

## STATISTICAL ANALYSIS PLAN

### ILLUMINATE-B: AN OPEN-LABEL STUDY TO EVALUATE THE EFFICACY, SAFETY, PHARMACOKINETICS, AND PHARMACODYNAMICS OF LUMASIRAN IN INFANTS AND YOUNG CHILDREN WITH PRIMARY HYPEROXALURIA TYPE 1

**Protocol Number:** ALN-GO1-004

**Protocol Version and Date:** Amendment 2: 04 May 2020  
Amendment 1: 09 August 2019  
Original: 08 November 2018

**Investigational Drug:** ALN-GO1 (Lumasiran)

**Phase:** Phase 3

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**Analysis Plan Version and Date:** Amendment 2: Version 3.0: 3 Aug 2020  
Amendment 1: Version 2.0: 12 Nov 2019  
Original: Version 1.0: 20 May 2019

<b>Confidentiality Statement</b>
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## APPROVAL SIGNATURE PAGE

### **ILLUMINATE-B: An Open-Label Study to Evaluate the Efficacy, Safety, Pharmacokinetics, and Pharmacodynamics of Lumasiran in Infants and Young Children with Primary Hyperoxaluria Type 1**

**Protocol Number:** ALN-GO1-004

**Analysis Plan Version and Date:** Version 3.0/3 Aug 2020

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## ABBREVIATIONS

Abbreviation	Definition
ADA	Anti-drug antibody
AE	Adverse event
AGT	Alanine-glyoxylate aminotransferase
<i>AGXT</i>	Alanine glyoxylate aminotransferase gene
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical Classification System
AUC	Area under the concentration-time curve
BMI	Body mass index
BSA	Body surface area
CDC	Center for disease control and prevention
CI	Confidence interval
C <sub>max</sub>	Maximum plasma concentration
CFR	Code of Federal Regulation
CL/F	Apparent clearance
CSR	Clinical study report
DMC	Data Monitoring Committee
ECG	Electrocardiogram
eCRF	Electronic case report form
eGFR	Estimated glomerular filtration rate
EOS	End of study
EOT	End of treatment
ET	Early termination
ESRD	End-stage renal disease
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GO	Glycolate oxidase
ICF	Informed consent form
LFT	Liver function test
LLOQ	Lower limit of quantitation
LLN	Lower limit of normal

Abbreviation	Definition
LOD	Limit of detection
MedDRA	Medical dictionary for regulatory activities
UOx:Cr	Oxalate:creatinine
PT	Preferred term
SAE	Serious adverse event
SAP	Statistical analysis plan
SC	Subcutaneous
SOC	System Organ Class
SMQ	Standardized MedDRA query
ULN	Upper limit of normal
WHO	World Health Organization
WNL	Within Normal Limit

## 1. INTRODUCTION

Primary hyperoxaluria type 1 (PH1) is a rare autosomal recessive disease characterized by excessive oxalate production by the liver and consequent hyperoxaluria. PH1 is caused by mutations in the alanine glyoxylate aminotransferase (*AGXT*) gene, which encodes the liver peroxisomal enzyme alanine-glyoxylate aminotransferase (AGT). As a consequence of AGT deficiency, glyoxylate accumulates and is oxidized to oxalate in the hepatocyte and ultimately transported to the kidneys for excretion. Oxalate, in the form of its calcium salt, is excreted almost entirely by the kidney. Due to its insolubility, calcium oxalate can crystallize readily in the urinary tract. In PH1, excess urinary oxalate results in recurrent nephrolithiasis and/or nephrocalcinosis, which can lead to pain, infections, progressive kidney disease and failure, along with reduced quality of life.[Cochat and Rumsby 2013] As renal function declines, elimination of oxalate is further reduced, such that oxalate levels in plasma rise and calcium oxalate subsequently accumulates in bone, vasculature, skin, retina, heart, and nervous system, resulting in severe end-organ damage. This devastating condition, systemic oxalosis, arises when the estimated glomerular filtration rate (eGFR) has declined to below 30 to 45 mL/min/1.73 m<sup>2</sup>. Without treatment, the disease progresses inexorably, and death from end-stage renal disease (ESRD) and/or complications of oxalosis is inevitable.[Cochat and Rumsby 2013; Harambat 2010; van der Hoeven 2012]

The ILLUMINATE-B Study (ALN-GO1-004) is an open-label phase 3 study designed to evaluate the efficacy and safety of subcutaneously administered lumasiran in infants and young children (<6 years of age) with PH1 and relatively preserved renal function. This statistical analysis plan (SAP) has been developed based on the Amendment 2 protocol of the ILLUMINATE-B study (dated 04 May 2020).

The analysis methods described in the protocol may be updated in this SAP. Any change to the data analysis methods described in the protocol, as well as the justification for the change, will be described in the SAP (Section 8) and clinical study report (CSR). Additional exploratory analyses of the data may be conducted when deemed appropriate.



## 2. STUDY OVERVIEW

### 2.1. Synopsis of Study Design

The ILLUMINATE-B Study (ALN-GO1-004) is a multicenter, multinational, open-label, single arm phase 3 study designed to evaluate the efficacy and safety of lumasiran in infants and young children (<6 years of age) with PH1 and relatively preserved renal function.

This study consists of 2 periods: a 6-month primary analysis period followed by a long-term extension period. During the 6-month primary analysis period, patients will undergo efficacy and safety assessments every 2 weeks for the first month and monthly thereafter. During the long-term extension period of up to 54 months, dosing will continue as listed in Table 1, with visits to the study site for evaluations occurring at least every 3 months and every 6 months for patients whose weight  $\geq 10$  kg after Month 24 visit.

Lumasiran will be administered as a subcutaneous (SC) injection with weight-based dosing for three body weight categories as listed in Table 1.

**Table 1: Loading Maintenance Doses of Lumasiran for Different Body Weight Categories**

Weight	Loading Dose (Day 1, Month 1, Month 2)	Maintenance Dose (Month 3 and Beyond)
0 to <10 kg	6.0 mg/kg monthly for 3 months	3.0 mg/kg monthly
$\geq 10$ to <20 kg	6.0 mg/kg monthly for 3 months	6.0 mg/kg every 3 months
$\geq 20$ kg	3.0 mg/kg monthly for 3 months	3.0 mg/kg every 3 months

For all patients, the dose will be based on body weight measurement obtained within 7 days prior to dosing.

Patients with weight increases that cross the threshold for the next weight-based dosing category (between 0 and less than 10kg (0-<10 kg) to greater or equal to 10kg and less than 20kg ( $\geq 10$ -<20 kg) to greater or equal to 20kg ( $\geq 20$  kg)) will follow the new dosing regimen for the remainder of the study or until the next dosing category threshold is reached (i.e., patients will not switch back to the lower-weight dosing schedule if their body weight subsequently decreases).

Patients in maintenance dosing who transition from 0-<10 kg to  $\geq 10$ -<20 kg will continue to receive monthly doses at 3.0 mg/kg until the next visit that coincides with the Schedule of Assessments for patients weighing  $\geq 10$ -<20 kg. Thereafter, patients will follow every-3-months dosing until the end of the study.

### 2.2. Randomization Methodology

Not applicable

### 2.3. Blinding

Not applicable

## **2.4. Data Monitoring Committee**

An independent Data Monitoring Committee (DMC) will oversee the safety and overall conduct of this study, providing input to the Sponsor. The DMC will operate under the rules of a charter that will be reviewed and approved by the DMC. Details are provided in the DMC Charter.

## **2.5. Study Procedures**

The schedule of assessments is described in the study protocol (Table 1 and Table 2).

### **3. ENDPOINTS**

#### **3.1. Primary Endpoint**

- Percent change in urinary oxalate excretion from baseline to Month 6
  - The primary endpoint is measured by the percent change from baseline in spot urinary oxalate:creatinine (UOx:Cr) ratios (mmol/mmol) from Month 3 to Month 6, averaged over these time points. Other measures for urinary oxalate excretion include 24-hour urinary oxalate corrected by BSA and 24-hour urinary UOx:Cr ratio, when available.

