Clinical Study Protocol
Oxalobacter formigenes (Oxabact)

An Open-Label Single-Arm Treatment Extension Study to Evaluate the Long-Term Efficacy and Safety of Oxabact for Patients with Primary Hyperoxaluria who Completed Study OC5-DB-02

Protocol Number	OC5-OL-02
Protocol Name	ePHex-OLE
EudraCT Number	2018-003576-12
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Sponsor:	OxThera Intellectual Property AB Regeringsgatan 111 SE-111 39 Stockholm Sweden
Sponsor signee:	Bastian Dehmel, MD, Chief Medical Officer
Protocol Date:	06 July 2020
Protocol Version:	2

Revision History

Rev.:	Version No.	Description:	Date:
000	1	New Document	07 NOV 2018
001	2	Amendment 1 • Tunisia added to the list of participating countries, France and Netherlands removed from the list. • Information on the completed OC5-OL-01 study was added to section 4.4.1.7. A new section 4.4.1.8 was added describing the OC5-DB-02 study. Table 3 was updated. • Update of section 4.4.1.9 *Named Patient Use*. • Implementation of a 4-week post-treatment safety follow-up and stool sample (mainly addressed in sections 6.2 and 6.3) • Addition of section 6.3.3 *Post-treatment follow-up* for clarification of safety follow-up and addition of a stool sample. A new definition for end of study is also detailed. • Exclusion criterion 7 has been modified to also include lactating women. • Update of section 11.4 *Reporting of Adverse Events* to harmonise with the Safety Management plan. • Update of section 11.5.3 to add information on the completed OC5-OL-01 study and ongoing OC5-DB-02 study. • Addition of section 11.10 that the Data and Safety Monitoring Board will review patients until the OC5-DB-02 study is completed. • Update of section 12.2 *Sample Size Calculation.* • Addition of subgroup analyses under section 12.8. • Minor corrections and clarifications.	06 JULY 2020

Study Protocol Approval

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Study title: **An Open-Label Single-Arm Treatment Extension Study to Evaluate the Long-Term Efficacy and Safety of Oxabact for Patients with Primary Hyperoxaluria who Completed Study OC5-DB-02**

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This protocol has been approved by:

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Signature: B. Dehmel

Date: 08.07.2020
(DD –MMM – YYYY)

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Procedures in Case of Emergency

Table 1: Emergency Contact Information

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1 SYNOPSIS

Name of Sponsor/Company
OxThera Intellectual Property AB.

Name of Investigational Product
Oxabact OC5.

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

Title of Study:
An open-label single-arm treatment extension study to evaluate the long-term efficacy and safety of Oxabact for patients with primary hyperoxaluria who completed study OC5-DB-02.

Planned number of sites/Countries
The study is planned to be conducted in the following countries:
Germany, United Kingdom, Belgium, Spain, USA and Tunisia.
8-10 sites will be participating.

Phase of development:
III

Objectives:
Primary:
- To evaluate the efficacy of Oxabact following two years continued treatment in subjects who have completed the Oxabact OC5-DB-02 (ePHex) study.

Secondary:
- To obtain additional safety data from two years continued treatment with Oxabact.

Methodology:
This single arm, open-label international multi-center study (OC5-OL-02, ePHex-OLE) will evaluate the efficacy and safety of Oxabact treatment for an additional two years in subjects with Primary Hyperoxaluria (PH) who have previously been treated with Oxabact or placebo in the ePHex study. Subjects who consent to participate will, upon completion of the ePHex study, be seamlessly transferred from the ePHex protocol to this study. Baseline in this protocol is defined as the baseline of the ePHex study, unless otherwise stated.

At the start of the ePHex study, subjects had maintained renal function but with an estimated Glomerular Filtration Rate (eGFR) below the lower limit of the normal range (<90 ml/min/1.73 m^2) and a total plasma oxalate concentration ≥10 µmol/L. Subjects will not be re-tested for inclusion into the present ePHex-OLE protocol but values will be measured for their second baseline at the last visit (week 52) in the ePHex study (this applies to all parameters except for echocardiography and ultrasound of the kidneys which are assessed at week 48). If there is no seamless transition (delay >1

month) from the ePHex study to the ePHex-OLE study, a new clinic visit 0 will take place with applicable measurements for the second baseline.

Study visits will be performed once every two months (bimonthly) during the first year of the study and once every three months (quarterly) during the second year of the study. Blood 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, ALP, bicarbonate, CRP, WBC and blood urea nitrogen.

Speckle Tracking Echocardiography (STE) as well as traditional echocardiographic examinations will be performed every six months during the study. Ultrasound of the kidney will be done once every 12 months. Quality of Life will be evaluated every six months using a questionnaire. Twenty-four-hour urine samples will be taken every fourth to sixth month, for determination of oxalate excretion, magnesium, phosphorus, citrate, calcium, glycolate, urea, creatinine, calcium oxalate crystals, pH, osmolality, and urinary volume. Stool samples will also be taken every sixth month and analysed for *O. formigenes* cell count. The urine and stool samples will be taken at the subject´s home.

Adverse events and concomitant medication will be monitored throughout the study. Information on kidney stone events and related symptoms occurring between first dosing in the ePHex-OLE study and end of study will be captured. Subjects will be followed for safety for 4 weeks after intake of last dose of study drug, and they will provide a stool sample 4 weeks after end of treatment. End of study is defined as when the safety follow-up (by telephone-call) and the post-treatment stool sample have been completed.

In the case of an acute kidney injury/kidney stone event occurring close to a scheduled visit, the visit will be rescheduled to ensure that the event does not adversely affect values (especially for eGFR).

Illustration of the Study Design:

	Treatment (24 months)											Post-treatment follow-up (4 weeks)
Month	0[1]	2	4	6	8	10	12	15	18	21	24	
Visit Number	0	1	2	3	4	5	6	7	8	9	10	NA
Clinic Visit	X	X	X	X	X	X	X	X	X	X	X	
eGFR[2]	X	X	X	X	X	X	X	X	X	X	X	
Plasma Oxalate	X	X	X	X	X	X	X	X	X	X	X	
Stone events[3]	X	X	X	X	X	X	X	X	X	X	X	X[5]
Echocard.	X			X			X		X		X	
Ultrasound	X						X				X	
Safety Labs[4]	X	X	X	X	X	X	X	X	X	X	X	
Stool	X			X			X		X		X	X[6]
24-hour urine	X		X		X		X		X		X	
Quality of Life	X			X			X		X		X	
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X[5]

[1] Month 0 (Visit 0; refererence time point) will be the same as the last visit (Week 52) in ePHex. For echocardiography of the heart and ultrasound of the kidneys, week 48 measurements in ePHex are considered Month 0. If there is no seamless transition (delay >1 month) from ePHex to ePHex-OLE, a new clinic visit 0 will take place with applicable measurements prior to first dose of open label Oxabact and further assessments should follow according to schedule.
[2] As determined by the Schwartz equation for children (age below 18), and CKD-EPI equation for adults (age 18 or above) based on serum creatinine.
[3] Kidney stone events and related symptoms will be captured at every visit, including occurrences in between visits.
[4] Safety Labs will include blood and urine sampling.
[5] A post-treatment safety follow-up will be performed as a telephone call (4 weeks after end of treatment phase).
[6] Subjects will provide a post-treatment follow-up stool (4 weeks after intake of last dose of study drug).

Number of Subjects (planned):
Approximately 16 subjects will be included in the study. This will be dependent on the number of subjects who complete the ePHex study and chose to participate in the ePHex-OLE study.

Inclusion Criteria:

1. Signed informed consent (as applicable for the age of the subject).
2. Participation in and completion of the ePHex study.
3. Subjects who had received vitamin B6 during the ePHex study should maintain a stable dose. Subjects not receiving vitamin B6 during the ePHex study must be willing to refrain from initiating pyridoxine during study participation.

Exclusion Criteria:

4. Inability to swallow size 4 capsules.
5. Use of antibiotics to which *O. formigenes* is sensitive.
6. Current treatment with a separate ascorbic acid preparation.

7. Pregnant women (or women who are planning to become pregnant) or lactating women.
8. Women of childbearing potential who are not using adequate contraceptive precautions.
9. 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).
10. Participation in any interventional study of another investigational product, biologic, device, or other agent 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 twice daily as one enteric-coated size-4 capsule with breakfast (in the morning) and dinner (in the evening). The dose will be NLT 10^9 colony forming units (CFU) per capsule.

Duration of Treatment:
Subjects will be treated with Oxabact for 24 months.

Criteria for Evaluation:
Efficacy evaluation will be based on the following parameters:

Primary Endpoint

- Change from baseline in kidney function (eGFR) after 12 and 24 months of open-label Oxabact treatment.

Key Secondary Endpoints

- Change from baseline in total plasma oxalate concentration after 12 and 24 months of open-label Oxabact treatment
- Frequency of kidney stones events after 12 and 24 months of open-label Oxabact 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

- Change from baseline in myocardial function as measured by Speckle Tracking and traditional echocardiography
- Change from baseline in free plasma oxalate concentration
- Change from baseline in urinary oxalate excretion
- 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, 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
The sample size is based on the number of subjects enrolled in the ongoing randomised, placebo-controlled double-blind ePHex study and who chose to participate in the ePHex-OLE study. The double-blind ePHex study plans to randomise approximately 22 subjects. It is anticipated that approximately 16 subjects will enter into the ePHex-OLE study and based on an anticipated 25% drop-out rate per year it is assumed that there will be approximately 12 patients completing the 12 month-visit, and 9 patients completing the 24 month-visit. Renal function in study subjects participating in the ePHex-OLE study will be analysed based on the randomised treatment assignment of subjects in the ePHex study using all data with a mixed effect repeated measures model (MRMM). Assuming a difference between treatment groups in eGFR change from baseline of 5 mL/min/1.73 m^2 with an SD of 3, the power will be greater than 90% with a sample size of 16 patients contributing with data.

Statistical Evaluation
The efficacy population (Full Analysis Set (FAS)) will include all subjects enrolled into the epHex-OLE study who receive at least one dose of open-label Oxabact treatment and have at least one efficacy assessment during the ePHex-OLE study. The safety population (Safety Analysis Set (SAF)) will include all subjects enrolled into the ePHex-OLE study who receive at least one dose of open-label Oxabact treatment.

Efficacy analyses, summaries and graphical presentations will include data from both the ePHex and ePHex-OLE studies based on the FAS, will be presented both for the overall population and according to the randomisation in the ePHex study. Safety summaries will include the safety data reported in the ePHex-OLE study and will be based on the SAF.

Baseline is defined as the baseline of the ePHex study. A second baseline will be calculated as the last measurement prior to open-label Oxabact treatment.

