calculated using the artsurv command in Stata and was based on the primary objective i.e. to assess the dose-response relationship between treatment group and the primary outcome, based on the following assumptions:

- Uniform recruitment over 24 months with allocation ratio fixed at 1 across all arms
- A maximum and minimum follow-up time of 36 and 24 months, respectively
- A cumulative drop-out rate of 10% at 24 months after study start
- Based on the available literature we assumed a risk of recurrence in the placebo group of 0.20 and 0.45 at 12 and 36 months after study start, respectively.
- Hazard ratios for the 12.5, 25 and 50 mg HCTZ doses of 0.90, 0.65 and 0.50, respectively. These effects were based on the literature review presented in Table 3.1-1 in the study protocol.
- Power was set to be at least 80% and alpha was fixed at a two-sided level of 0.05
- An unweighted log-rank test for linear trend with local alternatives.

2.4 Framework

The null hypothesis that there is no linear trend between dose and the survival function for the primary outcome (i.e. recurrence) will be tested against the alternative that there is a linear trend.

2.5 Statistical interim analyses and stopping guidance

There is no interim analysis planned.

2.6 Timing of final analysis

Analysis will start after completion of data export, preparation and validation as described in the paragraphs 3.1, 3.2 and 3.3 of the present document. Data validation starts at the beginning of the trial and goes on continuously until the validation of the last follow-up of the last patient.

The analysis will use all outcomes except for the dosage group variable as explained further in the paragraph 2.8.

2.7 Timing of outcome assessments

2.7.1 Participants recruited in the first 12 months of the recruitment period

Study Period	**Screening**		**Treatment period**								**Safety follow-up**
Visit	**1**	**2**	**3**	**4 or tel-ephone call**	**Tele-phone calls**	**5**	**Tele-phone calls**	**6**	**Tele-phone calls**	**7**	**Tele-phone call, EOS**
Visit time points	-14 to -1 day(s)	0	2 weeks	3 Months	6, 9 months	12 months	15, 18, 21 months	24 months/ EOT	27, 30, 33 months	36 months/ EOT	30 days after EOT
Allowed visit window		0	± 2 weeks	± 2 Weeks	± 4 weeks	± 4 weeks	± 4 weeks	± 4 weeks	± 4 weeks	± 2 weeks	± 2 weeks

2.7.2 Participants recruited ≥ 12 months after recruitment start

Study Period	**Screening**		**Treatment period**						**Safety follow-up**
Visit	**1**	**2**	**3**	**4 or tel-ephone call**	**Tele-phone call**	**5**	**Tele-phone call**	**6**	**Telephone call, EOS**
Visit time points	-14 to -1 day(s)	0	2 weeks	3 months	6, 9 months	12 months	15, 18, 21 months	24 months/ EOT	30 days after EOT
Allowed visit win-dow		0	± 2 weeks	± 2 weeks	± 4 weeks	± 4 weeks	± 4 weeks	± 4 weeks	± 2 weeks

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2.8 Blinding

All trial personnel is blinded to the assigned trial arm through all the stages of the study: recruitment, care of patients, trial assessment, monitoring - the only un-blinded people are 1) the independent statistician generating the randomization list; and 2) the personnel at the facility preparing the drug packs (Grosse Apotheke Dr. G. Bichsel AG, Interlaken, Switzerland). HCTZ and placebo are provided in identically looking capsules in plastic bottles with unique consecutive numbers.

Blinding will remain in place until the statistician codes the primary analysis of the primary and secondary outcomes and produces a dummy report of the primary analysis using a randomly generated group variable. The true group variable becomes open after the completion of the dummy report and gives place to the final report of all the analysis as well as the quality control by the independent statistician described in the paragraph 6.7.

3 Data management

3.1 Data export

Clinical study data are provided by the CTU Bern (University of Bern) in a database format (secuTrial®) and will be imported into R by the trial statistician for data preparation, validation and analysis.

3.2 Data validation

First line data validation is performed by the online eCRF system at real-time as defined in the data dictionary. Second line data validation and cleaning will be performed according to the SOP for data validation [3] with trimestrial Data Quality Review meetings during the data collection; and additionally after the completion of overall data collection, before database export for the final analysis.

3.3 Data preparation

We will derive the primary and the secondary outcomes from the data. Time will be calculated as days since randomization.

When recorded, calcium, citrate, oxalate, sodium and pH are measured in one (pH) or two (calcium, citrate, oxalate and sodium) 24-hour urine samples: 1 oil collection, 1 native collection. For the analysis, only the oil results are considered When recorded, heart rate, systolic and diastolic blood pressures are measured three times; the exact value will be approximated by the mean of these three measurements.

3.3.1 Categorization of data

3.3.1.1 Treatment groups:

Four treatment groups are considered, 12.5 mg, 25 mg or 50 mg HCTZ, or placebo.

3.3.1.2 Treatment centers:

There are twelve treatment centers in Switzerland:

- 7 Cantonal Hospitals: Aarau, Bellinzona, Chur, Lugano, Luzern, Sion and St. Gallen
- and 5 University Hospitals: Basel, Bern, Geneva, Lausanne, Zürich

3.3.1.3 Other

- Stratification group 1: 2 or 3 stone events within 10 years prior to randomization; stratification group 2: ≥ 4 stone events within 10 years prior to randomization.
- Baseline disease severity approximated by the number of stones within the last 10 years prior to randomization. The variable will be categorized into three groups using the first and the second tertiles as cut-offs. Patients with values below or equal to the first, second and third tertiles will be defined as patients with low, moderate disease and severe disease severity, respectively.
- Biochemical abnormality is defined as the occurrence of hypercalciuria.
- Hypercalciuria is defined as above 6.25 mmol/d (=250 mg/d) in women and above 7.5 mmol/d (=300 mg/d) in men.
- Biochemical abnormalities at baseline - hypercalciuria vs. no hypercalciuria in the baseline urine analysis.
- Hypertension is considered occurring when the systolic blood pressure exceeds 89 mmHg or when the diastolic blood pressure exceeds 139 mmHg or when the subject is treated by anti-hypertensive medication.