#### **3.2. Secondary Endpoints**

##### Extension Phase

- Percent change in urinary oxalate excretion from baseline
- Percentage of time that spot urinary UOx:Cr ratio is at or below the near-normalization threshold ( $\leq 1.5 \times \text{ULN}$  for age)

##### Duration of Study

- Absolute change in urinary oxalate excretion from baseline
- Change (percent and absolute) in plasma oxalate concentration from baseline
- Proportion of patients with urinary oxalate excretion  $\leq$  the upper limit of normal (ULN) for age and  $\leq 1.5 \times \text{ULN}$  for age
- Plasma PK parameters of lumasiran
- Change in estimated glomerular filtration rate (eGFR) from baseline

#### **3.3. Exploratory Endpoints**

- Change from baseline in nephrocalcinosis as assessed by renal ultrasound
- Change in frequency of renal stone events
- Change in urinary glycolate and plasma glycolate
- Change in growth parameters (z-scores) from baseline over time
- Changes in developmental milestones over time
- Changes in patient and/or caregiver experience as evaluated by a patient/caregiver survey
- Frequency and titer of ADA

#### **3.4. Safety Endpoint**

- Frequency of adverse events (AEs)

## 4. PATIENT POPULATION

### 4.1. Patient Definitions

The populations (analysis sets) are defined as follows:

- Safety Analysis Set: All patients who received any amount of lumasiran during study.
- Efficacy Analysis Set: All patients who received any amount of lumasiran and have at least one valid spot urinary UOx:Cr ratio value at baseline and at least one valid spot urinary UOx:Cr ratio value from assessment(s) at Month 3 through Month 6.
- Primary Interim Efficacy Analysis Set: Among first 16 enrolled patients, all patients who received any amount of lumasiran and have at least one valid spot urinary UOx:Cr ratio value at baseline and at least one valid spot urinary UOx:Cr ratio value from assessment(s) at Month 3 through Month 6
- PK Analysis Set: All patients who received one full dose of lumasiran and have at least one postdose blood sample for PK parameters and have evaluable PK data.

The Safety Analysis Set will be used for safety analyses and sensitivity analysis of efficacy. The Efficacy Analysis Set will be used to evaluate efficacy endpoints. The PK Analysis Sets will be used to conduct PK analyses of plasma lumasiran concentrations.

The primary analysis period is defined as the time period from the date/time of the first lumasiran dose administration until either the date/time of lumasiran dose administration at Month 6 or the date of the Month 6 visit (Day 169) for patients who discontinued treatment prematurely. Primary analysis will be conducted using all data collected in the Primary Analysis Period. All data up to the dosing at the Month 6 visit will be included in the Primary Analysis Period.

The extension period starts after the date/time of lumasiran dose administration at Month 6 until the end of the study. Analysis of Extension Phase will focus on long-term efficacy of lumasiran.

Duration of Study is defined as the time period from on or after the first date/time of lumasiran dose until the end of the study. Analyses of Duration of Study will summarize secondary endpoints using data collected during the entire study period. These analyses focus on long-term efficacy and safety effects of lumasiran during both the primary analysis period and the extension period.

### 4.2. Protocol Deviations

Protocol deviations will be classified into major or minor deviations by medical review. A major deviation is defined as a deviation that may significantly impact the completeness, accuracy and/or reliability of the trial data; that may significantly affect a patient's rights, safety and well-being (ICH.E3 R1 Structure and Contents of Clinical Study Reports Guidance for Industry, 2013). Deviations not classified as major will be assigned as minor. Major protocol deviations will be reviewed and approved by Alnylam prior to an interim or full database lock. All protocol deviations will be presented in a listing.

Major protocol deviations will be summarized in the CSR.

## 5. GENERAL STATISTICAL METHODS

### 5.1. Sample Size Justification

The planned enrollment for this study is 20 patients, including at least 1 patient <12 months of age with weight <10 kg at consent. Patients who discontinue lumasiran or stop participation in the study prior to Month 6 may be replaced.

The sample size was determined based on feasibility considerations, not power calculations.

### 5.2. General Methods

Continuous data will be described using descriptive statistics such as the number of observations (n), mean, standard deviation, standard error, median, quartiles, minimum, and maximum. For the summary statistics of all numerical variables unless otherwise specified, minimum and maximum will be displayed to the same level of precision as reported. Median and mean will be presented to the level of precision collected in the database plus one additional decimal. The standard deviation and standard error will be presented to the level of precision collected in the database plus 2 additional decimals. For any assessments with repeated collections at a given study visit (e.g. height) the mean will represent the value at that visit unless otherwise noted. For biomarker and/or lab data, if any value is recorded as <lower limit of quantitation (LLOQ) then the value used for calculations will be assigned a value of LLOQ.

Categorical and ordinal data will be described using the patient count and percentages in each category. When count data are presented, the percentage will not be presented when the count is zero.

Summary tables and figures for each study period will include all data collected during the corresponding period as defined in Section 4.1.

The per-patient listings will include all data collected during each study period. For per-patient listings of the duration of study, there will be a variable to indicate the period of the data collection (Primary Analysis Period or Extension Period).

Day 1 will be defined as the day of the first dose of lumasiran. Study day is calculated relative to the first dose date of lumasiran for all patients:

If the assessment date is after the date of first lumasiran dose, then the study day will be calculated as:

$$\text{Study Day} = \text{Date of assessment} - \text{date of first dose of lumasiran} + 1,$$

If the assessment date is before the date of the first dose of lumasiran, then the study day will be calculated as:

$$\text{Study Day} = \text{Date of assessment} - \text{date of first dose of lumasiran}$$

At a minimum, all listings will include the following variables:

- Baseline: relative to first dose of lumasiran (Section 5.4).
- Study Day: relative to first dose of lumasiran.
- Visit labels: visits based upon actual study visits (Section 5.6).

Summary statistics will be presented, as well as 2-sided 95% confidence interval (CI)s for primary/secondary endpoints or selected parameters, as described in the sections below.

All data recorded on the CRF will be displayed in data listings.

### **5.3. Computing Environment**

All statistical analyses will be performed using validated SAS statistical software Version 9.4 (or later), unless otherwise noted.

### **5.4. Baseline Definitions**

For spot urinary UOx:Cr ratio, baseline is defined as the mean of all spot urinary UOx:Cr ratio values collected prior to the first dose date/time of lumasiran. Baseline for all 24-hour urine parameters (i.e. 24-hour urinary oxalate corrected for BSA, etc) is defined as the median of all measurements collected prior to the first dose date/time of lumasiran. For all other PD parameters (i.e., plasma oxalate, etc.), baseline will use the mean of all measurements collected prior to the first dose date/time of lumasiran.

For other parameters, baseline is defined as the last non-missing value prior to the first dose date/time of lumasiran, unless otherwise specified. If multiple values are collected at a single visit (e.g. height, etc.), the mean of the values collected at the same visit will be used.

### **5.5. Missing Data**

Patients who discontinue the study prior to Month 6 will be encouraged to remain on study and complete their remaining clinical visits (excluding PK assessments) through the visit at Month 6 and only safety follow-up visits afterwards. All data collected regardless of whether it was collected before or after treatment discontinuation will be used for analysis. However, it is possible that data will remain missing.

For safety analyses, no date will be assigned to any AEs with fully missing dates and only partially missing dates will be imputed. An AE will be considered treatment emergent unless it can be unequivocally determined (from the partial onset date and/or a partial or complete stop date) that the event occurred prior to the first dose of lumasiran.

For medications with partial start or stop dates: the first day/month will be imputed for start date, and the last day/month will be imputed for stop date. For medications with a completely missing start date, the medications will be considered as started prior to the first dose of study drug in this study; medications will be classified as prior or both prior and concomitant depending on the medication stop dates. If any medications have a completely missing stop date, then the medication will be assumed as ongoing.

For partial dates of renal stone events (start and stop dates): the first day/month will be imputed for start date, and the last day/month will be imputed for stop date. It is not expected that renal stone dates will be completely missing.

Due to blood sample volume limitations related to patient age/size, missing data in blood sample tests are expected. For blood test parameters where fewer than 4 patients have available baseline values, no summary statistics will be provided, only by-patient listing will be provided.

## **5.6. Visit Windows**

For tabular and graphical summaries, all data will be tabulated and analyzed per the evaluation visit as recorded on the electronic case report forms (eCRF) even if the assessment is outside of window.

Unless otherwise specified, data collected at an unscheduled visit will be included in by-patient listings and/or spaghetti plot figures, but no assignment of the scheduled visit will be made for the purposes of summary tabulations. However, unscheduled study visits will be used in the calculation of baseline values if prior to first dosing and for any categorical shift tables (e.g. shift from baseline to worst post-baseline value).