All data evaluations will be descriptive in nature due to the limited sample size. The primary analysis in the ePHex-OLE will be based on the 24-month data, with summaries and graphical presentations (patient profiles and spaghetti/scatter plots), while other time-points presented will be considered as supportive.

Statistical analyses, beyond summaries and graphical presentations of the data collected, will only be performed as supportive if there is sufficient sample size. No

formal statistical testing will be done and therefore no correction for multiple comparisons will be necessary in the present analyses.

An interim analysis summarising both efficacy and safety will be performed when all subjects enrolled in the ePHex-OLE study have 12 months data.

Analysis of the Primary Endpoint
Graphical presentations of eGFR over time, observed and change from baseline values, will be done per individual patient as well as scatter and/or spaghetti plots.

Description of the data will also be provided including summaries and graphs of observed eGFR mean values (mean, standard deviation (SD)) and mean change from baseline and/or second baseline over time.

In addition, to describe if treatment with Oxabact presents a similar effect during the first 12 and 24 months in the (Oxabact – Oxabact (O - O) and Placebo – Oxabact (P – O)) groups, change from second baseline in eGFR over time will be presented as above.

As supportive analyses, and performed only if there is sufficient sample size, a MRMM will be used with a model including the following fixed effect factors: treatment group (O – O; P – O), baseline stratification factors at randomisation (PH type 2/3, 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, and the following fixed effect covariate: baseline eGFR value.

A second MRMM analysis will be used to evaluate change from Oxabact treatment initiation in eGFR to describe if Oxabact treatment present a similar effect during the first 12 and 24 months.

As a second analytical method approach will be provided as supportive analyses (i.e. Analysis of Covariance (ANCOVA) and/or Area under the curve (AUC) to evaluate the changes at 12 and 24 months. In addition, slopes will be calculated and summarised.

Data descriptions wil be based on observed data. Reasons for missing data and study drop-out will be described and evaluated. For the statistical analyses, and if sufficient data is available multiple imputations (MI)/last observation carried forward (LOCF) methods may be used to impute missing data, and if possible missing not at random (MNAR) may be evaluated with a pattern-mixture model (PMM).

Key secondary endpoints
Change from baseline in total plasma oxalate concentration will be analysed as done for the primary endpoint. Kidney stone events will be summarised along with time lost at work or school due to stone events.

Subgroup analyses
The results of this study will be presented primarily with descriptive graphical individual data presented over time and therefore no additional subgroup analyses will be needed for these. For summaries and/or any other analyses presented, subgroup analyses may be considered if there is sufficient sample size and for the following endpoints: eGFR, total and free plasma oxalate concentration, 24-hour urinary oxalate excretion, stone events, absolute count of *O. formigenes* per gram in stool, speckle tracking and traditional echocardiography). The subgroups are:

- 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 of the ePHex study).
- Subjects above or equal to and below 18 years of age at baseline of the ePHex study.
- 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 creatinine - based "Bedside Schwartz" equation (2009) for children below 18 years of age and creatinine - based CKD-EPI Equation (2009) for adults).
- Race.
- Gender.
- Progressors and non-progressors based on historic eGFR (i.e. eGFR prior to treatment start in the ePHex study
- Type of PH

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).

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
ANCOVA	Analysis of Covariance
AR	Autoregressive
AST	Aspartate Transaminase
AUC	Area under the curve
BUN	Blood Urea Nitrogen
CAPD	Continuous Ambulatory Peritoneal Dialysis
CCPD	Continuous Cycling Peritoneal Dialysis
CFU	Colony Forming Units
CKD	Chronic Kidney Disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CLKT	Combined liver/kidney transplantation
CRP	C-Reactive Protein
CS	Compound Symmetry
CTA	Clinical Trial Agreement
CTCAE	Common Terminology Criteria for Adverse Events
eCRF	Electronic Case Report Form
DSMB	Data and Safety Monitoring Board
e/a	Early to late ventricular filling velocity
e/é	Medial to lateral diastolic peak velocity
EENT	Eye, ear, nose, throat
eGFR	Estimated Glomerular Filtration Rate
ESKD	End Stage Kidney Disease
ESRD	End-Stage Renal Disease
FAS	Full Analysis Set

Abbreviation or Specialist Term	Explanation
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
ICH	International Council for Harmonization
IEC	Independent Ethics Committee
LOCF	Last Observation Carried Forward
LS	Least Square
MAR	Missing at Random
MCHC	Mean Cell Haemoglobin Concentration
MCV	Mean Cell Volume
MI	Multiple Imputations
MNAR	Missing Not at Random
MRMM	Mixed effect Repeated Measures Model
NLT	Not Less 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
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAF	Safety Analysis Set
SD	Standard Deviation
SE	Standard Error
SOC	System Organ Class
STE	Speckle Tracking Echocardiography
SUSAR	Suspected Unexpected Serious Adverse Reaction

Abbreviation or Specialist Term	Explanation
WBC	White Blood Cell Count

4 INTRODUCTION

4.1 Primary Hyperoxaluria

4.1.1 Aetiology, Clinical Features and Epidemiology

Primary hyperoxaluria (PH) type 1, 2 and 3 are rare autosomal recessive inborn errors of glyoxylate metabolism. PH type 1 is caused by deficient or absent activity of liver specific peroxisomal alanine/glyoxylate aminotransferase (AGT). In some patients with PH type 1, enzyme is present but mis-targeted to mitochondria where it is metabolically inactive. PH type 2 occurs as a result of deficient glyoxylate reductase-hydroxypyruvate reductase (GRHPR) enzyme activity. Oxalate overproduction in patients with PH type 3 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 characterised by severe hyperoxaluria and hyperoxalemia. Oxalate cannot be metabolised 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, the risk for ESRD is 50% by age 15 and 80% by age 30. The median age at death is around 30 (Hoppe *et al.*, 2009).

PH is an ultra-rare disease with a prevalence for PH type 1 of 1-3 per million and an incidence rate of approximately 1 per 120 000 births in Europe. PH type 1 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/kidney transplantation (CLKT). 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 40-50 years of age. 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. Eventually, the only curative therapy for PH type 1 is a CLKT at ESRD. For PH 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 lyophilised *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 hypothesised 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 mobilising 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 kidneys, especially on the tubular function, as well as other affected organs such as skin, skeleton and eyes.

Although the 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 PH (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 (GI) 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 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 the plasma to the GI 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 placebo-controlled, double-blind randomised 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 January 2015.

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

The placebo-controlled multicenter study ePHex (EudraCT No. 2017-000684-33), which is the basis for the present the ePHex-OLE study protocol, started in January 2018. This study plans to randomize 22 subjects aiming for 18 completers. All subjects who complete and give consent to the open-label follow-up study, will directly transition into the ePHex-OLE study.

Table 3 Summary of Clinical Studies

Study	Sites/Subjects	Study drug	Treatment duration	Outcome
CTIxOC.002 Phase I/II, open-label, non-comparative	Single site, n=9 PH type 1 5 males, 4 females Mean age 14, range 3-49	**OC2:** *Oxalobacter formigenes* frozen cell paste, containing 1000 mg (NLT 10^{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 1 5 males, 4 females Mean age 16, range 5-50	**OC3a*:** Enteric coated capsule, containing 137 mg (NLT 10^7 CFU) of lyophilised 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 10^7 CFU) of lyophilised 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 10^7 CFU) of lyophilised 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 10^7 CFU) of lyophilised 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
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 10^9 CFU) of lyophilised 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 heterogeneity 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	1 site, n=12 age range 4-53, Study completed (as of end January 2020).	**OC5#:** Enteric coated gelatin capsule, containing 80 mg (NLT 10^9 CFU) of lyophilised powder.	6 weeks +36 months	Study started in May 2014 and is completed (as of end January 2020). Twelve patients received study drug. Two-year interim analysis findings show decreased or

		One capsule was given orally b.i.d. with meals.		stable plasma oxalate and improved or stable myocardial function in patients who entered the continued treatment.
OC5-DB-02 (ePHex) Phase III, double-blind, placebo-controlled, multi-centre study	10 sites, 22 subjects planned to be randomised (aiming for 18 completers)	**OC5#:** Enteric coated gelatin capsule, containing 80 mg (NLT 10^9 CFU) of lyophilised powder. One capsule was given orally b.i.d. with meals.	12 months	Study started in January 2018 and is ongoing. 24 patients have been randomised.

**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 patients with serum creatinine <1 mg/dL (inclusion criteria) and three PH 1 patients in ESRD who were undergoing dialysis were included in this study (Hoppe *et al*., 2006).

This study showed that not less than (NLT) 10^{10} colony forming units (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 measurable 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 patients with ESRD. 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.

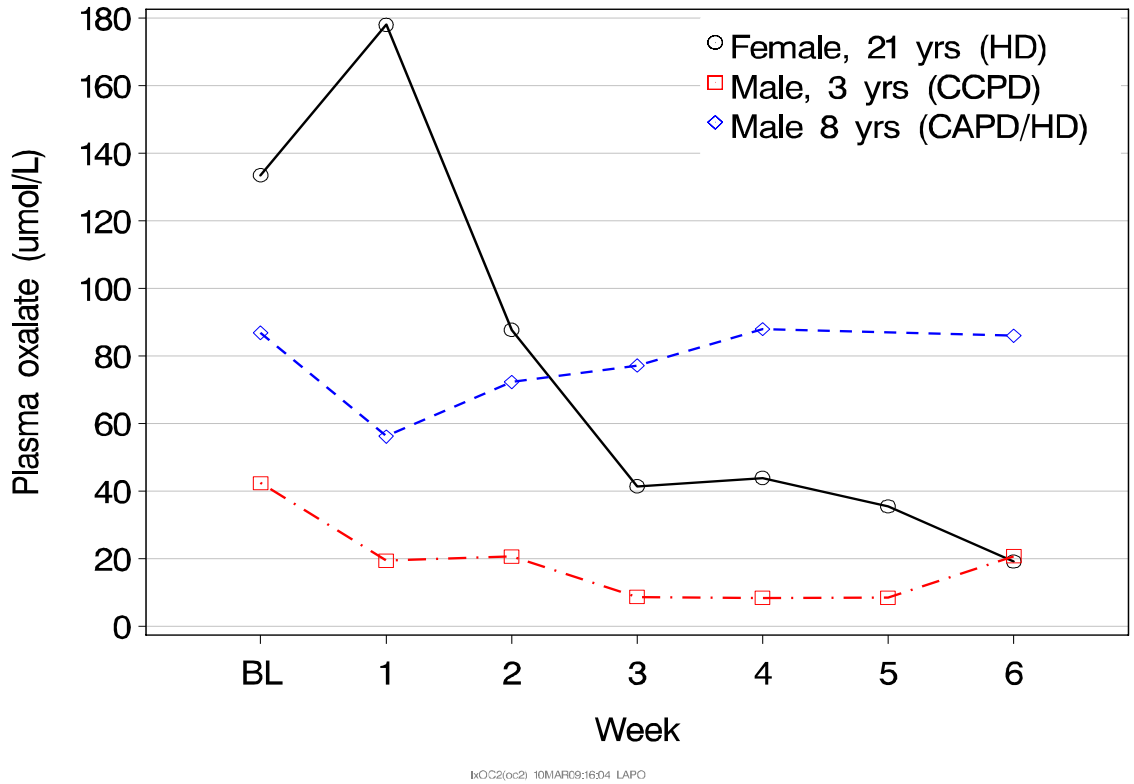

Figure 1 **Effect of *O. formigenes* Treatment on Plasma Oxalate Levels in Subjects with End-Stage Renal Disease Treated with *O. formigenes* Delivered as Frozen Cell Paste (OC2)**

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 lyophilised and administered as an enteric-coated capsule (OC3a active ingredient). The dose was NLT 10^7 CFU given twice a day for 4 weeks. In eight subjects with measurable 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 subject with ESRD. OC3a treatment was well tolerated (Hoppe *et al.*, 2006).