- Diabetes is defined as the occurrence of HbA1c 6.5% or higher; or treated (oral or sc antidiabetic or insulin
- Stone composition at baseline will be assessed from the composition of the last analyzed stone and categorized as
  (i) calcium oxalate (i.e. > 50% ca-ox)
  (ii) calcium phosphate (i.e. > 50% ca-p)
  (iii) other.

3.4 Outcome derivation

Table: Derivation of primary, secondary outcomes and some of the variables important for the primary and secondary analysis.

Outcome	Visit	eCRF sheet	Variable	Variable type	Derivation	Outcome type
Primary						
Stone event	Any visit or call	stone	stone_event	Binary: yes or no	none	Part of the primary outcome
Count of stone event per subject	Any visit or call	stone	sts_count	date	none	Part of the primary outcome
Date of the stone event (start date)	Any visit or call	stone	Sts_inc_date	date	none	Part of the primary outcome
HCTZ Dose group allocation[1]	Randomization	Separate blinded database		Group number	none	Part of the primary outcome
Secondary						
Total urine volume (ml), first collection	2,4,5,6,7	Urine Analysis	lab_ur1_tvol	Continuous, greater than 0: ml	none	Not an outcome without derivation
Total urine volume (ml), second collection	2,4,5,6,7	Urine Analysis	lab_ur2_tvol	Continuous, greater than 0: ml	none	Not an outcome without derivation
Duration (hrs) , first collection	2,4,5,6,7	Urine Analysis	lab_ur1_dur3	Continuous, greater than 0: hrs	none	Not an outcome without derivation
Duration (hrs) , second collection	2,4,5,6,7	Urine Analysis	lab_ur1_dur6	Continuous, greater than 0: hrs	none	Not an outcome without derivation
Urine Calcium, absolute excretion per 24hrs, first collection	2,4,5,6,7	Urine Analysis	lab_ur1_ca	Number continuous, greater than 0, detection limits are unknown	Absolute excretion per 24 hrs for calcium= lab_ur1_ca (mmol/l)* 1000ml*lab_ur1_tvol (ml)*24hrs/ lab_ur1_dur3	Secondary Continuous: mmol/24hrs

Outcome	Visit	eCRF sheet	Variable	Variable type	Derivation	Outcome type
Urine Calcium, absolute excretion per 24hrs, second collection	2,4,5,6,7	Urine Analysis	lab_ur2_ca	Number continuous, greater than 0, detection limits are unknown	Absolute excretion per 24 hrs for calcium= lab_ur2_ca (mmol/l)* 1000ml*lab_ur2_tvol (ml)*24hrs/ lab_ur1_dur6	Secondary Continuous: mmol/24hrs

[1] This variable will be un-blinded after the dummy report on primary and secondary analysis as described in paragraph 2.8

Urine Oxalate, absolute excretion per 24hrs, first collection	2,4,5,6,7	Urine Analysis	lab_ur1_oxa	Number continuous, greater than 0, detection limits are unknown µmol/l	Absolute excretion per 24 hrs for oxalate = lab_ur1_oxa (µmol/l)* 1000ml*lab_ur1_tvol (ml)*24hrs/ lab_ur1_dur3	Secondary Continuous: µmol/24hrs
Urine Oxalate, absolute excretion per 24hrs, second collection	2,4,5,6,7	Urine Analysis	lab_ur2_oxa	Number continuous, greater than 0, detection limits are unknown µmol/l	Absolute excretion per 24 hrs for oxalate = lab_ur2_oxa (µmol/l)* 1000ml*lab_ur2_tvol (ml)*24hrs/ lab_ur1_dur6	Secondary Continuous: µmol/24hrs
Urine Citrate, absolute excretion per 24hrs, first collection	2,4,5,6,7	Urine Analysis	lab_ur1_citr	Number continuous, greater than 0, detection limits are unknown mmol/l	Absolute excretion per 24 hrs for citrate = lab_ur1_citr (mmol/l)* 1000ml*lab_ur1_tvol (ml)*24hrs/ lab_ur1_dur3	Secondary Continuous: mmol/24hrs
Urine Citrate, absolute excretion per 24hrs, second collection	2,4,5,6,7	Urine Analysis	lab_ur2_citr	Number continuous, greater than 0, detection limits are unknown mmol/l	Absolute excretion per 24 hrs for citrate = lab_ur2_citr (mmol/l)* 1000ml*lab_ur2_tvol (ml)*24hrs/ lab_ur1_dur6	Secondary Continuous: mmol/24hrs
Urine Sodium, absolute excretion per 24hrs, first collection	2,4,5,6,7	Urine Analysis	lab_ur1_na	Number continuous, greater than 0, detection limits are unknown: mmol/l	Absolute excretion per 24 hrs for sodium = lab_ur1_na (mmol/l)* 1000ml*lab_ur1_tvol (ml)*24hrs/ lab_ur1_dur3	Secondary Continuous: mmol/24hrs
Urine Sodium, absolute excretion per 24hrs, second collection	2,4,5,6,7	Urine Analysis	lab_ur2_na	Number continuous, greater than 0, detection limits are unknown: mmol/l	Absolute excretion per 24 hrs for sodium = lab_ur2_na (mmol/l) * 1000ml * lab_ur2_tvol (ml) * 24hrs/ lab_ur1_dur6	Secondary Continuous: mmol/24hrs

3.5 Data sharing (if applicable)

Data will be shared as described in the Protocol: “Once results have been published, trial data will be accessible to external researchers and anonymized datasets corresponding to each publication will be made available. Investigators wishing to replicate the analyses or to do an individual patient meta-analysis may request the data from the steering committee. Access to data will be granted in an unbureaucratic way.”