## **5.7. Interim Analysis**

A primary interim analysis is planned when the first 16 enrolled patients complete Month 6 assessment or discontinue treatment (if applicable). The number of patients for the primary interim analysis is chosen due to the enrollment pattern and the need for evaluation of benefit risk in this population for regulatory submissions. It should be noted that this will also provide information on twice the number of patients originally planned to be enrolled in this study. No multiplicity adjustment is necessary, since no formal study hypothesis is being tested for this study.

Further interim analyses may be conducted for lumasiran regulatory submission purposes and/or publication requests.

## **5.8. Analysis for Primary Endpoint**

As this study will be ongoing at the time of the primary analysis, there will be an interim database lock when the last patient completes all Month 6 visit assessments. All data available as of the date of data cut for the interim database lock will be included in the interim CSR corresponding to the primary analysis period.

After the study is completed (i.e. all patients complete the long-term extension period and/or required safety follow-up visit(s)), the database will be locked, and all data will be summarized in the final CSR.

## 6. STUDY ANALYSES

### 6.1. Patient Disposition

Number and percentage of patients will be tabulated for the following categories:

- Enrolled (signed informed consent and met eligibility)
- Safety Analysis Set
- Efficacy Analysis Set
- Primary Interim Efficacy Analysis Set
- PK Analysis Set

Summaries of the number and percentage of patients who discontinued treatment and/or withdrew from the study will be presented, along with the primary reasons for discontinuation of treatment and/or withdrawal from the study. Additionally, the number of patients who completed the Month 6 visit will also be displayed. A patient is defined as having completed the Month 6 visit if the patient has at least one spot urinary UOx:Cr ratio result for the Month 6 visit.

The number and percentage of patients enrolled by region will be summarized.

Data listings of those patients who withdrew and/or discontinued treatment including the associated reasons will also be presented.

The numbers of patients in each dose group will be summarized for the duration of study.

Disposition will be summarized throughout the study.

### 6.2. Demographics and Baseline characteristics

Descriptive statistics of demographic characteristics including but not limited to : age (months, 2 decimals), weight (kg), weight category (0-<10kg, 10-<20kg, ≥20kg), gender, race, ethnicity, region, height (cm), and body mass index (BMI) will be presented. The modified z-score for all patients will be calculated and summarized for height, weight and body mass index (BMI) according to the Centers for Disease Control and Prevention (CDC) growth chart.[CDC National Center for Health Statistics 2019]

Additional baseline disease characteristics will be summarized using descriptive statistics. These include but are not limited to: spot urinary UOx:Cr ratio (mmol/mmol), plasma oxalate (umol/L), 24-hour oxalate excretion corrected for BSA (mmol/24hours/1.73m<sup>2</sup>), 24-hour urinary UOx:Cr ratio (mmol/mmol), eGFR (ml/min/1.73m<sup>2</sup>), spot urinary glycolate:creatinine ratio (mmol/mmol), plasma glycolate (umol/L), number of episodes with stone events in the past 12 months, and total number of patients reporting pyridoxine (vitamin B6) use prior to study entry. The collecting schedules are specified in the Schedules of Assessment in protocol.

### 6.3. Medical History

A complete medical and surgical history will be collected at the screening visit. The medical and surgical history will be coded using the Medical Dictionary for Regulatory Activities (MedDRA Version 21.1 or later).



Medical and surgical history will be summarized for the Safety Analysis Sets by system organ class, high level term and preferred term.

A tabular summary of historical PH1 disease characteristic information will be generated and include information on disease characteristics such as: time from diagnosis to first dose date (months), time from first symptoms to first dose date (months), time from first symptoms to diagnosis (months), number of siblings with PH1, reported history of disease events (e.g. pyelonephritis, renal stones, urinary tract infection). Reported medical history event rates may be adjusted based on age category, see Section 10.5.

Separate by-patient listings will be generated.

#### **6.4. Prior and Concomitant Medications**

Prior and concomitant medications will be coded using the World Health Organization (WHO) Drug Dictionary (September 2018 or later). Prior medications are defined as medications that were taken prior to the first dose of the study medication. Concomitant medications are defined as medications which were taken prior to and were ongoing while on lumasiran or medication(s) taken on or after the first dose date of lumasiran.

A tabular summary of the number and percentage of patients taking concomitant medications will be generated by anatomic therapeutic class (ATC) and preferred term. Data will be presented for the Safety Analysis Set.

By-patient listings will be generated separately for prior medications, concomitant medications and pyridoxine (vitamin B6).

Medications (Prior and Concomitant) will be summarized for the Safety Analysis Set.

## 7. STATISTICAL ANALYSIS

### 7.1. Primary Endpoint

The primary endpoint of this study is percent change in urinary oxalate excretion from baseline to the average of Month 3 to Month 6, which is measured by the primary outcome variable – percent change from baseline in spot urinary UOx:Cr ratio (mmol/mmol). The analysis will be conducted using the Efficacy Analysis Set.

Spot urinary UOx:Cr ratio is calculated from single-void urine collections. For all patients, triplicate single-void samples will be collected within 7 days prior to dosing, as specified in the Schedules of Assessment in the protocol. The mean value of these triplicate samples will be used as the baseline and postdose values in the analysis to account for the variability of the measure.

Despite historical data in healthy volunteers suggesting a natural decline in spot urinary UOx:Cr ratio in pediatrics, especially for children younger than 7 years old (Section 10.2.1), the effect of age on spot urinary UOx:Cr ratio in PH1 children is less clear (Section 10.2.2). Based on published data [Lieske 2005] at the time of writing of this SAP, it is considered that the potentially age-related decline in spot urinary UOx:Cr ratio level in children with PH1 is minimal, comparing with the anticipated treatment effect of lumasiran.

The primary analysis of the primary outcome variable will be performed using a restricted maximum likelihood (REML) based Mixed-effect Model Repeated Measures (MMRM) approach. Analysis will include fixed effects of scheduled visits and baseline spot urinary UOx:Cr ratio value (mmol/mmol), including patient as a random factor. An autoregressive (1) covariance structure will be used to model the within-patient error given the small sample size for this study. The primary estimate is the LS mean of the primary variable averaged over Month 3 to Month 6. This least square (LS) mean will be presented along with corresponding standard errors (SEMs), 95% confidence intervals (CI) and p-value from the model (i.e., testing against the null hypothesis of mean of primary outcome being equal to 0).

If the fit of the autoregressive (1) covariance structure matrix fails to converge, the primary estimate will be calculated as the LS mean of the primary outcome variable average across scheduled visits of Month 3 to Month 6 (based on available measures), according to analysis of covariance (ANCOVA) model with covariate of baseline spot urinary UOx:Cr ratio value (mmol/mmol). This LS mean will be presented along with corresponding SEMs, 95% CI and p-value from the model.

Descriptive statistics will also be generated at each scheduled visit. Mean (+/- SEM) figures of percent reduction will be plotted as well as individual spaghetti plots and listings. A table of the mean maximum percent reduction during the 6-months will also be generated.

Missing data on 24-hour urinary oxalate corrected for BSA are expected in the pediatric patients to be enrolled in this study. Existing correlation analysis based on PH1 patients older than 6 years old of 24-hour urinary oxalate corrected for BSA and spot urinary UOx:Cr ratio is included in Section 10.3.

#### Primary Interim Analysis

A similar analysis will be performed for the primary interim efficacy analysis set, i.e., among the first 16 enrolled patients, those who received any amount of lumasiran and have at least one

valid spot urinary UOx:Cr ratio value at baseline and at least one valid spot urinary UOx:Cr ratio value from assessment(s) from Month 3 through Month 6.

#### 7.1.1. Sensitivity Analysis

Sensitivity analyses include:

- Percent change from baseline in spot urinary UOx:Cr ratio by visits in Efficacy Analysis Set
- Percent change from baseline in spot urinary UOx:Cr ratio from Month 3 to Month 6 in Safety Analysis Set
- Percent change from baseline in ULN Ratio (ratio of measured spot urinary UOx:Cr to ULN) from Month 3 to Month 6 in Efficacy Analysis Set

Percent change from baseline in spot urinary UOx:Cr ratio by visits in Efficacy Analysis Set will be analyzed using MMRM, LS means with corresponding SEM and 95% CIs will be displayed.