4.4.1.3 OC3-DB-01

Based on the results from the phase I/II studies, OxThera transferred and scaled up manufacturing of OC3 (OC3b active ingredient). The third study was a phase II/III randomised, double-blind, placebo-controlled study that investigated safety and efficacy of lyophilised *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 randomised to receive NLT 10^7 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 randomised and placebo-controlled fashion (Hoppe *et al.*, 2011).

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 asked 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 patients given OC3b and -7% (SD=21%) in the placebo group; 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 patients treated with OC3b and -9% (SD=22%) in patients 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 AEs 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 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 to 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 patients with stage II and stage III chronic kidney disease (CKD). 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%) patients] did not show a ≥20% reduction in urinary oxalate to creatinine ratio during this open-label study (i.e., did not meet the 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, two of these subjects were non-responders and five 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 patients. 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 relatedness.

4.4.1.5 OC3-DB-02

In the phase II/III study OC3-DB-02, the study medication was administered as OC3b lyophilised 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 lyophilised 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 patient subsets, 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 24-hour oxalate 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 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 placebo. AEs were most frequently reported in the placebo group. The most frequently reported AEs 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 OC3 treatment. Overall, treatment with OC3 was safe and well tolerated (Milliner *et al.*, 2018).

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

The OC5-DB-01 study was a double-blind, randomised, placebo-controlled, multi-centre study to evaluate efficacy and safety of Oxabact OC5 in PH patients with maintained renal function (i.e., eGFR or a creatinine clearance of ≥40 mL/min normalised to 1.73m^2 body surface area). The subjects were randomised to treatment with Oxabact or placebo, and were administered one capsule twice a day for 8 - 10 weeks. Each dose of Oxabact corresponded to NLT 10^9 CFU *O. formigenes* and NLT 100 mmol/19 h of oxalate degrading capacity. The primary objective of the study was to evaluate the efficacy of the 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 randomised; 14 were randomised to receive Oxabact and 14 randomised to receive placebo. All 28 subjects fulfilled the Full Analysis Set (FAS) and Safety Analysis Set (SAS) criteria, received treatment and completed the study.

The primary analysis for the OC5-DB-01 study did not identify a statistically significant difference between OC5 treatment and placebo in terms of its effect on urinary oxalate excretion. After 8 weeks of 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 to placebo (p=0.00023). In all other secondary and exploratory endpoints, no significant differences were observed between OC5 and placebo (Hoppe et *al.*, 2017).

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 (LS) difference between OC5 and placebo was larger in the eGFR <90ml/ 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).

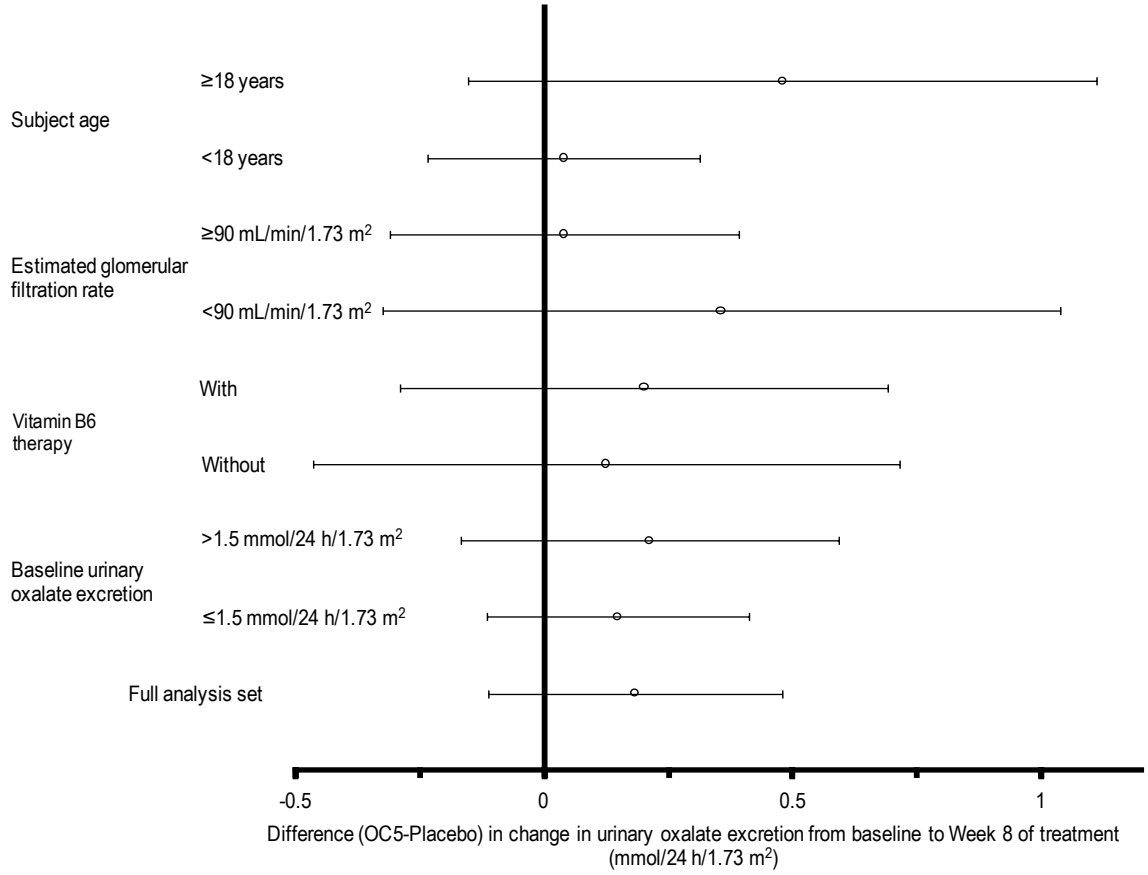

Figure 2 A Forest Plot of Difference in Change in Urinary Oxalate Excretion from Baseline to Week 8 of Treatment in Different Subgroups in the OC5-DB-01 Study

The figure displays estimate and 95 percent confidence intervals (FAS).

Despite a stratification of the two treatment groups for urinary oxalate excretion (< or ≥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 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 patients, p=0.04 (at study week 10). These findings suggest that the bacteria metabolise free oxalate that originates from the plasma, thereby supporting that enteric elimination of oxalate has occurred.

It is hypothesised, 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 OC5-treated subjects, which also suggests 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/III Study of OC5 (OC5-OL-01)

The OC5-OL-01 study is a phase II, open-label, multi-centre study to evaluate the efficacy and safety of Oxabact OC5 to reduce plasma oxalate in subjects with PH who are on dialysis. Subjects are 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 is approved. The study started in May 2014 and was completed (late January 2020).

Fourteen subjects were screened and 12 subjects have been enrolled in the study and received study drug. Eight subjects entered the continued treatment phase; 6 subjects completed 12 months, 5 subjects completed 24 months, and 3 subjects completed 36 months of continued treatment.

4.4.1.8 Phase III Study of OC5 (OC5-DB-02)

The OC5-DB-02 (ePHex) study is a phase III double-blind, placebo-controlled, randomised multi-centre study to evaluate the long-term efficacy and safety of Oxabact OC5 in PH patients with maintained renal function but with an eGFR below the lower limit of the normal range (<90 ml/min/1.73 m^2) and a total plasma oxalate concentration ≥10 µmol/L at baseline. The overall objective with the OC5-DB-02 study is to confirm a change from baseline in plasma oxalate concentration, improved/ stabilised kidney function and myocardial function (related to decreased oxalate crystal deposits in the heart) after 52 weeks of treatment with OC5 compared to placebo. In total, 22 subjects, aged 2 years and above, will be included in the study and randomised 1:1 to receive either OC5 or placebo. The study started in January 2018, is ongoing and is being conducted at ten sites in seven countries (Germany, France, Belgium, Spain,

United Kingdom, United States and Tunisia). Twenty-five patients have been randomized and 15 patients have completed the 52-week study (as of June 2020).

4.4.1.9 Named Patient Use

Oxabact has also been evaluated during named patient use in two 11-month-old girls with infantile oxalosis and ESRD at a clinical site in Germany (see Figure 3 and Figure 4). They received OC3b (via gastrostomy tube) twice a day up to 4 weeks during two treatment periods. Dialysis regimens remained 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).