4 Statistical principles

4.1 Confidence intervals and *P* values

All confidence intervals will be two-sided and relate to the 95% level and the two-sided significance level will be set at $\alpha = 0.05$.

4.2 Analysis populations

4.2.1 Full analysis set (FAS)

The full analysis set consists of all randomized patients regardless of whether they actually received the allocated intervention or not or any other protocol deviations. Patients will be analysed in the group they were randomized regardless of any cross-overs or loss to follow-up (intention-to-treat principle).

4.2.2 Per-protocol (PP)

The per-protocol population consists of all subjects in the FAS who did not have any protocol deviations that could confound the interpretation of analyses conducted on the FAS. The following are common major protocol deviations:

- Not receiving the allocated treatment.
- Not fulfilling the eligibility criteria.Failure to perform primary endpoint assessment, i.e. no visit/call performed

4.2.3 Dealing with non-compliance:

4.2.3.1 Definition of non-compliance

We will use two different definitions of non-compliance:

- First, we will consider as non-compliant participants who missed more than 20% of IMP during their study period (period when they were followed in the study).
- Second, we will only consider participants as non-compliant who missed more than 20% of IMP **voluntary** (i.e., without a medical indication as defined by the protocol) during their study period (period when they were followed in the study).

If the frequency of non-compliant patients exceeds 10%, additional per-protocol analyses that allow for more informative causal inferences [4] will be explored.

4.2.3.2 Assessment of non-compliance

Reasons for IMP stopping

Reasons for long temporary (more than 7 days in a row) and permanent IMP stops will be adjudicated and categorized as medical vs. voluntary. Short temporary stops (i.e., less than 7 days in a row) will be assumed to be voluntary.

Number of missed capsules
When a stopping period is reported (for long temporary & permanent stop), patients will be assumed to have missed 1 capsule/day.
In case of a short temporary stop, patients indicate the number of missed capsules in a categorical fashion. From this answer we will estimate the number of missed capsules since the last performed visit/call as follows:

Number of missed capsules, as reported by the patient	Estimated number of missed capsules since the last visit/call
1-5 tablets	3
6-10 tablets	8
11-20 tablets	16
more than 20	10 per each month since the last visit/call.

Capsules are assumed to be missing in a continuous period around the mid-point of the reporting period (i.e., [last visit/call – current visit]) with 1 missed capsule/day.

4.2.4 Safety population

In the safety analysis data set, patients will be included in the treatment group they actually received (at least one dose).

4.3 Estimands

The ICH E9 (R1) addendum on estimands and sensitivity analyses [1] defines different treatment estimators that are of interest in clinical trials. An estimand is the target of estimation to address the scientific question of interest posed by the trial objective. Attributes of an estimand include the population of interest, the outcome of interest, the specification of how intercurrent events such as cross-overs and non-compliance are reflected in the scientific question of interest, and the population-level summary for the outcome.

4.3.1 Treatment policy estimand

The estimand that addresses the main objective of this trial is based on the treatment policy strategy and resembles a classical intention-to-treat analysis.

We define the estimand with following attributes:

- Population: Full-Analysis Set
- Outcome:
    - The primary outcome i.e. stone recurrence defined as the composite of symptomatic and radiological recurrence
    - All secondary outcomes
- Effect measure:
    - Primary outcome: hazard ratio (odds ratio if needed, see section 6.1.1)
    - Secondary outcomes
        - Time-to-event: hazard ratio
        - Binary: odds ratio
        - Continuous: mean difference

- Handling of intercurrent events: cross-overs, drop-outs, non-adherence, or premature treatment stops are ignored

4.3.2 Additional estimands

Moreover, we will assess two additional estimands based on the per-protocol analysis sets:

- While-on-treatment estimand where medically justified treatment changes are ignored i.e. considered as part of the intended treatment
    - Population: per-protocol set according to definition 2 (see section 4.2.2)
    - Outcome:
        - The primary outcome i.e., stone recurrence defined as the composite of symptomatic and radiological recurrence
        - symptomatic stone recurrence
    - Effect measure: rate ratio
    - Handling of intercurrent events: subjects will be censored if they become non-compliant for a non-medically indicated, voluntary reason
- Hypothetical estimand where non-adherence is envisaged to not occur i.e. it reflects a scenario with perfect i.e. continuous treatment
    - Population: per-protocol set according to definition 1 (see section 4.2.2)
    - Outcome:
        - The primary outcome i.e., stone recurrence defined as the composite of symptomatic and radiological recurrence
        - symptomatic stone recurrence
    - Effect measure: rate ratio
    - Handling of intercurrent events: subjects will be censored if they become non-compliant for any reason

5 Trial Population

5.1 Screening data

No screening data were collected and the representativeness of trial sample cannot be described.

5.2 Eligibility

Individuals fulfilling all of the following inclusion criteria are eligible for the study:

- Informed Consent as documented by signature
- Age 18 years or older
- Recurrent kidney stone disease (≥ 2 stone events within the last 10 years prior to randomization)
- Any past kidney stone containing 50% or more of calcium oxalate, calcium phosphate or a mixture of both

The presence of any one of the following exclusion criteria will lead to exclusion of the individual:

1. Pharmacologic prevention for stone recurrence less than 3 months prior to randomization.
2. Patients with secondary causes of recurrent calcareous nephrolithiasis including:
    - Severe eating disorders (anorexia or bulimia);
    - Chronic inflammatory bowel disease, bariatric surgery, intestinal surgery with malabsorbtion or chronic diarrheal status;
    - Sarcoidosis;

- Primary hyperparathyroidism;
- Hyperthyroidism;
- Complete distal tubular acidosis;
- Active malignancy.

