Clinical Study Protocol
Oxalobacter formigenes (Oxabact)

A Phase III Double-Blind, Randomized Study to Evaluate the Long-Term Efficacy and Safety of Oxabact in Patients with Primary Hyperoxaluria.

Protocol Number	OC5-DB-02
Protocol Name	ePHex
EudraCT Number	2017-000684-33
IND Number	15881
NCT Number	NCT 03116685
Sponsor:	OxThera Intellectual Property AB Regeringsgatan 111 SE-111 39 Stockholm Sweden
Sponsor signee:	Bastian Dehmel, MD, Chief Medical Officer
Protocol Date:	08 January 2021
Protocol Version:	11

Revision History

Rev.:	Version No.	Description:	Date:
000	1	New document	2016-04-13
001	2	First draft revision in line with comments from EMA.	2016-06-29
002	3	Second draft revision	2016-12-28
003	4	Third draft revision	2017-01-23
004	5	Final protocol	2017-02-10
005	6	Amendment 1 – UK specific	2017-06-27
006	7	Amendment 2 • Spain and Belgium added to list of participating countries. • Clarified that a separate Clinical Trial Application will be issued for the follow-up study. • Safety analyses will be done at a central lab instead of at the local lab. • Clarified process for patients with AKI during baseline. • Clarified that in the event there is a need to know the identity of the drug given to a patient, the decision to unblind the treatment resides solely with the investigator. • Minor corrections and clarifications.	2017-09-21
007	8	Amendment 3. • Reporting of Pregnancy during the study added to the Safety section. • Information added about the Data and Safety Monitoring Board. • Sample size section updated. • Schedule of assessments updated.	2018-03-28

		• The previous numbering of references (superscription format) have been replaced by Author name and year. • Reference list corrected and updated in alphabetical order. • Minor corrections and clarifications.	
008	9	Amendment 4 • Secondary and exploratory endpoints have been reorganized. • *Frequency of kidney stone events* has been added under key secondary endpoints. • Information added that kidney stone events and related symptoms will be captured throughout the study. • Clarified that if an acute kidney injury/stone event occurred close to a scheduled visit, the visit would be rescheduled once the AKI/stone event was resolved. • Tunisia added to list of participating countries. • Statistical sections updated in line with revised endpoints. • Minor corrections and clarifications.	15 October 2018
009	9.1	Amendment 5 • Clarification that for determination of eligibility, estimated Glomerular Filtration Rate (eGFR) can be calculated using Schwartz or CKD-EPI equations that include serum creatinine and/or cystatin C. *Please note, that Amendment 5 was a country-specific Amendment for Spain and the US.*	06 Sept 2019
010	10	Amendment 6 • Includes revisions made in Amendment 5. • Implementation of post-treatment safety follow-up (mainly addressed in sections 6.2 and 6.3). Consequently, the definition of "End-of-Study" was updated and "End-of-Treatment" was defined. • Updated section 7.4. *Withdrawal criteria* to include "The subject requires dialysis"	19 Dec 2019

		• Updated list of blind data and added clarifications in section 8.4. • Updated section 11.4 *Reporting of Adverse Events* to harmonise with the Safety Management plan for this study (Vs 4.0 dated 24 Jun 2019). • Updated section 11.5.3 to refer to Investigator Brochure; this was to harmonise with the protocol of the OC5-OL-01 and OC5-OL-02 studies. • Added updates and clarifications in section 12 Statistical Methods including tests of hypotheses and significance levels, information on methods used to analyse endpoints and definition of treatment-emergent AEs. Added additional subgroups. • Minor updates, corrections and clarifications throughout the protocol.	
011	11	Amendment 7 • Added clinical success criteria in section 5.3 • Updated section 7.4. *Withdrawal criteria* to clarify which assessments should be done for early withdrawal. • Added 2 new other endpoints (Percent change from baseline in total plasma oxalate concentration and Subjects achieving 'near-normalization' in total plasma oxalate concentration) in section 10. • Added updates and clarifications in section 12 relating to hierarchial testing. • Updated OC5-OL-01 study information throughout the protocol. • Minor updates, corrections and clarifications throughout the protocol. (N.B. "Patient" has been replaced with "subject" throughout the protocol as appropriate).	08 Jan 2021

Study protocol approval

Protocol number: OC5-DB-02

Protocol Date: 08 January 2021

Protocol Version: 11

Study title: A phase III double-blind, randomized study to evaluate the long-term efficacy and safety of Oxabact in patients with primary hyperoxaluria.

Sponsor: OxThera Intellectual Property AB
Regeringsgatan 111
SE-111 39 Stockholm
Sweden

This protocol has been approved by:

Bastian Dehmel, MD
Chief Medical Officer
OxThera AB, Stockholm, Sweden
Sponsor's representative

Signature: B. Dehmel

Date: 12-JAN-2021
(DD –MMM – YYYY)

Dr Gesa Schalk
Coordinating Investigator
Kindernierenzentrum Bonn, Germany

Signature:

Date: 12-JAN-2021
(DD –MMM – YYYY)

Procedures in Case of Emergency

Table 1: Emergency Contact Information

Role in Study	Name	Telephone number	Fax number
Drug Safety / Medical Monitor	Yves Miclo	+44 1223 402660	+44 1223 413689
Clinical Study Leader at sponsor	Maria Norling	+46 73 987 00 78	

1 SYNOPSIS

Name of Sponsor/Company:
OxThera IP AB

Name of Investigational Product:
Oxabact (OC5)

Name of Active Ingredient:
Oxalobacter formigenes, strain HC-1

Title of Study:
A phase III double-blind, randomized study to evaluate the long-term efficacy and safety of Oxabact in patients with primary hyperoxaluria.

Planned Number of Sites/Countries
The study is planned to be conducted in the following countries:
Germany, USA, France, UK, the Netherlands, Spain, Belgium, Tunisia.
9-11 sites will be participating.

Phase of Development:
III

Objectives:
Primary:

- To evaluate the efficacy of Oxabact following 52 weeks treatment in patients with maintained kidney function but below the lower limit of the normal range (eGFR < 90 ml/min/1.73 m^2) and a total plasma oxalate concentration ≥ 10 µmol/L.

Secondary:

- To obtain additional safety data from 52 weeks continuous treatment with Oxabact.

Clinical Success Criteria for total plasma oxalate
Oxabact will be considered associated with a clinically meaningful treatment effect on total plasma oxalate, if the results meet the following criteria:

1. Statistically significant difference in change from baseline for total plasma oxalate concentration between Oxabact and placebo in favor of Oxabact after 52 weeks of treatment.

AND

2. Estimated difference in absolute change from baseline for total plasma oxalate concentration between Oxabact and placebo of ≥5 µmol/L after 52 weeks of treatment.
   OR

Estimated difference in percent change from baseline for total plasma oxalate concentration between Oxabact and placebo of ≥30% after 52 weeks of treatment.

AND

3. Greater percentage of subjects in the Oxabact arm than in the placebo arm achieving near-normalization of total plasma oxalate concentration (< 10 μmol/L) at least twice during weeks 24 to 52 of treatment.

Methodology:

This double-blind, randomized international multi-center study will evaluate the efficacy and safety of OC5 in patients with PH. The study will enrol subjects with maintained renal function but with an eGFR below the lower limit of the normal ranges (<90 ml/min/1.73 m^2) and a total plasma oxalate concentration ≥ 10 μmol/L.

During the screening/baseline period of up to 8 weeks, in total 3 visits will be made. Visit 1 will be the screening visit. Information on historical renal function (including eGFR, serum creatinine and equation used to calculate historic eGFR) and kidney stone events (prior to informed consent) will be collected at the screening visit. For subjects under 18 years of age and subjects that were <18 years up to 3 years prior to screening (i.e. for all subjects under 21 years at screening), height used to calculate historic eGFR will also be collected. During the baseline period, samples will be taken for analysis of plasma oxalate, serum creatinine and serum cystatine C. Serum will also be analysed for magnesium, phosphorus, citrate, calcium, glycolate, ALP, bicarbonate, CRP, WBC and blood urea nitrogen (BUN).

During the baseline period, two echocardiography (Speckle Tracking and traditional) examinations will be done as well as one ultrasound of the kidney. Patient Quality of Life will be evaluated using a questionnaire. Two 24-hour urine samples will be taken for determination of magnesium, phosphorus, citrate, calcium, glycolate, urea, creatinine, calcium oxalate crystals, pH, osmolality, urinary volume and oxalate excretion and one stool sample will be analysed for number of *O. formigenes*. The urine and stool samples will be taken at the subjects´s home during the baseline period. Information on stone events and related symptoms will be collected during visits 2 and 3 of the baseline period.

After the baseline period (at visit 3), eligible subjects will be randomized to start on twice daily dosing of Oxabact or placebo in a 1:1 ratio. Subjects will be stratified in a first step in an attempt to evenly distribute PH type 2/type 3 patients, if the subject is not PH type 2 or PH type 3, the subject will be stratified for baseline urinary oxalate excretion above or below or equal to 1.87 mmol/24h/1.73 m^2 (based on the mean of the two values from screening/baseline).

Randomized subjects will have visits to the clinic at week 8, 16, 24, 32, 40, 48 and 52. Plasma sample will be collected at week 8, 16, 24, 32, 40, 48 and 52. Subjects will be asked to provide 24-hour urine collections at week 8, 24, 40 and 52 and faecal samples at weeks 24, 40 and 52. Echocardiography will be performed at week 24 and 48 and ultrasound of the kidney at week 48. Safety evaluation will include physical examination and safety labs at week 8, 16, 24, 32, 40, 48 and 52. Patient Quality of Life will be evaluated using a questionnaire at week 8, 24, 40 and 52. After the treatment phase, subjects will be followed for safety for 2 weeks or until the day before first dose date in the follow-up study OC5-OL-02, whichever occurs first.

Adverse events and concomitant medication will be monitored throughout the study.
Information on kidney stone events and related symptoms occurring between signature of the informed consent form and the end of study will be captured.
In the case of an acute kidney injury (AKI) /kidney stone event occurring close to a scheduled visit, the visit will be rescheduled after resolution of the AKI/stone event.

Illustration of the Study Design:

	Screening/ Baseline (up to 8 weeks)			Treatment (52 weeks)								Post-treatment safety follow-up (up to 2 weeks)
Week	**-8-0**[1]			**0**	**8**	**16**	**24**	**32**	**40**	**48**	**52**	
Visit number	**1**	**2**	**3**	**TS**[2]	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**NA**
Plasma oxalate	X	X	X		X	X	X	X	X	X	X	
eGFR[3]	X	X	X		X	X	X	X	X	X	X	
Stone events[4,5]	X	X	X		X	X	X	X	X	X	X	X
Echocard.[6]	X		X				X			X		
Safety labs[7]	X	X	X		X	X	X	X	X	X	X	
Stool	X[8]						X		X		X	
24h urine collection	X	X			X		X		X		X	
Ultrasound			X							X		
Quality of Life	X[8]				X		X		X		X	
Adverse Events[5]					X	X	X	X	X	X	X	X

[1]All relevant assessments during screening/baseline will be completed during week -8 - 0, in total 2 visits will be done *before* baseline visit 3. The visits should be scheduled at least two weeks apart during the baseline period (visit 1, 2 and 3). After randomization of the subject, study drug will be ordered and shipped to the subject for start of treatment.

[2]Treatment Start (TS) - visit weeks in the Treatment period will be calculated starting from treatment day one.
[3]As determined by the Schwartz equation eGFR for children (age below 18), and CKD-EPI equation for adults (age 18 or above) based on serum creatinine. For determination of eligibility, eGFR can be calculated using Schwartz or

CKD-EPI equations that include serum creatinine and/or cystatin C.
[4]Kidney stone events and related symptoms will be captured at every visit, including occurrences in between visits.
[5]A post-treatment safety follow-up will be performed as a telephone call (see Section 6.3.4 for details).
[6]Two echocardiography examinations at least three weeks apart should be performed during the baseline period (week -8 – 0). The week 24 and 48 examination should be done within +/- 2 weeks of the clinic visit. If the images at week 24 and/or 48 do not meet quality criteria, the examination will be repeated within 4 weeks.
[7]Safety labs will include blood and urine sampling.
[8]Can be done anytime during screening/baseline, i.e. week -8 – 0.

Number of Subjects (planned):

Approximately 22 subjects will be randomized in the study to Oxabact or placebo in a 1:1 ratio; approximately 11 subjects will be treated with Oxabact and 11 subjects with placebo. This is to ensure that 18 subjects complete the 52-week treatment period. Screen failures may be rescreened at the discretion of the investigator.

Inclusion Criteria:

1. Signed informed consent (as applicable for the age of the subject)
2. A diagnosis of PH (as determined by standard diagnostic methods).
3. eGFR < 90 ml/min/1.73 m^2. The Schwartz equation will be used to estimate GFR for children (age below 18), and CKD-EPI equation will be used for adults (age 18 or above).
4. Plasma oxalate concentration ≥10 µmol/L in total plasma oxalate.
5. Male or female subjects ≥ 2 years of age.
6. Subjects receiving vitamin B6 must be receiving a stable dose for at least 3 months prior to screening and must not change the dose during the study. Subjects not receiving vitamin B6 at study entry must be willing to refrain from initiating pyridoxine during study participation.

Exclusion Criteria:

7. Inability to swallow size 4 capsules.
8. Subjects that have undergone transplantation (solid organ or bone marrow).
9. Subjects requiring dialysis or at immediate risk for kidney failure or expected to be in need of dialysis during the study period.
10. The existence of secondary hyperoxaluria, e.g. hyperoxaluria due to bariatric surgery or chronic gastrointestinal diseases such as cystic fibrosis, chronic inflammatory bowel disease and short-bowel syndrome.
11. Use of antibiotics to which *O. formigenes* is sensitive. (This includes current antibiotic use, or antibiotics use within 14 days of initiating study medication.)
12. Current treatment with a separate ascorbic acid preparation.
13. Pregnant women (or women who are planning to become pregnant) or lactating women.
14. Women of childbearing potential who are not using adequate contraceptive precautions.
15. Presence of a medical condition that the Investigator considers likely to make the subject susceptible to adverse effect of study treatment or unable to follow study procedures or any condition that is likely to interfere with the study drug mechanism of action (such as abnormal GI function).
16. Participation in any interventional study of another investigational product, biologic, device, or other agent within 60 days prior to the first dose of OC5 or not willing to forego other forms of investigational treatment during this study.

Investigational Product, Dosage and Mode of Administration:

The study drug consists of Oxabact. The dose will be administered orally as one enteric-coated size-4 capsule with breakfast and dinner twice daily. The dose will be NLT 1E+09 colony forming units (CFU) per capsule. Placebo capsules of same size and with the same bulking agent will be used in the study.

Duration of Treatment:

Subjects will be treated for 52 weeks with Oxabact or placebo.

The subjects will be offered to continue in an open-label follow-up protocol with Oxabact treatment directly after the 52 weeks treatment period.

Criteria for Evaluation:

Efficacy evaluation will be based on the following parameters:

Primary Endpoint

- Change from baseline in total plasma oxalate concentration after 52 weeks of treatment.

Key Secondary Endpoints

- Change from baseline in kidney function (eGFR) after 52 weeks of treatment.
- Frequency of kidney stones events after 52 weeks of treatment. Stone events are defined as:
  - Subject- or investigator reported symptoms, or
  - Stone passages or removals, or
  - Increase in number of stones assessed by ultrasound

Other Endpoints

- Percent change from baseline in total plasma oxalate concentration after 52 weeks of treatment.
- Subjects achieving 'near-normalization' of total plasma oxalate concentration (<10 µmol/L) at least twice during weeks 24 to 52 of treatment.
- Change from baseline in myocardial function as measured by Speckle Tracking and traditional echocardiography.
- Change from baseline in free plasma oxalate concentration after 52 weeks of treatment.

- ❖ Change from baseline in urinary oxalate excretion after 52 weeks of treatment.
- ❖ Change from baseline in grade of nephrocalcinosis as assessed by Ultrasound.
- ❖ Change in number of O. *formigenes* in stool.
- ❖ Association between change in number of O. *formigenes* in stool and change in total plasma oxalate concentration.
- ❖ Change from baseline in score of Quality of Life questionnaire.
- ❖ Change from baseline in markers for renal function, renal tubular capacity and inflammation:
  Urine: magnesium, phosphorus, citrate, calcium, glycolate, creatinine, urea, calcium oxalate crystals, pH, osmolality and urinary volume.
  Blood: magnesium, phosphorus, citrate, calcium, glycolate, BUN, ALP, bicarbonate, CRP, WBC, creatinine and cystatine C.

Safety:

Adverse events (AEs), vital signs, physical examination, hematology, clinical chemistry, urinalysis.

Statistical Methods:

Sample Size Calculation
In a previous OxThera study OC3-DB-02, with a total of 11 subjects (7 on active treatment, 4 on placebo) having a baseline eGFR <90 ml/min/1.73 m^2, a mean difference from baseline (screening) in total plasma oxalate concentration of - 4.964 (SD=5.219) µmol/L between active treatment versus placebo was observed after 24 weeks of treatment. Furthermore, OxThera study OC5-DB-01 showed a mean difference in total plasma oxalate of -5.299 (SD=3.235) after 8 weeks of treatment in a total of 9 subjects (7 active, 2 placebo) in subjects with baseline eGFR <90 ml/min/1.73 m^2.

Since for study OC5-DB-02 multiple plasma oxalate measurements will be taken at visits 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, the most appropriate statistical model for the primary endpoint is a mixed effect repeated measures model (MRMM). It is expected that at week 52 the difference may be larger than what has been observed in the previous study OC5-DB-01 at 8 weeks. Therefore, for the calculation a conservative mean difference of -5.50 with SD of 4.00 is assumed. Using the MRMM model in a two-sided approach with α=5%, β=10% (and thus power=90%), a 1:1 allocation ratio and assuming a within subject correlation of 0.70, the sample size is assessed at 9 subjects for each group. With this sample size, a difference of -6.00 with an SD of 4.50 may be considered to be statistically significant (Oxabact vs. Placebo). Considering 18 completers are needed based on this calculation, an estimate of 22 subjects will be randomized to account for possible drop-outs.

To account for the possiblity of higher than observed variability, please find below sample size estimates for various configurations.

Sample Size Calculation

Primary endpoint plasma oxalate

	Difference in plasma oxalate between Oxabact and Placebo					
SD	**-4.50**	**-5.00**	**-5.50**	**-6.00**	**-6.50**	**-7.00**
3.00	7:7	6:6	5:5	4:4	4:4	3:3
3.50	10:10	8:8	7:7	6:6	5:5	4:4
4.00	13:13	10:10	**9:9**	7:7	6:6	6:6
4.50	16:16	13:13	11:11	**9:9**	8:8	7:7
5.00	20:20	16:16	13:13	11:11	10:10	8:8
5.50	24:24	19:19	16:16	14:14	12:12	10:10

Key secondary endpoint eGFR

Using a MRMM model in a two-sided approach with α=5%, and a within subject correlation of 0.70, the sample size evaluated at 9 subjects for each group yields approximately 90% power to identify a difference (SD) of 4 (3) ml/min/1.73m^2 in eGFR change from baseline at week 52 between the treatment arms.

Primary Endpoint

Change from baseline in total plasma oxalate concentration will be calculated at each visit as the visit value minus the mean of the three measurements during baseline. Analysis of total plasma oxalate will be considered as the primary analysis. The primary statistical analysis will be performed using a mixed-effect repeated-measures model analysis based on the full analysis set (FAS) with a model that includes the following fixed effects: treatment group, baseline total plasma oxalate value, baseline stratum (PH type 2, PH type 3, not PH type 2 or 3 and baseline urinary oxalate excretion $\leq$ 1.87 mmol/24h/1.73 m^2, not PH type 2 or 3 and baseline urinary oxalate excretion > 1.87 mmol/24h/1.73 m^2), week, and week-by-treatment interaction. The primary comparison will be between the Week 52 changes from baseline in OC5 and placebo. Unstructured covariance matrix will be used in the model. An autoregressive of order 1 (AR (1)) variance covariance matrix will be used for the within subject variation in the MRMM. In case there is a convergence problem, alternative variance covariance matrix structures will be used.

Mean total plasma values over time for both treatment groups will be plotted in addition to a plot with the data over time presenting one line for each subject. Additionally, and for each subject a plot presenting both total plasma oxalate and eGFR over time will be produced to illustrate how total plasma oxalate and eGFR change together.

A sensitivity analysis will also be performed using multiple imputations (MI) prior to applying the MRMM.

An additional sensitivity analyses to assess the robustness of the primary analysis to possible deviations from the missing at random (MAR) assumption will be performed. A pattern-mixture model (PMM) will be used to evaluate the possibility of the missing pattern being missing not at random (MNAR).

Additionally, a second analytical method approach will be provided as supportive analyses (i.e. ANCOVA and/or AUC) and will be further defined in the SAP.

Secondarily the same analysis will be produced based on the per protocol analysis (PP) set.

Key Secondary Endpoints

Change from baseline in kidney function (i.e. eGFR slope) after 52 weeks of treatment will be analysed in the same way as the primary endpoint. Estimated GFR will be calculated for the primary analysis based on the 2009 creatinine-based "Schwartz bedside" equation (for children below 18 years of age) and 2009 creatinine-based CKD-EPI equation for adults. Historical renal function will be assessed in the context of on-study renal function to determine the degree of progression of renal function decline (i.e. the slope) before and during the study.

Kidney stone events after 52 weeks of treatment will be summarized using descriptive statistics. Incidence rates and 95% CIs of kidney stone events (defined as the number of events divided by the total person-years) will be calculated along with the number of stone events and the number of subjects with a stone event. Treatment groups will be compared.

Other Endpoints

- Percent change from baseline in total plasma oxalate concentration after 52 weeks of treatment will be analyzed in the same way as the primary endpoint.
- Subjects achieving 'near-normalization' of total plasma oxalate concentration (<10 µmol/L) at least twice during weeks 24 to 52 of treatment will be summarized. The treatment arms will be compared using the stratified generalized Cochran-Mantel-Haenszel test of general association including the appropriate stratification factors. Exact 95% CIs will be calculated for each treatment arm, and the common odds ratio across strata for the treatment arms. Wilson's score method with continuity correction will be used to calculate a 95% CI for the difference in rates.
- Change from baseline in myocardial function as measured by Speckle Tracking and traditional echocardiography will be summarized descriptively over time.
- Change from baseline in free plasma oxalate after 52 weeks of treatment will be analysed in the same way as the primary endpoint.
- Change from baseline in urinary oxalate excretion after 52 weeks of treatment will be analysed in the same way as the primary endpoint.
- Change from baseline in grade of nephrocalcinosis as assessed by ultrasound will be summarized.