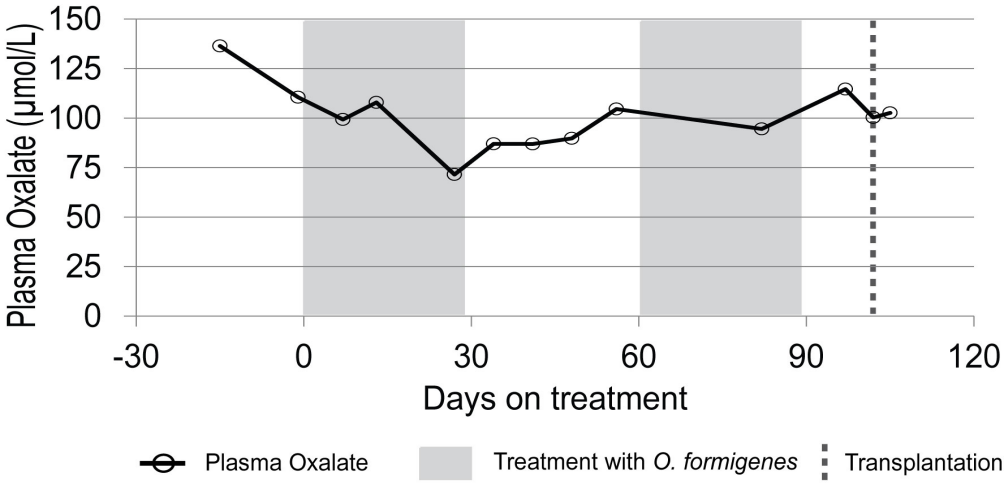

Figure 3 Follow up of Plasma Oxalate Levels in Patient 1 During Treatment with *Oxalobacter formigenes*

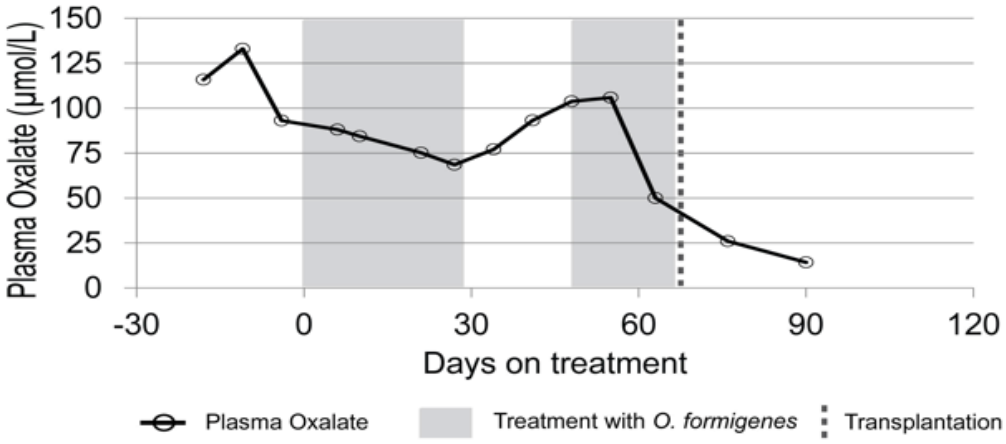

Figure 4 Follow up of Plasma Oxalate Levels in Patient 2 During Treatment with *Oxalobacter formigenes*

Named patient 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 for up to 8 weeks has 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 named patient use.

OC5 has been provided under named patient use to a 3-month-old female patient with infantile oxalosis in Germany. She developed CKD shortly after birth and was treated with daily peritoneal and 3 times/week haemodialysis. Treatment with OC5 (in buffer solution) started in December 2017, and was ongoing for almost two years. During the course of treatment with OC5 in addition with maintenance dialysis, there have been indications that disease progression has stabilised. Moreover, plasma oxalate decreased by approximately 35% during OC5 treatment. The patient recently received a combined liver/kidney transplantation at the age of 2 years. It appears, that the transplantation was successful and the patient is currently doing well, with further reductions in plasma oxalate following transplantation. OC5 has been well tolerated by the patient (Pape *et al.*, 2020).

4.5 Rationale for the ePHex-OLE Study

The ePHex-OLE (OC5-OL-02) study is an open-label extension of the parent double-blind, placebo-controlled, randomised ePHex study (OC5-DB-02). The overall objective of the ePHex study is to confirm a change from baseline in plasma oxalate concentration, improved/ stabilised kidney function and myocardial function (related to decreased oxalate crystal deposits in the heart) after 52 weeks of treatment with OC5 compared to placebo.

4.5.1 Overall Objective

The overall objective of the ePHex-OLE study is to evaluate efficacy and safety of Oxabact treatment for an additional two years in subjects with PH who have previously been treated with Oxabact or placebo in the ePHex study. The primary aim is to investigate any progression in kidney function assessed by eGFR after 12 and 24 months of open-label Oxabact treatment. Key secondary objectives are evaluation of the frequency of kidney stone events and the effect of Oxabact on total plasma oxalate after 12 and 24 months of open-label Oxabact. Other endpoints include evaluation of myocardial function together with reduction in free plasma oxalate concentration, urine oxalate excretion and Quality of Life after open-label Oxabact treatment.

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 findings from the OC5-DB-01 study and the completed OC5-OL-01 study 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 OC5-treated subjects. The *O. formigenes*-mediated enteric elimination process would then shift the equilibrium between crystallised 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. 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 common perception that calcium oxalate stones do not dissolve, these findings would support the hypothesis of *in vivo* oxalate stone dissolution.

When treated with *O. 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 12 to 24 months of treatment with Oxabact would be needed to see a treatment effect with improved kidney function (eGFR) in this patient population.

In the 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, plasma oxalate concentration and deposits in various organs build 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 the ePHex-OLE study is, based on previous findings, that with a longer treatment time (2 years) with the OC5 product, there is an improvement in kidney function over time in treated subjects. This outcome would strongly support 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 optimisation of excipients used for the freeze-drying process to develop the new and 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 Oxabact OC3 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 optimised excipients and relative amounts of these and cells, the product is now also better protected in the lyophilisation process. A higher number of viable cells recover from OC5 lyophilised 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 lyophilised 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 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.

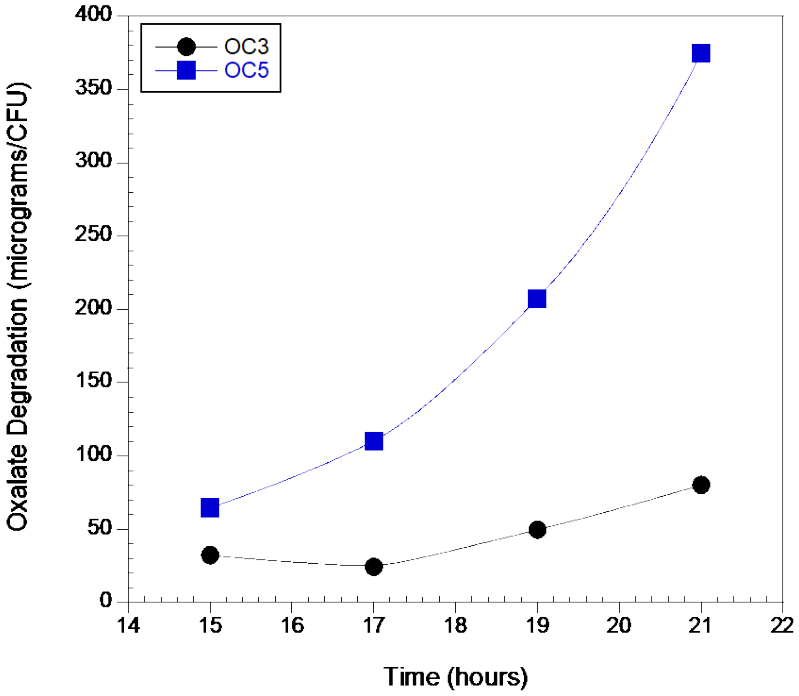

Figure 5 Oxalate Degrading Activity of OC5 Compared to OC3

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 ePHex-OLE study will administer NLT 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 GI 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 ten times throughout the study treatment period. 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 examination will be performed four times during the study treatment period. Subjects will have an ultrasound of the kidneys taken two times during the study treatment period.

Study medication will be administrated orally two times per day as one capsule with breakfast (in the morning) and one capsule with dinner (in the evening) for 24 months. The capsule size is quite small (size 4) and should be relatively easy to swallow even for younger subjects.

Subjects are requested to provide four faecal samples and five 24-hour urine samples during the study treatment period. Subjects will provide a post-treatment follow-up stool sample 4 weeks after intake of last dose of study drug. Faecal and urine samples will be collected at the 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 AEs of the ePHex-OLE 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 have 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 AE profile similar to placebo, even for GI 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.

Data collected to date for the completed studies OC5-DB-01 and OC5-OL-01 and the ongoing ePHex study indicate, that the OC5 product is safe and well tolerated. No related severe or serious AEs have been reported so far in these studies for Oxabact, see section 11.5. Analysis of potential changes in microbiota due to treatment with OC5 during the OC5-DB-01 and OC5-OL-01 studies 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 are 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 every two to three months during the additional two year-period (every two months during year 1, every three months during year 2) of conduct for study-specific visits in order to monitor AEs 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. 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 *O. formigenes* is a promising potential treatment for patients with PH. Clear beneficial effects of *O. 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 to 12 subjects in the completed OC5-OL-01 dialysis study (for up to 36 months). Administration of *O. formigenes* as a frozen cell paste, capsule or buffered powder for suspension was well tolerated in subjects with PH. No SUSARs have been reported in any of these studies for Oxabact, and Oxabact has had a favourable safety profile.

The ePHex-OLE study should provide valuable information on the ability of the improved OC5 Oxabact product to reduce calcium oxalate deposits, lower the level of plasma oxalate and confer clinical benefit in subjects with PH. It will also generate further safety information on the product. The 2-year treatment time is expected to be sufficient to start to reduce the widespread oxalate deposits in the body and to stabilise/improve the kidney function (eGFR) and myocardial function.

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. Currently, there are no approved therapeutic treatments available for PH. Therapeutic options are limited and (except for the crystallisation inhibitors) all interventions aim at reducing urinary oxalate concentrations. 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 two larger placebo-controlled studies (OC3-DB-01 and OC3-DB-02) found that the OC3 product was safe

and well tolerated with an AE profile similar to the placebo. Safety data from the completed blinded OC5-DB-01 study and the 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 trial for treatment in subjects with PH is considered favourable.

5 TRIAL OBJECTIVES AND PURPOSE

5.1 Primary Objective

- To evaluate the efficacy of Oxabact following two years continued open-label treatment in subjects who have previously participated in and completed the (randomised, placebo-controlled, double-blind) the ePHex study.

5.2 Secondary Objectives

- To obtain additional safety data from two years continued open-label treatment with Oxabact.

6 INVESTIGATIONAL PLAN

6.1 Background and Rationale

This study is an open-label, single arm, multi-centre study to evaluate the efficacy and safety of the long-term use (two years of open-label Oxabact treatment) of OC5 (*O. formigenes*) to stabilise/improve kidney function, stabilise/reduce plasma oxalate concentration, and to reduce oxalate deposits in PH patients. Included subjects will be the subjects who underwent treatment (administered either Oxabact or placebo) in the ePHex study and consented to participate in the ePHex-OLE study. At the start of the ePHex study, subjects had 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 at baseline. These criteria will not be re-tested for inclusion into the ePHex-OLE study protocol.

It is hypothesised that daily administration of *O. formigenes* facilitates the secretion of endogenously produced oxalate via the GI tract. Enteric elimination of oxalate may help to reduce oxalate deposits and eventually decrease the plasma oxalate level, thereby improving eGFR and kidney function as well as progression of myocardial function.