3. Patients with the following medications. According to the Protocol' criteria for withdrawal/ discontinuation of participants, the "IMP will be discontinued only if the patient took the medications listed in the exclusion criteria for more than 4 months":
   - Thiazide or loop diuretics;
   - Carbonic anhydrase inhibitors (including topiramate) ;
   - Xanthine oxidase inhibitors (febuxostat or allopurinol);
   - Alkali, including potassium citrate or sodium bicarbonate;
   - Treatment with 1,25-OH Vitamin D (calcitriol);
   - Calcium supplementation;
   - Bisphosphonates;
   - Denusomab;
   - Teriparatide;
   - Glucocorticoids.
4. Obstructive uropathy, if not treated successfully.
5. Urinary tract infection, if not treated successfully.
6. Chronic kidney disease (defined as CKD-EPI eGFR < 30 mL/min per 1,73 m2 body surface area for more than 3 months).
7. Patients with a kidney transplant.
8. > 3 gout arthritis episodes within one year prior to randomisation or gout arthritis requiring uric acid lowering therapy.
9. Cystinuria at screening.
10. Hypokalemia (blood potassium level < 3 mmol/L) at screening.
11. Hyponatremia (blood sodium level < 125 mmol/L) at screening.
12. Pregnant and lactating women [pregnancy test to be performed for women of child-bearing potential (defined as women who are not surgically sterilized/ hysterectomized, and/ or who are postmenonpausal for less than 12 months)].
13. Previous (within 3 months prior to randomization) or concomitant participation in another interventional clinical trial.
14. Inability to understand and follow the protocol.
15. Known allergy to the study drug.

5.3 Recruitment

A CONSORT patient flow diagram will be completed following the CONSORT 2010 standards (http://www.consort-statement.org/consort-2010).

The flow chart will consider specifically:

- Ns assessed for eligibility
- Ns eligible and not included into the trial
- Ns randomized
- Ns allocated to intervention
- Ns receiving allocated intervention
- Ns not receiving allocated intervention (with reasons)
- Ns discontinued intervention (with reasons)

- Ns lost to follow up (with reasons)
- Ns analyzed
- Ns excluded from primary analysis

5.4 Baseline patient characteristics

The patient characteristics of the FAS at baseline will be presented in a table, stratified by treatment arm, as number and percentage or mean and standard deviation for categorical and normally distributed continuous variables, respectively. For data severely deviating from a normal distribution, we will present median and interquartile ranges. No statistical comparisons of patient characteristics at baseline will be performed.

Total	Placebo n=	12.5mg HCTZ n=	25mg HCTZ n=	50 mg HCTZ n=
DEMOGRAPHICS				
Age, years (mean ± SD)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Sex (Female, n [%])	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
DISEASE SEVERITY AND STONE COMPOSITION				
Number of previous stone events within the 10 years prior to randomization	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
– [2,3] stones	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
– ≥4 stones	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
Number of previous stone events within the 10 years prior to randomization	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
– First tertile	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
– Second tertile	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
– Third tertile	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
Stone composition:				
– calcium oxalate (i.e. > 50% ca-ox)	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
– calcium phosphate (i.e. > 50% ca-p)	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
– other.	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
PHYSICAL EXAMINATION & VITAL SIGNS				
BMI	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Systolic blood pressure [2]	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Diastolic blood pressure[2]	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Heart rate[2]	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
HABITS & CARDIOVASCULAR RISK FACTORS				
Smoking				
– Never	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
– Current	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)

[2] Values averaged over 3 measurements

– Former	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
Alcohol consumption (unit/day)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Hypertension	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
Treated for hypertension (yes, n [%])	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
Diabetes (yes, n [%])	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
Treated for diabetes (yes, n [%])	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
Gout arthritis (yes, n [%])	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
RENAL SYSTEM				
Lower urinary tract infection(s) in the past (yes, n [%])	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
Upper urinary tract infection(s) in the past (yes, n [%])	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
Kidney malformations/Urinary tract malformations (yes, n [%])	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)
BLOOD ANALYSIS (SERUM)				
Potassium (K) (mmol/l)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Sodium (Na) (mmol/l)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Calcium (mmol/l)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Magnesium (mmol/l)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Bicarbonate (mmol/l)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Haemoglobin A1c (%)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Creatinine (umol/l)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Uric acid (µmol/l)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Total Cholesterol (mmol/l)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
HDL Cholesterol (mmol/l)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
LDL Cholesterol (mmol/l)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Triglycerides (mmol/l)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
URINE ANALYSIS (FIRST 24 HR COLLECTION, with oil and paraffin)				
Urine volume (l /24 h)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Calcium (mmol/24 h)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Sodium (mmol/24 h)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Citrate (mmol/24 h)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
Oxalate (mmol/24 h)	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
pH	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x	xx.x ± xx.x
BIOCHEMICAL ABNORMALITIES (urine based)				
Hypercalciuria (urine calcium > 7.5 mmol/d in men or > 6.25 mmol/d in women) (yes, n [%])	xx (xx.x)	xx (xx.x)	xx (xx.x)	xx (xx.x)

5.5 Adherence and protocol deviations

At each follow-up visit, participants are asked whether they were able to take their study medication every day. For each visit, we will present the number and percentage of patients who were or were not able to take their study medication every day. Among the patients who were not able to take their study medication every day, we will present the percentages of the following categories: missed 1-5 capsules, 6-10 capsules, 11-20 capsules, more than 20 capsules of the 91 expected in the last 3 months.

The common major protocol deviations are listed in the paragraph 4.2.2 above. The number of patients presenting each of these deviations will be summarized in a table.