Percent change from baseline in spot urinary UOx:Cr ratio in Safety Analysis Sets will be analyzed using MMRM including all scheduled visits. LS mean with corresponding SEM and 95% CIs will be displayed.

To further adjust for any potentially confounding effect of natural maturation, the analysis of the ratio of spot urinary UOx:Cr ratio to the reference value (age-matched ULN established from the ULNs published in [Matos 1999]) that is established for healthy children of varying age will also be performed. Percent change from baseline in ULN Ratio from Month 3 to Month 6 in Efficacy Analysis Set will be analyzed using MMRM, and LS means with corresponding SEM and 95% CIs will be displayed. A sensitivity analysis will be conducted with the [REDACTED] (Bayesian) ULN (See SAP Addendum Version 1.0)

#### 7.1.2. Analyses of 24-Hour Urinary Oxalate Excretion

24-hour urinary oxalate analysis will be conducted to assess the robustness of the primary outcome. Only valid 24-hour urinary oxalate values (see Section 6.2.1.1 of the protocol) will be considered for analysis for patients in Efficacy Analysis Set. The correlation between spot urine and 24-hour collection urine values maybe investigated. The analyses for 24-hour urinary oxalate corrected for BSA and 24-hour urinary UOx:Cr ratio will be carried out with data as observed. Percent change from baseline of 24h urinary oxalate corrected for BSA and 24h UOx:Cr ratio through Month 6 will be summarized.

### 7.2. Subgroup Analysis

Subgroup analyses will include analyses of the primary endpoint by age groups and weight-based dosing groups defined according to the baseline values:

Age group: 0-<1 year, 1-<6 years;

Weight-based dosing category: 0-<10 kg, ≥10-<20 kg, and ≥20 kg.

For primary analysis period, weight-based dosing category will be determined by initial dose level; for extension phase, dosing category may be analyzed both by initial dose level and by the dose level received for the longest duration.

Other subgroups may be examined, if deemed appropriate.

Subgroup analysis will be conducted for primary analysis of spot urinary UOx:Cr ratio in Efficacy Analysis Set to further understand the treatment effects. Descriptive statistics will be reported for the subgroups.

The subgroup analyses may be performed for secondary endpoints in Section 7.3.

### 7.3. Secondary Endpoints

For efficacy secondary endpoints, the Efficacy Analysis Set will be used; for PK secondary endpoints, the PK Analysis Set will be used.

Secondary endpoints in this study are as follows:

#### Extension Period (Month 6 to End of Study)

Percent change in spot urinary UOx:Cr ratio from baseline for all post-Month 6 visits will be summarized descriptively using the Efficacy Analysis Set. The analyses for 24-hour urinary oxalate corrected for BSA and 24-hour urinary UOx:Cr ratio will be carried out using Extension Period data as well.

#### Duration of Study (Baseline to End of Study)

Absolute changes in spot urinary UOx:Cr ratio and percent and absolute change in plasma oxalate from baseline using Efficacy Analysis Set will be performed similarly to primary analysis. In addition, percent and absolute change in plasma oxalate from baseline will also be analyzed using only patients in the Efficacy Analysis Set whose baseline plasma oxalate  $\geq 1.5 \times \text{LLOQ}$ . LS means by postdose visits with corresponding SEM and 95% CIs will be displayed for the primary analysis period.

For continuous endpoints (including eGFR), descriptive statistics will be generated at each scheduled visit. Mean ( $\pm$  SEM) figures of actual values and changes from baseline will be plotted as well as individual spaghetti plots and listings. For binary endpoints, the number and associated percent of patients who met each threshold (e.g.  $\leq \text{ULN}$  or  $\leq 1.5 \times \text{ULN}$ ) at visits will be presented.

To demonstrate durability of the effect of lumasiran, for binary endpoints, (e.g. proportion of patients with spot urinary UOx:Cr ratio level  $\leq \text{ULN}$  or  $\leq 1.5 \times \text{ULN}$ ), tabular summaries of the number and percent of patients who met the threshold at post-baseline visits will be presented.

An analysis of the percentage of time that spot urinary UOx:Cr ratio is at or below the near-normalization threshold for the duration of study will be summarized. A patient is considered to have met the threshold if the spot urinary UOx:Cr ratio is  $\leq 1.5 \times \text{ULN}$  at a post-baseline visit.

For each patient, the percentage of time that spot urinary UOx:Cr ratio is at or below the near-normalization threshold of  $\leq 1.5 \times \text{ULN}$  will be calculated as follows:

$$\frac{\text{cumulative time at or below near normalization threshold}}{\text{cumulative time of valid assessments}} \times 100$$

where cumulative months in near-normalization will be defined as the summation across all intervals that met the near-normal threshold and cumulative months of valid assessments will be defined as the summation across all valid post-baseline collections.

Given that the sample collection varies during the study, the calculation of percentage of time will include the following considerations:

- Time will include all follow-up assessments until study end or premature treatment discontinuation, whichever occurs first.
- A linear interpolation method will be used to determine the bookend dates at which the patient crossed into the normal threshold and crossed out of the normal threshold

Descriptive statistics of percentage of time at or below the threshold (among the subset of patients who had at least one post-baseline value that met threshold) will be presented. In addition, the number of patients and associated percentage of patients in each category (e.g. <25%, ≥25% to <50%, ≥50% to <75%, ≥75% of time at or below the threshold) will also be presented.

Descriptive statistics of eGFR at and after 6 months of lumasiran will be presented using the Efficacy Analysis Set and Safety Analysis Set. Shift tables of eGFR categories from baseline to post-baseline visits and an overall worst post-baseline will also be generated

To demonstrate durability of the treatment effect of lumasiran, additional secondary endpoints may be assessed.

## 7.4. Exploratory Efficacy Analysis

Efficacy exploratory endpoints will be analyzed using the Efficacy Analysis Set generally.

To evaluate the changes in nephrocalcinosis, a renal ultrasound will be performed at baseline and 6, 12, 24, 36, 48 and 60 months. The ultrasound will measure the grade of medullary nephrocalcinosis (range: 0 to 3) where a higher grade indicates greater severity.

Changes in the grade of medullary nephrocalcinosis will be categorized in 3 groups ('no change', 'worsening' or 'improving'). Shifts in the grade of nephrocalcinosis from baseline to each post-baseline visit will be summarized. The distribution of patients by extent of change in nephrocalcinosis grade (i.e., number of grades by which nephrocalcinosis improved or worsened) from baseline to each post-baseline visit will also be summarized. At each post-baseline visit, the number and associated

percentage of patients in the following 4 categories of the overall change (i.e., accounting for both kidneys), 'no change', 'improving', 'worsening', and 'indeterminate (one kidney improving and one worsening)' will be presented. Descriptive statistics will also be generated.

To evaluate the changes in renal stone events, these events will be captured at baseline (i.e. historical information) and throughout the study. A renal stone event is defined as an event which includes at least one of the following: visit to health care provider because of a renal stone, medication for renal colic, stone passage, and macroscopic hematuria due to a renal stone.

Total number of patients with a renal stone event, total number of renal stone events and rate of renal stone events during each study period will be calculated. The rate is calculated as total

number of renal stone events divided by total number of on-study days of patients during the respective period. And 95% confidence intervals will be presented.

Changes in other PD markers (such as spot urinary and plasma glycolate) will be explored. Descriptive statistics of actual, absolute change and percent change in levels of these markers will be generated at baseline and each post-baseline visit (as applicable).

Change in growth parameters (z-scores) from baseline over time and changes in developmental milestones over time will be summarized descriptively. The achievement of developmental milestones over time is assessed using Vineland Adaptive Behavior Scales. Composite scores for developmental milestones are collected and summarized using Vineland Adaptive Behavior Scales, second version (Vineland-II). Vineland-II consists of 5 domains each with subdomains. The adaptive levels associated with the domain and subdomain composite scores will be categorized and shift tables will be generated, details in Section 10.5. Vineland-II scores of each domain, subdomain, and the adaptive levels will also be presented in by-patient listing.

Changes in healthcare resource use will be listed and patient experience, and caregiver experience will be summarized descriptively, details in Section 10.6.

Analysis of ADA is included in Section 7.8.