A 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 subjects 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 subjects developed ESRD. The most important progression factors for kidney deterioration in subjects with 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 an urinary oxalate excretion of >1.87 mmol/1.73 m^2 per 24hr

Scientific evidence in support of eGFR change

The recently published KHI consensus paper on "Endpoints for clinical trials in Primary Hyperoxaluria" highlights the importance of preserving renal function and summarises the available literature on the rate of kidney function decline in patients with PH (Milliner *et al*, 2020):

"*In PH loss of kidney function can begin in infancy and early childhood. Among those with the most severe form, PH1, 50% will progress to ESKD by 33 years of age, and nearly all by 60 years of age (Hopp et al., 2015). In most patients, renal function is not typically lost at a rapid rate. In a study by Tang et al. 2015, the rate of loss of renal function in a mixed group of PH1, PH2, and PH3 patients ranged from -0.4 ml/min/1.73 m^2/year in those with no or prevalent nephrocalcinosis to -1.2 ml/min/1.73 m^2/year in those with incident nephrocalcinosis. A small study (Milliner et al., 2001) reported a change of -1.7 ml/min/1.73m2/year in PH1 and -1.04 ml/min/1.73m^2/year in PH2. Fargue et al. (2009) reported a median decrease of -1.0 ml/min/1.73m^2/year in 19 children with PH1 who were older than 2 years of age at diagnosis. Clinical experience suggests higher rates of GFR decline in those PH patients with more advanced CKD, particularly CKD stages 3b to 4. Further, rapid declines in kidney function leading to ESKD over weeks to months occasionally occur in PH patients, sometimes precipitated by acute events such as dehydration or an obstructive kidney stone (Cochat and Rumsby 2013; Zhao et al., 2016).*"

Particularly on the last note, unpublished data (presented at the PH workshop organized by the Oxalosis & Hyperoxaluria Foundation in June 2018) from the Mayo Clinic Rare Kidney Stone Consortium (RKSC) registry showed that the rate of decline heavily depends on where patients are in their course of the disease. The analysis involved 157 patients with PH without ESRD at the time of PH diagnosis and without complete response to pyridoxine. 32 patients developed ESRD in the follow-up period of observation. Median follow-up time in patients who had at least one urinary oxalate value obtained between PH diagnosis and incident ESRD or death was 15.4 years. The mean estimated eGFR decline in the 32 patients who developed ESRD was -0.822 ml/min/1.73 m^2/year for several years until about -3.2 years before the event of ESRD. At that time point, the slope rapidly steepened to about -9 ml/min/1.73 m^2/year (see Figure 6). Mechanisms explaining the accelerated decline in eGFR are not entirely clear but could include infection and dehydration as well as more rapid calcium oxalate crystallization in the kidney as plasma oxalate levels rise markedly.

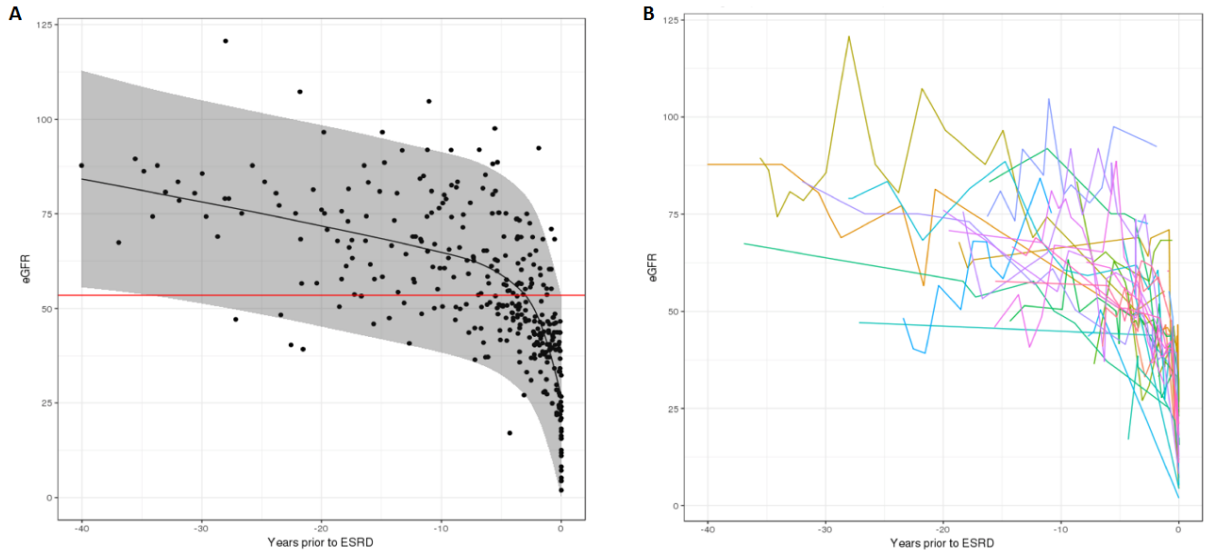

Figure 6 Plots of eGFR versus years prior to ESRD from 32 patients with follow-up ESRD, with piecewise regression line and 95% prediction intervals. Break point is –3.2 years. Red line is eGFR of 55.8 ml/min/1.73m^2, upper limit of prediction interval at time of ESRD (n=356, Figure "A"). Figure "B" shows individual patient profiles.

It is noteworthy that the analysis (Figure 6) included only patients who developed ESRD at some point during the follow-up period of observation and it does not describe the general eGFR rate across the entire population with PH in the RKSC registry. However, the data clearly demonstrate how heterogeneous the disease progresses in individual patients (Figure 6) and how much the part of the slope that any individual patient currently occupies when entering a prospective clinical trial will determine the rate of progression observed during the trial.

6.2 Overall Study Design and Plan

Following the ePHex study, subjects will be treated with OC5 for 2 years (24 months) in the ePHex-OLE study (Figure 7).

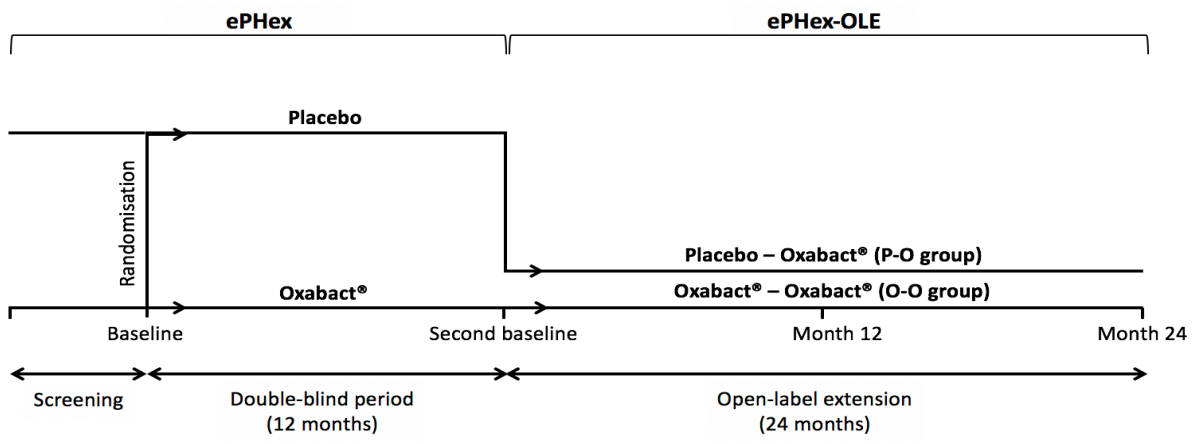

Figure 7 Study Design of the ePHex (OC5-DB-02) study and the ePHex-OLE (OC5-OL-02) study

P-O group: subjects who were randomised to placebo in the ePHex study followed by Oxabact open-label treatment for 24 months in the ePHex-OLE study; O-O group: subjects who were randomised to Oxabact in the ePHex study and continued on Oxabact open-label treatment for 24 months in the ePHex-OLE study.

Baseline, unless otherwise stated, is defined as the baseline of the ePHex study. An additional baseline, specific to the ePHex-OLE study (Figure 7), will be calculated at the start of the ePHex-OLE study prior to open-label Oxabact treatment for subjects who were randomised to placebo in the ePHex study (P-O group). This additional baseline is considered the *second baseline*.

Visit 0 (Month 0; start of the ePHex-OLE study) will be the same as the last visit (Week 52) in the ePHex study. A second baseline value for all parameters will be recorded. Provided there is a seamless transition from the ePHex study to the ePHex-OLE study, second baseline values (Visit 0 for the the ePHex-OLE study) will correspond to the respective value taken from the last visit in the ePHex study (week 52). For operational reasons, baseline values for echocardiography and renal ultrasound data will be derived from the assessment at week 48 in the ePHex study. If there is no seamless transition (delay >1 month) from the last dose of study drug in the ePHex study, a new clinic visit 0 (Month 0) will take place with applicable second baseline measurements for all parameters prior to first dose of open label Oxabact and further assessments should follow according to schedule of assessments.

During the study period, subjects will have visits every two to three months, i.e., at months 2, 4, 6, 8, 10, 12, 15, 18, 21 and 24. During these visits, plasma oxalate and safety labs will be taken. Subjects will be asked to provide stool samples four times (months 6, 12, 18 and 24) and 24-hour urine five times (months 4, 8, 12, 18 and 24). In addition, subjects will provide a post-treatment follow-up stool sample 4 weeks after intake of last dose of study drug. Echocardiography (STE and traditional) will be assessed four times (months 6, 12, 18 and 24). Ultrasound of the kidney will be done two times (months 12 and 24). Quality of Life will be evaluated by a questionnaire during months

6, 12, 18 and 24 (Table 5). Information on kidney stone events and related symptoms will be captured throughout the study.

Adverse events and concomitant medication will be monitored throughout the study.

Safety evaluation will include physical examination, vital signs and safety labs. Monitoring of AEs, concomitant medication and compliance with the administration of study drug will be performed at each visit. In addition, there will be a 4-week safety follow-up after intake of the last dose of study drug. Furthermore, questions on kidney stone events and related symptoms will also be asked throughout the 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 below.