5.6 Withdrawal/follow-up

The number of withdrawal and loss to follow-up at each visit in the control and in the treatment arm will be presented in a CONSORT patient flow diagram (for details see 5.3 above).

6 Analysis

6.1 Outcome definitions

6.1.1 Primary outcome

The primary outcome of the trial is the relationship between dose group and stone recurrence during study treatment (up to 36 months). We will analyze stone recurrence as time to event outcome.

The primary outcome at the patient level is stone recurrence during study treatment. Stone recurrence is the composite of symptomatic and radiological recurrence.

Symptomatic stone recurrence is defined as visible passage of a stone with or without accompanying typical symptoms such as flank/ loin pain and hematuria or a symptomatic or asymptomatic stone requiring urological intervention for stone removal. In case of potential stone passages without visible stones, it will be up to the local investigators to evaluate patients' symptoms and determine whether a stone passage occurred or the symptoms were rather due to a different cause.

Radiological stone recurrence as assessed by low-dose non-intravenous (iv) contrast CT imaging is defined as the appearance of new caliculi or enlargement of preexisting caliculi with reference to the baseline CT performed at visit 3 (2 weeks) to the end of study treatment. The processing and evaluation of the CT images is described in a specific SOP (SOP_CT_V1.3).

6.1.1.1 Outcome derivation:

Stone events:
Radiological stone recurrence will be assessed by comparing baseline to end of study CT. CT must be performed in a time window of three months.
When the CT are performed on time, we will consider (i) the time under observation as the interval [randomization, last performed visit], (ii) the date of (eventual) radiological event(s) as the date of the last performed visit.

In case of deviation from the study plan we will adopt different strategies:

- when the baseline CT was performed more than three months before or after the randomization: the radiological assessment will be considered as missing.
- when the end of study CT was performed more than three months after the last visit: the radiological assessment will be considered as missing.
- when the end of study CT was performed more than three months before the last visit: the time under observation will be calculated as = (date of end of study CT + 3 months) – randomization date. In case of a radiological event, we will consider the date of the event to be equal to the date of end of study CT + 3 months). Every symptomatic event occurring after (date of end of study CT + 3 months) will not be considered for the analysis.

According to protocol (v1.7), stone events occurring during the first 6 weeks after randomization will not be considered as events for the analysis.

In case of multiple stones events occurring on the same day, we will assume the events to be separated by 1h each.

Missing data

In cases where no symptomatic stone is reported and the radiological stone recurrence is not assessed (e.g. because of missing or not-on time CT) we will use different strategies.

- For the main analysis we will assume that, at the time of censoring no radiological stone occurred.
- In a first sensitivity analysis, we will assume the occurrence of radiological stone 1h before censoring (i.e., worst case scenario).
- In a second sensitivity analysis we will remove all the patients without a radiological stone assessment regardless of the occurrence (or not) of symptomatic stones.

6.1.2 Secondary outcomes

Secondary outcomes include:

- The number of stone recurrence(s)
- The individual components of the composite primary outcome:
    - Symptomatic stone recurrence (assessed as described for the primary outcome)
    - Number of symptomatic stone recurrence(s)
    - Radiologic stone recurrence(s)
    - Number of radiologic stone recurrence(s)
- Changes in urinary biochemistry: urinary volume[3], calcium, citrate, oxalate, sodium and pH at visits 4 (3 months), 5 (12 months), 6 (24 months) and 7 (36 months).

6.1.2.1 Outcome derivation:

As for the primary outcome:

- Radiological results will only be considered as missing when the baseline CT was not performed in a window of +/- 3months around the randomization date, and/or the end-of-study CT was performed more than 3months after the last visit.
- Symptomatic stones occurring during the first 6 weeks after randomization or more than three months after the end of study CT, will not be considered for the analysis of number of stone recurrence, symptomatic stone recurrence and number of symptomatic stone recurrence(s).

Because of technical limitations, the number of radiologic stone recurrence could not be assessed directly, we will use the number of new radiological stone as a proxy of this outcome.

The total number of stone recurrence will be assessed as: N(symptomatic stones) + N(new radiologic stones).

For the biochemical outcomes we will use the urine collected with addition of thymol and paraffin oil (i.e., oil measurements).

[3] Urinary volume per se is not biochemistry, however it is an absolute prerequisite for properly evaluating the eventual biochemistry changes as for equal microelement excretions per 24 hours its urinary concentrations might differ depending on volume.

6.1.3 Assessment of safety outcomes

6.1.3.1 Serious adverse events (SAEs)

SAEs will be collected, fully investigated and documented in the source documents and the eCRF for all participants from the date of ICF signature until the last protocol-specific procedure has been completed, including a safety follow-up period of 30 days.

Adverse events (AEs) of special interest

For this trial, (non-serious) AEs will not be documented in the eCRF with exception of the following events "of special interest" occurring from the date of randomization (visit 2) until visit 7 (36 months):

- Hypokalemia, defined as blood potassium level < 3 mmol/L;
- Hyponatremia, defined as blood sodium level < 125 mmol/L;
- Hypomagnesemia, defined as blood magnesium level < 0.5 mmol/L;
- Blood creatinine > 150% of baseline creatinine;
- Newly developed overt diabetes;
- Allergic reaction of skin if considered by the local investigator to be potentially related to the study medication;
- Gouty arthritis if recurrence > 3 times per year or requiring Uric acid lowering therapy.

To assess the AEs of special interest the following blood biochemistry parameters are collected as safety outcome: potassium, sodium, magnesium, creatinine, glucose (fasting), HbA1c at visits 4 (3 months), 5 (12 months), 6 (24 months) and 7 (36 months). These parameters will be assessed according to the protocol. Moreover, we will perform the additional analysis as below in the paragraph 6.2.5.