- Change in number of O. *formigenes* in stool will be based on number of *O. formigenes* at week 52 compared to baseline in the active group versus placebo.
- Association between change in number of *O. formigenes* in stool and change in total plasma oxalate concentration will be evaluated.
- Change from baseline in score of Quality of Life scores as measured by SF36V2 or CHQ/PF50 questionnaires, depending on age, will be summarized over time and compared descriptively between the two treatment groups
- Change from baseline in markers for renal function, renal tubular capacity and inflammation:
  Urine: magnesium, phosphorus, citrate, calcium, glycolate, creatinine, urea, calcium oxalate crystals, pH, osmolality and urinary volume.
  Blood: magnesium, phosphorus, citrate, calcium, glycolate, BUN, ALP, bicarbonate, CRP, WBC, creatinine and cystatine C.

Subgroup Analyses
Subgroup analyses for total and free oxalate concentration, eGFR, urine oxalate, stone events, *O. formigenes* in stool, Speckle Tracking and traditional echocardiography) will be performed in the following subgroups of subjects, provided there is sufficient sample size:

- Subjects with a baseline urinary oxalate excretion above and equal to or below 1.87 mmol/L/24h/1.73 m^2 respectively (mean of the two values during screening/baseline).
- Subjects above or equal to and below 18 years of age at baseline.
- Subjects with a baseline eGFR above or equal to and below 60 ml/min/1.73m^2 respectively (mean of the obtained values during screening/baseline calculated by the 2009 creatinine-based "Schwartz bedside" equation for children (below 18 years of age) and the 2009 creatinine-based CKD-EPI equation for adults).
- Race.
- Gender.
- Progressors and non-progressors based on historic eGFR (i.e. eGFR prior to treatment start)
- Use of vitamin B6 at baseline

Statistical Method Safety:

The safety will be summarized using descriptive statistics based on frequency, seriousness, relationship and severity of treatment-emergent AEs. Clinically relevant changes in safety laboratory parameters, vital signs, and physical examinations will also be summarized.

2 TABLE OF CONTENTS, LIST OF TABLES, AND LIST OF FIGURES

2.1 List of Tables

2.2 List of Figures

3 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

The following abbreviations and specialist terms are used in this study protocol.

Table 2: Abbreviations and Specialist Terms

Abbreviation or Specialist Term	Explanation
AE	Adverse Event
AGT	Alanine/glyoxylate aminotransferase
AKI	Acute Kidney Injury
ALP	Alkaline phosphatase
ALT	Alanine Transaminase
AST	Aspartate Transaminase
BUN	Blood Urea Nitrogen
CAPD	Continuous Ambulatory Peritoneal Dialysis
CCPD	Continuous Cycling Peritoneal Dialysis
CFU	Colony Forming Units
DSMB	Data and Safety Monitoring Board
eCRF	Electronic Case Report Form
e/a	Early to late ventricular filling velocity
e/é	Medial to lateral diastolic peak velocity
eGFR	Estimated Glomerular Filtration Rate
EOS	End of study
EOT	End of treatment
ESRD	End-Stage Renal Disease
FAS	Full Analysis Set
FDD	First Dose Date
FSH	Follicle Stimulating Hormone
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
GI	Gastrointestinal
GRHPR	Glyoxylate reductase / hydroxypyruvate reductase
HD	Haemodialysis
HOGA	4-hydroxy-2-oxoglutarate aldolase
ICF	Informed Consent Form

Abbreviation or Specialist Term	Explanation
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
LDD	Last Dose Date
MAR	Missing at random
MCHC	Mean Cell Haemoglobin Concentration
MCV	Mean Cell Volume
MI	Multiple Imputation
MNAR	Missing not at random
MRMM	Mixed-effect repeated measures model
NLT	Not Less Than
NMT	Not More Than
OC2	Old investigational Drug, evaluated in earlier clinical studies.
OC3	Old Investigational Drug, evaluated in earlier clinical studies.
OC5	Investigational Drug
PH	Primary Hyperoxaluria
PI	Principal Investigator The investigator who leads the study conduct at an individual study centre.
PMM	Pattern-mixture model
PP	Per Protocol
SAE	Serious Adverse Event
SOC	System Organ Class
STE	Speckle Tracking Echocardiography
SUSAR	Suspected Unexpected Serious Adverse Reaction
WBC	White Blood Cell Count

4 INTRODUCTION

4.1 Primary Hyperoxaluria

4.1.1 Aetiology, Clinical Features and Epidemiology

Primary hyperoxaluria (PH) type I, II and III are rare autosomal recessive inborn errors of glyoxylate metabolism. PH type I is caused by deficient or absent activity of liver specific peroxisomal alanine/glyoxylate aminotransferase (AGT). In some patients with PH type I, enzyme is present but mis-targeted to mitochondria where it is metabolically inactive. PH type II occurs as a result of deficient glyoxylate reductase-hydroxypyruvate reductase (GRHPR) enzyme activity. Oxalate overproduction in patients with PH type III is caused by loss-of-function of the mitochondrial 4-hydroxy-2-oxoglutarate aldolase (HOGA) enzyme (Cochat and Rumsby, 2013).

All types of PH are characterized by severe hyperoxaluria and hyperoxalemia. Oxalate cannot be metabolized by human cells and is eliminated through the intestines and the kidneys as an end product of metabolism. Oxalate is freely filtered at the glomerulus, reabsorbed in proximal tubules and secreted by the distal tubules. Urinary oxalate excretion levels in PH patients are extremely high (>1.0 mmol/day/1.73m^2) as compared to normal levels (< 0.5 mmol/day). Oxalate precipitates with calcium at µmolar concentrations, and high oxalate concentration levels damage the renal parenchymal cells both as free oxalate and as calcium-oxalate crystals (Cochat and Rumsby, 2013). In addition to calcium-oxalate deposition, chronic hyperoxaluria and calcium oxalate crystals are associated with parenchymal inflammation and interstitial fibrosis (Cochat and Rumsby, 2013; Hoppe *et al*., 2009).

In PH, marked hyperoxaluria is present from birth. There is however a marked interfamilial as well as intrafamilial heterogeneity of disease expression. The individual hepatic oxalate production and time for exposure to high oxalate (age) are important factors for progression of the disease (Zhao *et al*., 2016). The majority of patients are symptomatic during childhood and in most cases before 10 years of age. Some patients suffer from the severe infantile oxalosis and reach end-stage renal disease (ESRD) before one year of age. In some cases, however, the disease may go unrecognized until patients reach 30-50 years of age. Overall, approximately 30% of PH patients have reached ESRD by age of 15 (Rare Kidney Stone Consortium, 2013). Untreated, PH1 leads to death in most patients, but even with currently available treatments average life expectancy is shorter than in the general population (Cochat *et al*., 1995; Hoppe *et al*., 2009; Van der Hoeven *et al.,* 2012).

PH is an ultra-rare disease with a prevalence for PH type I of 1-3 per million and an incidence rate of approximately 1 per 120 000 births in Europe. PH type I is the most severe and the most common variant, accounting for 70-80% of all known cases. Available prevalence and incidence rates may however be underestimated because of the diagnosis being delayed or overlooked (Cochat and Rumsby, 2013). Early diagnosis, molecular subtyping and prompt initiation of conservative treatment are of vital importance for PH patients.

The clinical hallmark of the disease is recurrent calcium-oxalate urolithiasis and/or nephrocalcinosis with progressive decline in renal function. PH type 1 patients with ESRD receive dialysis while waiting for combined liver and kidney transplantation. So far dialysis has not been shown to overcome the problems caused by ongoing oxalate production and deposition at extra-renal sites. Even the most intensified dialysis regimen is not able to cope with the increasing oxalosis that often leads to multiple organ dysfunction including ischemic ulcers of the skin,

metabolic bone disease, refractory anaemia, cardiomyopathy and cardiac conduction system abnormalities causing severe morbidity and mortality (Cochat and Rumsby, 2013; Hoppe, 2012).

PH type 2 is a less severe disease, and these patients reach ESRD later in life, around age 40-50 years. PH type 3 is the mildest form of the disease with severe recurrent kidney stones, but as of today no known patients with ESRD.

4.1.2 Unmet Medical Need

PH is a seriously debilitating and life-threatening disease with a high unmet medical need. There is currently no approved pharmaceutical therapy for PH available on the market. Eventually the only curative therapy for PH type 1 is a combined kidney and liver transplantation at ESRD. For type 2 and 3, recurrent kidney transplantation is the only temporary treatment.

A medical treatment, which can enhance or contribute to the removal of oxalate could be of immense importance in management of these patients.

4.2 Oxabact

The active study drug Oxabact consists of lyophilized *Oxalobacter formigenes* strain HC-1 in an enteric-coated capsule for oral administration. Oxabact comprises *O. formigenes* derived from a human strain HC-1.

O. formigenes is a strict anaerobe that relies exclusively on oxalate as a substrate to obtain energy for its survival and growth. Three proteins involved in oxalate degradation have been purified, their genes isolated and expressed to understand the physiological significance of this bacterium (Stewart *et al*., 2004). It is currently believed to be the most efficient oxalate degrading enzymatic system that operates at neutral pH. *O. formigenes* is a part of the normal intestinal flora in humans; it is non-pathogenic and has never been isolated systemically as a pathogen. *O. formigenes* is also hypothesized to interact with the oxalate transporter proteins in the intestinal wall enhancing the transport of oxalate from plasma to the intestinal lumen.

4.3 Oxabact Treatment for PH Patients

Oxalobacter formigenes, given orally, has the potential to modify the course of PH by enhancing enteric elimination of oxalate (transfer of oxalate from plasma to the intestine), thereby potentially mobilizing the oxalate stores and decreasing total body burden and serum levels of oxalate. This then could have significant benefit on the heart, the joints and the kidney, especially on the tubular function, as well as other affected organs such as skin, skeleton and eyes.

Although kidneys are believed to be the principal route for oxalate excretion, considerable intestinal excretion of oxalate has been shown in animal models. Colonic secretion of oxalate is an extra-renal route for oxalate elimination in rats with hyperoxalemia with or without chronic renal failure, which is also a clinical feature seen in patients with Primary Hyperoxaluria (Hatch and Freel, 2005). Administration of the bacteria *O. formigenes* is proven to create a trans-epithelial gradient for oxalate flux from the blood stream over to the small intestines, to increase degradation of oxalate in the gastrointestinal tract, thus promoting enteric elimination of oxalate. The bacteria have also been shown to actively promote enteric elimination of oxalate through its secretion of a signal to epithelial cells to transport oxalate from plasma to intestines in animal models (Hatch *et al*., 2011)

Incremental secretion of oxalate into the gut can also be maintained by constantly degrading the secreted oxalate with the help of *O. formigenes* in the gastrointestinal (GI) tract. Thus *O. formigenes* treatment is a potential therapy to promote the removal of endogenously produced plasma oxalate by enteric elimination, thereby lowering the body burden of oxalate in PH patients. It is also possible, that *O. formigenes*, can act to dissolve and remove oxalate deposits in the body. By enhancing transport of free oxalate from plasma to the gastrointestinal tract, *O. formigenes* can shift the equilibrium between solid and free oxalate in plasma and start a dissolution process, thereby releasing free oxalate from systemic deposits. In the long-term this will reduce systemic and tubular deposits in the patients and should result in a significant clinical benefit for all patients in all affected organs and tissues.

4.4 Clinical Experience with Oxabact

Five clinical studies in PH have been performed with older Oxabact products (OC2 and OC3): two phase I/II studies and two phase II/III studies, plus an open-label extension study following the first phase II/III study, see Table 3.

In addition, a recent placebo-controlled, double blind randomized Phase I/II study (OC5-DB-01: EudraCT No. 2012-005606-22) has been performed with the new OC5 product in 8 clinical sites in Europe. This study enrolled 28 PH patients with maintained renal function and evaluated the efficacy and safety of Oxabact OC5 to reduce urinary oxalate and plasma oxalate in patients with PH. OC5-DB-01 started in December 2013 and the study ended in Jan 2015.

An open-label phase II study with OC5 (OC5-OL-01, EudraCT No. 2013- 004368-74) to evaluate the efficacy and safety of Oxabact (OC5) to reduce plasma oxalate levels in patients who are on dialysis was completed in January 2020, see further Table 3.

Table 3: Summary of Clinical Studies

Study	Sites/Subjects	Study Drug	Treatment Time	Outcome
CTIxOC.002 Phase I/II, open-label, non-comparative	– Single site, n=9 – PH type I – 5 males, 4 females – Mean age 14, range 3-49	**OC2:** *Oxalobacter formigenes* frozen cell paste, containing 1000 mg (NLT 1E+10 CFU), given orally b.i.d. with meals.	4 weeks	– Reduction in plasma oxalate in some subjects on dialysis – No safety concerns
CTIxOC.002 A 2-3 Phase I/II, open-label, non-comparative	– Single site, n=9 – PH type I – 5 males, 4 females – Mean age 16, range 5-50	**OC3a*:** Enteric coated capsule, containing 137 mg (NLT 1E+07 CFU) of lyophilized powder. One capsule was given orally b.i.d. with meals.	4 weeks	– Significant reduction in urinary oxalate in nearly all subjects – No safety concerns
OC3-DB-01 Phase II/III, double-blind, placebo-controlled, multi-centre	– 9 sites, n=42 – PH type 1/2 – 19 males, 23 females – Mean age 13, range 6-39	**OC3b**:** Enteric coated capsule, containing 137 mg (NLT 1E+07 CFU) of lyophilized powder. One capsule was given orally b.i.d. with meals.	24 weeks	– Post hoc analyses showed trends toward reduction in urinary oxalate – No safety concerns – Questionable 24-hour urine collections
OC3-OL-01 Phase II/III, open-label, non-comparative	– 8 sites, n=37 – PH type 1/2 – 16 males, 21 females – Mean age 14, range 6-38	**OC3b**:** Enteric coated HPMC capsule, containing 137 mg (NLT 1E+07 CFU) of lyophilized powder. One capsule was given orally b.i.d. with meals.	12-24 weeks	– No trends towards reduction in urinary oxalate – No safety concerns – Questionable 24-hour urine collections
OC3-DB-02 Phase II/III, double-blind, placebo-controlled, multi-centre	– 3 sites, n=36 – PH type 1/2 – 17 males, 17 females – Mean age 22, range 3-62	**OC3b buffer**:** Buffer formulation, containing 500 mg (NLT 1E+07 CFU) of lyophilized powder. Reconstituted with bicarbonate buffer, given orally b.i.d. before meals.	24 weeks	– No trends towards reduction in urinary oxalate – No safety concerns – Improved 24-hour urine collections

Study	Sites/Subjects	Study Drug	Treatment Time	Outcome
OC5-DB-01 Phase I/II, double-blind, placebo-controlled, multi-centre	– 8 sites, n=28 – PH type 1 / 2 / 3 – 15 males, 13 females – Mean age 14.5, range 3-27	**OC5#:** Enteric coated gelatin capsule, containing 80 mg (NLT 1E+09 CFU) of lyophilized powder. One capsule was given orally b.i.d. with meals.	8-10 weeks	– No significant difference for primary endpoint (urinary oxalate excretion) – Marked patient heterogenity with indication that oxalate deposits may be dissolving in OC5-treated subjects – Post hoc analyses identified significant differences in some parameters including kidney function markers – No safety concerns
OC5-OL-01 Phase I/II, open-label, non-comparative, multi-centre	– 3 sites – 12 PH patients with ESRD treated with dialysis enrolled; 8 subjects entered continued treatment phase	**OC5#:** Enteric coated gelatin capsule, containing 80 mg (NLT 1E+09 CFU) of lyophilized powder. One capsule was given orally b.i.d. with meals.	6 weeks +36 months	– Study started in May 2014 and ended in January 2020. – 12 subjects received study drug; 8 subjects entered the continued treatment period. 6 subjects completed 12 months, 5 subjects completed 24 months and 3 subjects completed 36 months of continued treatment. MRMM analysis showed decreased total plasma oxalate levels over time and there were signs of improved or stabilised myocardial function in subjects who have received continued treatment.

**OC3a: OC3 active ingredient prior to technology transfer and scale-up*
***OC3b: OC3 active ingredient after technology transfer and scale-up*
#OC5: OC5 active ingredient after process development and transfer

4.4.1 Clinical Experience in PH Patients

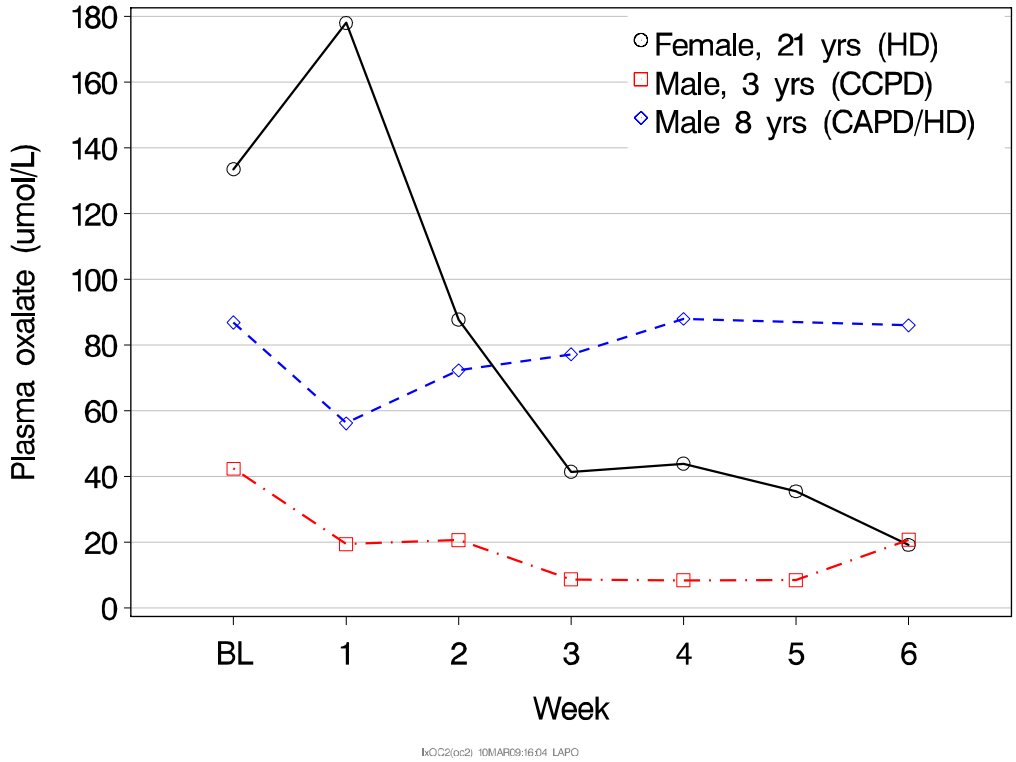
4.4.1.1 CTIx.002

The first phase I/II study (CTIx.0002) was conducted in 9 subjects using a frozen cell paste formulation of *O. formigenes* (OC2). Subjects were given two doses of OC2 per day for four weeks. Six subjects with serum creatinine <1 mg/dL (inclusion criteria) and three PH I patients in ESRD who were undergoing dialysis were included in this study (Hoppe *et al*., 2006).

This study showed that NLT 1E+10 CFU administered twice a day for 4 weeks resulted in a 20% (range 6-43%) reduction in 24-hour urinary oxalate excretion in six subjects with measureable urine (p=0.0313, Wilcoxon rank-sum test). Overall, the median reduction in plasma oxalate levels was 47% (p=0.0195). The decrease in plasma oxalate was substantial (>30 µmol/L) in 2 of the 3 subjects with end-stage renal disease. OC2 treatment was well tolerated in all subjects in the study.

Figure 1 shows the change in plasma oxalate in the subjects in ESRD. Pre-dialysis plasma oxalate measurements were made once a week. A substantial decrease in plasma oxalate was observed in two of the subjects in ESRD and no safety concerns were observed (Hoppe *et al*., 2006). In one of the subjects (Female 21 years on HD) the treatment was prolonged to 5 weeks since the therapeutic effect was quite significant and this subject also reported amelioration of clinical symptoms under therapy with less pain due to oxalate osteopathy.

Figure 1: Effect of O. *formigenes* Treatment on Plasma Oxalate Levels in Subjects with End-Stage Renal Disease Treated with *O. formigenes* Delivered as Frozen Cell Paste (OC2)



Note: The eight year-old male did not take all study medication per protocol after second week of treatment. HD=Haemodialysis, CCPD=Continuous Cycling Peritoneal Dialysis; CAPD=Chronic Ambulatory Peritoneal Dialysis.

4.4.1.2 CTIx.002 Amendments 2 and 3

The second phase I/II study was a single arm, single centre study conducted under an amendment of the same protocol (CTIx.002 A2-3) and enrolled 9 subjects. In this study, *O. formigenes* was lyophilized and administered as an enteric-coated capsule (OC3a active ingredient). The dose was NLT 1E+07 CFU given twice a day for 4 weeks. In eight subjects with measureable urine, there was a reduction in 24-hour urinary oxalate excretion by a median 58% (p=0.0078, Wilcoxon rank-sum test), ranging from 4% to 95%. Plasma oxalate decreased overall by a median 26% and the reduction ranged from 22% to 84% in 6 of these subjects. The plasma oxalate decreased by 26 μmol/L in the subjects with end-stage renal disease. OC3a treatment was well tolerated.

4.4.1.3 OC3-DB-01

Based upon results from the phase I/II studies, OxThera transferred and scaled up the manufacturing of OC3 (OC3b active ingredient). The third study was a phase II/III randomized, double blind, placebo-controlled study that investigated the safety and efficacy of lyophilized *O. formigenes* administered as an enteric-coated capsule. In contrast to the preceding single center studies, this study was conducted at 9 sites in France, the Netherlands, United Kingdom, Germany, and the United States. Of the 42 subjects enrolled, 19 were randomized to receive NLT 1E+07 CFU OC3b and 23 to placebo. Study drug was administered twice daily for 24 weeks. It should be noted that this study was the first large international study to evaluate a new therapy in subjects with PH in a randomized and placebo-controlled fashion.

Analysis of the prospectively defined primary endpoint change in urinary oxalate excretion showed no difference between the OC3b and placebo treatment groups on percentage change from baseline to Week 24 in urinary oxalate excretion. No treatment effect was seen in analyses of secondary endpoints.

In an attempt to understand the lack of treatment effect in this larger study, an independent expert committee of clinicians was tasked to review the adequacy of the 24-hour urine data. They determined that despite the detailed instructions provided to the sites, the sites varied in quality of the urine collection, handling and shipping. Also, some subjects had a large amount of variability in their 24-hour urinary creatinine measurements across collections in the study indicating poor compliance. After the data review, the expert committee established stricter eligibility criteria for valid urine samples, and developed rules for post hoc analyses.