## 7.5. Pharmacodynamic Analysis

Analyses of pharmacodynamic (PD) endpoints based on urinary excretion and blood samples (i.e., spot urinary UOx:Cr ratio, 24hr urinary oxalate corrected for BSA, plasma oxalate, etc.) are included in primary/secondary/exploratory endpoints and described in Section 7.1 to Section 7.4. All PD analysis will be based on Efficacy Analysis Set.

## 7.6. Pharmacokinetic Analysis

Pharmacokinetic analyses will be conducted using noncompartmental methods. PK parameters will be calculated using a validated version of Phoenix® WinNonlin. All PK analysis will be based on PK Analysis Set.

Population PK analysis is planned for all patients in the study and will be described in a separate population PK analysis plan.

## 7.7. Safety Analyses

Primary safety analysis is a descriptive summary of frequency of AEs using the Safety Analysis Set. The primary safety parameter is treatment emergent Adverse Events (AEs). A treatment emergent AE will be defined as an AE that occurs or worsens on or after the first dose date/time of lumasiran through 84 days after the last dose of lumasiran. In addition, any AE that worsened in intensity or was subsequently considered related to study drug will be considered treatment emergent. Other safety parameters will include vital signs, ECGs, clinical laboratory assessments and physical exams.

Safety analysis will be conducted both at Month 6 interim analysis and end of study. AE analysis may be generated for potential interim analyses if applicable.

### 7.7.1. Adverse Events

AEs will be classified using the Medical Dictionary for Regulatory Activities (MedDRA Version 21.1 or later) and displayed in tables using System Organ Class (SOC) and Preferred Terms (PT). All treatment emergent AEs hereafter will be referred to as AEs in this document.

An overview table of AEs will be generated. The overview table will include the number and percentage of patients in categories such as, but not limited to:

- At least 1 AE
  - At least 1 drug related AE
- At least 1 severe AE
  - At least 1 drug related severe AE
- At least 1 Serious Adverse Event (SAE)
  - At least 1 drug related SAE
- At least 1 AE leading to treatment discontinuation
  - At least 1 drug related AE leading to treatment discontinuation
- At least 1 AE leading to study withdrawal
  - At least 1 drug related AE leading to study withdrawal
- Death

Tabulations by System Organ Class (SOC) and Preferred Term (PT) displaying the number of patients (percentage) and total events will be produced for the following tables:

- All AEs
- Severe AEs
- AEs by Maximum Severity
- AEs related to treatment
- AEs related to treatment by Maximum Severity
- All SAEs
- SAEs related to treatment
- AEs leading to treatment discontinuation
- AEs leading to treatment interruption
- AEs leading to study withdrawal

Tabulations by PT in decreasing order of frequency within the Safety Analysis Set will be generated for the following tables:

- All AEs
- All SAEs

- AEs related to treatment
- SAEs related to treatment

There will also be an All AE table generated displaying rates of AEs adjusted for exposure-time during the respective period.

Patients who report multiple occurrences of the same AE (preferred term) will be classified according to the most severe occurrence or most related.

Separate listings will be provided for deaths, SAEs, and AEs leading to discontinuation of study drug, dose interruption, or withdrawal from study. By-patient AE listings will be provided.

Additional summaries of injection site reactions (ISRs) and AEs mapping to the Drug related hepatic disorders Standardized MedDRA Query (SMQ) will be generated as described below. In addition, other SMQs or AE groupings may be evaluated.

**Injection Site Reactions (ISRs):** AEs mapping to the High-Level Term (HLT) Injection Site Reactions using MedDRA dictionary will be included in the summary. Frequency (percentages) of ISRs by HLT and PT will be generated. A separate listing will be generated to display all patients who reported ISRs. In addition, a table of the number of patients with at least 1 ISR, total number of injections, total number of injections complicated by ISRs and corresponding % of injections complicated by ISR with the most common signs and symptoms reported due to ISRs will be generated.

**Hepatic AEs, including Liver Function Test (LFT) abnormalities:** Analysis of hepatic AEs will include AEs mapping to the SMQ Drug-related hepatic disorders – comprehensive search (includes all narrow and broad terms). Frequency (percentages) of hepatic AEs will be summarized by SOC and PT. A separate listing will be generated of all patients reporting these events.

#### 7.7.2. Dosing and Extent of Exposure

Summaries of exposure will include the following characteristics such as, but not limited to: the total number of doses received per patient, mean number of doses per patient, cumulative number of doses received, duration of drug exposure (months), the total dose administered (mg) and total volume administered (mL).

Definition of drug exposure (days) will be defined as the minimum of [Exposure time= (date of last exposure – date of first dose +1)] where date of last exposure will represent either the date of the last dose administered dose +84, analysis cut-off date or end of study date. The exposure during the primary analysis period will be right censored; that is, the date of the last exposure will not be later than the first date of dosing in the Extension Period.

Dose interruptions and compliance are not taken into account.

#### 7.7.3. Clinical Laboratory Data

Clinical laboratory parameters will be expressed in Standard International (SI) units. Laboratory data collected and recorded as below the lower limit of quantitation (LLOQ) will be set to the LLOQ for calculation of summary statistics. Key laboratory parameters will be graded according to NCI CTCAE v5.0.



Clinical laboratory data by visit in summary tables will use data from central laboratory.

eDISH plots and the summary tables and listings for abnormality for clinical laboratory data will be analyzed in two ways; one using only central laboratory data and one using both central and local laboratory data by the following rules;

- Central laboratory data at both scheduled and unscheduled visits will be used.
- If central laboratory data at a visit is missing, local laboratory data will be used.
- If a patient has both local and central laboratory data at the same visit, the central data will be used.

Shift tables will be generated to summarize shifts from baseline category to the worst post-baseline category with directionality specified (e.g. hyper and hypo).

Clinical laboratory tests with normal ranges will be classified as Low, Normal, and High. For these tests, abnormal values will be flagged in the listings with H when the value is higher than the upper limit of the reference ranges and with L when the value is lower than the lower limit of the reference ranges.

For hematology and chemistry labs, summary tables of potentially clinically significant abnormalities may also be provided.

- All laboratory data will be presented in data listings. The listing of the clinical laboratory data will display both local and central laboratory data. Out of range laboratory results will be identified in listings.

**LFTs:** A listing will be generated for all patients with abnormal liver function tests as defined by alanine aminotransferase (ALT)>3xULN, aspartate aminotransferase (AST)>3xULN or total bilirubin >2x ULN at any visit.

A tabular summary for LFTs will be generated to summarize the number and associated percentage of patients in each of the categories at any post-baseline visit:

- ALT: Within normal limit (WNL),  $1 < ALT \leq 3$ ,  $3 < ALT \leq 5$ ,  $5 < ALT \leq 10$ ,  $10 < ALT \leq 20$ ,  $ALT > 20 \times ULN$
- AST: WNL,  $1 < AST \leq 3$ ,  $3 < AST \leq 5$ ,  $5 < AST \leq 10$ ,  $10 < AST \leq 20$ ,  $AST > 20 \times ULN$
- ALT or AST: WNL,  $1 < ALT \text{ or } AST \leq 3$ ,  $3 < ALT \text{ or } AST \leq 5$ ,  $5 < ALT \text{ or } AST \leq 10$ ,  $10 < ALT \text{ or } AST \leq 20$ ,  $ALT \text{ or } AST > 20 \times ULN$
- ALP: WNL,  $1 < ALP \leq 1.5 \times ULN$ ,  $ALP > 1.5 \times ULN$
- Total Bilirubin: WNL,  $1 < \text{Total Bilirubin} \leq 1.5$ ,  $1.5 < \text{Total Bilirubin} \leq 2$ ,  $2 < \text{Total Bilirubin} \leq 3$ ,  $3 < \text{Total Bilirubin} \leq 5$  and  $\text{Total Bilirubin} > 5 \times ULN$

eDISH plots of peak bilirubin at any time versus peak ALT or AST at any time will also be presented.

#### 7.7.4. Vital Signs

A summary table of potentially clinically significant shifts in vital signs may be generated.

A summary table of descriptive statistics of modified z-scores of patient height, weight, and BMI by visits will be generated. A tabular summary of the proportion of patients with z-scores at various cut-points (i.e.  $\geq \pm 2$  SD) will also be generated.

A by-patient listing of vital sign data will be generated. Per patient plots of z-scores over time may also be generated.