6.1.4 Assessments in participants who prematurely stop the study

Participants who permanently discontinue the study treatment will undergo all protocol-specified study visits and assessments. If study visits are not possible, a telephone consultation will be performed to determine if relevant health events/ endpoints have occurred.

6.2 Analysis methods

6.2.1 Primary analysis

The CTU Bern statistician will perform the analysis of the study. The statistician will be first blinded to the group allocation, then un-blinded as described in the paragraph 2.8. In the primary analysis, all patients will be analysed using the FAS according to the intention-to-treat principle. All effect measures will be accompanied by 95% confidence intervals and all p-values will be two-sided without adjustment for multiple testing.

Primary outcome

Using the below approach, we will address the primary objective of the trial, i.e. test for any dose-response relationship between dosage group and stone recurrence.

Investigate the treatment effect on the occurrence (or not) of stone recurrence.

We will test for a linear trend using a log-rank test stratified for the number of stones (<4 vs. ≥ 4 within 10 years prior to randomization) and present Kaplan-Meier curves by treatment dose. We will then calculate rate ratios between dosage groups using the Mantel–Cox method, stratified for number of stones at baseline (<4 vs. ≥ 4).
In a sensitivity analysis we will analyze the primary outcome as a binary outcome (i.e. occurrence of any symptomatic or radiologic stone recurrence yes/no, see section 6.2.3). We will consider the comparisons between placebo and each of the three active trial arms as exploratory, because the trial size does not provide enough statistical power to detect differences between each active arm and the placebo group.

6.2.1.1 Secondary outcomes

The total number of stone recurrence will be analysed using a generalized linear model including the treatment dose as categorical variable and adjusted for the number of stone events at baseline (<4 vs. ≥ 4). Depending on the distribution of the number of stones, we will use a negative binomial or a zero inflated negative binomial regression. In case of zero inflation we will investigate the effect of treatment on both the count and the logit part of the model.

Symptomatic recurrence will be analyzed as the primary outcome.

Number of symptomatic stone recurrence(s) will be analyzed as the total number of stone recurrence(s) (see above)

Radiologic recurrence will analyzed both as a binary outcome (i.e. yes/no) and then as a count variable using the methodology described for the total number of stone recurrence(s).

Changes in urinary biochemistry from baseline to visits 4 (3 months), 5 (12 months), 6 (24 months) and 7 (36 months):

- urine volume,
- calcium,
- citrate,
- oxalate,
- sodium,
- pH,

will be analysed using a repeated-measures random-effects linear regression model in order to assess differences between treatment groups over time. Models will include fixed effects for the intervention group (continuous), the baseline value (continuous), the time point (categorical), an interaction term between group and time point, and an indicator for number of stones (<4 vs. ≥ 4), and a random intercept for the patient.

$$OUTCOME_{ij} \sim OUTCOME_{0j} + ARM_j + TIME_i + ARM_j * TIME_i + STRAT_j + (1|j),$$

where j is for subject and i is for visit number, i=0 being screening visit.

If the timing of urinary analysis is highly variable among patients, we will not consider time as a discrete but as a continuous variable.

6.2.2 Secondary analyses

In a secondary per-protocol analysis, we will evaluate primary and secondary outcomes based on the PP analysis set.

We will use a multivariable Cox-model to assess the impact of baseline disease severity (<4 vs. ≥ 4 within 10 years prior to randomization) on stone recurrence; the impact of biochemical abnormalities on

stone recurrence; and the impact of stone composition on stone recurrence using the categorization of exploratory variables from the paragraph 3.3.1.

6.2.3 Sensitivity analyses

In case of undetermined results for the primary outcome (i.e., no symptomatic stone and no radiologic assessment), the primary analysis will be done by

(I) Assuming occurrence of a radiological stone 1h before censoring
(II) Removing patients with a missing radiologic assessment, regardless of the occurrence (or not) of symptomatic stones

For the primary outcome as well as for the number of symptomatic stone we will present a Kaplan-Meier curve including the events occurring in the first 6 weeks of the study and perform a landmark analysis at the 6 weeks landmark point.

We will analyze the primary outcome as a binary outcome (i.e. occurrence of any symptomatic or radiologic stone recurrence yes/no). To do so we will use a logistic regression model including the treatment dose as categorical variable and adjusted for the number of stone events at baseline (<4 vs. ≥ 4) and the time under observation.

We will compare the FAS and PP analysis based on multiple imputations with the analysis of all available cases.

We will assess the sensitivity of time-to-first-event approach (log-rank test, Kaplan-Meier curves and the Mantel-Cox rate ratios) comparing it with multiple event models or frailty (count) models or marginal count models.

6.2.4 Subgroup analyses

The following baseline characteristics may modify the expected treatment effect:

- Baseline disease severity (defined by cut-offs at highest, middle and lowest tertiles in stone frequencies within the last 10 years prior to randomization.)

- Biochemical abnormalities (hypercalciuria vs. no hypercalciuria)

- Stone composition of the last analyzed stone at baseline (calcium oxalate, calcium phosphate and other).

Although power may be too low for the definitive results, we will perform subgroup analyses for the primary outcome as well as for the occurrence of symptomatic stone outcomes according to the subpopulations above defined. All subgroup analyses will be accompanied by tests for interactions.

6.2.5 Additional analyses

- In the secondary analysis of the paragraph 6.2.2 in case of a relevant number of patients with multiple symptomatic stone events (i.e. more than 5% of patients with more than one event), we will consider a shared-frailty Cox model for multiple recurrent events.
- We will additionally address the primary objective of the trial, i.e. the dose-response relationship for dosage groups and the stone recurrence hazard during the study by considering treatment dose as continuous variable and fitting a Cox proportional hazards model stratified for the number of stone (<4 vs. ≥ 4). In this model, we will assess the linear versus non-linear dependence of the dosage by introducing fractional polynomial dosage terms and evaluating their significance.
- In order to assess differences between treatment groups and occurrence of adverse events of special interest over time we will additionally analyze the changes in continuous safety outcomes from baseline to discharge (visits 4, 5, 6 and 7) using a repeated-measures linear random-effects model.