Table 5 Schedule of Assessments

	Treatment (24 Months)											Post-treatment follow-up (4 weeks)[8, 9]
Month	0[1]	2	4	6	8	10	12	15	18	21	24	
Day (+/- 7 days)	0	61	122	183	243	304	365	456	547	638	730	
Visit Number[2]	0	1	2	3	4	5	6	7	8	9	10	NA
Clinic Visit	X	X	X	X	X	X	X	X	X	X	X	
Incl/Excl criteria	X											
Vital signs	X	X	X	X	X	X	X	X	X	X	X	
Physical exam.	X	X	X	X	X	X	X	X	X	X	X	
Conc. Med.	X	X	X	X	X	X	X	X	X	X	X	X
eGFR[3]	X	X	X	X	X	X	X	X	X	X	X	
Plasma Oxalate	X	X	X	X	X	X	X	X	X	X	X	
Stone events[4,]	X	X	X	X	X	X	X	X	X	X	X	X
Echocard.[5]	X			X			X		X		X	
Ultrasound	X						X				X	
Safety Labs[6]	X	X	X	X	X	X	X	X	X	X	X	
Stool	X			X			X		X		X	X
24-hour urine	X		X		X		X		X		X	
Quality of Life	X			X			X		X		X	
Pregancy test[7]	X										X	
Review Adverse events	X	X	X	X	X	X	X	X	X	X	X	X
Drug dispense/ Accountability	X	X	X	X	X	X	X	X	X	X	X	

[1] Month 0 (Visit 0; refererence time point) will be the same as the last visit (Week 52) in ePHex. For echocardiography of the heart and ultrasound of the kidneys, week 48 measurements in ePHex are considered Month 0. If there is no seamless transition (delay >1 month) from ePHex, a new clinic visit 0 (Month 0) will take place with applicable measurements prior to first dose of open label Oxabact and further assessments should follow according to schedule.
[2] Visit window during treatment period +/- 7 days. 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). The days on which each visit should occur are detailed in Table 6.
[3] As determined by the Schwartz equation for children (age below 18), and CKD-EPI equation for adults (age 18 or above) based on serum creatinine.
[4] Kidney stone events and related symptoms will be captured at every visit, including events in between visits.
[5] Echocardiography to be done within +/- 2 weeks of the clinic visit at months 6, 12 and 18. If the images fail quality criteria, the examination will be repeated within 4 weeks.
[6] Safety Labs will include blood and urine sampling.
[7] If applicable.
[8] A post-treatment safety follow-up will be performed as a telephone call (the safety follow-up period will cover a duration of four weeks and the telephone call will be made maximum 3 working days after the safety follow-up period).
[9] Subjects will provide a post-treatment follow-up stool sample 4 weeks (plus maximum 3 working days) after intake of last dose of study drug.

6.3.1 Study Start

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 subjects who are considered for participation in the study and who have signed the Informed Consent in an Inclusion log. Subjects who are not transitioning from the ePHex study to the ePHEx-OLE study should also be listed together with the reason for not participating in the ePHex-OLE study (if possible). The inclusion and exclusion criteria should be reviewed. Included subjects will be the eligible subjects who completed the ePHex study and consented to participate in the ePHex-OLE study. Completers in the ePHex study are defined as:

- Subjects who completed 52 weeks of treatment and finished the final assessments, or
- Subjects who performed the assessments as required for baseline Visit 0, if the ePHex study is terminated early by the Sponsor.

The following will be recorded for the ePHex-OLE study:
- Any clinically relevant medical and/or surgical event (including stone events) or disease
- Prior/concomitant medication and any non-medication therapy (e.g. physiotherapy) from completion of the ePHex study to the first dosing in the ePHex-OLE study.

The start of the ePHex-OLE study (Month 0/Visit 0) will be the same as the last visit (week 52) in the ePHex study. Thus, second baseline to be identified as the last available measurement prior to the first dosing of ePHex-OLE open label study drug, will include the values measured at week 52 in the ePHex study for all parameters except for echocardiography and renal ultrasound data. For operational reasons, second baseline values for echocardiography and ultrasound data correspond to the values measured at week 48 of the ePHex study. In addition, age will be assessed at the start of ePHex-OLE. Other demographic data will be retrieved from the ePHex study based on the subject ID. Collection of demographic data will comply with local requirements regarding patient confidentiality in the respective countries.

At the start of the ePHex-OLE study, vital signs will be assessed and a complete physical examination (if not a seamless transition) (see section 11.7 and 11.8 for details on what this includes) will be performed. For women of childbearing potential, a pregnancy test will be made at visit 0.

6.3.1.1 Study Start in Case of Delayed Transition from the ePHex study to ePHex-OLE study

If there is no seamless transition (delay >1 month) from the last intake of study drug in the ePHex study to the start of the ePHex-OLE study, a new clinic visit 0 will take place with applicable second baseline measurements. Further assessments should follow according to schedule of assessments (Table 5).

6.3.2 Treatment Period

During the two-year period, the subject will be followed by visits at months 2, 4, 6, 8, 10, 12, 15, 18, 21 and 24 according to Table 5. For operational purposes, months can be converted to days as follows (Table 6):

Table 6: Conversion of Months to Days

Month	0	2	4	6	8	10	12	15	18	21	24
Day	0	61	122	182	243	304	365	456	547	638	730

At the visits, blood samples for eGFR, plasma oxalate and safety labs will be taken. At each visit, vital signs will be taken and subjects will have a physical examination (see sections 11.7 and 11.8 for details).

Subject Quality of Life will be evaluated using a questionnaire at months 6, 12, 18 and 24.

Echocardiography (STE and traditional) will be performed at months 6, 12, 18 and 24. Ultrasound of the kidney will be done once every 12 months.

Subjects will be asked to provide five 24-hour urine samples (months 4, 8, 12, 18 and 24) and four faecal samples (months 6, 12, 18 and 24) during the treatment period. These collections will be done at the subject´s home and should be scheduled in connection with the visit (+/- 7 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 AEs and any changes in concomitant medications. Information on the occurrence of any stone event and related symptoms will be collected at each visit. 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 AE as defined in Section 11.1.)

For women of childbearing potential, a pregnancy test will be made at the last clinical visit.

The subject will receive shipment of study drug once every second week. 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 clinic visit month 24.

In case any acute kidney stone event should occur during the study period, 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 patient can be rescheduled at the investigator's discretion to the study timetable.

The ePHex study defined blind data will remain blind in the ePHex-OLE study until the ePHex study results have been released.

6.3.3 Post-treatment follow-up

Previous clinical experience with Oxabact suggests that the bacteria only transiently colonise the gastrointestinal tract and fecal recovery generally drops below detectable levels one to two weeks after end of study treatment (Hoppe *et al.* 2006).

A post-treatment safety follow-up will cover a duration of 4 weeks after the last dose of study treatment. 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 end of last dose of study treament. 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 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.

Subjects will provide a post-treatment follow-up stool sample 4 weeks (plus maximum 3 working days) after intake of last dose of study drug.

End of study is defined as when the safety follow-up telephone-call and the post-treatment stool sample have been completed.

6.3.4 Electronic Patient Reported Outcome

A web-based interface (ViedocMe) for electronic patient reported outcome (ePRO) will be used continuously during the study. Here, subjects will record details on collections of urine and faeces, as well as Quality of Life. 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 Patient's Homes

Collections of faeces and urine will be performed at the subjects' 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 subjects' 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 PATIENTS

7.1 Subject Inclusion Criteria

1. Signed informed consent (as applicable for the age of the subject).
2. Participation in and completion of the ePHex study.
3. Subjects who had received vitamin B6 during the ePHex study should maintain a stable dose. Subjects not receiving vitamin B6 during the ePHex study must be willing to refrain from initiating pyridoxine during study participation.

7.2 Subject Exclusion Criteria

4. Inability to swallow size 4 capsules.
5. Use of antibiotics to which *O. formigenes* is sensitive.
6. Current treatment with a separate ascorbic acid preparation.
7. Pregnant women (or women who are planning to become pregnant) or lactating women.
8. Women of childbearing potential who are not using adequate contraceptive precautions.
9. 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).
10. Participation in any interventional study of another investigational product, biologic, device, or other agent 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 sterilisation of the partner (vasectomy for 6 months minimum);

    c. combined (oestrogen 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;

    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 sterilisation procedures at least 6 months prior to the first dose:
    a. hysteroscopic sterilisation;
    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 requirement for contraception is applied for male subjects.

Females of childbearing potential will have a pregnancy test at the end of the study. 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 treatment in the study if:

- Any clinically significant 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 Prinicpal Investigator in consultation with OxThera may discontinue the subject in case of a major protocol violation.
- The subject requires dialysis.

Any subject who withdraws prematurely from the study treatment will be asked to complete all study assessments scheduled for the month 12 or 24 visit at the time of withdrawal and will be followed for safety for 4 weeks after the last intake of study drug. Subjects will provide a stool sample 4 weeks after end of treatment.

Patients 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 from the study and the reason for withdrawal from treatment and from the study will be recorded in the source data and eCRF.

Withdrawn subjects will not be re-entered into the study.

8 TREATMENT OF SUBJECTS

8.1 Description of Study Treatment

The study drug consists of Oxabact OC5 as the active treatment. The study drug is supplied as enteric-coated size 4 capsules. One capsule shall be administered orally with water twice daily with breakfast (in the morning) and dinner (in the evening). 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 as part of the ePHex study documentation. If a seamless transition (delay >1 month) from the ePHex study to the ePHex-OLE study should not be possible, information on medication used from completion of the ePHex study to first dosing in the ePHex-OLE study will be recorded. Any historical use of vitamin B6 and any other relevant medication as judged by the investigator should also be recorded. Subjects may continue any medications they are receiving at study entry for underlying medical conditions. The medications will be recorded at screening of the ePHex study and changes will be noted throughout the study.

8.2.1 Concomitant Treatment with Vitamin B6

Subjects who had received vitamin B6 (pyroxidine) during the ePHex study should maintain a stable dose. Subjects not receiving vitamin B6 during the ePHex study must be willing to refrain from initiating pyridoxine during participation in the ePHex-OLE study. 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

Prohibited medications are:

- Ascorbic acid preparations: Ascorbic acid preparations must not be used during the study 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

Compliance to study treatment will be checked with the subject at each visit to the clinic. Any missed doses or doses lost during handling should be registered in the eCRF and/or source documentation.

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 at the hospital pharmacy based on dispensed and returned drug for an overall check of subject compliance.

8.4 Open-label

Subjects will be identified with a unique identification number consisting of country and site identification number and consecutive subject number which they had been assigned at the first screening visit of the ePHex study. Subjects will receive study drug in an open-label manner.

9 STUDY DRUG MATERIALS AND MANAGEMENT

9.1 Study Drug

OC5 is supplied as enteric-coated, size 4, gelatine capsules. One capsule contains NLT 10^9 CFU and has the capacity to metabolise NLT 100 mmol oxalate per 19 hours. Details on the product are described in Table 7 below:

Table 7 Details of the Study Drug in the ePHex-OLE Study

Parameter	High Dose
Active Substance	Lyophilised *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 initially contain 18 capsules. During the course of the study, there will be a transition from tubes containing 18 capsules to tubes containing 16 capsules. The overage medication in each tube will be reduced from 4 to 2 extra capsules in each 2-week supply.