#### **7.7.5. Physical Examinations**

Full physical examinations and symptom-directed physical examinations will be conducted throughout the study. Clinically significant abnormalities observed during the physical examination are recorded on the medical history or AE eCRF as appropriate.

A separate listing per patient will be generated to display the date and time of the physical exam.

#### **7.7.6. Electrocardiogram**

Rhythm from single Electrocardiogram (ECG) parameter will be collected. The number and percentage of patients with normal, abnormal, clinically significant abnormal results, and specified abnormalities at baseline and each timepoint will be summarized.

A listing of all ECG data will be provided.

#### **7.8. Anti-Drug Antibody**

The number, titer and associated percentage of patients who are ADA positive at baseline and also, at any post-baseline visit will be summarized. A listing of AEs for patients with ADA positivity will be provided.

A listing of all ADA data will be provided.

#### **7.9. COVID-19 Pandemic Impact Analyses**

Additional data were collected to characterize the impact of the COVID-19 pandemic on general study conduct, disposition, and quality of life, and subsequently, additional analyses and summaries will be provided in acknowledgement of multiple regulatory guidances (FDA Guidance on Conduct of Clinical Trials of Medical Products during COVID-19 Pandemic: Guidance for Industry, Investigators, and Institutional Review Boards, US Food and Drug Administration, 2020; Guidance on the Management of Clinical Trials during the COVID-19 (Coronavirus) Pandemic, European Medicines Agency, 2020; Points to consider on implications of Coronavirus disease (COVID-19) on methodological aspects of ongoing clinical trials, European Medicines Agency, 2020; Statistical Considerations for Clinical Trials During the COVID-19 Public Health Emergency, US Food and Drug Administration, 2020).

##### **7.9.1 General impact**

Patients who discontinue treatment or stop study participation due to COVID-19 pandemic will be included in patient disposition summaries. Impact on study participation due to COVID-19 pandemic, including visit completion, visit location changes, and study drug dosing and location changes, will be summarized overall on the patient level, and overall and by visit on the event level.

### **7.9.2 Impact on Efficacy**

COVID-19 pandemic impacted efficacy analyses are not carried out for the primary analysis since efficacy assessments (e.g., blood and urine samples for PD, renal ultrasound data) missed due to COVID-19 pandemic are minimal.

However, the final analyses will include COVID-19 pandemic impacted efficacy analyses. The analyses will evaluate the impact of changes in the visit windows for the efficacy assessments made in the protocol amendment 2 (dated 04 May 2020) due to COVID-19 pandemic. Missing efficacy data due to the COVID-19 pandemic will be summarized.

### **7.9.3 Impact on Adverse Events**

Events will be considered during the COVID-19 pandemic if the event occurs on or after first confirmed case of COVID-19 based on the country where the study site is located, described in Appendix 10.7.

An overall summary of AEs mapping to a COVID-19 custom query will include the number and percentage of patients, as well as events and event rates, with any AE, any AE related to study drug, any severe AE, any severe AE related to study drug, any SAE, any SAE related to study drug, any AE leading to treatment discontinuation, any study drug related AE leading to treatment discontinuation, any AE leading to study withdrawal, any study drug related AE leading to study withdrawal, and any deaths. AEs mapping to the COVID-19 custom query will be summarized by high level term and preferred term. Due to the evolving nature of COVID-19-related MedDRA terminology, the COVID-19 custom query will be based on the latest information available at the specified analysis timepoints.

An overall summary of AEs by COVID-19 pandemic phase (i.e., pre-, during, and post) will include the number and percentage of patients, as well as events and event rates, with any AE and any SAE.

### **7.9.4 Other Impacts**

Treatment duration will also be summarized by pandemic phase. Protocol deviation due to COVID-19 pandemic will be summarized and will be indicated in the listing of protocol deviation. Adverse event, study drug exposure, and efficacy listings will include identification of assessments occurring during the pandemic. For patients reporting an AE mapped to the COVID-19 custom query, AEs and prior and concomitant medications will also be presented in separate data listings. Additionally, patient profiles will be provided.

## 8. CHANGES TO PLANNED ANALYSES

Summary of Changes from SAP V2.0	Rationale
Section 7.9 and Appendix 10.7 are added	To add the analysis plan for COVID-19 pandemic impact
Remove the imputation for analyses of 24-Hour Urinary Oxalate Excretion in Section 7.1.2	24-hour urine data are only available from few patients. Imputation planned initially will not lead meaningful results and thus will not be carried out
The definition of PK Analysis Set is changed in Section 4.1	PK Analysis Set is defined using all patients who received “one full” dose rather than “any amount” dose of lumasiran to account patients with adequate study drug exposure
Add additional analysis for changes in plasma oxalate for patients whose baseline POx $\geq$ 1.5xLLOQ in Section 7.3	The LLOQ of the plasma oxalate assay is 5.55 $\mu\text{mol/L}$ . Due to the inability of quantify oxalate level below LLOQ by the assay, additional analysis is added so that meaningful reduction can be quantified
Change clinical laboratory data source and detailed analysis plan in Section 7.7.3	To align with the change in Protocol Amendment 2.0, the data handling for both central and local clinical laboratory data is added
Various editorial changes	To improve clarity

## 9. REFERENCES

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## 10. APPENDICES

### 10.1. Urinary Oxalate calculation

#### 10.1.1. Spot Urinary Oxalate:Creatinine Ratio

Spot urinary UOx:Cr ratio is calculated using single urine void with spot urinary oxalate normalized by creatinine concentration to account for differences in urinary dilution. The unit of spot urinary UOx:Cr ratio is mmol/mmol.

#### 10.1.2. 24-Hour Urinary Oxalate Corrected for BSA

24-hour urinary oxalate (mmol/24hrs/1.73m<sup>2</sup>) corrected for BSA at each visit per patient is calculated as follows:

$$\text{Urinary oxalate concentration (umol/L)/1000 (umol/mmol)*24hr urinary volume (mL)/1000 (mL/L)*24 hours/actual collection hours*1.73/(BSA)}$$

where BSA is calculated using the Mosteller formula:

$$\text{BSA} = \text{square root (mean height (cm)*weight (kg)/3600) at the visit.}$$

Only valid 24-hour urinary oxalate values will be considered for analysis. (note: valid is defined as: creatinine  $\geq 5$  mg/kg, duration of collection 18 to 26 hours, no missing voids and the sample was not collected within 14 days after the most recent dialysis session, if applicable).

### 10.2. Spot Urinary UOx:Cr Ratio Given Natural Growth

#### 10.2.1. Natural decline of spot urinary UOx:Cr ratio in healthy pediatric population

Cross sectional studies, where the spot urinary UOx:Cr ratios were compared across broad age ranges (e.g., 1 - 6 months, 6 – 12 months, 1 – 2 years), showed that in young children over the age range of 0 to about 8 years, the urinary UOx:Cr ratio declines with increasing age. Data from healthy children ages 1 month to 17 years old (N=386) showed a decline in the 95<sup>th</sup> percentile of the spot urinary UOx:Cr ratio.[Matos 1999]. The decline in the UOx:Cr ratio appears to plateau after 7 years of age.[Matos 1999] A similar publication using data from 188 children from birth to 16 years old showed that the mean (and 5 – 95<sup>th</sup> percentiles) of the UOx:Cr ratio declined with increasing age, with no further decline observed in children ages 9 and above.[Leumann 1990]