Models will include fixed effects for the intervention group (continuous), the baseline value (continuous), the time point (categorical or continuous), an interaction term between group and time point, and baseline stratification for the number of stones (<4 vs. ≥ 4), and a random intercept for the patient:

$$OUTCOME_{ij} \sim OUTCOME_{0j} + ARM_j + TIME_i + ARM_j * TIME_i + STRAT_j + (1|j),$$

where j is for subject and i is for visit number, $i = 0$ being screening visit. The following variables from blood serum will be subject to this analysis: sodium, potassium, magnesium, creatinine, glucose (fasting), HbA1c, total cholesterol, LDL and HDL cholesterol, and triglycerides - In repeated-measures random-effects models of continuous secondary outcomes, we will also consider to use the treatment group categorical and the time point continuous, and will assess different relationships between the continuous variables and the outcome of interest using fractional polynomial functions.

6.2.6 Causal inferences for PP analysis

Analysis will be conducted separately for the first and second definition of compliance (see section 4.2.3).

Subject will be regarded as dependently censored at the day a subject withdraws, is lost to FUP or at the first time a subject becomes non-compliant. We will structure our dataset into time intervals. At each time interval a subject will be considered as non-compliant if he or she was non-compliant before or if the cumulative number of (voluntary) missed capsules reached 20% of the cumulative number of expected number of taken capsules. If possible, we will use all the observed event or treatment censoring times in the sample to define the intervals. Otherwise we will consider three months intervals.

We will then use the inverse probability of censoring weighting technique (IPCW) to recreate an unbiased scenario where

- subjects remain on their therapy always (using definition 1 of non-compliance), i.e. subjects will be censored if they become non-compliant for any reason
- subjects remain on their therapy until discountinuation of the therapy becomes medically indicated (using definition 2 of non-compliance), i.e. subjects will only be censored if they become non-compliant for a non-medically indicated, voluntary reason

IPCW weights corresponding to the inverse probability of censoring, will be calculated separately for the first and second definition of non-adherence. Depending on the frequency and pattern of censoring the probability of censoring will be calculated either as the product of the probability of being censored for “classical reasons” (i.e., lost to FUP, withdraw) and the probability of being “non-compliant”; or as the probability of being censored for whatever reason (i.e., lost to FUP, withdraw or non-adherence).

We will use a logistic model or a Cox proportional hazards model for censoring to calculate the probabilities. This model will include baseline and time-dependent prognostic factors as covariates. Baseline predictors will include the randomization group as well as baseline variables (as described in the BL table section 5.4). Time dependent covariates will notably include the blood and urine values, urinary biochemistry (i.e., urinary volume, calcium, citrate, oxalate, sodium and pH), blood pressure and values, heart rate, number of AE and SAE event.

6.2.7 Assessment of statistical assumptions

For all models, we will perform model validation i.e. check for the goodness-of-fit. In case of deviations from the applied models, to achieve a good fit to the data we will apply 1) common transformations and 2) non-parametric models.

6.3 Interim analyses

No formal interim analysis is planned.

6.4 Missing data

For the primary outcome, drop-outs will be censored at the last available visit.

For count data assessed over time (i.e., number of symptomatic, and total stone recurrence), we will adjust observation time if patients drop out.

For changes in urinary biochemistry from baseline to visits 4 (3 months), 5 (12 months), 6 (24 months) and 7 (36 months) we will use repeated-measures models that implicitly account for missing data as long as the baseline and at least one follow-up value is available.

If missing data are problematic for the primary analyses of primary or secondary outcomes, multiple imputation will be used as sensitivity analysis. Missing data will be considered as problematic where their proportions for individual variables exceed 10%.

Missing data will be handled by multiple imputation including the primary outcome in the multiple imputation model besides other baseline and follow-up characteristics.

We will assume missingness at random. Each outcome will be imputed separately. We will use relevant baseline variables, the respective outcome measure and relevant variables at all time-points as well as the treatment indicator as predictors in the imputation models. Age, BMI, systolic and diastolic blood pressure, heart rate, alcohol consumption, blood analysis values (i.e. potassium, magnesium, glucose, creatinine, uric acid and cholesterol) and urine analysis values (i.e. calcium, creatinine, citrate, oxalate and PH) will be used as continuous, the other variables will be treated as categorical. Binary variables with a frequency of less than 5% will be omitted. We will use multiple imputation by chained equation. In total, fifty imputed data sets will be generated, which will be analyzed as described by van Buuren [2].

6.5 Harms

Safety analysis will include a descriptive summary of pre-specified AEs of special interest, all SAEs, and vital signs. All safety endpoints will be reported separately for each treatment group based on the safety analysis data set.

No formal statistical testing will be applied.

6.6 Statistical software

We will use the R statistical software to calculate outcome and co-variate values, and to perform all analyses. We will list the R version and all used packages in the statistical report.

6.7 Quality control

A second statistician will double code the primary analysis from the raw data.

6.8 Changes from the protocol

The SAP is consistent with principle features of the statistical methods described in the protocol. Any deviation from the protocol is detailed hereunder with reason.