Results from the post hoc analyses showed that the mean percentage change in urinary oxalate to creatinine ratio (mmol/mol) was -21% (SD=23%) in subjects given OC3b and -7% (SD=21%) in placebo, a difference of -14% (p=0.056, Student's t-test). Analysis of data from the two largest sites (n=20), showed a mean percentage change of -37% (SD=17%) from baseline in subjects treated with OC3b and -9% (SD=22%) in subjects treated with placebo, corresponding to a difference of -28% (p=0.006). These two sites were also the most experienced in the conduct of clinical studies in this patient group.

OC3b was safe and well tolerated with a similar distribution of adverse events (AE) in the active treatment and placebo groups. The most commonly reported individual AE were headache, nausea and vomiting.

4.4.1.4 OC3-OL-01

This was an open-label extension, single-arm, 6-month study evaluating the safety of OC3b with long term exposure in subjects who had completed participation in the double-blind, placebo-controlled efficacy study (OC3-DB-01). Subjects with less than 20% reduction in

urinary oxalate levels at week 12 compared to time of screening in the OC3-DB-01 study (i.e., at entry into the qualifying study OC3-DB-01) were withdrawn from the study.

A greater proportion of subjects who received placebo in the OC3-DB-01 study [13 (65.0%)] experienced a stone event (renal stones and/or signs and symptoms of renal stones) compared with those who had received OC3b [5 (31.3%)]. The mean incidence of stone events, adjusted for a 48-week study period, was 0.46 (SD: 0.89) and 0.84 (SD: 1.00) in subjects who had previously received treatment with OC3 or placebo respectively. The validity of these differences should however be considered due to the inherent difficulties in defining a "new" stone event in this subject population, the relatively short follow-up time and varying time of exposure to study drug.

Overall, estimated glomerular filtration rate (eGFR) did not change during the study. Similar results were seen in subjects with Stage II and Stage III chronic kidney disease. Changes in this parameter were not expected during this relatively short follow-up.

Overall, there were no clinically relevant changes in free plasma oxalate during the study.

The majority of subjects [24 (85.7%) subjects] did not show a ≥20% reduction in urinary oxalate to creatinine ratio during this open-label (OL) study (i.e. did not meet responder criteria). Among the seven subjects that were given OC3 for 48 weeks (DB and OL study combined) the mean reduction in urinary oxalate was 24%. At the end of study, 2 of these subjects were non-responders and 5 remained responders. The corresponding reduction in urinary oxalate among the seven subjects, who received placebo for 24 weeks during the DB study followed by 24 weeks of OC3b treatment in this OL study, was 23%; however, the reduction during the preceding 24 weeks of placebo treatment was 19% for this subgroup of subjects. Three of these subjects had an additional reduction of ≥20% during the OL study alone.

Overall, OC3b was safe and well tolerated in the OL extension study with no unexpected safety issues raised and the adverse events reported were essentially evenly distributed between subjects who had previously received OC3 treatment compared with those who had received placebo in terms of severity, seriousness and relationship.

4.4.1.5 OC3-DB-02

In phase II/III OC3-DB-02 the study medication was administered as OC3b lyophilized powder suspended in water together with a bicarbonate buffer (OC3b buffer), and administered concomitantly with 10-20 mg esomeprazole (Nexium granules) taken with water once daily. The reason for using a buffer formulation was to increase the dose administrated, to have an earlier release of the bacteria in the GI tract, and to reduce the time to recovery of lyophilized bacteria.

The primary efficacy analysis showed no reduction in urinary oxalate excretion following treatment with OC3 for 24 weeks compared to placebo. Both treatments showed a slight decrease in the urinary oxalate compared to baseline. The reduction of the urinary oxalate was analyzed in several subsets of subjects, including subject populations based on disease characteristics, age and region. Decreases in urine oxalate were seen in the OC3b group as well as in the placebo group. A significant treatment difference in favor of OC3b treatment was not found. OC3b treatment showed no effect on the overall proportion of responders following 24 weeks of treatment. The percentage responders in the OC3 and placebo group was 25% (5/20) and 42% (5/12), respectively.

Overall, the day-to-day variability of urinary oxalate and creatinine measurements was much improved in this study as compared to OC3-DB-01. Results were basically similar when looking at oxalate per creatinine ratio or oxalate 24-hour excretion, which indicates that the 24-hour collections were of good quality. In addition to implementing urine acceptance criteria, the instructions to subjects and study personnel were much improved in this study.

OC3 treatment did not result in a clinically significant reduction of the plasma oxalate levels, although the active group had a decrease in plasma oxalate concentration of -12% whereas the placebo group had an increase of 7%.

OC3b was safe and well tolerated with an AE profile similar to the placebo. AE were most frequently reported in the placebo group. The most frequently reported AE were infections and gastrointestinal disorders. The majority of AEs were of mild or moderate intensity and considered by the investigator to be not related to the OC3 treatment. Overall, treatment with OC3 was safe and well tolerated.

4.4.1.6 Phase I/II Study of OC5 (OC5-DB-01)

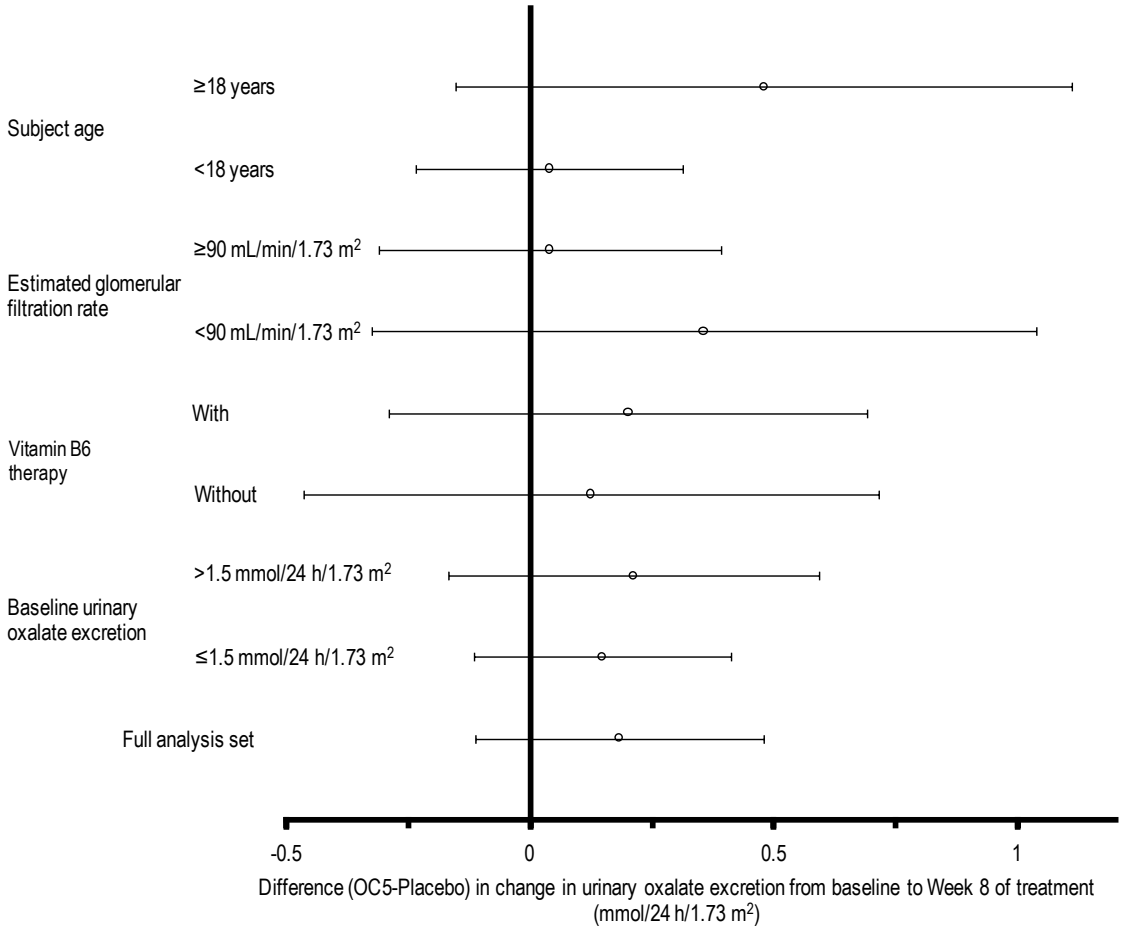
The OC5-DB-01 study was a double-blind, randomized, placebo-controlled, multi-centre study to evaluate the efficacy and safety of Oxabact OC5 in PH patients with maintained renal function (i.e. eGFR or a creatinine clearance of ≥ 40 mL/min normalized to 1.73m^2 body surface area). The subjects were randomized to treatment with Oxabact or placebo, and were administered one capsule twice a day for 8 - 10 weeks. Each dose of Oxabact corresponded to not less than 10^9 CFU *O. formigenes* and not less than 100 mmol/19 h of oxalate degrading capacity. The primary objective of the study was to evaluate the efficacy of Oxabact drug product to reduce urinary oxalate excretion following 8 - 10 weeks of treatment.

In total, 44 subjects from 8 sites in Germany, France, and the United Kingdom were screened for inclusion in the study. 28 of these were randomized; 14 were randomized to receive Oxabact and 14 randomized to receive placebo. All 28 subjects fulfilled the FAS and Safety set criteria, received the treatment and completed the study.

The primary analysis for the OC5-DB-01 study did not identify a statistically significant difference between the OC5 treatment and placebo in terms of its effect on urinary oxalate excretion. After 8 weeks' treatment, the change in urinary oxalate excretion appeared slightly higher (0.182 mmol/24 h/1.73 m^2) in the OC5 group than in the placebo group; this difference did not achieve statistical significance (p=0.22). OC5 treatment did lead to a statistically significant increase in the number of *O. formigenes* cells in the faeces of subjects compared with placebo (p=0.00023). In all other secondary and exploratory endpoints, no significant differences were observed between OC5 and placebo.

Subgroup analysis of the primary endpoint based on baseline urinary oxalate excretion, baseline eGFR, age or concomitant use of vitamin B6 therapy did not reveal any statistically significant differences between the treatment groups, but the magnitude of the least square difference between OC5 and placebo was larger in the eGFR<90 ml/min/1.73 m^2 subgroup than in the eGFR≥90 ml/min/1.73 m^2 subgroup (0.356 vs 0.040 mmol/24 h/1.73 m^2) and larger in the subgroup of subjects aged 18 years or older than in the subgroup of subjects younger than 18 years (0.480 vs 0.039 mmol/24 h/1.73 m^2). A Forest plot of difference in change in urinary oxalate excretion from baseline to Week 8 of treatment in different subgroups is shown in the figure below (Figure 2).

Figure 2: A Forest Plot of Difference in Change in Urinary Oxalate Excretion from Baseline to Week 8 of Treatment in Different Subgroups in the OC5-DB-01 Study.



The figure displays estimate and 95 percent confidence intervals (Full analysis set).

Despite a stratification of the two treatment groups for urinary oxalate excretion (below or equal to/above 1.5 mmol/24 h/1.73 m^2), the subjects in the OC5 group had a more pronounced reduction in renal function than the placebo subjects at baseline. Mean baseline eGFR was lower in the OC5 group than in the placebo group (97.5±38.7 versus 123.1±45.4 mL/min/1.73 m^2), and renal and urinary disorders were more common in the OC5 group (11 cases versus eight cases). Most notably, four subjects in the OC5 group had a history of chronic renal failure, whereas no subjects in the placebo group had been affected by this condition.

There was a negative correlation between plasma oxalate and eGFR in the full population at baseline ($r = -0.508$, $p < 0.007$) indicating that plasma oxalate concentration gradually increases with a decreasing kidney function in PH patients.

In light of the observed difference in renal function between the two groups, ad hoc analyses were conducted to investigate the relationship between efficacy parameters and indicators of renal health.

An analysis of the change in urinary oxalate excretion per urinary creatinine excretion showed a small (+5.4 mg oxalate/g creatinine), but statistically significant increase of oxalate excretion in the active group versus placebo, p=0.023 (FAS).

Significant correlations were observed between renal function and the effect of OC5 treatment on urinary oxalate concentration. Overall, the OC5-induced increase in urinary oxalate excretion was larger in subjects with reduced renal function. The analyses also demonstrated that plasma oxalate concentration decreased as the number of *O. formigenes* increased in the OC5 treated subjects, p=0.04 (at study week 10).

These findings suggest that the bacteria metabolise free oxalate that originates from plasma, thereby supporting that enteric elimination of oxalate has occurred.

It is hypothesized that this reduction in free plasma oxalate shifts the endogenous equilibrium between free plasma oxalate, plasma protein-associated oxalate and tissue-deposited oxalate and forces a dissolution of oxalate deposits in plasma-proteins, vessels and kidneys which in turn leads to increased excretion of urinary oxalate. An analogous relationship between plasma urate levels and solid urate dissolution has been observed in patients with gout, where a reduction of serum uric acid to normal levels resulted in disappearance of urate crystals from synovial fluid (Pascual and Sivera, 2007). Because oxalate deposits are expected to be more pronounced in patients with reduced renal function, this hypothesis also explains why the effect of OC5 treatment was greater in subjects with more advanced kidney disease. The study also showed a statistically significant decrease in urine output in the OC5-treated subjects which also suggest that *O. formigenes* confers some beneficial effect on water reabsorption and urine concentrating-ability in the renal tubules of these subjects.

4.4.1.7 Phase II Study of OC5 (OC5-OL-01)

The OC5-OL-01 study was a phase 2, open-label, multi-centre study to evaluate the efficacy and safety of Oxabact OC5 to reduce plasma oxalate in patients with primary hyperoxaluria who are on dialysis. Subjects were treated for 6 weeks with study drug, with 4 weeks of baseline measurements prior to initiation of study medication and 4 weeks of measurement after drug administration. In Germany, the protocol was amended to allow a 36-month treatment period after the first 14 weeks of the study and in France a 12-month treatment period was approved. The study started in May 2014 and ended in January 2020.

Fourteen subjects were screened and 12 subjects were enrolled in the study and received study drug. Eight subjects entered the continued phase of the study. Six subjects completed 12 months of treatment, 5 subjects completed 24 months of treatment and 3 subjects completed 36 months of treatment. A Mixed-effect Repeated Measures Model (MRMM) analysis showed a statistically significant decline in total plasma oxalate through time. Results of traditional and Speckle-Tracking echocardiography (STE) indicated that cardiac function substantially improved in 2 subjects who had impaired cardiac function at baseline, was stable within the normal range in the other subjects, and did not deteriorate in any subjects. Importantly, these observations occurred in the absence of clinically significant changes to the dialysis regimen. The safety profile was overall favourable, even in this severely ill study population.

4.4.1.8 Compassionate Use

Oxabact has also been evaluated during compassionate use in two 11-month-old girls with infantile oxalosis and ESRD (see Figure 3 and Figure 4). They received OC3b (administered via a gastrostomy tube) twice a day up to 4 weeks during two treatment periods. Dialysis regimens were unchanged. Plasma oxalate levels decreased from >110 µmol/L before to 72 µmol/L following treatment in patient 1, and from >90 to 69 µmol/L (first treatment period) and to 50 µmol/L (second treatment period) in patient 2 (Hoppe *et al*., 2011).

Figure 3: Follow Up of Plasma Oxalate Levels in Patient 1 during Treatment with *Oxalobacter formigenes*

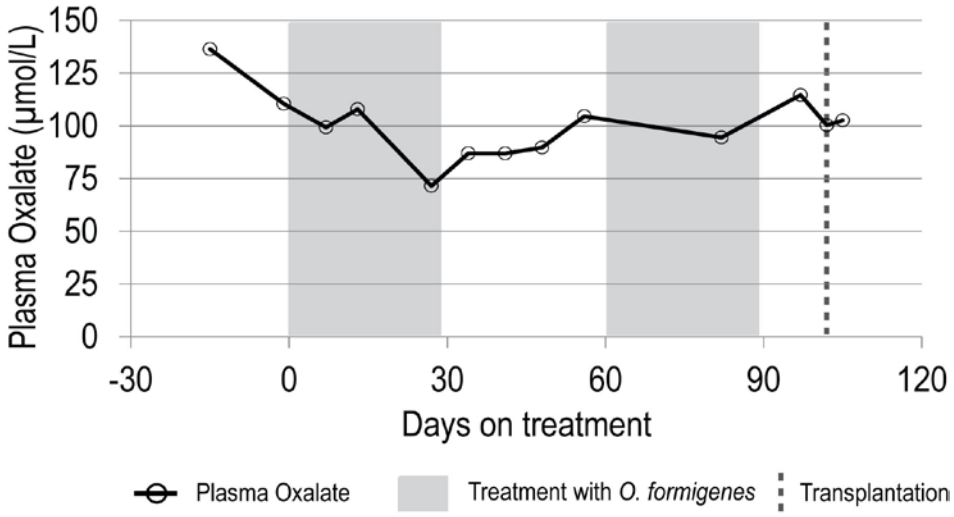
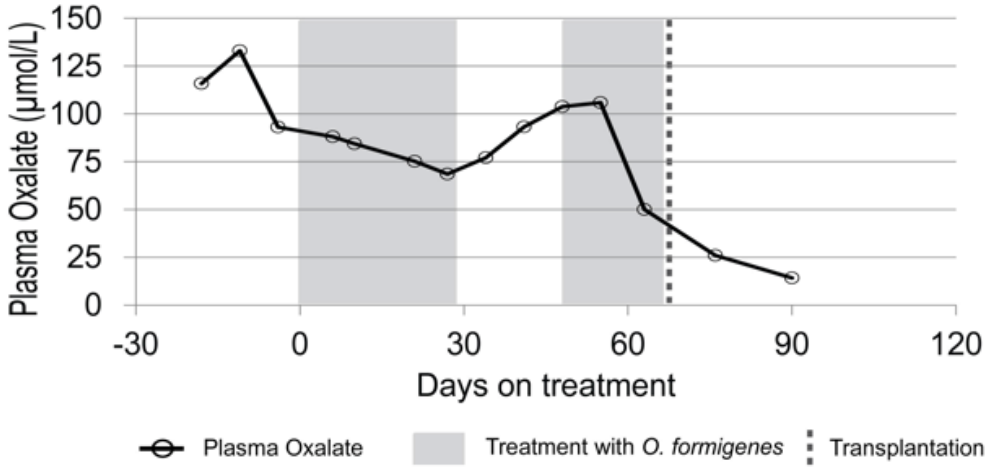


Figure 4: Follow Up of Plasma Oxalate Levels in Patient 2 during Treatment with *Oxalobacter formigenes*



Compassionate use of OC3b (broken capsules, 137-276 mg, mixed with phosphate buffer, reconstituted with 50-100 mL water and administered via gastric tube) twice a day up to 8 weeks have been evaluated in two other patients with infantile oxalosis at two other sites in the United States and the United Kingdom, without any reduction in plasma oxalate (unpublished data). It has recently been concluded that the buffer used for administration of drug was sub-optimal for survival of *O. formigenes.*

OC3b and OC3 buffer were well tolerated and no serious side effects were reported during compassionate use.

OC5 has been provided under named patient use to a female patient with infantile oxalosis in Germany. She developed chronic kidney disease shortly after birth and was treated with daily

peritoneal and 3 times/week haemodialysis. Treatment with OC5 (in buffer solution) started in December 2017, when the child was 3 months old and continued for 22 months, until 2 weeks after the patient had undergone a successful combined liver-kidney transplantation. Plasma oxalate was approximately 130 µmol/L at initiation of OC5 treatment, and overall a 35 % was seen (to around 80 µmol/L) after 16 months of treatment. Nephrocalcinosis and macular oxalate deposits did not deteriorate, and the disease progression was slowed down during the treatment period. The OC5 treatment was well-tolerated and no related adverse events were reported. (Pape *et al*.; 2020).

4.5 Rationale for Current Phase III Study

4.5.1 Overall Objective

The overall objective of this study is to evaluate the efficacy of long-term treatment with Oxabact in PH patients with maintained kidney function but with an eGFR below normal ranges (<90 ml/min/1.73 m^2) at baseline and a total plasma oxalate concentration ≥ 10 µmol/L.

A comprehensive review of the data from the completed OC5-DB-01 study revealed a clear difference between the OC5 and placebo groups, including some significant effects in kidney function for OC5 treated subjects. The study findings suggest that, as a result of *O. formigenes* metabolising GI oxalate and enhancing transport of plasma oxalate into the GI tract, systemic oxalate deposits in the body start to dissolve in the OC5 treated subjects. The *O. formigenes* mediated enteric elimination process would then shift the equilibrium between crystallized and/or protein-associated oxalate and free oxalate towards the free oxalate in plasma, thereby releasing free oxalate from plasma proteins and from systemic deposits in the body. Furthermore, a recent publication (Sivaguru *et al*, 2018) demonstrated that calcium oxalate stones undergo multiple events of dissolution as they crystallise and grow within the kidney. Contrary to the perception that calcium oxalate stones do not dissolve, these findings would support the hypothesis of *in vivo* oxalate stone dissolution.

When treated with *Oxalobacter formigenes*, subjects are expected to gradually deplete systemic oxalate deposits over time. The treatment duration needed for plasma oxalate levels to start to decrease would then depend on the amount of deposits at baseline for each individual subject. It is expected that 52 weeks treatment would be needed to see a treatment effect in this patient population.

In blood, a large portion of the plasma oxalate is associated to proteins and a number of proteins such as albumin have been shown to interact with calcium oxalate during crystal formation (Hatch *et al*., 1977; Aggarwal *et al*., 2013). The intricate interplay between biological molecules and calcium oxalate crystals is an emerging research field, and may play a role in tissue deposit formation in blood vessels, in the heart and in the kidneys.

Available study results from earlier Oxabact studies obtained to date also demonstrate that the patient population is extremely heterogeneous with subjects exhibiting high variability in their baseline characteristics and their stage of disease progression. Data shows that while kidney function is deteriorating in the subjects, plasma oxalate concentration and deposits in various organs are building up proportionally. In the recent OC5-DB-01 study there was a negative correlation between plasma oxalate and eGFR in the full population at baseline ($r = -0.508$, $p < 0.007$).

In the previous double-blind multicentre study OC3-DB-02, there was a statistically significant difference in change in plasma oxalate between treated and placebo subjects in the group of subjects with a baseline eGFR <90 ml/min/1.73 m^2 after 24 weeks of treatment with OC3.

The rationale for this study is, based on these findings, to prove that with a longer treatment time with the new, more potent OC5 product, there is a decrease in plasma oxalate concentration and calcium oxalate deposits over time in treated subjects. This outcome would prove the hypothesis that Oxabact is eliminating plasma oxalate via enteric elimination into the gut and is providing a clinical benefit by dissolution of calcium oxalate crystals.

4.5.2 OC5 Development

OxThera has made significant improvements in the Oxabact formulation to develop the new OC5 product.