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 patients. 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 by the subject 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 kidney function (eGFR) after 12 and 24 months of open-label Oxabact treatment.

Key Secondary Endpoints

- Change from baseline in total plasma oxalate concentration after 12 and 24 months of open-label Oxabact treatment
- Frequency of kidney stones events after 12 and 24 months of open-label Oxabact treatment. Kidney stone events are defined as:
  - Subject- or investigator-reported symptoms, or
  - Stone passages or removals, or
  - Increase in the number of kidney stones as assessed by ultrasound.

Other endpoints

- Change from baseline in myocardial function parameters as measured by Speckle Tracking and traditional echocardiography
- Change from baseline in free plasma oxalate concentration
- Change from baseline in urinary oxalate excretion
- 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, BUN, ALP, bicarbonate, CRP, WBC, creatinine and cystatine C.

10.1 Kidney Function Parameters

Samples for eGFR calculations will be processed at the clinical site and analysed at a central lab (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.

10.2 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 40 mms. 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 could be defined as the occurrence of one 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 classified 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, the investigator can decide whether or not this should be reported as a separate stone event.

Assements of kidney 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 baseline of the ePHex study. During the study period of the ePHex-OLE study, information will be collected concerning occurrence of self-reported kidney stone event and related symptoms. This information will be captured by completing the applicable items on the Adverse Event eCRF. (Please note this is a data entry convention only and does not replace the per protocol definition of an Adverse Event as defined in Section 11.1).

10.3 Plasma Oxalate

Samples for plasma oxalate will be collected during each clinical visit (i.e., every two to three months).

Samples for total plasma oxalate will be processed at the clinical site and analysed at Academic Medical Center (AMC), Amsterdam, the Netherlands. 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).

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 ePHex-OLE 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.

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; Lagies *et al*., 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 four times 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 (except for visit 10).

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

STE evaluations will include 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, e/é, e/a, Left Ventricular End Diastolic Dimension, Fractional shortening, Tricuspid Regurgitation by Doppler as an indicator of right ventricular/pulmonary pressure.

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 (18 years or older) and the CHQ/PF50 for subjects aged 5-<18 years and their parents (per age at the ePHex-study

entry). The same questionnaire will be completed by the subjects/parents during the clinical visits at months 6, 12, 18 and 24.

10.6 Other Parameters for Evaluation of 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 *O. 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 the 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.

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 standardised 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 according to reference article published by Boyce *et al*. (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, calcium, glycolate, magnesium, citrate, urea, creatinine, phosphorus excretion, pH, osmolality and urine volume will be taken at patient´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, BUN, ALP, bicarbonate, CRP, WBC, creatinine and cystatine C. 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, then they should not be recorded as AEs. If the symptoms are worse than what is normally experienced, then they should be recorded as AEs.

An AE does **not** include:

• Symptoms of the underlying disease (with the 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 hospitalisation, 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:

- Results in the subject's death
- Is life-threatening*
- Requires inpatient hospitalisation or prolongation of existing hospitalisation**
- Results in persistent or significant disability/incapacity; or
- Results in 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 patient's death. Life threatening does not mean that had an AE occurred in a more severe form it might have caused death.

  ** Hospitalisation requires overnight 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 patient 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 8). Events classified as certain, probable/ likely or possible will be considered related to study drug.

Table 8 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 recognised 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 patients 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, patient 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; hospitalisation or prolongation of hospitalisation 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. The reporting shall continue during the course of the study.

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 patients.

Unresolved AEs that occurred and were recorded during the ePHex study will not be recorded separately in the ePHex-OLE study unless they change in severity grade, seriousness or relatedness. Furthermore, any medical events (including stone events) that occur in the time interval after the safety follow-up in the ePHex study but before the first intake of study drug in the ePHex-OLE study, should be reported in the Medical History page for the ePHex-OLE study.

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

SAEs are to be reported 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, ProductLife 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.

11.5 Anticipated Adverse Events

11.5.1 Review of Available Data

OC3 has been evaluated in several clinical studies in subjects 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 patients may experience headache and gastrointestinal symptoms including abdominal pain, constipation, nausea, vomiting, diarrhoea, and flatulence.

No treatment related Serious Adverse Events were reported in subjects 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 randomised 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 subject's last visit was in January 2015. No serious related adverse events were reported in the trial.

The AEs are summarised in Table 9 and Table 10. 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 9 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 patients; m: Number of mentions

Table 10 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 were 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 patients in the OC5 group than in the placebo group (four subjects [29%] versus no patients [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 patients 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 OC5-OL-01 Study and OC5-DB-02 Study

The OC5-OL-01 study started in May 2014 and was completed late January 2020. Fourteen subjects were screened and 12 subjects have been enrolled in the study and received study drug. This study includes late-stage patients with ESRD who are on dialysis. Most of these patients are on a waiting list for transplantation. Eight subjects continued into the continued treatment phase of the study. Six patients completed the 12-months, five patients completed the 24-months and three patients completed the 36-months continued treatment period. There have been no severe nor serious related AEs for Oxabact in the dialysis study.

In the ongoing, double-blind OC5-DB-02 study (started in January 2018) in patients with maintained but reduced kidney function (eGFR <90ml/min/1.73m^2), 25 patients have been randomised, and 15 patients have completed the study. There have been 12 SAEs reported to-date (in six subjects); none of them was considered related to study drug (as of June 2020).

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 hospitalisation 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 *O. 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 metabolised 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 36 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/or plasma formate).

Formate accumulation in plasma is the main reason for acidosis in early, uncomplicated stages of metabolic acidosis. Metabolic acidosis is characterised 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 or blood pH will serve as an indication of elevated levels of formate. The ePHex study will investigate these early signs 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.
- Pregnancy test for women of childbearing potential at baseline and month 24.
- 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 at every clinic visit.

11.7 Vital Sign Measurements

Vital sign measurements on study include temperature and blood pressure (systolic and diastolic in a supine position), heart rate, respiratory rate (assessed after 5 minutes resting in a supine position), weight and height. 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 patients where the Schwartz equation is used to determine eGFR. This would apply to children (patients under 18 years of age at the screening visit of the ePHex study) and to any patient who turn 18 either during the ePHex study or the ePHex-OLE study periods. For patients who are adults at the screening visit of the ePHex study, height for the ePHex-OLE study will be retrieved from the ePHex database using the subject ID.

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 Investigator's 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 hours and send the form to ProductLife Limited. Administration of study medication should immediately be stopped, i.e. the subject will be withdrawn from the study treatment. The subject must be followed until birth or termination of pregnancy Generally, follow up will occur within 6 to 8 weeks

following the estimated delivery date (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 parent ePHex study consisting of independent clinical and statistical experts. There is no DSMB designated for the ePHex-OLE study. However, the DSMB will review patients that are transferred into the ePHex-OLE study until the parent ePHex study has been completed. The DSMB will periodically review efficacy data, 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.

12 STATISTICAL METHODS

12.1 Study Populations

The Efficacy Population (Full Analysis Set, FAS) will include of all subjects enrolled into the ePHex-OLE study who receive at least one dose of open-label Oxabact treatment, and have at least one efficacy assessment during the ePHex-OLE study.

The Safety Population (Safety Analysis Set, SAF) will include all subjects enrolled into the ePHex-OLE study who receive at least one dose of open-label Oxabact treatment.

12.2 Sample Size Calculation

The sample size for this study is based on the number of subjects enrolled in the ongoing randomised, placebo-controlled double-blind ePHex study and who choose to participate in the ePHex-OLE study. The double-blind ePHex study plans to randomise approximately 22 subjects (11 subjects to Oxabact and 11 subjects to placebo). It is anticipated that approximately 18 subjects will complete the ePHex study, and approximately 16 subjects will enter into the ePHex-OLE study. Based on an anticipated 25% drop-out rate per year, it is assumed that there will be approximately 12 patients completing the 12-month-visit, and 9 patients completing the 24-month-visit. The observation period for the primary analyses of eGFR extends to 12 months in ePHex and 24 months in the ePHex-OLE study resulting in a maximum follow-up of 36 months for completers.

A supporting power analysis using a repeated measurements model in a two-sided approach with α=5%, a 1:1 allocation ratio and assuming a within patient correlation of 0,70, showed that a power greater than 90% is achieved if a difference between treatment groups of 5 ml/min/1.73 m^2 with a SD of 3 ml/min/1.73 m^2 is found. With a slightly lower difference of 4 mL/min/1.73 m^2 and a SD of 3 mL/min/1.73 m^2, or a difference of 5 mL/min/1.73 m^2 with a larger SD of 4 mL/min/1.73 m^2, the power is still above 80% (Table 11).

Table 11 Power Calculations

Standard deviation	Difference between treatment groups in eGFR (mL/min/1.73 m²)			
	3	**4**	**5**	**6**
3	0,65635	0,88298	0,97601	0,99715
4	0,42540	0,65635	0,83968	0,94338
5	0,29375	0,47211	0,65635	0,80922

12.3 Statistical Evaluation

All statistical analyses will be further described in a Statistical Analysis Plan (SAP). Efficacy analyses will be based on the FAS and safety analyses will be based on the SAF. Efficacy analyses, summaries and graphical presentations will include data from both the ePHex study and the ePHex-OLE study. Safety summaries will include the safety data reported in the ePHex-OLE study, unless otherwise stated.

Continuous parameters will be summarised by number of subjects reporting the parameter n, mean, standard deviation, median, Q1 and Q3, minimum, and maximum. Categorical parameters will be summarised by count and percentage.

Summaries will be primarily based on the FAS population and according to the randomisation in the ePHex study (Oxabact – Oxabact (O - O) and Placebo – Oxabact (P – O)). For the safety analyses, subjects will be included according to actual treatment received in the ePHex-OLE study.

Patients randomised to Oxabact in the ePHex study, the O – O group, would have, at the end of the ePHex-OLE study, a total of maximum three years of Oxabact treatment (1 year in the ePHex study and 2 years in the ePHex-OLE study). Patients randomised to placebo in the ePHex study, the P – O group, would have, at the end of the ePHex-OLE study, a total of maximum two years Oxabact treatment (none in the ePHex study and 2 years in the ePHex-OLE study).

Baseline used in the analyses, unless otherwise stated, is defined as the baseline of the ePHex study. Baseline eGFR is defined as the mean of the three measurements taken prior to randomisation in the ePHex study.