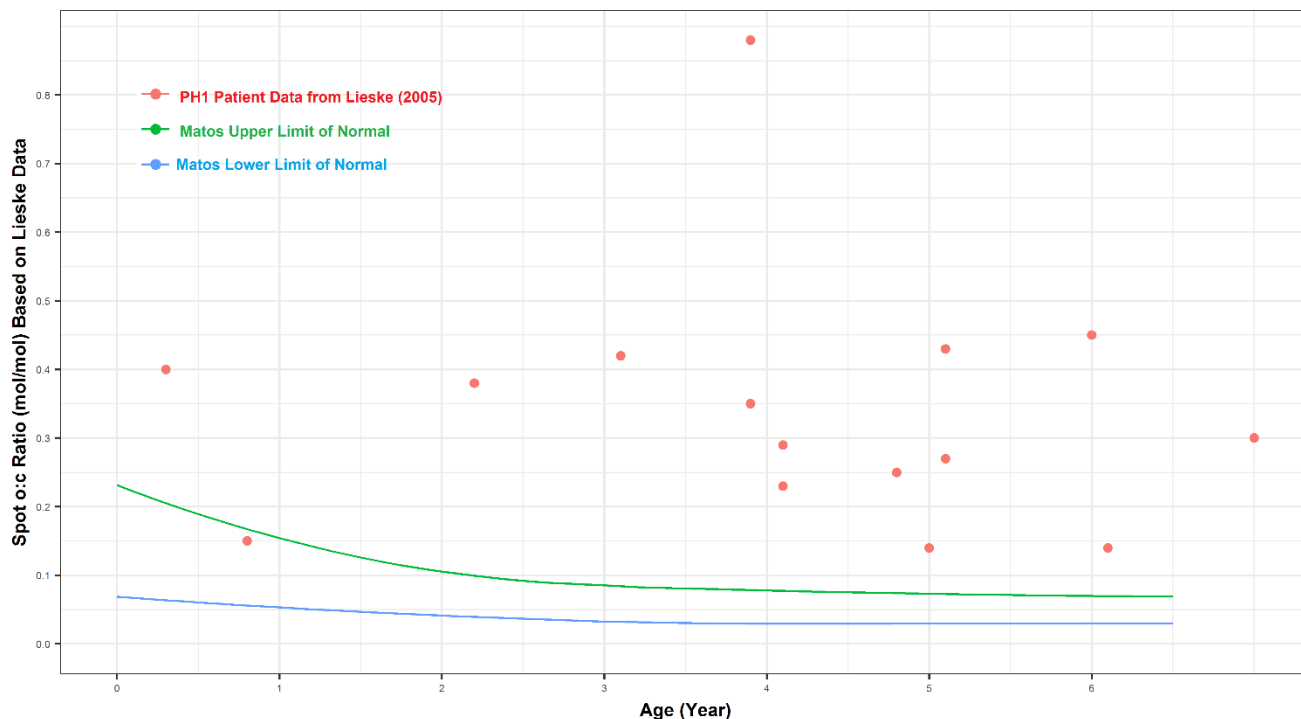
On the other hand, longitudinal data based on 30 healthy infants where intensive random urine samples were collected starting from day 2 of birth with additional samples collected on weeks 2, 8, 16, 24 and 36 show that the mean UOx:Cr ratio stays stable over a period of 8 months (day 2 to week 36).[Morgenstern 1993] These findings are consistent with the results of another cross-sectional study where the mean (5 – 95<sup>th</sup> percentile) of UOx:Cr ratios stayed stable from birth up to 1 year of age.[Reusz 1995] Relative to other cross-sectional studies, Reusz compared UOx:Cr ratios using age categories with narrow age ranges.[Reusz 1995]

### 10.2.2. Effect of Age on Spot urinary UOx:Cr ratio in pediatric PH1 patients

There is limited longitudinal data of spot urinary UOx:Cr ratio in pediatric PH1 patients. Lieske reported spot urinary UOx:Cr ratio from 35 PH1 patients across ages from international registry of primary hyperoxaluria.[Lieske 2005] Of these patients, 15 children are under 7 years old, with 2 less than 2 years old.

To check whether similar decline is observed in PH1 patients as in selected studies involving unaffected children, single values of individual patient spot UOx:Cr (plotted in Figure 1) were read from Lieske's paper using Digitize (R package). Lieske's data are shown in Figure 1 along with the reference curves for Lower limit of normal (LLN) and ULN identified by Matos' publication. All patients except for one patient under 1 year old had spot urinary UOx:Cr ratios above the Matos ULN.

**Figure 1: Spot urinary UOx:Cr ratio (mmol/mmol) in children with PH1 based on [Lieske 2005] Data**



### 10.3. Urinary Excretion Correlation Between Spot and 24-Hour Values

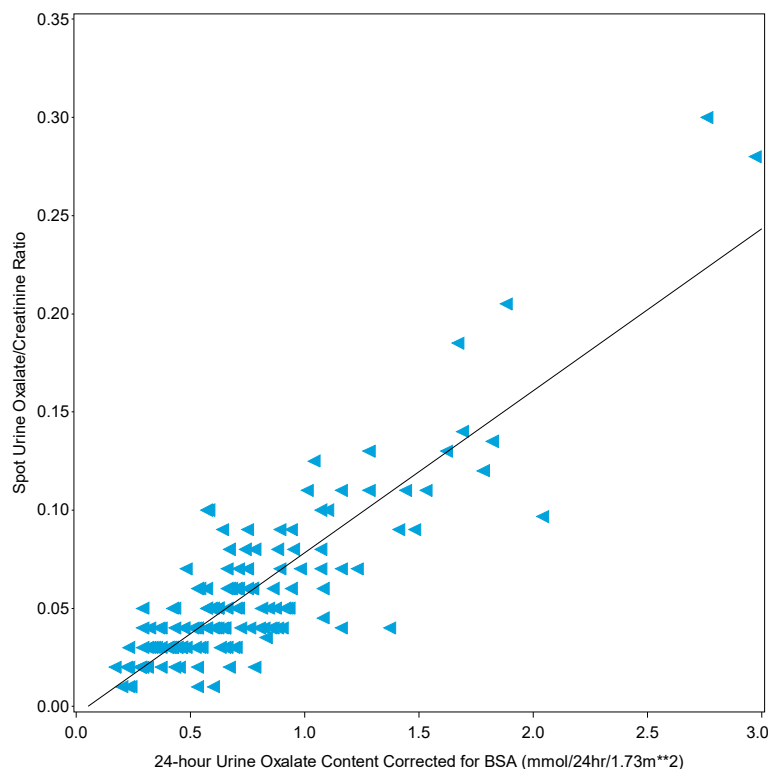
The correlation between percent reduction from baseline in spot urinary UOx:Cr ratio and 24-hour urinary oxalate excretion relative to baseline was evaluated based on ALN-GO1-001 data collected on August 18, 2018. Four patients were excluded due to a laboratory processing issue leading to potentially erroneous results in the UOx:Cr ratios from spot samples. A total of 156 paired samples from 16 patients are included in the analysis. The correlation between time-matched UOx:Cr ratios from spot urinary samples and oxalate excretion from 24-hour urinary collections was strong, with a Pearson correlation coefficient of 0.86 (95% CI: [0.82, 0.90]).

**Table 2: Percent Reduction from Baseline over Time of Spot Urinary Oxalate: Creatinine Ratios Vs 24-Hour Urinary Oxalate Excretion in Study 001B**

Study Day		24-hour Collections Urinary Oxalate Excretion	Spot Urinary Oxalate:Creatinine Ratio
	N	Mean % Reduction (SEM)	Mean % Reduction (SEM)
Day 29	14	49.8 (5.2)	51.9 (6.8)
Day 57	15	63.3 (4.2)	59.3 (5.7)
Day 85	13	63.2 (4.4)	60.9 (6.7)
Day 113	13	70.8 (4.1)	60.0 (6.0)
Day 141	13	70.9 (3.0)	63.9 (6.6)
Day 169	11	63.3 (5.0)	70.2 (4.0)

The observed mean percent reduction and standard error are similar in both urinary sample types (Table 2). The data also show sustained reduction over time in both urinary sample types.