Process development has resulted in improvements of the culture conditions, cell harvesting procedures and optimization of excipients used for the freeze-drying process to develop the new highly concentrated product OC5. As compared to OC3b, the OC5 product has hundred-fold higher concentration of viable cells and these cells show a significantly higher recovery rate than OC3b.

OC5 is derived from the same cGMP Master Cell Bank as the earlier product. However, the cell bank has been sub-cloned to meet stringent requirements with regard to viable cell count and oxalate degrading activity. Furthermore, culture conditions and harvest criteria in the fermentation process have been refined such that the cells are harvested at exponential phase rather than at the stationary phase. Together with optimized excipients and relative amounts of these and cells, the product is now also better protected in the lyophilization process. A higher number of viable cells recover from OC5 lyophilized powder than what was previously obtained from OC3b.

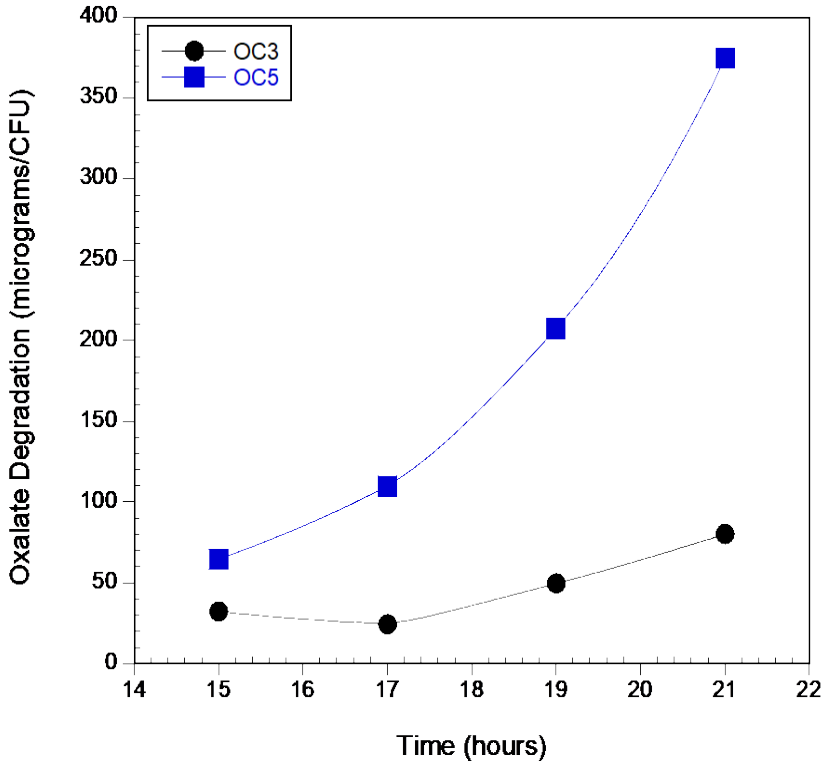
In addition, product-related analytical methods have been refined since the previous production process. The viable cell count assay (CFU/g) has been improved to show the number of viable cells faster than previously. The oxalate degrading activity assay (moles oxalate degraded/g or CFU at 19h) has also been improved to measure activity at the recovered stage. One OC5 capsule contains not less than 10^9 CFU and has the capacity to degrade not less than 100 mmol oxalate/capsule at 19h.

The higher concentration of viable cells and higher oxalate degrading activity in OC5 allows for use of less lyophilized powder per dose. Thus, a smaller capsule, size 4 instead of size 2, is used for the OC5 product.

OC5 is formulated as enteric-coated capsules designed to protect the *O. formigenes* bacteria from gastric juice and to deliver the active ingredient to its natural habitat and site of action, the small intestines. The disintegration time for the coated OC5 capsules is shortened in comparison to the OC3b capsules to better mimic the disintegration profile of a previous version of the product, OC3a capsules.

As can be seen in Figure 5, the OC5 material begins replicating much quicker than does the OC3b material. Hence, the new product shows superior activity. In essence, with an improved cell bank, improved harvesting technique and improved freeze-drying method, the quality of the product has been greatly improved.

Figure 5: Oxalate Degrading Activity of OC5 Compared to OC3



4.5.3 Dose Justification

Based on toxicity studies it is within the NOAEL to administer up to 9 x 10^{11} CFU of OC5 twice daily to human subjects. The OC5-DB-02 study will administer a 10^{9} CFU twice daily dose to all subjects.

The oxalate degrading capacity of Oxabact is limited by the availability of endogenous oxalate. The typical daily production of endogenous oxalate is 4–7 mmol/1.73m^2 in PH 1 patients. The optimal (i.e. *in vitro*) oxalate degrading capacity of the OC5 Oxabact capsules according to drug product specifications is approximately 15-25 times higher than the daily endogenous production of oxalate in PH patients (i.e. ≥100 mmol/capsule/19h). However, the amount of available oxalate cannot support this capacity. Since endogenous oxalate is limited, no safety concerns or difference in safety is anticipated with varying oxalate degrading capacity.

It is necessary to provide an exaggerated dose of Oxabact to ensure continuous delivery of sufficient viable *O. formigenes* to the relevant part of the gastrointestinal tract. The bacteria need to survive transit through the stomach and upper small intestine and withstand the dilution effect from the normal gut microbiota. It is a competitive environment particularly given that *O. formigenes* are anaerobic and utilise only oxalate as an energy source. The dosing strategy supports the use of the same dose across age groups.

4.6 Overall Risk and Benefit Assessment

4.6.1 Burden

Subjects will attend clinical visits at ten times throughout the study. This will involve time travelling to the hospital and the time required to see the investigator/study nurse. Blood samples will be collected during the clinical visits for safety laboratory evaluations and for plasma oxalate analysis. Approximately 20 mL of blood will be needed for the safety lab sample and approximately 5 mL for the plasma oxalate sample. Echocardiography will be performed four times during the study. Subjects will have an ultrasound of the kidneys taken two times during the study.

Study medication will be administrated orally with breakfast and dinner as one capsule two times per day, for 52 weeks. The capsule size is quite small (size 4) and should be relatively easy to swallow even for younger subjects. The subjects will be asked to record the intake of the first and last dose of study drug in a diary.

Subjects are requested to provide four faecal samples and six 24-hour urine samples during the study. Faecal and urine samples will be collected at subjects' homes, a designated courier will pickup collections at times agreed with the subjects even during weekends if requested. Samples will be sent from the subject´s home directly to the central lab.

4.6.2 Risk Threshold

Detailed information on the anticipated adverse events of the OC5-DB-02 study is outlined in Section 11.5. In brief these potential risks may include:

- Displacement of indigenous *O. formigenes* or changes in the normal gut microbiota
- Infection risks
- Elevated plasma formate
- Gastrointestinal symptoms

While the potential for these risks does exist, non-clinical and clinical data to date has not indicated that the Oxabact product is associated with any changes in the gut microbiota, local or systemic infection or elevated plasma formate levels. Previous studies with OC3 (OC3-DB-01, OC3-DB-02) have also found that the OC3 product was safe and well tolerated with an adverse event profile similar to the placebo, even for gastrointestinal symptoms. While the OC5 product is more concentrated than the OC3 product, the safety profile has been shown to remain favourable, as supported by the pre-clinical and clinical studies to date.

The bridging toxicology study (DP-1002), which included 28 consecutive days of twice daily oral administration by capsule of OC3 at 2 x 2E+08 CFU/day or OC5 at 2 x 9E+09 CFU/day to male and female rats was well tolerated with no test article-related changes or differences being noted between the two product formulations. The safety profile for OC5 was comparable to OC3, i.e. no changes observed on clinical observation, laboratory parameters, or histopathology considered specific to the treatment with OC5.

Data collected to date for the completed OC5-DB-01 study and the current OC5-OL-01 study indicate that the OC5 product is safe and well tolerated. No related severe or serious adverse events have been reported so far in these ongoing studies for Oxabact, see Section 11.5. Analysis of potential change in microbiota due to treatment with OC5 during the OC5-DB-01 study showed that no change in the microbiota could be detected during treatment with OC5.

Overall, the safety data accrued to date would suggest that the OC5 drug product is safe and well tolerated and appears to have a similar safety profile as the earlier OC3 drug product.

In conclusion, the burden and risk threshold is deemed to be acceptable for the study and many efforts have been made to minimise burden and potential risks to the subjects. Furthermore, the subjects will visit the clinic ten times during the study for study specific visits in order to monitor adverse events and to make sure the subject is able to follow study procedures.

4.6.3 Benefits

PH is a devastating, life-threatening disease for which there are no approved pharmaceutical therapies available on the market. It is particularly emotive since it primarily affects a paediatric population. Consequently, there is a significant unmet medical need in treating this disease. Oral administration of *Oxalobacter formigenes* is a promising potential treatment for patients with PH. Clear beneficial effects of *Oxalobacter formigenes* have been demonstrated in animal models of PH and in earlier Phase I/II clinical studies.

Based on analysis of the non-clinical and clinical safety data generated to date, Oxabact has been well tolerated. The older OC2 and OC3 Oxabact products have been given to more than 80 subjects for time periods of 4 weeks to 12 months (with the majority of subjects having been treated for 6 months). In addition, the OC5 product has been administered to 14 subjects for 8-10 weeks in the OC5-DB-01 study and for up to 36 months to 12 subjects in the ongoing OC5-OL-01 study. Administration of *O. formigenes* as a frozen cell paste, capsule or buffered powder for suspension was well tolerated in subjects with Primary hyperoxaluria. No SUSARs have been reported in any of these studies for Oxabact, and Oxabact has had a favourable safety profile.

The OC5-DB-02 study should provide valuable information on the ability of the improved OC5 Oxabact product to reduce calcium oxalate deposits and eventually lower the level of plasma oxalate and confer clinical benefit in patients with PH. It will also generate further safety information on the product. The 52-week treatment time is expected to be enough to start to reduce the widespread oxalate deposits in the body and eventually also decrease plasma oxalate levels.

4.6.4 Benefit: Risk Assessment

There is an unmistaken need for additional therapeutic measures to treat patients with PH. These patients are at high risk for kidney damage due to over-production of oxalate and currently there are limited treatments available for this disease. The efficacy of Oxabact in PH patients is still to be proven.

All non-clinical and clinical safety data to date indicate that Oxabact has been well tolerated. There have been no SUSARs for Oxabact reported in any clinical study with *O. formigenes*. Older studies with OC2 and OC3 drug product have been performed in over 80 subjects with treatment times of 4 weeks to 12 months. The 2 larger placebo-controlled studies (OC3-DB-01 and OC3-DB-02) found that the OC3 product was safe and well tolerated with an adverse event profile similar to the placebo. Safety data from the completed blinded OC5-DB-01 study and the ongoing open-label OC5-OL-01 study suggest that OC5 has a favourable safety profile and would not be expected to differ from that of the earlier OC3 drug product.

Thus, based on the knowledge with regard to the mechanism of action for OC5, a naturally occurring and non-pathogenic bacteria that relies exclusively on oxalate for its metabolism within the gut, and the available non-clinical and clinical data, the Sponsor believes that the benefit/risk evaluation of conducting this Phase III trial for treatment in patients with PH is considered favourable.

All in all, the potential clinical benefit to the target population (and study participants in this trial) is deemed to outweigh the potential risks implied and the study is therefore medically and ethically justifiable.

5 TRIAL OBJECTIVES AND PURPOSE

5.1 Primary Objective

- To evaluate the efficacy of Oxabact following 52 weeks treatment in patients with maintained kidney function but below the lower limit of the normal range (eGFR < 90 ml/min/1.73 m^2) and a total plasma oxalate concentration ≥ 10 µmol/L.

5.2 Secondary Objectives

- To obtain additional safety data from up to 52 weeks continuous treatment with Oxabact.

5.3 Clinical Success Criteria for total plasma oxalate

Oxabact will be considered associated with a clinically meaningful treatment effect on total plasma oxalate, if the results meet the following criteria:

1. Statistically significant difference in change from baseline for total plasma oxalate concentration between Oxabact and placebo in favor of Oxabact after 52 weeks of treatment.

AND

2. Estimated difference of an absolute change from baseline for total plasma oxalate concentration between Oxabact and placebo of ≥5 µmol/L after 52 weeks of treatment.
   OR
   Estimated difference in percent change from baseline for total plasma oxalate concentration between Oxabact and placebo of ≥30% after 52 weeks of treatment.

AND

3. Greater percentage of subjects in the Oxabact arm than in the placebo arm achieving near-normalization of total plasma oxalate concentration (< 10 µmol/L) at least twice during weeks 24 to 52 of treatment.

6 INVESTIGATIONAL PLAN

This study is a phase III, double-blind placebo-controlled, randomized, multi-centre study to evaluate the efficacy and safety of OC5 (*O. formigenes*) to stabilise/reduce plasma oxalate concentration, to stabilise/improve kidney function and to reduce oxalate deposits in PH patients. Enrolled subjects will have a maintained kidney function but below normal range (eGFR < 90 ml/min/1.73 m^2) and a total plasma oxalate concentration ≥ 10 µmol/L.

It is hypothesized that daily administration of *O. formigenes* facilitates the secretion of endogenously produced oxalate via the GI tract. The enteric elimination of oxalate may help to reduce the oxalate deposits and eventually decrease the plasma oxalate level.

6.1 Background and Rationale

A recent natural history study in PH patients included in the Rare Kidney Stone Consortium (RKSC) Registry (Zhao *et al.,* 2016), studied the key determinants for renal outcome in this patient population. The patients included in the analysis had a mean eGFR of 73 ml/min/1.73 m^2. During a median follow-up of 3.9 years (1.0, 12.8), 20% of patients developed End-Stage-Renal-Disease (ESRD). The most important progression factors for kidney deterioration in patients with Primary hyperoxaluria (PH) from this registry study are detailed in Table 4.

Table 4: Factors Univariately Associated with Incident ESRD among PH Patients without ESRD at Diagnosis

Variable	Hazard Ratio (95% CI)	p-Value
PH1	13.17 (3.19-54.38)	<0.001
Age at diagnosis	1.02 (1.00 to 1.04)	0.02
Uox, mmol/1.73 m^2 per 24h	1.13 (0.94 to 1.37)	0.20
Uox, mmol/1.73 m^2 per 24h (Q4)*	3.40 (1.40 to 7.90)	0.01
eGFR, ml/min per 1.73 m^2	0.96 (0.94 to 0.99)	0.002

*Q4: Quartile 4 related to a urinary oxalate excretion of > 1.87 mmol/1.73 m^2 per 24hr

Univariate analyses showed that the most important parameters governing the progression to ESRD in PH-patients were type of PH, highly elevated urinary oxalate excretion (the highest quartile Q4 with above 1.87 mmol/1.73 m^2 per 24hr), age at diagnosis and eGFR at diagnosis.

This protocol will include subjects with an eGFR below 90mL/min/1.73 m^2 and a total plasma oxalate concentration of ≥ 10 µmol/L. The rationale for these inclusion criteria is based on available data from the previous studies with OC3 and OC5 and the above referenced analysis of registry data for PH patients. The following key points have been considered in determining the selection of subjects.

- eGFR at diagnosis is statistically significantly inversely correlated to risk for ESRD (Zhao *et al*., 2016). The lower the eGFR, the higher the risk for progression to ESRD. In the selected patient population with an eGFR below 90 ml/min/1.73 m^2 the natural disease progression is at a stage where a decline in kidney function (resulting in increased plasma oxalate) would be measurable over a 52-week study. The endpoints are selected to determine whether Oxabact treatment could halt or delay the disease

progression i.e. stabilise or reduce plasma oxalate and stabilise or improve kidney function over time.

- The selected patient population would have increased levels of plasma oxalate at baseline, as a consequence of the declining eGFR, and thus it would be possible to study not only a stabilization, but also a decrease in plasma oxalate concentration during 52 weeks treatment with Oxabact in these subjects.

It is evident from current knowledge that baseline kidney function is an important factor determining the rate of disease progression and that there is a clear correlation between plasma oxalate concentration and kidney function. Consequently, it is important to harmonise the treatment groups considering these parameters.

The following measures have been taken to prevent potential imbalances in the treatment arms:

- Subjects with an eGFR below 90 ml/min per 1.73 m^2 and plasma oxalate ≥ 10 µmol/L will be included in the study, in order to harmonise the study population with regard to stage of disease.
- Subjects will be stratified at randomization firstly in an attempt to evenly distribute any PH type 2/type 3 patients and secondly to evenly distribute PH1 patients with a baseline Urinary oxalate (Uox) excretion more or less than 1.87 mmol/1.73m^2 per day between the treatment groups.

In order to further define the patient population, the endpoints will be analysed for sub-groups of subjects determined by the subject´s baseline oxalate excretion (urinary oxalate excretion more or less than (or equal to) 1.87 mmol/24hr/1.73m^2), high and low age (greater (or equal to) or less than 18 years) and high and low eGFR (more (or equal to) or less than 60 ml/min/1.73m^2). Since these parameters seems to be the most important drivers of the disease progression, the subgroups of subjects with a high baseline urinary oxalate excretion, high age and a low baseline eGFR would be expected to show a more pronounced treatment response over 12 months.

6.2 Overall Study Design and Plan

The study subjects will be monitored for up to 8 weeks prior to initiation of study medication, in order to obtain a robust baseline for each subject. Subjects will be treated for 52 weeks with OC5 or placebo. After the treatment phase, subjects will be followed for safety for 2 weeks or until the day before first dose in the follow-up study OC5-OL-02, whichever occurs first.

During the screening/baseline period, three visits in total will be done. Information on historical renal function (including eGFR, serum creatinine and the equation used to calculate historical eGFR) and kidney stone events (prior to informed consent) will be collected. For subjects under 18 years of age and subjects that were <18 years up to 3 years prior to screening (i.e. for all subjects under 21 years at screening), height used to calculate historic eGFR will also be collected.

The impact of stone events on days work/school attendance will be documented. During each of the screening/baseline visits, blood samples will be taken for safety labs, plasma oxalate and kidney function analyses. Subjects will have two Echocardiography examinations (Speckle Tracking and traditional echocardiography). Ultrasound of the kidney will be performed once during the baseline period. Subjects will be asked to provide one faecal and two 24-hour urine samples. These samples will be taken at the subject´s home. Subjects will be asked to complete a questionnaire evaluating their Quality of Life once during the baseline period.

Eligibility for plasma oxalate and eGFR will be based on the mean value of visit 1 and 2. For determination of eligibility, eGFR can be calculated using Schwartz or CKD-EPI equations that include serum creatinine and/or cystatin C.

For the urinary oxalate excretion, a mean value will be determined for visit 1 and 2 (screening/baseline period) and used for stratification/randomization.

Following screening and baseline evaluations, eligible subjects will be randomized 1:1 to receive either OC5 or placebo twice daily for 52 weeks.

During the treatment period, subjects will visit the clinic at weeks 8, 16, 24, 32, 40, 48 and 52. During these visits, blood sample for analyses of safety labs and plasma oxalate and kidney function will be taken and patient Quality of Life will be evaluated by a questionnaire during weeks 8, 24, 40 and 52. Speckle Tracking and traditional echocardiography will be done at two occasions during the treatment period, in weeks 24 and 48. Subjects will be asked to provide stool at three time points (weeks 24, 40 and 52) and 24-hour urine samples at four time points (weeks 8, 24, 40 and 52). Ultrasound of the kidney will be done at the end of the study in week 48. Information on kidney stone events and related symptoms occurring between signature of the informed consent form and the end of study will be captured (Please see Figure 6 for details of the OC5-DB-02 study design).

The subjects s will be offered to continue in an open-label follow-up protocol (OC5-OL-02, ePHex-OLE) with Oxabact treatment directly after the 52-weeks treatment period. A separate Clinical Trial Protocol and subsequent Clinical Trial Application will be issued for this study.

Figure 6: Study Design for OC5-DB-02 Study

	Screening/ Baseline (up to 8 weeks)			Treatment (52 weeks)								Post-treatment safety follow-up (up to 2 weeks)
Week	**-8-0**[1]			**0**	**8**	**16**	**24**	**32**	**40**	**48**	**52**	
Visit number	**1**	**2**	**3**	**TS**[2]	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**NA**
Plasma oxalate	X	X	X		X	X	X	X	X	X	X	
eGFR[3]	X	X	X		X	X	X	X	X	X	X	
Stone events[4,5]	X	X	X		X	X	X	X	X	X	X	X
Echocard.[6]	X		X				X			X		
Safety labs[7]	X	X	X		X	X	X	X	X	X	X	
Stool	X[8]						X		X		X	
24h urine collection	X	X			X		X		X		X	
Ultrasound			X							X		
Quality of Life	X[8]				X		X		X		X	
Adverse Events[5]					X	X	X	X	X	X	X	X

[1]All relevant assessments during screening/baseline will be completed during week -8 - 0, in total 2 visits will be done *before* baseline visit 3. The visits should be scheduled at least two weeks apart during the baseline period (visit 1, 2 and 3). After randomization of the subject, study drug will be ordered and shipped to the subject for start of treatment.

[2]Treatment Start (TS) - visit weeks in the treatment period will be calculated starting from treatment day one.

[3]As determined by the Schwartz equation eGFR for children (age below 18), and CKD-EPI equation for adults (age 18 or above) based on serum creatinine. For determination of eligibility, eGFR can be calculated using Schwartz or CKD-EPI equations that include serum creatinine and/or cystatin C.

[4]Kidney stone events and related symptoms will be captured at every visit, including occurrences in between visits.

[5]A post-treatment safety follow-up will be performed as a telephone call (see Section 6.3.4 for details).

[6]Two echocardiography examinations at least three weeks apart should be performed during the baseline period (week -8 – 0). The week 24 and 48 examination should be done within +/- 2 weeks of the clinic visit. If the images at week 24 and/or 48 do not meet quality criteria, the examination will be repeated within 4 weeks.

[7]Safety labs will include blood and urine sampling.

[8]Can be done anytime during screening/baseline, i.e. week -8 – 0.

Safety evaluation will include physical examination, vital signs and safety labs at baseline and at weeks 8, 16, 24, 32, 40, 48 and 52. Adverse events will be monitored throughout the study.

For the course of the study, the subjects should be instructed to maintain their normal diet and fluid intake as prescribed under their standard clinical care.

Monitoring of adverse events, concomitant medication and compliance with the administration of study drug will be performed at least at each visit. Furthermore, questions on kidney stone events and related symptoms will also be asked throughout the study.

After the intake of the last dose of study treatment (Last Dose Date; LDD), subjects will be followed for safety (see Section 6.3.4.1 for details on the post-treatment safety follow-up). The LDD is considered the End of Treatment (EOT); the end of the safety follow-up period is considered End of Study (EOS). Adverse events, including kidney stone events and related symptoms, are recorded throughout the entire treatment phase and safety follow-up, i.e. until EOS (Figure 7); AE recording is described in detail in Section 11.4.