Header	Change	Reason
Additional secondary outcomes	Overall, symptomatic and radiologic stone recurrence will be analyzed as count outcomes. The total number of stone recurrence will be assessed as: N(symptomatic stones) + N(radiologic stones) and analyzed using a generalized linear model.	The total number of stone is a measure of interest.
Change in secondary outcomes	Radiologic recurrence will be analyzed both as a binary outcome (i.e. yes/no) as well as a count outcome, but not as a time to event outcome.	Radiological recurrence is assessed by scan at the end of the study: we don't know the exact time of each event.
Change in the analysis of the primary outcome	Instead of reporting the hazard ratios between dosage groups using the Cox proportional hazards model, we will calculate the rate ratios with Mantel–Cox method	Proportional hazard assumption will probably not be fulfilled, and rate ratio is the statistics corresponding to the log-rank test (i.e., test used for testing the occurrence of a linear trend)
Change in diabetes definition	Diabetes is defined as the occurrence of HbA1c 6.5% or higher; or treated (oral or sc antidiabetic or insulin).	Fasting status during blood draw was not consistently reported during study visits. Hence glucose measurements will not be suitable for diabetes definition.
Change in derivation of secondary outcome	The number of radiologic stone recurrence will be approximated by the number of new radiological stones.	The number of radiological stone recurrence cannot be assessed

6.9 References

1. European Medicines Agency, C.f.H.M.P., *Draft ICH E9 (R1) addendum on estimands and sensitivity analysis in clinical trials to the guideline on statistical principles for clinical trials, step 2b.* 2017.
2. Rubin, D.B., *Multiple imputation for nonresponse in surveys*. Wiley series in probability and mathematical statistics Applied probability and statistics. 1987, New York etc.: John Wiley.
3. SOP Statistical data validation, CS_STA_SOP_02, version 03, 11.09.2018
4. https://arxiv.org/abs/1911.06030

Revision history

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Revision history

Revision	Justification	Timing
1.0	First version	06.11.2018
2.0	**Definition**: Definitions of symptomatic and radiologic stone recurrence updated according to the last version of the protocol (i.e. 1.7). **Derivation & analysis of the primary outcome** 1. According to protocol (v1.7), stones occurring during the first 6 weeks after randomization will not be considered as events for the analysis. 2. Time to event analysis: In case of radiological recurrence, we will assume the date of the event to be equal to the date of the end of study CT. In case of multiple radiological stones, we will consider them as multiple events separated by 1h each. In case of a violation of the proportional hazard assumption, we will analyze the outcome as a binary outcome (i.e. occurrence of any symptomatic or radiologic stone recurrence yes/no). **Secondary outcomes** – Stone recurrence will be analyzed as a count outcome. The total number of stone recurrence will be assessed as: N(symptomatic stones) + N(radiologic stones) and analyzed using a generalized linear model. – Symptomatic recurrence will be analyzed as time to event as well as count outcome.	27.04.2020 Data still blinded; change implemented without inspection of data

	– Radiologic recurrence will be analyzed both as a binary outcome (i.e. yes/no) as well as a count outcome, but not as a time to event outcome. – Changes in urinary biochemistry : If the timing of urinary analysis is highly variable among patients, we will not consider time as a discrete but as a continuous variable.	
3.0	**Definition of the PP population** The following issues are not considered anymore as major protocol deviations: – Failure to perform primary endpoint assessment, i.e. assessment not done. We will only remove patients who didn't perform any visit/call: patients for whom neither the occurrence of symptomatic events nor the occurrence of radiological stone events have been assessed. – Missing treatment doses: we will use inverse probability censoring weighting methods to deal with non-compliance.	3.5.2021 Data still blinded; change implemented without inspection of data
3.0 updated in 4.0	**Derivation & analysis of the primary outcome** 1. Derivation Symptomatic events occurring more than 3 months after the end-of-study CT, will not be considered for the analysis Radiological results will only be used for analysis when the baseline CT was performed in a window of +/- 3 months around the randomization date, and the end-of-study CT less than 3 months after the last visit. In case of radiological recurrence, we will assume the date of the event to be equal to the date of the last performed visit or, if earlier, to the date of the end-of-study CT+3 months. In cases where no symptomatic stone events are reported and the radiological stone recurrence is not assessed (e.g. because of no CT performed at the end of study) we will use different strategies. - For the main analysis we will assume that, at the time of censoring no radiological stone occurred - In a first sensitivity analysis, we will assume the occurrence of a radiological stone 1h before censoring (i.e., worst case scenario)	3.5.2021 updated on 9.11.21 Data still blinded; change implemented without inspection of data

	- In a second sensitivity analysis we will remove all the patients without a radiological stone assessment.	
3.0	2. Analysis Instead of reporting the hazard ratios between dosage groups using the Cox proportional hazards model, we will calculate rate ratios with the Mantel–Cox method.	3.5.2021 Data still blinded; change implemented without inspection of data
4.0	**Derivation of the secondary outcomes** Radiological results will only be used for analysis when the baseline CT was performed in a window of +/- 3 months around the randomization date, and the end-of-study CT less than 3 months after the last visit. Symptomatic events occurring more than 3 months after the end-of-study CT, will not be considered for the analysis The number of radiologic stone recurrence will be approximated by the number of new radiological stones. For the biochemical outcomes, we will use the results of the 24 h urines collected with thymol and paraffin oil (i.e., oil measurements).	9.11.2021 Data still blinded; change implemented without inspection of data
3.0	**Sensitivity analysis** The primary outcome (i.e., occurrence of any symptomatic or radiologic stone recurrence yes/no) will be analyzed as a binary outcome in a sensitivity analysis.	3.5.2021 Data still blinded; change implemented without inspection of data
4.0	For the primary outcome as well as for the number of symptomatic stone events we will present a Kaplan-Meier curve including the events occurring in the first 6 weeks of the study and perform a landmark analysis at the 6 weeks landmark point.	9.11.2021 Data still blinded; change implemented without inspection of data
4.0	**Definition** Diabetes is defined as the occurrence of HbA1c 6.5% or higher; or treated (oral or sc antidiabetic or insulin).	9.11.2021 Data still blinded; change implemented without inspection of data