A second baseline will be defined for subjects who were randomised to the placebo treatment in the ePHex study, as the last measurement prior to open-label Oxabact treatment in the ePHex-OLE study.

The primary analysis in the ePHex-OLE study will be based on both the 12- and the 24-months data while other timepoints presented will be considered as supportive.

As the population is highly heterogenous, primarily individual subject profiles and spaghetti and/or scatter plots will be produced for all indicated parameters over time.

All descriptions of the data, including summaries and graphical presentations, will also be presented for both mean observed values and mean changes from baseline (mean and standard deviation, or medians and quartiles) over time. Whenever possible, LS means, LS mean differences and corresponding SEs or 95% confidence intervals may also be presented.

An interim analysis summarising both efficacy and safety will be performed when all subjects enrolled in the ePHex-OLE study have 12-months data.

All data evaluations will be descriptive in nature due to the limited sample size at both 12 and 24 months. Considering this, and the fact that PH is a highly heterogenous disease population, statistical analyses proposed, beyond summaries and graphical presentations of the data collected, will only be performed as supportive and depending on the sample size. No formal statistical testing will be done and therefore no correction for multiple comparisons will be necessary in the present analyses.

12.4 Demographics and Baseline Characteristics

Demographic variables and other baseline characteristics will be summarised. These summaries will be produced for the efficacy population and the safety population. Both baseline in the ePHex study and second baseline in the ePHex-OLE study will be described. Baseline distributions will be examined and any potential impact of differences found will be evaluated and discussed. Demographics and baseline characteristics will also be described for patients included in the FAS of the ePHex study but not participating in the ePHex-OLE study.

12.5 Primary Endpoint

Kidney function measurements of eGFR will be calculated primarily based on the creatinine - based "Bedside Schwartz" equation (2009) (for children below 18 years of age) and the creatinine - based CKD-EPI equation (2009). For subjects reporting age 18 to 23 years old at screening visit 1 of ePHex the mean of child and adult equations will be used. Subjects eGFR will be calculated using the same equation used at the ePHex study baseline throughout the study. Supportive summaries may use alternative eGFR equations.

Change from baseline in kidney function (eGFR) over time (including both the ePHex and the ePHex-OLE study) will be calculated as each visit value minus the value at baseline (ePHex). Description of eGFR and change from baseline in eGFR values after 24 months of open-label Oxabact treatment will be considered the primary analysis, although 12 months will also be presented. Graphical presentations of the eGFR data over time will therefore be done based on the observed data per individual patient as well as scatter and/or spaghetti plots.

In addition, to describe if Oxabact treatment has a similar effect during the first 12 and 24 months in the O – O and P – O groups, change from second baseline in eGFR over time will be summarised as above.

Additional description of the data will be provided including summaries and graphs of observed eGFR mean values (mean, SD) and mean change from baseline and/or second baseline over time.

12.5.1 Change from Baseline in eGFR Supportive Analyses MRMM

A supportive additional analysis of the data may also be performed, whenever possible, using Mixed Effect Repeated Measures Model (MRMM) analysis based on the FAS with a model including fixed effect factors: treatment group (O – O; P – O), baseline stratification factors at randomisation (PH type 2/3, 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, and the following fixed effect covariates: baseline eGFR value.

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.

A descriptive comparison will be presented at 12 and 24 months in change from baseline between O – O and P – O treatment groups as measured by the treatment effect factor. Results will be summarised including LS mean change from baseline in eGFR at months 12 and 24 (SE) in both treatment groups along with treatment group difference (LS mean difference) and corresponding 95% confidence interval. The same results will be presented also for the other timepoints.

To describe if Oxabact treatment has a similar effect during the first 12 and 24 months in the O – O and P – O groups, a second MRMM analysis on change from baseline in eGFR in the O – O group and change from second baseline in the P – O group based on the FAS will be used with a model including fixed effect factors: treatment group (O – O; P – O), week, and week-by-treatment interaction, and the following fixed effect covariates: baseline eGFR (baseline for the O - O group and second baseline for the P – O group).

12.5.2 Change from Baseline in eGFR Supportive Analyses

A secondary method of analyses, corresponding to the MRMM above, will be done using an ANCOVA model with treatment group, baseline/second baseline stratum, and baseline/second baseline as covariates and change from baseline/second baseline in eGFR at 12 and 24 months as a dependent variable. Supportive analyses based on AUC may also be performed and further described in the SAP.

12.5.3 Change from Baseline in eGFR Supportive Summaries of Slopes

Another description of the data based on slopes will be provided. For each patient, a separate regression analysis will be calculated for eGFR observed values over time and individual subject slopes identified. Slopes will be then summarised primarily with descriptive statistics. In order to describe differences a non-parametric analysis is proposed, the Wilcoxon rank-sum test may be used for comparisons, medians and quartiles can be provided, estimates for the median of groups differences or within group differences, Hodges-Lehmann approach estimate of location shift and the 2-tailed Moses 95% confidence intervals.

12.6 Key Secondary Endpoints

12.6.1 Total Plasma Oxalate

Change from baseline in total plasma oxalate concentration will be analysed similar to the primary endpoint. Total Pox observed minimum and maximum post baseline values and change from baseline will also be summarised and plotted. Additionally, a combined plot for key secondary endpoint total plasma oxalate and primary endpoint eGFR over time will be created using a double-axis display. This plot will be created for

each patient, but also a summary plot presenting both parameters' mean values over time per treatment group will be made.

12.6.2 Kidney Stone Events

Frequency of kidney stone events after 12 and 24 months of treatment. Kidney stone events are defined as:

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

Kidney stone events after 12 and 24 months of open-label Oxabact treatment will be summarised using descriptive statistics. Incidence rates of kidney stone events (defined as the number of events divided by the total person-years) will be calculated along with the number of subjects with a stone event. Risk ratio and risk difference between the two treatment groups will be presented in combination with a 95% CI.

Time lost at work or school due to kidney stone events will be summarised.

12.7 Other Endpoints

- Change from baseline in myocardial function parameters as measured by Speckle Tracking and traditional echocardiography) will be summarised descriptively over time after 12 and 24 months of open-label Oxabact treatment.

  STE results will be evaluated over time for global longitudinal strain, including segmental changes, short-axis myocardial function, rotational displacement and apical sparing patterns. Traditional echocardiographic parameters will also be evaluated over time (e.g. Ejection Fraction, End Diastolic Volume, e/é, 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. Change from baseline in free plasma oxalate after 12 and 24 months of open-label Oxabact treatment will be described similar to the primary endpoint.
- Change from baseline in urinary oxalate excretion after 12 and 24 months of open-label Oxabact treatment will be described.
- Grade of nephrocalcinosis after 12 and 24 months of open-label Oxabact treatment will be summarised. Grade of nephrocalcinosis will be assessed by ultrasound images using grades 0-3.
- Number of *O. formigenes* during the study will be summarised over time. Summaries will present both observed and change from baseline values overall and by genotype (genotype 1, and genotype 2). In the P – O group, change from second baseline will be used instead. The association between *O. formigenes* in

stool and total plasma oxalate concentration will be evaluated using graphical displays.

- Change from baseline in Quality of Life scores as measured by SF36V2 or CHQ/PF50 questionnaires, depending on age, will be summarised over time in the two treatment groups.
- Change from baseline after 12 and 24 months of open-label Oxabact treatment in markers for renal function, renal tubular capacity and inflammation in urine and plasma will be described. Remaining time-points during the study will be presented as supportive.

12.8 Subgroup Analysis

The results of the study will be presented primarily with descriptive individual data graphically presented over time and therefore no additional subgroup analyses will be needed for these. For summaries and/or any other analyses presented the following subgroups will be considered, only if there is sufficient sample size and for the following endpoints: eGFR, total and free plasma oxalate concentration, 24-hour urinary oxalate excretion, stone events, absolute count of *O. formigenes* per gram in stool, speckle tracking and traditional echocardiography). The subgroups are:

- Subjects with a baseline 24-hour 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 of the ePHex study).
- Subjects above or equal to and below 18 years of age at baseline of the ePHex study.
- 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 creatinine - based "Bedside Schwartz" equation (2009) for children below 18 years of age and the creatinine - based CKD-EPI equation (2009) for adults).
- Race.
- Gender.
- Progressors and non-progressors based on historic eGFR (i.e. eGFR prior to treatment start in the ePHex study).
- Type of PH

12.9 Safety

Adverse events will be categorised by System Organ Class (SOC) and Preferred Term from MedDRA. Safety data will be summarised for the overall study population SAF and also according to the actual treatment received in the ePHex study O – O and P – O. AEs will be considered to be treatment emergent if the event occurs on or after the first administration of open label Oxabact treatment and up to 4 weeks after the last dose date of open-label Oxabact treatment of the ePHex-OLE study.

The incidence of AEs will be summarised using descriptive statistics, by SOC, preferred term and severity grade (CTCAE) for all treatment emergent, serious, treatment-related, and serious treatment-related AEs. Summaries will be provided separately for AEs leading to investigational product discontinuation and deaths (severity grade 5).

Changes in safety laboratory parameters, vital signs and physical examinations will also be summarised. Graphical representations may also be presented for parameters of interest. Listings of SUSARs, AEs and SAEs will be provided along with narratives.

12.10 Other Data

Subjects starting dialysis will be described.

12.11 Handling of Missing Data

Maximum likelihood, as used in MRMM, is a preferred method to address missing data considered at least missing at random (MAR). Depending upon the limited sample size, additional sensitivity analyses may be performed using LOCF or MI. As both MRMM and MI assume MAR data, the extent, pattern and reasons for missing data will be evaluated and described, if feasible. If necessary for the supportive analyses, MNAR can be evaluated using a pattern-mixture model (PMM), whenever possible.

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, completion of the ePHex study
- 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 patients 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 patients 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 patients 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 patients 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 ICFs 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/ICF 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 authorisation 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.

Concomitant medication, any medical history and Adverse Events will be coded using standardised 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, patients 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 patients' records and source document for the purposes of monitoring, auditing, or inspection by OxThera, authorised representatives of OxThera, Regulatory Authorities and IECs.

As part of the required content of the informed consent, patients 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 authorisation, 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 summarised 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 patients 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 patients 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 patients. All changes to the final study protocol must be documented in a written protocol amendment.

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21 APPENDICES

Appendix 1: Study Protocol Approval by Investigator

Appendix 1: Study Protocol Approval by Investigator

Protocol number: OC5-OL-02

Protocol Date: 06 July 2020

Protocol Version: 2

Study title: An open-label single-arm treatment extension study to evaluate the long-term efficacy and safety of Oxabact for patients with primary hyperoxaluria who completed study OC5-DB-02

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