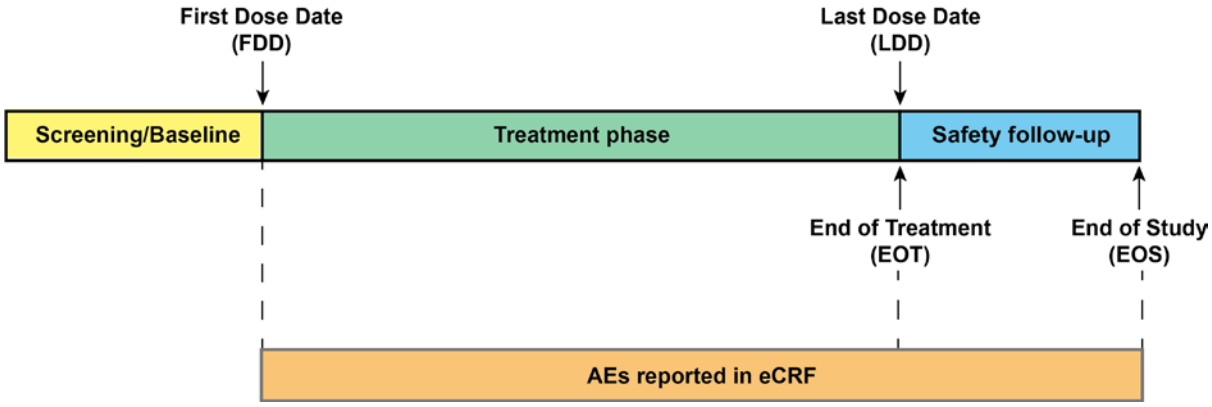


Figure 7: Illustration of Safety Reporting in the ePHex Study

6.3 Schedule of Assessments

The assessments to be performed during the study are described in Table 5, and the procedures are further described in Sections 6.3.1– 6.3.4.

Table 5: Schedule of Assessments

Study period:	Screening/Baseline[1] Week -8 - 0			Treatment Week 0-52[2]								Post-treatment safety follow-up
Week:				0	8	16	24	32	40	48	52	
Visit number[3]	1	2	3	TS	4	5	6	7	8	9	10	
Incl/Excl criteria[4]	X		X									
Demographics	X											
Vital signs	X				X	X	X	X	X	X	X	
Physical exam	X				X	X	X	X	X	X	X	
PH Med History	X											
Medical history	X	X	X									
Concomitant med[5]	X	X	X		X	X	X	X	X	X	X	X
Pregnancy test (if appl)			X								X	
Plasma oxalate	X	X	X		X	X	X	X	X	X	X	
eGFR[6]	X	X	X		X	X	X	X	X	X	X	
Stone events[5,7]	X	X	X		X	X	X	X	X	X	X	X
Safety Labs[8]	X	X	X		X	X	X	X	X	X	X	
Echocard.	X[9]		X[9]				X[10]			X[10]		
Ultrasound			X							X		
Stool	X[11]						X		X		X	
24 hr urine	X	X			X		X		X		X	
Review of Adverse Events[5]					X	X	X	X	X	X	X	X
Quality of Life	X[11]				X		X		X		X	
Drug dispense/ Accountability[12]			X		X	X	X	X	X	X	X	

1. All relevant assessments during screening/baseline will be completed during week -8 - 0, in total 2 visits will be done before baseline visit 3. The visits should be scheduled at least two weeks apart during the baseline period (visit 1, 2 and 3). After randomization of the subject, study drug will be

ordered and shipped to the subject for start of treatment. Treatment will start as soon as the first delivery of study drug to the subject has been done.

2. Treatment weeks will be planned from treatment day 1, i.e. visit week 8 will be scheduled at treatment day 1 + 56 days.
3. Visit window during treatment period +/- 3 days (except for baseline period). In the case of an acute kidney injury occurring close to a scheduled visit, the visit will be rescheduled to ensure that the AKI does not adversely affect values (especially for eGFR).
4. Eligibility criteria for plasma oxalate and eGFR samples taken at visit 1 and 2, will be evaluated during Baseline at visit 3. For determination of eligibility, eGFR can be calculated using Schwartz or CKD-EPI equations that include serum creatinine and/or cystatin C.
5. A post-treatment safety follow-up will be performed as a telephone call (see Section 6.3.4 for details).
6. As determined by the Schwartz equation for children (age below 18), and CKD-EPI equation for adults (age 18 or above). As detailed in point 4 above, eGFR equations including serum creatinine and/or cystatin C can be used for determination of eligibility.
7. Kidney stone events and related symptoms will be captured at every visit, including occurrences in between visits.
8. Safety labs include: haematology analysis, clinical chemistry analysis and urinalysis. FSH analysis for postmenopausal women will be done at screening.
9. Two examinations to be done during week -8 – 0 at least three weeks apart.
10. Echocardiography to be done within +/- 2 weeks of the clinic visit at week 24 and 48. If the images fail quality criteria, the examination will be repeated within 4 weeks. Any repeat examination for the week 48 visit should be done before end of treatment at week 52.
11. Can be done anytime during screening/baseline, i.e. week -8 – 0.
12. First study drug dispense to subject will be arranged when enrollment in the study has been confirmed for the subject. During treatment period, study drug will be shipped to the subject every other week.

6.3.1 Screening

Patient Information must be given and the Informed Consent form (ICF) must be signed prior to any study related procedures being performed. The investigator shall list all patients who are considered for participation in the study and who have signed the ICF on a Screening and Inclusion log. Pre-screening activities may be conducted if required. The inclusion and exclusion criteria should be reviewed. Demographics and baseline data will be collected.

These include:

- Date of birth, height, weight.
- Physical examination and Vital Signs.
- PH medical history (including diagnosis, renal function, kidney stone events).
- Other relevant medical history, including concurrent illnesses and symptoms.
- Prior/Concomitant medication and any non-medication therapy (e.g. physiotherapy) used in the 60 days prior to ICF signature. Any historical use of vitamin B6 and any other relevant medication, as judged by the investigator, should also be recorded.

Subject demographic details collected will comply with local requirements regarding patient confidentiality in applicable countries.

Historic renal function parameters (e.g. serum creatinine, eGFR including the equation used to calculate historic eGFR), if available, will be collected to determine the rate of renal function decline in years before participation in study OC5-DB-02. For subjects under 18 years of age and subjects that were <18 years up to 3 years prior to screening (i.e. all subjects under 21 years at screening), height used to calculate historic eGFR will also be collected. Information on historical stone events and related symptoms for the past 3 years will also be collected (visit 1). The impact of stone events on days work/school attendance will be documented.

A complete physical examination will be performed and will include assessment of: general appearance, gastrointestinal system, dermatological system, EENT (eyes, ear, nose, throat), head and neck, cardiovascular system, respiratory system, musculo-skeletal system, peripheral nervous system. Additional examinations may be done at the Investigators discretion.

Vital signs assessed on study include temperature and blood pressure (systolic and diastolic), heart rate and respiratory rate assessed after 5 minutes resting in a supine position. Weight will be measured using a calibrated scale with the subject lightly clothed and shoes off. Height will be measured using a calibrated wall mounted stadiometer. Height should be measured at each visit for all subjects where the Schwartz equation is used to determine eGFR. This would apply to children (subjects under 18 years of age) and to any subject who turn 18 during the study period. For subjects who are adults at study start, height will only be recorded at screening.

The following samples should be taken and sent for analysis:

- Haematology analyses, clinical chemistry analyses, FSH analysis for post-menopausal women.
- Urine sample for urinalysis.
- Plasma sample.

The subject (and parents when applicable) will be instructed how to collect 24-hour urine and faeces samples, and dates for collection will be scheduled. See Section 6.4 for further

information on collections at subject´s home. For the course of the study, the subject should be instructed to maintain their normal diet and fluid intake as prescribed under their standard clinical care. The subject will be asked to avoid taking ascorbic acid preparations or multivitamin preparations during the study period. The subject will be scheduled for an additional two visits during the baseline period.

6.3.2 Baseline

During the baseline period of up to 8 weeks, plasma oxalate sampling will be done at 3 occasions (including the screening visit). The blood sample will be processed at the clinical site and the acidified plasma will be shipped frozen to the central laboratory (Academical Medical Center, Amsterdam, the Netherlands, AMC) for determination of total oxalate using the Isotope dilution Gas Chromatography with mass selective detection (GC-MSD). The mean of the two first plasma oxalate results (total plasma oxalate) during the baseline period will be reviewed for the eligibility criteria concerning plasma oxalate.

For urinary oxalate excretion, a mean value will be determined for visit 1 and 2 (screening/baseline period) and used for stratification/randomization.

A separate sample will be processed at the site with ultrafiltration, acidification and sent frozen to AMC, to be analysed for free plasma oxalate concentration with GC-MSD.

Plasma samples will also be sent to central laboratory BARC (BARC Europe, Ghent, Belgium for EU and Tunisia sites; and BARC USA, NY, USA for North-American sites) for analysis of serum creatinine for calculation of eGFR. Plasma samples will also be analysed for some of the exploratory analytes as specified in the lab manual.

Patient Quality of Life will be evaluated using a questionnaire once during the baseline period.

Safety labs will be taken at 3 occasions during baseline (including the screening visit) and analysed at central lab (BARC). Safety urinalysis will be analysed locally. Pregnancy test for women of childbearing potential will be done at visit 3 at central lab (BARC). Subjects will have two Echocardiography examinations done at visits 1 and 3. Ultrasound of the kidneys will be done once during baseline (visit 3). Information on kidney stone events and their impact on days work/school attendance will be collected during baseline (visit 2 and 3). Subjects will also be asked to provide two 24-hour urine samples and one faecal sample.

The faeces sample will be collected at the subject´s home and shipped for analysis at Institut fur Mikroökologie, Herborn, Germany. The 24-hour urine sample will also be taken at the subject´s home and shipped to The Doctors Laboratory (TDL), London, United Kingdom.

Any changes in concomitant medication will be recorded in the eCRF during the baseline period. At visit 3 each subject's eligibility will be reviewed to assure that no exclusionary conditions have developed since the screening visit. The mean result from the two eGFR values (visit 1 and 2) calculated using the Schwartz/CKD-EPI equations based on creatinine and/or cystatin C will be reviewed for the eligibility criteria concerning eGFR at this point. If the eGFR values vary more than 50% between the two samplings, the subject will be evaluated for Acute Kidney Injury (AKI). If AKI is confirmed, the subject should not be randomized and needs to be stabilized first. When the kidney status is stable (as confirmed by routine local measurements/investigator decision), the subject may perform a new baseline period to verify eligibility. If eligible, the subject will be randomized. If the subject experiences another AKI during the baseline period, the subject should not be enrolled. A repeat baseline period may be performed only once.

For subjects who fail any of the inclusion or exclusion criteria, applicable parts of the eCRF should be completed (inclusion/exclusion criteria including corresponding data, and reason for the subject not being eligible). Subjects deemed not eligible for treatment should be recorded as screen failures on the Screening and Inclusion log. However, at the discretion of the investigator, a subject who fails screening may be re-evaluated (re-screened) at a later timepoint.

After verification of the inclusion and exclusion criteria, the subject will be randomized and assigned a randomization number. Subjects will be randomized 1:1 to receive either OC5 or placebo twice daily for 52 weeks.

At the clinical visit 3, the subject and/or the parents will be instructed about the administration procedures for the study drug. See Section 8 regarding treatment of subjects.

After randomization of the subject, study drug will be ordered and shipped to the site. Treatment will start as soon as the first shipment of study drug has been delivered to the subject. The following visit dates, during the treatment period, will be calculated from the first day of treatment.

6.3.3 Treatment Period

During the 52-week treatment period, the subject will be followed by visits at week 8, 16, 24, 32, 40, 48 and 52 according to Table 5. End of treatment (EOT) is defined as the day of last intake of study drug (Last dose date (LDD)).

At the visits blood samples for determination of plasma oxalate, kidney function and safety labs will be taken. Subjects will have a physical examination and vital signs will be taken. Patient Quality of Life will be evaluated using a questionnaire at weeks 8, 24, 40 and 52.

At week 24 and 48, Echocardiography (Speckle Tracking and traditional echocardiography) will be performed. Ultrasound of the kidneys will be performed at week 48.

Subjects will be asked to provide four 24-hour urine samples, at weeks 8, 24, 40 and 52, and three faecal samples, at weeks 24, 40 and 52, during the treatment period. These collections will be done at the subject´s home and should be scheduled in connection with the visit (+/- 3 days). The collections may be planned to occur during a weekend if preferred by the subject/parents.

At each visit the subject will be assessed for the presence or absence of Adverse Events and any changes in concomitant medications. Information on the occurrence of any stone event and related symptoms will be collected at each visit (from the time of signed informed consent form until the end of the study). Days missed at school/work due to a stone event will also be documented. Information on stone events will be captured by completing the Adverse Event eCRF. (Please note this is a data entry convention and does not replace the per protocol definition of an Adverse Event as defined in Section 11.1.).

Pregnancy test will be repeated at the last study visit for women of childbearing potential.

The subject will receive shipment of study drug once every second week during the treatment period. Remaining study drug and empty containers will be returned to the clinic or site pharmacy continuously during the study at the clinic visits, as soon as new supply for the current treatment weeks has been received. The final return of study drug/empty containers will be done at the last clinic visit.

In case an acute kidney injury/stone event should occur during the study treatment, the subject should be treated according to standard hospital care. The subject should not attend a visit if

they are experiencing an acute kidney injury/stone event since this may affect study values (particularly the eGFR value). When the event has been resolved the subject can be rescheduled to the study timetable.

6.3.4 Follow Up

The subject will be offered to continue in an open-label follow-up protocol (ePHex-OLE, OC5-OL-02) with Oxabact treatment directly after the 52-week treatment period. A separate Clinical Trial Protocol has been developed and Clinical Trial Applications have been or will be submitted/approved for this study.

6.3.4.1 Post-Treatment Safety Follow-Up

Previous clinical experience with Oxabact® suggests that the bacteria only transiently colonise the gastrointestinal tract and faecal recovery generally drops below detectable levels 1-2 weeks after end of treatment (Hoppe *et al*. 2006). All subjects who complete study treatment in ePHex, withdraw or were withdrawn from the study prematurely, will be followed for safety.

This post-treatment safety follow-up period starts the day after LDD in ePHex and ends:

- Fourteen days after LDD in ePHex, *or*
- The day before the first dose of study drug in the extension study ePHex-OLE has been taken,

whichever occurs first.

The follow-up will be performed as a telephone call maximum three working days after the end of the safety follow-up period. The subject will be asked for any AEs (incl. kidney stone events) appearing after completion of ePHex study treatment (i.e. after LDD). Additionally, any AEs that were present at the last completed study visit should be followed-up. Concomitant medication taken during the safety follow-up period should also be recorded.

Adverse Drug Reactions, which are unresolved at the time of the safety follow-up call, should be followed by the Investigator until the event has resolved or, if persistent, has been assessed as “chronic” or “stable”.

6.3.5 Electronic Patient Reported Outcome

A web-based interface for electronic patient reported outcome (ePRO) will be used continuously during the study. Subjects will record details on collections of urine and faeces, Quality of Life as well as the first and last dose of study drug intake here. Subjects will regularly receive reminders to record this data, and to follow study procedures with study drug and specimen collection.

6.4 Specimen Collection at Subjects’ Homes

The collections of faeces and urine will be performed by the subjects in their home environment, and should be performed according to Table 5. The sponsor will provide collection kits and a Patient Handbook describing the collection procedures. Collection kits will either be taken home from the clinic by the subject or delivered to subject’s home by a courier. Pick-up of collections will be done by courier.

6.5 Diet

For the course of the study, the subjects will be instructed to maintain their normal diet and fluid intake as prescribed under their standard clinical care. They will be asked to avoid making

significant changes in their diet during the study specifically related to high oxalate foods and fluid intake.

The subjects will be asked to refrain from ascorbic acid preparations or multivitamin preparations during the study.

7 SELECTION AND WITHDRAWAL OF SUBJECTS

7.1 Subject Inclusion Criteria

1. Signed informed consent (as applicable for the age of the subject).
2. A diagnosis of PH (as determined by standard diagnostic methods).
3. eGFR < 90 ml/min/1.73 m^2. The Schwartz equation will be used to estimate GFR for children (age below 18), and CKD-EPI equation will be used for adults (age 18 or above).
4. Plasma oxalate concentration ≥10 µmol/L in total plasma oxalate.
5. Male or female subjects ≥ 2 years of age.
6. Subjects receiving vitamin B6 must be receiving a stable dose for at least 3 months prior to screening and must not change the dose during the study. Subjects not receiving vitamin B6 at study entry must be willing to refrain from initiating pyridoxine during study participation.

7.2 Subject Exclusion Criteria

7. Inability to swallow size 4 capsules.
8. Subjects that have undergone transplantation (solid organ or bone marrow).
9. Subjects requiring dialysis or at immediate risk for kidney failure or in expected to be in need of dialysis during the study period.
10. The existence of secondary hyperoxaluria, e.g. hyperoxaluria due to bariatric surgery or chronic gastrointestinal diseases such as cystic fibrosis, chronic inflammatory bowel disease and short-bowel syndrome.
11. Use of antibiotics to which *O. formigenes* is sensitive. (This includes current antibiotic use, or antibiotics use within 14 days of initiating study medication.)
12. Current treatment with a separate ascorbic acid preparation.
13. Pregnant women (or women who are planning to become pregnant) or lactating women.
14. Women of childbearing potential who are not using adequate contraceptive precautions. Please see Section 7.3 regarding requirements for contraception
15. Presence of a medical condition that the Investigator considers likely to make the subject susceptible to adverse effect of study treatment or unable to follow study procedures, or any condition that is likely to interfere with the study drug mechanism of action (such as abnormal GI function).
16. Participation in any interventional study of another investigational product, biologic, device, or other agent within 60 days prior to the first dose of OC5 or not willing to forego other forms of investigational treatment during this study.

7.3 Contraceptive Precautions

The following requirements should be followed for women of childbearing potential.

1. Females of childbearing potential will be included if they are either sexually inactive (sexually abstinent for 14 days prior to the first dose continuing through 28 days after the last dose), or using one of the following highly effective contraceptive methods (i.e. results in <1% failure rate when used consistently and correctly) during this trial:
    a. intrauterine device (IUD);
    b. surgical sterilization of the partner (vasectomy for 6 months minimum);
    c. combined (estrogen or progestogen containing) hormonal contraception associated with the inhibition of ovulation (either oral, intravaginal, or transdermal);
    d. progestogen only hormonal contraception associated with the inhibition of ovulation (either oral, injectable, or implantable);
    e. intrauterine hormone releasing system (IUS);
    f. bilateral tubal occlusion.

    Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. In this trial abstinence is only acceptable if in line with the subjects preferred and usual lifestyle.

    Periodic abstinence (calendar, symptothermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhoea method (LAM) are not acceptable methods of contraception. As well, female condom and male condom should not be used together.
2. Females of childbearing potential agree to remain sexually inactive or to keep the same birth control method for at least 28 days following the last dose.
3. A female of non-childbearing potential must have undergone one of the following sterilization procedures at least 6 months prior to the first dose:
    a. hysteroscopic sterilization;
    b. bilateral tubal ligation or bilateral salpingectomy;
    c. hysterectomy;
    d. bilateral oophorectomy;

    or be postmenopausal with amenorrhea for at least 1 year prior to the first dose and follicle stimulating hormone (FSH) serum levels consistent with postmenopausal status.

Since the study drug consist of commensal bacteria, which are a natural part of the microbiota, the risk of excretion via the semen or any negative effects from such excretion is highly unlikely. Therefore, no requirements for contraception is applied for male subjects.

Females of childbearing potential will have a pregnancy test at baseline and at the end of the study at week 52. Additional information concerning participants becoming pregnant during the study can be found in Section 11.9.

7.4 Subject Withdrawal Criteria

If a subject fails to return for a scheduled study visit, the investigator will make a reasonable effort to contact the subject and determine why the subject failed to return and to schedule a new study visit. Any information obtained during this contact will be documented in the study records.

A study subject will be discontinued from study treatment if:

- Any clinically significant adverse event (AE), laboratory abnormality, illness, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the subject.
- OxThera or the Principal Investigator in consultation with OxThera may discontinue the subject at any time for medical and/or administrative reasons.
- The Principal Investigator in consultation with OxThera may discontinue the subject in case there is a major protocol violation.
- The subject requires dialysis.

Any subject who withdraws prematurely from study treatment will be asked to complete all study assessments described for the week 48 and week 52 clinical visits (combined) and will be followed for safety for two weeks after the last intake of study drug.

Subjects who withdraw consent from participating in the study at any time, will end the study. They will not have any further assessment and no further study data will be collected.

For any subject who has withdrawn, the date of withdrawal and the reason for withdrawal from treatment and/or from the study will be recorded in the source data and eCRF.

A subject who fails screening may be re-evaluated (re-screened) at a later timepoint. Withdrawn subjects should not be re-entered into the study. Subjects that are withdrawn due to major protocol violations will be summarized in the clinical study report. Additional subjects may be enrolled at the discretion of the Sponsor.

8 TREATMENT OF SUBJECTS

8.1 Description of Study Treatment

The study drug consists of OC5 as the active treatment and placebo with the same appearance. The study drug is supplied as enteric-coated capsules. One capsule shall be administered orally with water twice daily with breakfast and dinner. The study product is described in more detail in Section 9.

8.2 Concomitant Medications

Any medications including over-the-counter medications or herbal supplements will be recorded as concomitant drug therapy on the case report form. Prior/concomitant medication used in the 60 days prior to signing of the ICF will be recorded. Any historical use of vitamin B6 and any other relevant medication as judged by the investigator should also be recorded. Subjects will continue any medications they are receiving at study entry for underlying medical conditions and the medications will be recorded at screening and changes will be noted throughout the study, i.e. until EOS).

8.2.1 Concomitant Treatment with Vitamin B6

Subjects receiving vitamin B6 (pyridoxine) prior to study entry must be on a stable dose for at least 3 months prior to the screening visit. Subjects who are not receiving vitamin B6 at study entry will not be allowed to initiate vitamin B6 during study participation. Subjects will be encouraged not to change the dosing of vitamin B6 or discontinue vitamin B6 during study participation except for any safety events considered related to vitamin B6.

8.2.2 Prohibited Medications

Subjects are not allowed to receive any other investigational therapies during or within 60 days prior to study participation.