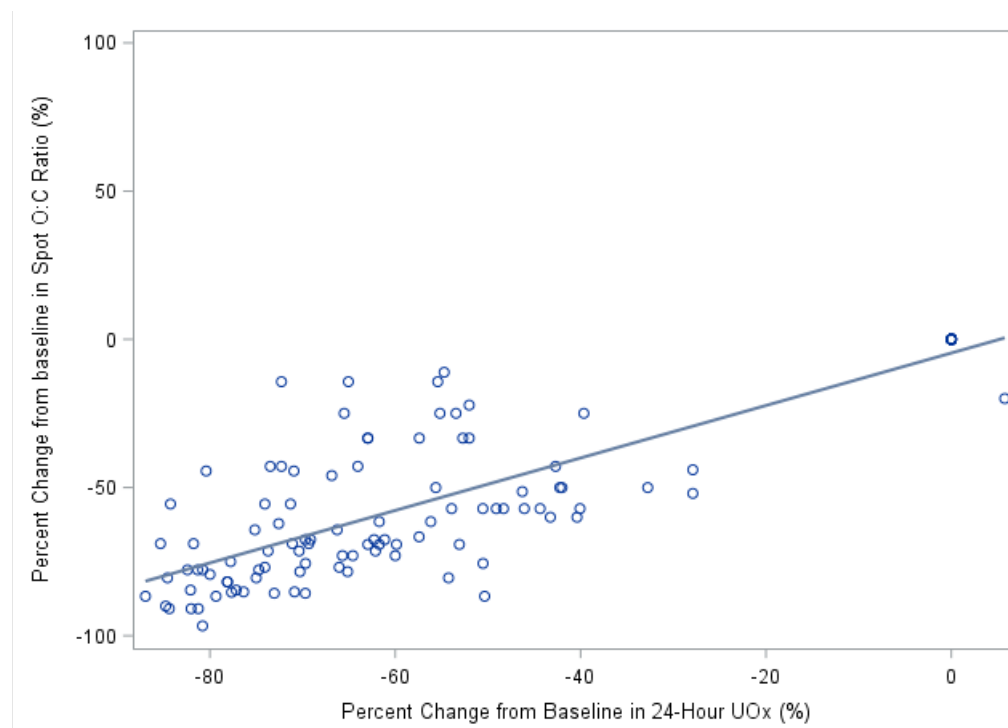
**Figure 2: Scatterplot of Spot Urinary Oxalate:Creatinine Ratios vs 24-hour Urinary Oxalate Excretion in Study 001B**



Time-matched paired samples are plotted from 20 patients enrolled in Part B, ALN-GO1-001 demonstrating a linear relationship. Regression: spot ratio =  $-0.004 + 0.083 \times 24\text{-hour urinary oxalate}$ ;  $R^2=0.774$ .

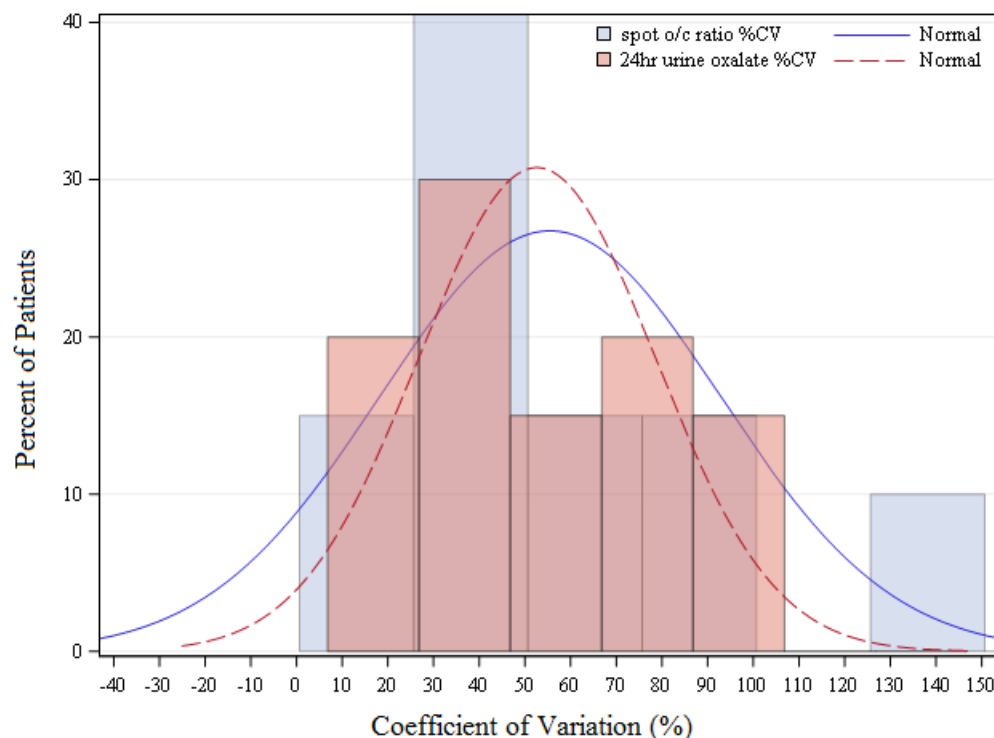


**Figure 3: Scatterplot of Percent Change from Baseline in Spot Urinary Oxalate: Creatinine Ratios vs 24-hour Urinary Oxalate Excretion in Study 001B**



Time-matched paired samples are plotted from 16 patients enrolled in Part B, ALN-GO1-001 demonstrating a linear relationship. Regression: spot ratio =  $-4.625 + 0.885 \times 24\text{-hour urinary oxalate}$ ;  $R^2=0.656$ .

**Figure 4: Percent Coefficient of Variation Comparison of Spot Urinary Oxalate: Creatinine and 24-Hour Urinary Oxalate Excretion**



For each patient, the percent coefficient of variation was calculated using  $(\text{standard deviation} / \text{mean}) * 100\%$ . A histogram of the results is presented for spot urinary (blue) and 24-hour urinary excretion (red). The fitted curves represent the normal distribution for each of the sample types.

**Table 3: Observed Percent Coefficient of Variation from Spot Urinary Oxalate: Creatinine Ratios vs 24-Hour Urinary Oxalate Excretion**

Urinary Sample	N	Mean	95% CL Mean	Std Dev	Min	Max
Spot Ox/Cr ratio CV (%)	20	55.5	[38.06, 72.96]	37.29	13.2	143.5
24hr oxalate CV (%)	20	52.6	[40.47, 64.75]	25.93	16.8	101.2
CV Difference (%)	20	2.9	[-7.07, 12.87]	4.76	-43.1	50.0

The population mean %CV is presented for both sample types. In addition, the mean of the differences between spot urinary samples and 24-hour collections is also presented.

Histograms depicting the coefficients of variation for spot urinary and 24-hour urinary samples are plotted, the mean and standard deviation for these coefficients of variation are similar (Figure 4 and Table 3). The correlation between the spot urinary UOx:Cr ratios and 24-hour urinary oxalate excretion coefficients of variation was strong, with a Pearson correlation coefficient of 0.83 [0.62, 0.93]. The difference between the coefficients of variation was 2.9% [-7.1%, 12.9%]. Therefore, the analysis of the data suggests that the correlation of variation for spot urinary samples is similar to that observed for 24-hour urinary samples. This supports the previous findings that the variations between the urinary sample types are minimal.

## 10.4. Normal Range Analysis

Normalization rate will be based on normal range analysis of PD parameters (i.e. spot urinary UOx:Cr ratio, 24-hour oxalate corrected BSA, etc.) from [REDACTED] healthy volunteer data. ULNs will be defined in the separate normal range report that will be finalized before database lock.

## 10.5. Medical History Adjustment

Baseline disease history within the preceding year is required at enrollment. For infants less than one year old, reported values may need to be adjusted to represent a medically meaningful baseline value. Below is an example of the adjustment criteria to be used in such situations, for the outcome of renal stone events:

1. Patients less than 6 months old, baseline records will be presented in by-patient listings, however will not be used in summary tables
2. Patients ( $\geq 0.5$  and  $< 1$  year old): Annualized rate will be calculated from reported data; i.e. if a patient of 8 months old has had 2 renal stone events prior to screening, his/her renal stone event rate at baseline will be  $2/(8/12) = 3$  events per year
3. Patients ( $\geq 1$  year old): records at screening for the preceding year will be used to calculate baseline annual stone event rate without justification

## 10.6. Category of Vineland Adaptive Behavior Scale

Vineland adaptive behavior composite scores are collected and summarized using Vineland-II. The adaptive level of the composite scores can be categorized from “Low” to “High” in [Table 4](#).

**Table 4: Adaptive Level of Vineland Composite Scores**

Adaptive Level	v-Scale	Standard Score
Low	1 to 9	20 to 70
Moderately Low	10 to 12	71 to 85
Adequate	13 to 17	86 to 114
Moderately High	18 to 20	115 to 129
High	21 to 24	130 to 160

## 10.7. COVID-19 Pandemic Phase Start Dates by Country

Table 5 is as reported by the World Health Organization.

**Table 5: COVID-19 Pandemic Phase Start Dates by Country**

Country	Date of 1 <sup>st</sup> Confirmed Case
France	2020-01-24
Germany	2020-01-28
Israel	2020-02-27
United Kingdom	2020-01-31
United States	2020-01-20