Other prohibited medications are:

- Ascorbic acid preparations: Ascorbic acid preparations must not be used during the study period (screening/baseline and treatment period) as it may affect the measurement of urinary oxalate.
- Long-term use of antibiotics: Subjects that require long-term use (more than 3 consecutive weeks) of antibiotics to which *O. formigenes* is sensitive during the treatment period will discontinue study drug and be followed for safety.
- For subjects that require short-term use of antibiotics during the treatment period, care should be taken to primarily choose antibiotics to which *O. formigenes* is resistant. In case of repeated short-term use (more than one course) an evaluation will be made to assess the risk for interference with the study drug treatment.
    - *O. formigenes* has shown resistance to antibiotics that affect the cell envelope, such as ceftizoxime, imipenem, ampicillin, amoxicillin and penicillin.
    - *O. formigenes* has shown sensitivity to antibiotics that are protein synthesis inhibitors, such as chloramphenicol, doxycycline, erythromycin, and tetracycline.

8.3 Treatment Compliance

Subjects will be provided with a diary to record the intake of the first and last dose of study drug. Compliance to study treatment will also be checked with the subjects at each visit to the clinic.

Unused medication will be returned to the clinic or site pharmacy at the next clinical visit, or the visit thereafter if new supply has not yet been received by the subject. Accountability will be done for the unused medication for an overall check of subject compliance.

8.4 Randomization and Blinding

At the screening visit, the subjects will be identified with a site-specific consecutive screening number consisting of site identification number and subject number. After completed screening procedures and eligibility check, the subjects will be assigned to blinded OC5 or placebo in a 1:1 ratio according to a randomization list created by an independent statistician. The randomization list and treatment assignments will not be accessible until database-lock and unblinding. Subjects will be stratified, prior to randomization, firstly in an attempt to obtain an even distribution of PH type 2/type 3 patients and secondly to stratify the PH type 1 patients for a baseline urinary oxalate excretion above or below (or equal to) 1.87 mmol/24h/1.73 m^2 (based on the mean of the two first visits during screening).

Randomization will be performed via a centralized procedure.

If a subject discontinues the study, the subject number will not be re-used by another subject.

Capsules with study medication will all have the same appearance to enable the double-blind study design.

The following analytes/parameters on treatment, which may reveal a treatment-induced effect, will be defined as blind data and will remain blind to both subjects and sites until the study ends, the database is locked and the unblinding has occurred:

- Plasma oxalate
- Quantity of *O. formigenes* in faeces
- Urinary oxalate and urinary calcium
- Echocardiography (Speckle Tracking and traditional echocardiography) and ultrasound: During image analysis, Echocardiography and ultrasound central readers will also carry their assessments unaware of any clinical data from the study subjects.

This data (except for quantity of *O. formigenes* in faeces) will also remain blind to the study team but may be subject to blind data quality review at the earliest at LPLV.

9 STUDY DRUG MATERIALS AND MANAGEMENT

9.1 Study Drug

OC5 is supplied as enteric-coated, size 4, gelatine capsules. One capsule contains NLT 1E+09 CFU and has the capacity to metabolise not less than 100 mmol oxalate per 19 hours. Details on the product are described in Table 6 below.

Table 6: Details of the Study Drug in the OC5-DB-02 Study

Parameter	High Dose
Active Substance	Lyophilized *O. formigenes*, strain HC-1
Name	Oxabact (OC5)
Route of Administration	Oral
Dose form	Enteric-coated capsule
Viable cell count	$1.0 * 10^9 – 5.0 * 10^{10}$ CFU/dose
Oxalate degrading capacity	>100 mmol/capsule
Excipients	Oligofructose, maltodextrin, alginate, sucrose, microcrystalline cellulose

9.2 Study Drug Packaging and Labelling

Capsules will be filled into sealed aluminium tubes. Each tube will contain 18 capsules.

The label, including subject number, will be placed on the aluminium tube and on secondary packaging (transparent bag) holding the aluminium tubes.

The product will be labelled to meet national regulatory requirements.

9.3 Study Drug Storage

Study medication provided by the sponsor must be stored in a temperature-controlled freezer (-20°C +/- 5°C), in a locked area at site pharmacies before dispensing and delivery to subjects. Study medication should be stored refrigerated (2°C to 8°C) at the subject's home, current stability data supports up to 4 weeks refrigerated storage.

9.4 Study Drug Accountability, Handling and Disposal

OxThera will supply study medication through the designated drug depot to all sites in the study.

The drug depot will complete a drug inventory log to document receipt and distribution of study drug. They will send study drug to the site pharmacies as a controlled shipment at -20°C +/- 5°C. The site pharmacies will store study medication in a temperature- controlled freezer (-20°C +/- 5°C), in a locked area. The site pharmacies will distribute study drug to subjects and will inform subjects that study capsules should be stored refrigerated (2°C to 8°C) at their homes in the securely sealed tubes. Subjects will be supplied with sufficient capsules for dosing every second week.

Unused study medication and empty vials will be returned to the clinic or site pharmacy at the next scheduled visit to the clinic after each delivery of new supply. The pharmacy will

document receipt of unused study medication. Any surplus of study medication will be reviewed and properly documented by the study monitor, and returned to the distributor.

Investigational product accidentally destroyed in transit, in subject´s home or at the study site should be accounted for and documented.

10 ASSESSMENT OF EFFICACY

The efficacy parameters to be assessed are:

Primary Endpoint:

- Change from baseline in total plasma oxalate concentration after 52 weeks of treatment.

Key Secondary:

- Change from baseline in kidney function after 52 weeks of treatment.
- Frequency of kidney stones events after 52 weeks of treatment. Stone events are defined as:
  - Subject- or investigator reported symptoms, or
  - Stone passages or removals, or
  - Increase in number of stones assessed by ultrasound.

Other Endpoints

- Percent change from baseline in total plasma oxalate concentration after 52 weeks of treatment.
- Subjects achieving 'near-normalization' of total plasma oxalate concentration (<10 µmol/L) at least twice during weeks 24 to 52 of treatment.
- Change from baseline in myocardial function as measured by Speckle Tracking and traditional echocardiography.
- Change from baseline in free plasma oxalate concentration after 52 weeks of treatment.
- Change from baseline in urinary oxalate excretion after 52 weeks of treatment.
- Change from baseline in grade of nephrocalcinosis as assessed by Ultrasound
- Change in number of O. *formigenes* in stool.
- Association between change in number of *O. formigenes* in stool and change in total plasma oxalate concentration.
- Change from baseline in score of Quality of Life questionnaire.
- Change from baseline in markers for renal function, renal tubular capacity and inflammation:
  Urine: magnesium, phosphorus, citrate, calcium, glycolate, creatinine, urea, calcium oxalate crystals, pH, osmolality and urinary volume.
  Blood: magnesium, phosphorus, citrate, calcium, glycolate, BUN, ALP, bicarbonate, CRP, WBC, creatinine and cystatine C.

10.1 Plasma Oxalate

Samples for plasma oxalate will be collected during clinical visits, as specified in Table 5. The mean of the two values at Visit 1 and 2 will be the basis for eligibility criteria, whereas the mean of the three values obtained at Visit 1, 2 and 3 will be used as the mean baseline plasma oxalate value for statistical evaluation.

Samples for total plasma oxalate will be processed at the clinical site and analysed at Academic Medical Center, Amsterdam, the Netherlands (AMC). Each site will be provided with kits and supplies for collection, processing and shipping of blood samples for determination of total plasma oxalate. Complete instructions for the collection, processing, storage and shipping of sample will be provided in the site manual.

Total plasma oxalate will be measured using isotope dilution gas chromatography with mass selective detection (GC-MSD). The measurement of plasma oxalate has analytical and biological variability. The analytical variability for the total plasma oxalate assay as performed by AMC is very low and the accuracy is within 1 µmol/L. The biological variability is higher and may be related to the actual hydration status for the subject.

Ultrafiltered, acidified plasma samples will also be analysed for free plasma oxalate with GC-MSD at AMC.

The ratio between the two different results will be monitored throughout the study.

Earlier studies on Oxabact used either an enzymatic assay for plasma oxalate where the oxalate available for enzymatic reaction is measured, or a standard plasma oxalate Ion Chromatography (IC) method where a 10 kDa ultra filtration step prior to acidification of the sample separates the protein-bound oxalate from the sample. Earlier study results should therefore be interpreted with this fact in mind. In contrast, since the sample preparation for analysis of total plasma oxalate does not include an ultra filtration step prior to acidification, the method measures the total amount of plasma oxalate. Since the major portion of the plasma oxalate is likely protein-associated (Hatch, 1990; Hatch *et al*., 1977) both ultrafiltered samples and samples without ultrafiltration will be analysed in the OC5-DB-02 study, complementary to each other. By using both sample preparation methods in the study for analysis of plasma oxalate, the ratio between the change in free plasma oxalate and the change in total plasma oxalate can be evaluated as an indicator of change in protein associated oxalate, as well as the two different parameters independently.

A positive treatment effect on plasma oxalate would be a statistically significant difference in change from baseline in total plasma oxalate concentration after 52 weeks of treatment. The relationship between change in *O. formigenes* and change in total plasma oxalate will also be evaluated.

Change from baseline in total plasma oxalate concentration after 52 weeks treatment will also be evaluated in the sub-groups of subjects with a urinary oxalate excretion above and below or equal to 1.87 mmol/L/24h/1.73 m^2, age above or equal to and below 18 years of age and an eGFR above or equal to and below 60 ml/min/1.73 m^2.

10.2 Kidney Function Parameters

Change from baseline in kidney function (eGFR) will be evaluated after 52 weeks treatment. The mean of the two values taken at visit 1 and 2 will be the basis for the eligibility criteria. For determination of eligibility eGFR will be evaluated using Schwartz or CKD-EPI equations that include serum creatinine and/or cystatin.

Change in kidney function will be evaluated based on eGFR calculation using the 2009 creatinine-based “Schwartz bedside” equation (for children below 18 years of age) (Schwartz *et al*., 2009) and 2009 creatinine-based CKD-EPI equation for adults (Levey *et al*., 2009). Subjects who turn 18 during the study period will continuously be evaluated using the Schwartz equation, ie the equation used at baseline will be kept throughout the study.

Samples for eGFR calculations will be processed at the clinical site and analysed at central lab (Cerba Research [previously called BARC]). Each site will be provided with kits and supplies for collection, processing and shipping of blood samples for determination of serum creatinine and cystatine C. Complete instructions for the collection, processing, storage and shipping of sample will be provided in the site manual.

10.3 Kidney Stone Events

Kidney stones are hard deposits made of minerals and salts that form inside the kidneys. Most kidney stones (approximately 80%) are calcium stones, usually in the form of calcium oxalate. Stones can also be composed of struvite, uric acid or cystine. Stones vary in size and shape, ranging from a few mms up to 40mms. Patients can typically pass the smaller stones in the urine. However, larger stones (e.g. > 10mm) may require lithotripsy and surgical or endoscopic removal. A kidney stone may not cause symptoms until it moves around within the kidney, passes into the ureter, bladder, or urethra.

Stone Events

In this study, kidney stone events are defined as follows:

- A stone event is defined as the occurrence of one or more of the following symptoms due to a kidney stone that may or may not require medical intervention:
  - Abdominal, flank or groin pain, sometimes associated with nausea and vomiting.
  - Macroscopic hematuria (visible blood in the urine).
  - Urinary tract infection (cloudy or foul-smelling urine, more frequent and/or painful urination than normal, persistent need to urinate and/or urinating small amounts).
- Stone events may also be defined by subject-reported stone passage or by medical procedures to remove identified kidney stones (e.g. lithotripsy, endoscopy, surgery).
- An increase in number of kidney stones as seen in a kidney ultrasound would also be defined as a stone event.

Duration between stone events: if symptoms of a stone event occur simultaneously or close in time to another kidney stone event symptom, the investigator can decide whether or not this should be reported as the same stone event or as a new, separate stone event.

Assessments of stone events will be collected by ultrasound (renal imaging) (see Section 10.6.2 below) as well as subject-reported stone events or symptoms of events (see definition above). Historical kidney stone events and related symptoms for the past three years preceding study entry will be asked at screening. During the baseline and treatment period, information will be collected concerning occurrence of self-reported kidney stone events and related symptoms. This information will be captured by completing the Adverse Event eCRF at each visit throughout the study.

Frequency of kidney stone events will be evaluated after 52 weeks of treatment.

10.4 Speckle Tracking and Traditional Echocardiography

Speckle Tracking Echocardiography (STE) is currently evaluated as a method to detect and quantify effects of oxalate deposits in the heart muscle (Lagies *et al*., 2013, 2019). STE is considered to be a feasible tool for discovering subtle changes of myocardial performance and identifying PH patients at risk for serious cardiac damage.

Speckle Tracking and traditional echocardiography will be performed twice during screening/baseline and at week 24 and 48 (+/- 2 weeks) during the treatment period. The examination will be performed locally using specific equipment as detailed in the site manual. Images will be interpreted centrally in a blinded manner. If the images are not meeting the quality criteria, the examination will be repeated within 4 weeks of the initial examination. For the week 48 examination, a repeat examination, if needed, should be done before end of treatment in week 52.

Complete instructions for the collection, processing, storage and transfer of images will be provided in the imaging manual.

STE results will be evaluated for changes in global longitudinal strain, including segmental changes, short-axis myocardial function, rotational displacement and apical sparing patterns. Traditional echocardiographic parameters will also be evaluated (e.g. Ejection Fraction, End Diastolic Volume, Medial to Lateral Diastolic Peak Velocity (e/é), Early to Late Ventricular Filling Velocity (e/a), Left Ventricular End Diastolic Dimension, Fractional shortening, Tricuspid Regurgitation by Doppler as an indicator of right ventricular/pulmonary pressure.). A positive treatment effect would be an improved myocardial function based on the above-mentioned parameters, decreased global longitudinal strain, improved rotational displacement and reduced/removed apical sparing pattern. The treatment effect on STE parameters will be compared to traditional echocardiography parameters of left and right ventricular function to determine which assessment of cardiac function is more sensitive in these subjects.

10.5 Quality of Life Questionnaire

A questionnaire will be used to evaluate the Quality of Life for the participating subjects. The SF36v2 questionnaire will be used for the adult subjects and the CHQ/CHQ/PF50 for subjects 5-18 years and their parents. The questionnaire will be completed by the subjects/parents during screening/baseline and during the clinical visits at weeks 8, 24, 40 and 52.

10.6 Other Parameters for Evaluation of Treatment Effect

The additional parameters described below will also be analysed for treatment effect.

10.6.1 Quantification of *O. formigenes*

The possibility to monitor the natural occurrence of *O. formigenes* bacteria and the presence of the *O. formigenes* drug before, during and after treatment with OC5 is an important tool for control of the pharmacodynamics of the drug. To date, two different genotypes of naturally occurring *Oxalobacter formigenes* have been identified, genotype 1 and 2 (groups I and II). Oxabact, or OC5, is of the strain HC-1 of the *Oxalobacter formigenes* genotype 1. A real-time quantitative PCR assay will be used that permits determination of the numbers of both *O. formigenes* genotype 1 and genotype 2 in faecal samples.

Faecal samples will be collected at subject's home and shipped to central laboratory MVZ Institut für Mikroökologie GmbH, Herborn, Germany.

A positive treatment effect on the result from analysis of number of *O. formigenes* should show an increased number of *O. formigenes* genotype 1 during treatment period. Earlier studies have shown that *O. formigenes* genotype 2 have increased in the OC5 treated group, but not in the placebo group indicating a transfer of oxalate from plasma to the intestine mediated by OC5 treatment. *O. formigenes* genotype 2 will also be monitored for this reason.

The association between change in *O. formigenes* and change in total plasma oxalate will also be evaluated.

10.6.2 Ultrasound

Ultrasound of the kidneys will be done at the site hospital. Images will be sent for central reading in a blinded manner. The examination will be standard and described in detail in the imaging manual.

Ultrasound images will be evaluated to determine the grade of nephrocalcinosis and the amount of kidney stones.

For nephrocalcinosis, the grading system using the ultrasound will be using 0-3 grades (Boyce *et al*., 2013)

• grade 0: no echogenicity

• grade 1: mild echogenicity around medullary pyramid borders

• grade 2: moderate echogenicity around and inside pyramids

• grade 3: severe echogenicity of entire pyramids

10.6.3 Twenty-Four-Hour Urine Samples

Twenty-four-hour urine samples for analysis of urinary oxalate, urinary calcium, urinary glycolate, urinary magnesium, urine citrate, urine urea, urine creatinine, urine phosphorus excretion, pH, osmolality and urine volume will be taken at subject´s home and sent to central laboratory TDL, London, UK. Urine calcium oxalate crystals and pH will also be analysed from this sample. Urinary oxalate will be analysed with a colorimetric enzymatic assay using oxalate oxidase, which oxidizes oxalate to carbon dioxide and hydrogen peroxide. The analyses will be described in more detail in the lab manual.

10.6.4 Plasma Sample – Other Endpoints

Plasma samples will also be analysed for magnesium, phosphorus, citrate, calcium, glycolate, BUN, ALP, bicarbonate, CRP, WBC, creatinine and cystatine C for evaluation of other endpoints. The details of sampling and analysis of these parameters will be outlined in the lab manual.

11 ASSESSMENT OF SAFETY

The safety parameters to be assessed are:

- Adverse Events
- Laboratory safety measurements
- Vital signs
- Physical examination

11.1 Definition of Adverse Events

Adverse Event (AE)

An AE is any untoward medical occurrence in a patient or clinical study subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including 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. AEs therefore include e.g. worsening of a pre-existing illness and any injury or accident. This refers also to symptoms due to a pre-existing allergy, e.g. if seasonal allergy symptoms are within what is normally experienced, they should not be recorded as adverse events. If the symptoms are worse than what is normally experienced, then they should be recorded as adverse events.

An AE does **not** include:

• Symptoms of the underlying disease (with exception of kidney stone events) that might be reasonably anticipated to come and go, or progress, given the nature and severity of the condition. However, if the progression of the disease escalates, resulting in hospitalization, is life-threatening, or is fatal, then progression of the disease should be reported as an AE of serious nature;

• Expected variations in severity of disease signs and symptoms that have previously been reported in the subject's medical history;

• Pre-planned medical or surgical procedures (e.g., surgery, tooth extraction, or transfusion) [Note: The condition that leads to the procedure may be an AE];

• Overdose of study drug without any clinical signs or symptoms; or

• Clinically significant laboratory values. If abnormal laboratory values are accompanied by abnormal signs or symptoms, the signs or symptoms are considered an AE and should be recorded as such. Abnormal laboratory values associated with the underlying disease are not an AE unless the values unexpectedly worsen. Abnormal laboratory values will be recorded in the study database.

Adverse Drug Reaction (ADR)

An AE is defined as an adverse drug reaction (ADR) if further analyses prove that the AE is caused or partially caused by the investigational product. This includes interaction, overdosing, abuse and development of addiction. Expected ADRs are also possible events due to the substance class of the investigational drug, expected from analogue conclusions or theoretical considerations related to toxicological, pharmacological or kinetic characteristics.

Serious Adverse Event (SAE)

A Serious Adverse Event (SAE) is one that suggests a significant hazard, contraindication, side effect or precaution that results in:

- the subject's death
- is life-threatening*
- requires inpatient hospitalization or prolongation of existing hospitalization **
- persistent or significant disability/incapacity; or
- congenital anomaly/birth defect
- corresponds to another important medical event as determined by the Investigator.

* Life threatening means that the subject was at immediate risk of death from the AE as it occurred, or it is suspected that the use or continued use of the investigational product would result in the subject's death. Life threatening does not mean that had an AE occurred in a more severe form it might have caused death.

** Hospitalization requires over-night stay at the hospital. Outpatient treatment in an emergency room is not itself a SAE. Hospital admission and/or operations planned before or during a study are not considered SAEs if the illness or disease existed before the subject was enrolled in the study, provided that it did not deteriorate in an unexpected way during the study.

Suspected Unexpected Serious Adverse Reaction (SUSAR)

Suspected Unexpected Serious Adverse Reaction (SUSAR) is a suspected unexpected serious adverse reaction.

In cases of doubt on this issue, it is suggested that there should be a predisposition to report rather than not to report (see Section 11.3).

11.2 Relationship to Study Drug

The following relationships to study drug will be used in the study (in accordance with the WHO-UMC Causality Categories, Table 7). Events classified as certain, probable/likely or possible will be considered related to study drug.

Table 7: WHO-UMC Causality Categories

Causality Term	Assessment Criteria
Certain	• Event or laboratory test abnormality, with plausible time relationship to drug intake • Cannot be explained by disease or other drugs • Response to withdrawal plausible (pharmacologically, pathologically) • Event definitive pharmacologically or phenomenologically (i.e. an objective and specific medical disorder or a recognized pharmacological phenomenon) • Rechallenge satisfactory, if necessary

Probable/ Likely	• Event or laboratory test abnormality, with reasonable time relationship to drug intake • Unlikely to be attributed to disease or other drugs • Response to withdrawal clinically reasonable • Rechallenge not required
Possible	• Event or laboratory test abnormality, with reasonable time relationship to drug intake • Could also be explained by disease or other drugs • Information on drug withdrawal may be lacking or unclear
Unlikely	• Event or laboratory test abnormality, with a time to drug intake that makes a relationship improbable (but not impossible) • Disease or other drugs provide plausible explanations
Conditional/ Unclassified	• Event or laboratory test abnormality • More data for proper assessment needed, or • Additional data under examination
Unassessable/ Unclassifiable	• Report suggesting an adverse reaction • Cannot be judged because information is insufficient or contradictory • Data cannot be supplemented or verified

11.3 Recording Adverse Events

Each subject will be questioned about AEs at each visit/ following initiation of treatment. The question asked will be "Since your last visit have you had any health problems?" The information can also be obtained from signs and symptoms detected during each examination, observed by the study personnel or spontaneous reports from the study subjects or by lab results.

The investigator is to record in the eCRFs all directly observed AEs, all AEs as a response of the open question and all AEs spontaneously reported by the subject during the study.

The investigator will record all AEs by:

- Description of event (recorded in standard medical terminology and avoiding abbreviations),
- Start and end date,
- Intensity/grade*
- Seriousness (serious or not serious, according to definition),
- Causal relationship, (certain, probable/likely, possible, unlikely, conditional/unclassified, unassessable/unclassifiable)
- Action taken, (none, treatment required, study drug interrupted, subject withdrawn, other)
- Outcome of the AE (recovered, recovered with sequelae, death, not recovered)

The Sponsor or delegate will code all AEs and SAEs using MedDRA.

* For each reported AE, the intensity (grade) will be recorded. The following grades of intensity are to be used (CTCAE version 4.0):

Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL*.

Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL**.

Grade 4: Life-threatening consequences; urgent intervention indicated.

Grade 5: Death related to AE.

A Semi-colon indicates 'or' within the description of the grade.

*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

**Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

If the intensity /grade changes within 24 hours the maximum intensity should be recorded. If the intensity changes over a longer period of time, the changes should be recorded in the eCRF.

11.4 Reporting Adverse Events

Adverse Event reporting for each subject shall start at the initiation of study treatment, (i.e. from FDD). The reporting shall continue during the course of the study (i.e. until EOS).

Thus, reported AEs include:

- all AEs that occur during the treatment phase
- all AEs that occur during the safety follow-up period

Spontaneously reported events by study subjects in between planned visits shall also be reported. AEs should be reported for all subjects, independent of treatment assignment.

NON-SERIOUS AEs are to be reported in the eCRF.

SAEs are to be reported by the investigator within 24 hours of awareness. The eCRF and the initial SAE form (provided in the Investigational Site File) are both to be completed within this time, and the latter submitted to ProductLife Department via fax (please see SAE report form for fax number) or email (safety@productlife-group.com).

In case of any question related to SAE reporting please contact Drug Safety Physician/ Medical Monitor, see contact information in Table 1. All SAEs must be reported whether or not considered drug related. The site is required to send the available SAE information even if the data is incomplete.

After receipt of the initial report, ProducLife will forward the information to OxThera within 24 hours. ProductLife Pharmacovigilance Department will work with OxThera to review the information received and contact the site to request any missing information/amendments needed. When follow-up information is obtained by the investigator it should also be forwarded

to the Medical Monitor within 24 hours. The report should be marked "Follow-up report". Any follow-up received should be treated in the same way as initial reports.

The investigator will submit copies of SAE reports to the independent ethics committee concerned as required by local regulations. All serious and unexpected adverse events will be reported to the European authorities as per regulations.

Adverse Drug Reactions, which are unresolved at the end of the safety follow-up period should be followed by the Investigator until the event has resolved or, if persistent, has been assessed as "chronic" or "stable". This data is not recorded in the eCRF.

An Unexpected Adverse Reaction is any adverse reaction, of which the specificity or severity is not specified in the current Investigator's Brochure for the study drug.

A SUSAR is a Suspected Unexpected Serious Adverse Reaction. All SUSARs that are possibly, probably or definitely related to OC5 are subject to expedited reporting to Regulatory Authorities, Ethic Committees and participating investigators in accordance with local requirements in force and the ICH guidelines for Good Clinical Practice (GCP) and the EU Directive 2001/20/EC. ProductLife Pharmacovigilance Department will be responsible for ensuring expedited reporting of SUSARs.

11.4.1 Emergency Unblinding

The investigator is responsible for ensuring that there are procedures and expertise available to handle emergencies during the study. In the event the investigator considers it necessary to know the identity of the study substance to manage a specific serious adverse event, the treatment may be un-blinded for that particular subject. The responsibility to break the treatment code in emergency situations resides solely with the investigator and the investigator has unrestricted and immediate access to emergency envelopes available at the site to break the treatment code. The investigator shall immediately inform the Medical Monitor if the treatment code is broken.

11.5 Anticipated Adverse Events

11.5.1 Review of Available Data

OC3 has been evaluated in several clinical studies in patients with PH; one 28-day phase I/II study (OC3a), one 24-week double blind placebo-controlled multicentre international phase II/III clinical study including a 24-week open-label extension study (OC3b), and one 24-week double-blind placebo-controlled multicentre international phase II/III clinical study (OC3b buffer formulation). OC5 has been evaluated in one double-blind placebo-controlled multicentre international phase II study and one ongoing phase II open-label study, see Table 3. In summary OC3 and OC5 have been evaluated in over 100 subjects receiving a dose of up to 10^9 CFU twice a day. The continuous exposure to study drug ranged from 4 weeks up to 3 years. Both OC3 and OC5 were considered safe and well tolerated in all clinical studies.

Review of the safety data from the complete studies indicate that subjects may experience headache and gastrointestinal symptoms including abdominal pain, constipation, nausea, vomiting, diarrhoea, and flatulence.

No treatment related Serious Adverse Events for Oxabact were reported in patients with PH. In all studies, the majority of adverse events were reported as mild and unlikely related to the treatment. In the placebo-controlled studies, the adverse events were equally distributed between OC3/OC5 and placebo.

11.5.2 Safety Data for OC5-DB-01 Study

Twenty-eight subjects were randomized in the OC5-DB-01 trial, and half of them were treated with active drug. The first subject was enrolled in December 2013 and the last patient last visit (LPLV) was in January 2015. No serious related adverse events were reported in the trial.

The AEs are summarized in Table 8 and Table 9. In general, the treatment groups appeared to be comparable, although some slight differences could be noted. No SAE was reported in the placebo treatment group, whereas three subjects in the OC5 treatment group reported a SAE. Slightly higher number of subjects reported Renal and urinary disorders in the OC5 treatment group (n=4) as compared to the placebo treatment group (n=0). Slightly higher number of subjects reported Nervous system disorders in the placebo treatment group (n=5, whereof all reported headache) as compared to the OC5 treatment group (n=1, reported both headache and dizziness).

Table 8: Overview of Adverse Events for Study OC5-DB-01

	OC5 (N=14)		**Placebo (N=14)**		**Total (N=28)**	
	n (%)	**m**	**n (%)**	**m**	**n (%)**	**m**
Any adverse events	10 (71.4%)	41	12 (85.7%)	26	22 (78.6%)	67
Any serious adverse events	3 (21.4%)	3	0	0	3 (10.7%)	3
Adverse events by relationship						
NOT RELATED	9 (64.3%)	26	10 (71.4%)	15	19 (67.9%)	41
POSSIBLE	6 (42.9%)	14	6 (42.9%)	10	12 (42.9%)	24
PROBABLE	0	0	0	0	0	0
DEFINITELY	1 (7.1%)	1	1 (7.1%)	1	2 (7.1%)	2
Adverse events by intensity						
MILD	9 (64.3%)	28	12 (85.7%)	23	21 (75.0%)	51
MODERATE	6 (42.9%)	11	2 (14.3%)	2	8 (28.6%)	13
SEVERE	2 (14.3%)	2	1 (7.1%)	1	3 (10.7%)	3
Adverse event leading to withdrawal	0	0	0	0	0	0
Adverse event leading to death	0	0	0	0	0	0

n: Number of subjects; m: Number of mentions

Table 9: Summary of Types of Adverse Events Experienced in OC5-DB-01 (Safety Analysis Set)

	OC5 (N=14)		Placebo (N=14)		Total (N=28)	
	n (%)	m	n (%)	m	n (%)	m
Any adverse event	10 (71.4)	41	12 (85.7)	26	22 (78.6)	67
Gastrointestinal disorders	6 (42.9)	14	5 (35.7)	7	11 (39.3)	21
Infections and infestations	3 (21.4)	3	4 (28.6)	5	7 (25.0)	8
Nervous system disorders	1 (7.1)	2	5 (35.7)	5	6 (21.4)	7
Respiratory, thoracic and mediastinal disorders	2 (14.3)	3	3 (21.4)	4	5 (17.9)	7
Renal and urinary disorders	4 (28.6)	7	0	0	4 (14.3)	7
Renal colic	2 (14.3)	3	0	0	2 (7.1)	3
Renal pain	2 (14.3)	2	0	0	2 (7.1)	2
Calculus urethral	1 (7.1)	1	0	0	1 (3.6)	1
Pyelonephritis	1 (7.1)	1	0	0	1 (3.6)	1
General disorders and administration site conditions	3 (21.4)	6	1 (7.1)	1	4 (14.3)	7
Skin and subcutaneous tissue disorders	3 (21.4)	3	0	0	3 (10.7)	3
Musculoskeletal and connective tissue disorders	1 (7.1)	1	1 (7.1)	1	2 (7.1)	2
Injury, poisoning and procedural complications	1 (7.1)	1	0	0	1 (3.6)	1
Psychiatric disorders	1 (7.1)	1	0	0	1 (3.6)	1
Ear and labyrinth disorders	0	0	1 (7.1)	1	1 (3.6)	1
Investigations	0	0	1 (7.1)	1	1 (3.6)	1
Reproductive system and breast disorders	0	0	1 (7.1)	1	1 (3.6)	1

n: Number of subjects; m: Number of mentions
Adverse events are coded according to MedDRA version 16.1
Percentage is based on number of subjects in safety analysis set

Ten subjects (71%) in the OC5 group experienced an AE, which was similar to the number in the Placebo group (12; 86%). A greater number of individual AEs were mentioned in the OC5 group than in the Placebo group (41 versus 26). Four subjects in the OC5 group experienced renal and urinary disorders (seven mentions in total); no AEs in this system organ class were reported in the Placebo group.

The occurrence of AEs was similar in the two groups. Although more renal and urinary disorders were experienced by subjects in the OC5 group than in the Placebo group (four subjects [29%] versus no subjects [0%]), only one occurrence was considered related to the treatment (Subject SCR01-0002; kidney pains, moderate, possibly related). The subjects in the OC5 group had a more pronounced reduction in renal function than the placebo subjects at baseline. Mean baseline eGFR was lower in the OC5 group than in the placebo group (97.5±38.7 versus 123.1±45.4 mL/min/1.73 m^2), and renal and urinary disorders were more common in the OC5 group (11 cases versus eight cases). Most notably, four subjects in the OC5 group had a history of chronic renal failure, whereas no subjects in the placebo group had been affected by this condition.

Two AEs were judged to be definitely related to treatment. Subject SCR01-0001 (OC5) experienced a case of mild rumbling stomach, which began the day that treatment commenced and resolved the day after treatment ended. Subject SCR07-0004 (Placebo) experienced a case of mild diarrhoea the day after treatment started.

Four AEs were of at least moderate severity and judged to be possibly related to the treatment: a moderate case of gastroenteritis (OC5; SCR01-0018), the aforementioned case of kidney pains (OC5; SCR01-0002), a moderate case of increased bowel movements (OC5; SCR03-0005) and a case of severe headaches (Placebo; SCR07-0004). No AEs required changes of dose.

11.5.3 Safety Data for the OC5-OL-01 Study

The OC5-OL-01 study started in May 2014 and ended in January 2020. Fourteen subjects were screened and 12 subjects were enrolled in the study and received study drug. This study included late-stage patients with ESRD who were on dialysis. Most of these subjects were on a waiting list for transplantation. Eight subjects continued into the continued treatment phase of the study. There were no severe nor serious related adverse events for Oxabact in this study.

Overall the safety data accrued to date would suggest that the OC5 drug product is safe and well tolerated and appears to have a similar safety profile as the earlier OC3 drug product. Further safety information of the OC5-OL-01, OC5-DB-02 and OC5-OL-02 studies has been detailed in the current version of the Oxabact Investigator's Brochure.

11.5.4 Systemic Infections due to *O. formigenes*

O. formigenes is a commensal bacterium in the intestinal tract and is a strict anaerobe. Following an extensive search of the literature, there have been no reports of local or systemic infections where *O. formigenes* have been isolated. Preparations of *O. formigenes* have been administered to rats, pigs and humans without any apparent toxicity or side effects including local or systemic infections. *O. formigenes* is dependent on oxalate as carbon source and cannot use any other carbohydrate source. In the completed clinical studies, no subject experienced any infection due to *O. formigenes*.

Any subject who develops signs and symptoms of bacteremia requiring hospitalization should be evaluated to identify the source of infection (e.g. lung, GI tract, meningeal). Evaluation for the source of bacterial infection should include clinical and laboratory tests and cultures for common pathogens. Empiric antibiotic treatment should include coverage for common pathogens. It is recommended that an Infectious Diseases Specialist be consulted to assist in the assessment and management of the subject.

O. formigenes has shown sensitivity to chloramphenicol, doxycycline, erythromycin, and tetracycline. In the event of unexpected infection caused by the study medication erythromycin is the recommended first line antibiotic therapy in children and adults, and tetracycline is the recommended second line antibiotic therapy in older children and adults. If common pathogens have been excluded or if the infection is not responding to erythromycin or tetracycline the study medication should be stopped until further evaluation.

11.5.5 Elevated Levels of Plasma Formate

Since *Oxalobacter formigenes* converts oxalate to formate in the intestine, there may theoretically be a possibility of elevated plasma formate levels following administration of high doses of OC5. However, formate is metabolized by a number of bacteria in the gut microbiota. Formate is a metabolite of methanol responsible for the toxicity observed with methanol poisoning. Typically, toxic effects require prolonged exposure to elevated plasma formate levels.

The potential for elevated plasma formate levels following administration of OC5 is theoretically limited due to the limited availability of endogenous oxalate. Over 100 subjects have been exposed to date to Oxabact (OC3 and OC5) with doses up to NLT 10^9 CFU in studies up to 12 months. There have been no signs or symptoms of elevated formate plasma levels, metabolic acidosis or methanol poisoning. The main manifestations of methanol poisoning are metabolic acidosis and ocular toxicity. Any subject who develops signs and symptoms of metabolic acidosis should be evaluated to identify the source (e.g. evaluation of serum bicarbonate, blood pH, anion gap, osmolality gap and plasma formate).

Formate accumulation in plasma is the main reason for acidosis in early, uncomplicated stages of metabolic acidosis. Metabolic acidosis is characterized by low blood pH (arterial pH < 7.38 or venous pH < 7.34) or low levels of serum bicarbonate, HCO_3^-(< 18 mmol/L). Decreased levels of serum bicarbonate will serve as an indication of elevated levels of formate. The OC5-DB-02 study will investigate this early sign of elevated plasma formate as part of routine safety labs. Low levels of serum bicarbonate should trigger determination of the anion gap and the osmotic gap in order to confirm or rule out the diagnosis of metabolic acidosis due to elevated formate levels.

If metabolic acidosis is diagnosed, the acidosis should be corrected as quickly as possible and therapy provided, if appropriate. If a diagnosis due to elevated formate levels cannot be excluded or if the metabolic acidosis cannot be corrected by standard of care the study medication should be stopped until further evaluation. Further evaluation should then include levels of plasma formate and visual disturbance test.

11.6 Laboratory Safety Measurements

The laboratory safety tests include:

- Haematology: Erythrocytes, Leucocytes, Lymphocytes, Monocytes, Neutrophils, Basophils, Eosinophils, Platelets, Haemoglobin, Haematocrit, MCV, MCHC.
- Chemistry: Blood Urea Nitrogen (BUN), electrolytes (Na+, K+, Mg++, Ca++, HCO_3^-, Cl), glucose, albumin, alkaline phosphatase, ALT, AST, total bilirubin, and total protein.
- FSH for postmenopausal women (see Section 7.3) at the screening visit only.
- Pregnancy test for women of childbearing potential at baseline and week 52.
- Random Urine (Urinalysis): protein, glucose, pH.

Laboratory parameters for safety assessment from haematology and chemistry will be assessed at the central laboratory BARC. Urinalysis will be done at the local lab at each clinic. Laboratory safety tests will be performed as specified in Table 5.

11.7 Vital Sign Measurements

Vital sign measurements on study include temperature and blood pressure (systolic and diastolic), heart rate, respiratory rate (assessed after 5 minutes resting in a supine position) and weight. Weight will be measured using a calibrated scale with the subject lightly clothed and shoes off. Height will be measured using a calibrated wall mounted stadiometer. Height should be reported for all subjects in whom the Schwartz equation is used to determine eGFR. This would apply to children (subjects under 18 years of age) and to any subject who turn 18 during the study period. For subjects who are adults at study start, height will only be recorded at screening.

11.8 Physical Examinations

Physical examinations include appearance, gastrointestinal system, dermatological system, EENT, head and neck, cardiovascular system, respiratory system, musculo-skeletal system, peripheral nervous system. Additional examinations may be done at the Investigators discretion.

11.9 Pregnancy

If a subject becomes pregnant during the course of the study the subject must immediately contact the Principal Investigator who should complete the pregnancy form and report the pregnancy within 24 h and send the form to ProductLife Limited. The administration of study medication should immediately be stopped, i.e. the subject will be withdrawn from study treatment. The subject must be followed until birth and the birth outcome must be documented within 6 to 8 weeks on the pregnancy form (detailed instruction can be found in the Safety Management Plan).

11.10 Data and Safety Monitoring Board

The Data and Safety Monitoring Board (DSMB) is the primary data and safety advisory group for the Sponsor (OxThera) consisting of independent clinical and statistical experts for study OC5-DB-02. Treatment with Oxabact has been shown to be well tolerated with no particular safety concerns in six completed studies, and one ongoing interventional study.

The DSMB will periodically review safety data, evaluate excess adverse effects and judge whether the overall integrity and conduct of the trial remain acceptable, and make recommendations to the Sponsor. The DSMB will meet shortly after study start, and then have regular meetings every 3-4 months. During the meetings, the DSMB will review general safety data (reported AEs, SAEs, SUSARs, safety labs and other safety variables that may be applicable) and will not be unblinded to treatment assignment, unless specifically requested by the DSMB. The DSMB will discuss their findings in closed sessions and convey their recommendations to the Sponsor in an open session in which the sponsor attends to make necessary decisions from a safety perspective.

The DSMB will be authorized to make recommendations to the Sponsor regarding safety issues, study conduct, and modifications to or termination of the study in the event that significant safety concerns arise during the study conduct. There will be no pre-defined discontinuation rules or formal statistical analyses performed as part of the DSMB to assess termination of the trial for efficacy or futility.

The DSMB will be advisory to the clinical trial leadership group at the Sponsor. The Sponsor will be responsible for promptly reviewing the DSMB recommendations, formulating decisions whether to continue or terminate the trial, and determine whether amendments to the protocol or changes in study conduct are required from a safety perspective.

12 STATISTICAL METHODS

12.1 Study Populations

The efficacy population (Full Analysis Set, FAS) will include all randomized subjects who had at least baseline, one post-baseline assessment and received at least one dose of study medication.

The evaluable population (Per Protocol Analysis Set) will include all randomized subjects included in the FAS who have taken at least 80% of scheduled doses of study medication, as judged by dispensation and return of study drug, and who provide at least two eligible measurements of plasma oxalate during treatment. Protocol deviations will be evaluated, and any further exclusions from PP will be decided prior to database lock and unblinding.

The primary and secondary efficacy analyses will be based on the efficacy population as well as the evaluable population respectively and as randomized. The efficacy population will be the primary population for efficacy analyses.

The safety population will consist of all randomized subjects who receive at least one dose of study drug. Safety analyses will be based on the safety population and as treated.

12.2 Tests of Hypotheses and Significance Levels

The mixed effect repeated measures model will be utilized to compare the treatment arms with respect to the primary endpoint with the null hypothesis that there is no effect of treatment (active treatment versus placebo) and the alternative hypothesis that there is an effect of treatment (active treatment versus placebo).

> H_0: There is no effect of treatment (active versus placebo) on the 52-week change from baseline for total plasma oxalate.
>
> H_A: There is an effect of of treatment (active versus placebo) on the 52-week change from baseline for total plasma oxalate.

Statistical analyses will be performed using a two-sided significance level of 0.05.

A fixed sequence stepwise multiple testing procedure at the 0.05 level will be performed in the pre-specified order of 1) plasma oxalate, 2) eGFR and 3) kidney stone events. When the hypothesis tested for an endpoint is statistically significant at the 0.05 level then the subsequent test will be performed at the 0.05 level. When a hypothesis test is not rejected at the 0.05 level then the subsequent analyses will only be considered descriptive.

12.3 Sample Size Calculation

In a previous OxThera study OC3-DB-02, with a total of 11 subjects (7 on active treatment, 4 on placebo) having a baseline eGFR <90 ml/min/1.73 m^2, a mean difference from baseline (screening) in total plasma oxalate concentration of - 4.964 (SD=5.219) µmol/L between active treatment versus placebo was observed after 24 weeks of treatment. Furthermore, OxThera study OC5-DB-01 showed a mean difference in total plasma oxalate of -5.299 (SD=3.235) after 8 weeks of treatment in a total of 9 subjects (7 active, 2 placebo) in subjects with baseline eGFR <90 ml/min/1.73 m^2.

Since for study OC5-DB-02 multiple plasma oxalate measurements will be taken at visits 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, the most appropriate statistical model for the primary endpoint is a mixed-effect repeated measures model. It is expected that at week 52 the difference may be

larger than what has been observed in the previous study OC5-DB-01 at 8 weeks. Therefore, for the calculation a conservative mean difference of -5.50 with SD of 4.00 is assumed. Using the MRMM model in a two-sided approach with α=5%, β=10% (and thus power=90%), a 1:1 allocation ratio and assuming a within subject correlation of 0.70, the sample size is assessed at 9 subjects for each group. With this sample size, a difference of -6.00 with an SD of 4.50 may be considered to be statistically significant (Oxabact vs. Placebo). Considering 18 completers are needed based on this calculation, an estimate of 22 subjects will be randomized to account for possible drop-outs.

To account for the possiblity of higher than observed variability, please find below Table 10 sample size estimates for various configurations.

Table 10: Sample Size Calculation Primary Endpoint

	Difference in plasma oxalate between Oxabact and Placebo					
SD	**-4.50**	**-5.00**	**-5.50**	**-6.00**	**-6.50**	**-7.00**
3.00	7:7	6:6	5:5	4:4	4:4	3:3
3.50	10:10	8:8	7:7	6:6	5:5	4:4
4.00	13:13	10:10	9:9	7:7	6:6	6:6
4.50	16:16	13:13	11:11	9:9	8:8	7:7
5.00	20:20	16:16	13:13	11:11	10:10	8:8
5.50	24:24	19:19	16:16	14:14	12:12	10:10

Considering the anticipated 18 completers using a MRMM model in a two-sided approach with α=5%, and a within subject correlation of 0.70, the sample size evaluated at 9 subjects for each group yield approximately 90% power to identify a difference (SD) of 4 (3) ml/min/1.73m^2 in eGFR change from baseline at week 52 between the treatment arms (Table 11).

Table 11: Power Calculations for Change from Baseline in eGFR

	Difference in eGFR between Oxabact and Placebo					
SD	**1**	**2**	**3**	**4**	**5**	**6**
3.00	0,12786	0,37707	0,69504	0,90883	0,98450	0,99856
4.00	0,08973	0,23426	0,45726	0,69504	0,87028	0,95953
5.00	0,07133	0,16555	0,31637	0,50645	0,69504	0,84237
6.00	0,06078	0,12786	0,23426	0,37707	0,53923	0,69504
7.00	0,05402	0,10488	0,18371	0,29169	0,42248	0,56250
8.00	0,04935	0,08973	0,15065	0,23426	0,33866	0,45726

Due to the limited number of patients in this rare disease population, and the limited amount of historical data, it is not possible to define the expected magnitude of change during the study period. Analysis of available data from the OC3-DB-02 study gives an indication of the change in the full population over six months treatment as indicated above, although the older less potent OC3 study drug was used in this study. Furthermore, the PH population is very heterogeneous, and subjects will likely show an individual response to treatment.

12.4 Primary Endpoint

Change from baseline in total plasma oxalate concentration will be calculated at each visit as the visit value minus the mean of the three measurements during baseline. The primary statistical analysis will be performed using MRMM analysis based on the FAS with a model that includes the following fixed effects: treatment group, baseline plasma oxalate value, randomization stratum (PH type 2, PH type 3, not PH type 2 or 3 and baseline urinary oxalate excretion ≤ 1.87 mmol/24h/1.73 m^2, not PH type 2 or 3 and baseline urinary oxalate excretion > 1.87 mmol/24h/1.73 m^2), week, and week-by-treatment interaction. The primary comparison will be between the Week 52 changes from baseline in OC5 and placebo. An autoregressive of order 1 (AR (1)) variance covariance matrix will be used for the within subject variation in the MRMM model. In case there is a convergence problem the following variance covariance matrix structures will be used in the order of 1) unstructured, 2) Toeplitz, and 3) compound symmetry (CS). The first (co)variance structure which does not have convergence problem will be the one used for the analysis. Comparisons at other weeks will also be performed respectively.

Values and change from baseline over time will be summarized. Mean total plasma values over time for both treatment groups will be plotted in addition to a plot with the data over time presenting one line for each subject. Total plasma oxalate observed minimum and maximum post baseline values and change from baseline will also be summarized and plotted. Additionally, and for each subject a plot presenting both total plasma oxalate and eGFR over time will be produced to ilustrate how total plasma oxalate and eGFR change together.

A sensitivity analysis will also be performed using MI prior to applying the MRMM analysis.

As both maximum likelihood (used in the MRMM) and MI assume at least MAR missing data will be evaluated and described (Ratitch *et al.,* 2013). To assess the robustness of the primary analysis to possible deviations from the MAR assumption a PMM will be used to support the evaluation of data with missingness mechanism not at random, MNAR.

Additionally, a second analytical method approach will be provided as supportive analyses (i.e. ANCOVA and/or AUC) and will be further defined in the SAP.

Secondarily the same analysis will be produced based on the PP analysis set.

12.5 Key Secondary Endpoints

- Change from baseline in kidney function (i.e. eGFR slope) after 52 weeks of treatment will be analysed in the same way as the primary endpoint. Estimated GFR will be calculated for the primary analysis based on the 2009 creatinine-based "Schwartz bedside" equation (for children below 18 years of age) and the 2009 creatinine-based CKD-EPI equation for adults. For subjects between 18 and 23 years (both inclusive) of age at screening visit 1, eGFR will be calculated as the average of the eGFR values based on the pediatric and adult equations (Ng *et al.,* 2018). The mean value of the three values obtained at visit 1, 2 and 3 will be considered as the baseline eGFR value for the statistical evaluation. Subjects will continuously be evaluated using the same eGFR equation as when entering visit 1. Difference in slopes using the proposed time-by-treatment interaction will be presented as well. LS means will be determined for each visit. Supportive summaries will use alternative eGFR equations based on the Cystatin-C based CKiD/CKD-EPI 2012 equations without modification for subjects between 18 and 23 years. Historical renal function will be assessed in the context of on-study renal

function to determine the degree of progression of renal function decline (i.e. the slope) before and during the study.

- Frequency of kidney stone events defined as
    - Subject- or investigator reported symptoms, or
    - Stone passages or removals, or
    - Increase in number of stones assessed by ultrasound

  after 52 weeks of treatment.

Kidney stone events after 52 weeks of treatment will be summarized using descriptive statistics. Incidence rates and 95% CIs of kidney stone events (defined as the number of events divided by the total person-years) will be calculated along with the number of stone events and the number of subjects with a stone event. Treatment groups will be compared.

12.6 Other Endpoints

- Percent change from baseline in total plasma oxalate concentration after 52 weeks of treatment will be analyzed in the same way as the primary endpoint.
- Subjects achieving 'near-normalization' of total plasma oxalate concentration (<10 µmol/L) at least twice during weeks 24 to 52 of treatment will be summarized. The treatment arms will be compared using the stratified generalized Cochran-Mantel-Haenszel test of general association including the appropriate stratification factors. Exact 95% CIs will be calculated for each treatment arm, and the common odds ratio across strata for the treatment arms. Wilson's score method with continuity correction will be used to calculate a 95% CI for the difference in rates.
- Change from baseline in myocardial function as measured by Speckle Tracking and traditional echocardiography will be summarized descriptively over time.
- Change from baseline in free plasma oxalate after 52 weeks of treatment will be analysed in the same way as the primary endpoint.
- Change from baseline in urinary oxalate excretion after 52 weeks of treatment.
- Change from baseline in grade of nephocalcinosis as assessed by Ultrasound
- Change in number of O. *formigenes* stool will be based on number of O. *formigenes* at week 52 compared to baseline in the active group versus placebo. The measurement at week 24 will be used as supportive data. Remaining time-points during the study will be used descriptively. Both O. *formigenes* genotype 1 and genotype 2 will be analysed.
- Association between change in O. *formigenes* in stool and change in total plasma oxalate concentration will be evaluated in the same way. The measurement at week 24 will be used as supportive data. The method of analysis will be the same as for the primary and secondary endpoints. Remaining time-points during the study will be presented descriptively.
- Change from baseline in score of Quality of Life scores as measured by SF36V2 or CHQ/PF50 questionnaires, depending on age, will be summarized over time and compared descriptively between the two treatment groups.
- Change from baseline in markers for renal function, renal tubular capacity and inflammation in urine and plasma will be analysed as the difference between absolute change from baseline in the active group and absolute change from baseline in the

placebo group. The analysis will be based on the week 52 value compared to the mean of the three values from baseline for plasma-based analysis and the two values from the baseline 24 h urine collections for the urinary based analysis. The measurement at week 24 will be used as supportive data. The method of analysis will be the same as for the primary and secondary endpoints. Remaining time-points during the study will be presented descriptively.

12.7 Subgroup Analysis

Aspects on Subgroup Analysis Based on Previous Studies

A sub-group analysis has been made based on pooled historical data from treatment with OC3 (OC3-DB-02) and OC5 (OC5-DB-01) where the combined patient population was divided into sub-groups based on kidney function, plasma oxalate concentration and urinary oxalate excretion at baseline. The purpose of the sub-group analysis was to see whether there were any detectable differences with regard to treatment response between the groups.

Although the number of subjects in each group is small, the analysis showed that:

- subjects with high Urinary oxalate excretion (above 1.87 mmol/24h per 1.73 m^2)
- subjects older than18 years
- subjects with eGFR <60 ml/min/1.73 m^2

tend to show a more pronounced treatment response measured as change in plasma oxalate concentration and/or change in eGFR over time. The primary and secondary endpoints will therefore be analysed also in these sub-groups of subjects.

Subgroup Analysis

Subgroup analyses of the following endpoints will be performed, total and free oxalate concentration, eGFR, urine oxalate, stone events, *O. formigenes* in stool, Speckle-Tracking and traditional echocardiography. The analyses will be based on the following subgroups provided there is sufficient sample size:

- Subjects with a baseline urinary oxalate excretion above and equal to or below 1.87 mmol/L/24h/1.73 m^2 respectively (mean of the two values during screening/baseline).
- Subjects above or equal to and below 18 years of age at baseline.
- Subjects with a baseline eGFR above or equal to and below 60 ml/min/1.73m^2 respectively (mean of the obtained values during screening/baseline calculated by the Schwartz CKiD (2009 creatinine-based “bedside Schwartz” equation for children (below 18 years of age) and 2009 creatinine-based CKD-EPI equation for adults).
- Race.
- Gender.
- Progressors and non-progressors based on historic eGFR (i.e. eGFR prior to treatment start).
- Use of vitamin B6 at baseline.

For the subgroup analyses, only the primary method of analysis will be used. Subgroups may also be analysed using alternative eGFR equations. No formal testing of subgroups will be performed due to small sample sizes.

12.8 Safety

The safety will be assessed through collection and analyses of adverse events (AEs), baseline medical conditions, laboratory tests, vital sign data and physical examinations. Listings of AE's and SAE's will be provided along with narratives. Adverse events will be categorized by SOC and Preferred Term from MedDRA. The focus of the adverse event summaries will be on treatment-emergent adverse events defined as occurring from the first dose in ePHex until;

- up to 14 days after the last dose of double-blind treatment, or
- the first day of open-label Oxabact treatment of ePHex-OLE,

whichever occurs earlier. Treatment-emergent adverse events will be summarized using counts and percentages overall, serious, by severity, and by relationship to study product.

Values over time and changes from baseline and shifts from baseline will be summarized by treatment group. Continuous parameters will be summarized by number of non-missing observations, mean, standard deviation, median, minimum, and maximum. Categorical parameters will be summarized by count and percent.

12.9 Randomization

This is a randomized, placebo-controlled double-blind multi-centre study. Subjects will be randomly assigned to OC5 or placebo in a 1:1 ratio. Subjects will be stratified firstly in an attempt to achieve an even distribution of PH type 2/type 3 patients and secondly to stratify the PH type 1 patients for a baseline urinary oxalate excretion above or below or equal to 1.87 mmol/24h/1.73 m^2 (based on the mean of the first two visits from screening/baseline).

12.10 Demographics and Baseline Characteristics

Demographic variables and other baseline characteristics will be summarized using descriptive statistics (mean, standard deviation, median, Q1, Q3, minimum and maximum) for continuous variables and counts and percentages for categorical variables. These summaries will be produced for the efficacy population, evaluable population and safety population.

12.11 Statistical Analysis

All statistical analyses will be further defined in a Statistical Analysis Plan (SAP). Any deviations from the SAP will be described and justified in the Clinical Study Report.

12.11.1 Data Imputation

Both maximun likelihood, as used in MRMM, and MI are the preferred methods to address missing data considered at least missing at randon. MRMM is the preferred method and uses all available data to provide unbiased estimates and therefore no imputation is required. Additional sensitivity analyses will be performed based on MI prior to the MRMM analyses and PMM. The extent and reasons for missing data will be evaluated.

13 DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

The investigator/institution should permit study-related monitoring, audits, IEC review and regulatory inspections, and should provide direct access to the source data/medical record.

The monitor should verify that each subject has consented in writing to direct access to the original medical record/source data (by the use of written patient information and signed informed consent). During the monitoring, the data recorded in the eCRFs by the investigator will be checked for consistency with the source documents/medical record by the study monitor (source data verification). Any discrepancies of data should be documented and explained in the monitoring reports.

For each subject, the medical records should include the minimum following information:

- subject number and study id
- date for information given, signing informed consent, screening
- treatments given, including investigational product(s)
- visits to the clinic
- AE/SAE, if any
- concomitant medication
- time and reason for discontinuation, if any

There are data that are recorded only on the eCRF, which are associated with protocol-specific procedures and not with normal clinical care practice. For such clinical data the investigator would not be expected to duplicate the information. Source data location will then be specified in the Investigator file.

14 QUALITY CONTROL AND QUALITY ASSURANCE

14.1 Monitoring and Audits

Monitoring of the study will be arranged by the sponsor according to GCP guidelines. Monitoring visits will be performed regularly to the study sites during the study, to ensure that all aspects of the protocol are followed. Source documents will be reviewed for verification of consistency with data recorded on the Case Report Forms and product accountability will be performed as a part of the monitoring visits.

The study site may also be subject to quality assurance audits by the sponsor/CRO as well as inspection by the appropriate regulatory agencies.

It is important that the investigator and their relevant personnel are available during the monitoring visits and possible audits/inspections, that study related records are made available, and that sufficient time is devoted to the monitoring process.

14.2 Site Personnel

Investigators and other key personnel shall provide curriculum vitae or equivalent, that will confirm their suitability for the clinical study. All investigators and key personnel should be listed together with their responsibilities in the study on a signature and delegation log.

It is the responsibility of the Investigator to ensure that all personnel involved in the study are fully informed of all relevant aspects of the study, including detailed knowledge of and training in all procedures to be followed.

15 ETHICS AND REGULATORY REQUIREMENTS

15.1 Ethics Review

It is the responsibility of the investigator to obtain written approval of the study protocol (incl. the patient information and informed consent) and subsequent protocol amendments from the IEC. The investigator should file all correspondence with the IEC, and a list of the IEC composition (names and position) should be filed in the Investigator File. A copy of the IEC approval should be forwarded to the sponsor.

15.2 Ethical Conduct of the Study

The study will be performed in accordance with the recommendations guiding physicians in biomedical research involving human subjects adopted by the 18th World Medical Assembly, Helsinki, Finland, 1964 and later revisions and applicable ICH GCP guidelines.

The investigator is responsible for complying with all reporting procedures applicable to their IEC.

15.3 Written Informed Consent

It is the responsibility of the investigator to give each subject, prior to inclusion in the study, full and adequate verbal and written information regarding the purpose and procedures of the study and the possible risks involved. The subjects must be informed about their right to withdraw from the study at any time, and that such withdrawal will not affect their future medical care, treatment or benefits to which the subject is otherwise entitled. The subjects should be informed that the results will be stored and analysed in a computer, maintaining confidentiality in accordance with local data protection laws.

Furthermore, it is the responsibility of the investigator to obtain signed informed consent from all subjects prior to initiation of any study-related activity. As the study involves children, age specific patient information sheets and parent information sheets should be prepared, and subject and/or parental consenting should depend on the age of the subject. The investigator or his/her designated representative, who gave the verbal and written information about the study to the subject, must also sign the informed consent form. A copy of the written patient information must be handed to each subject, to bring home. The investigator will confirm the receipt of informed consent from each subject by a recording in the eCRF. The signed Informed Consent forms should be filed by the Investigator in the Investigator File for possible future audits and inspections.

The investigator must always use the current IEC approved Patient Information/Informed Consent Form and it must not be changed without prior discussion with the sponsor and approval from the IEC.

15.4 Regulatory Requirements

The study will be performed in compliance with national regulatory requirements. As with the IEC, clinical trial authorization from the appropriate Regulatory Authority(ies) must be sought and obtained (as applicable to local country regulations), prior to the start of the study. The investigational product for this study will not be shipped to a study site until a copy of the applicable Regulatory Authority approval has been received by the sponsor. In addition, the Regulatory Authority(ies) must approve amendments (as instructed by OxThera), receive

SUSAR reports and annual safety updates or as required by local country regulations, and be notified of the end of the trial.

16 DATA HANDLING AND RECORDKEEPING

16.1 Case Report Forms

Study data for all subjects will be collected in a confidential fashion using an electronic Case Report Form (eCRF). All the information required by the protocol must be documented and any omissions explained. The investigator must review all eCRF entries for completeness and accuracy. Source documents, including all demographic and medical information, eCRFs and informed consent form for each subject in the study must be maintained by the Investigator. All information in the eCRFs must be traceable to the original source documents.

The eCRF must be signed and dated by the investigator who takes responsibility for the accuracy, completeness and legibility of the data reported to the sponsor in the eCRFs.

Medical history, concomitant medication and Adverse Events will be coded using standardized medical dictionaries (medical dictionary for regulatory activities; MedDRA).

16.2 Retention of Records

All essential documents must be safely retained by the investigator for at least 2 years following the date a marketing application is approved for the drug, for the indication for which it is being investigated; or if no application is filed or if the application is not approved for such indication for 15 years after the investigation is discontinued and the regulatory authorities are notified.

16.3 Protection of Personal Data

The completion of the Study involves the collection and processing of Personal Data. All processing of Personal Data at the clinic and by the sponsor must be carried out in accordance with national legislation concerning the protection of Personal Data.

The investigator must ensure that the subject's privacy is maintained. On the eCRF or other documents submitted to OxThera, subjects will be identified by a subject ID number only. Documents that are not submitted to OxThera (e.g., signed informed consent form) should be kept in a strictly confidential file by the investigator.

The investigator shall permit direct access to subjects' records and source document for the purposes of monitoring, auditing, or inspection by OxThera, authorized representatives of OxThera, Regulatory Authorities and IECs.

As part of the required content of the informed consent, subjects will be informed that their records may be reviewed by OxThera's designee and by regulatory agencies. Should access to medical record require a separate waiver or authorization, it is the Investigator's responsibility to obtain such permission from the subject in writing before the subject is entered into the study.

17 FINANCING, INDEMNIFICATION AND INSURANCE

The Clinical Trial Agreement (CTA) outlines the compensation and payment terms of the study. The CTA must be signed before the start of the study. If there are differences between the CTA and the Protocol regarding certain rights and obligations the CTA is the prevailing document. Indemnification is covered by the CTA between the sponsor and the Institution.

OxThera has civil liability insurance, which covers this study in all participating countries.

18 CONFIDENTIALITY, INTELLECTUAL PROPERTY AND PUBLICATION POLICY

Investigator's, Institution's and OxThera IP AB's obligations regarding intellectual property, confidentiality and publication are described in detail in the Clinical Trial Agreement. They can be summarized as follows;

All information (whatever form) disclosed by OxThera to Institution, or generated pursuant to this study, shall be deemed to be confidential information. Except as required by applicable law, Institution shall not use or disclose to any party OxThera 's confidential information received pursuant to this study or otherwise, without the prior written consent of OxThera. All data generated or arising from the performance of the study shall be the exclusive property of OxThera.

It is intended to publish the results of the study as a whole once all subjects have completed the study and the study has been analysed. If there is a publication, the Investigator that has the highest number of treated subjects will be the lead author, unless otherwise agreed. The investigator may not publish the results of their cohort of subjects until the full study has been submitted for publication. The investigator may not submit for publication or present the results of this study without allowing OxThera 30 days in which to review and comment on the pre-publication manuscript. The investigator may not submit the results of the study for publication without the prior consent of OxThera, unless the review period has passed and there has been no reaction from the sponsor.

19 CHANGES TO THE STUDY PROTOCOL

The investigator should not implement any deviation from or changes to the protocol without agreement with the sponsor and prior review and documented approval from the IEC and Regulatory Authorities, except where necessary to eliminate an immediate hazard to the subjects. All changes to the final study protocol must be documented in a written protocol amendment.

20 REFERENCES

Aggarwal KP *et al*. Nehprolithiasis: Molecular Mechanism of Renal Stone Formation and the Critical Role Played by Modulators. BioMed Res Int Vol 2013; Article ID 292953.

Boyce AM *et al*. Ultrasound is superior to computed tomography for assessment of medullary nephrocalcinosis in hypoparathyroidism. J Clin Endocrin Metab, 2013; 98: 989-994.

Cochat, P. *et al.* Epidemiology of primary hyperoxaluria type 1, Nephrol Dial Transplantation, 1995; 10(Suppl 8), pp. 3–7.

Cochat P and Rumsby G. Primary Hyperoxaluria, N Engl J Med 2013; 369:649-58.

Hatch M *et al.,* New Enzymic Method for Serum Oxalate Determination. Clin Chem, 1977; 23(1):76-7.

Hatch M., Spectrophotometric determination of oxalate in whole blood. Clin Chim Acta, 1990; 193:199-202.

Hatch M and Freel RW. Intestinal transport of an obdurate anion: oxalate. Urol Res, 2005; 33:1-16.

Hatch M. *et al*. Enteric ox elimination is induced and ox is normalized in a mouse model of PH following intestinal colonization with *Oxalobacter*. AJPGLP, 2011; 300: G461-G469

Hoppe B *et al*. Oxalobacter formigenes: a potential tool for the treatment of primary hyperoxaluria type 1. Kidney Intl, 2006; 70:1305–11.

Hoppe B, Beck B and Milliner D. The Primary Hyperoxalurias. Kidney Intl, 2009; 75: 1264-71.

Hoppe B *et al*. Reduction of plasma oxalate levels by oral application of Oxalobacter formigenes in 2 patients with infantile oxalosis. Am J Kidney Dis, 2011; 58(3):453-5.

Hoppe B. An update on primary hyperoxaluria. Nat Rev Nephrol, 2012; 8:467-75.

Lagies R *et al.* Apical Sparing of Longitudinal Strain, Left Ventricular Rotational Abnormalities, and Short-Axis Dysfunction in Primary Hyperoxaluria Type 1. Circulation: Heart Failure. 2013; 6: e45-e47.

Lagies, R. *et al*. Subclinical myocardial disease in patients with primary hyperoxaluria and preserved left ventricular ejection fraction: a two-dimensional speckle-tracking imaging study, Pediatric Nephrology (Berlin, Germany), 2019; 34(12), pp. 2591–2600

Levey AS *et al*. A New Equation to Estimate Glomerular Filtration Rate. Ann Intern Med. 2009, 150(9): 604–612.

Ng DK *et al*. Combination of pediatric and adult formulas for valid glomerular filtration rate estimates in young adults with a history of pediatric chronic kidney disease. Kidney Intl, 2018; 94(1): 170-177.

Pape, L. *et al*. *Oxalobacter formigenes* treatment combined with intensive dialysis lowers plasma oxalate and halts disease progression in a patient with severe infantile oxalosis, Pediatric Nephrology 2020, 35: 1121-1124.

Pascual E and Sivera F, Time required for disappearance of urate crystals from synovial fluid after successful hypouricaemic treatment relates to the duration of gout. Ann Rheum Dis. 2007; 66:1056-8.

Rare Kidney Stone Consortium (2013) Primary Hyperoxaluria. Available at: https://www.rarekidneystones.org/hyperoxaluria/physicians.html (Accessed: 24 September 2019).

Ratitch B, OKelly, Tosiello M. Missing data in clinical trials: from clinical assumptions to statistical analysis using pattern mixture models. Pharma Stat. 2013; 12: 337-47.

Sivaguru M *et al*. Geobiology reveals how human kidney stones dissolves in vivo. Scientific Reports 2018; 8: 13731 (DOI:10.1038/s41598-018-31890-9).

Schwartz GJ *et al*. New equations to estimate GFR in children with CKD. J Am Soc Nephrol 2009, 20:629–637.

Stewart CS, Duncan SH and Cave DR. Oxalobacter formigenes and its role in oxalate metabolism in human gut. FEMS Microbiol Lett 2004; 230:1-7.

van der Hoeven SM *et al*. Primary hyperoxaluria Type 1, a too often missed diagnosis and potentially treatable cause of end-stage renal disease in adults: results of the Dutch cohort. Nephrol Dial Transplant 2012, 27: 3855-3862.s

Zhao F et al., Predictors of Incident ESRD among Patients with Primary Hyperoxaluria Presenting Prior to Kidney Failure. Clin J Am Soc Nephrol. 2016; 11(1):119-26.

21 APPENDICES

Appendix 1: Study protocol approval by investigator

Appendix 1: Study protocol approval by investigator

Protocol number: OC5-DB-02

Protocol Date: 08 January 2021

Protocol Version: 11

Study title: A phase III double-blind, randomized study to evaluate the long-term efficacy and safety of Oxabact in patients with primary hyperoxaluria

Sponsor: OxThera Intellectual Property AB
Regeringsgatan 111
SE-111 39 Stockholm
Sweden

I, the undersigned, have read and understand the protocol specified above, and agree on the contents. The Study Protocol, the Clinical Trial Agreement/ Financial Agreement, and GCP Guidelines will serve as a basis for co-operation in this study.

Investigator: _____

Affiliation: _____

_____

Signature: _____

Date: _____
DD –MMM – YYYY