NCT #: NCT04581629

# Statistical Analysis Plan (SAP)

| | |
|---|---|
| **Protocol Title:** | A Phase IIb, Open-label Dose-ranging Study Evaluating the Safety, Tolerability, Pharmacodynamics and Pharmacokinetics, and Efficacy of CLTX-305 in Autosomal Dominant Hypocalcemia Type 1 (ADH1). |
| **Protocol Version No./Date:** | Amendment 10/18-May-2023 |
| **SAP Version No./Date:** | 1.0 / 12-Jan-2022<br>2.0 / 9-Jan-2023<br>3.0 / 26-Jun-2023 |

## 1.0 Approvals

| **Sponsor** | |
|---|---|
| **Sponsor Name:** | Calcilytix Therapeutics, Inc |
| **Author/ Title:** | [redacted] |
| **Signature /Date:** | [redacted] |
| **Reviewer/ Title:** | [redacted] |
| **Signature /Date:** | [redacted] |
| | |
| **Reviewer / Title:** | [redacted] |
| **Signature /Date:** | [redacted] |

# 2.0 Change History

| Version/Date | Change Log |
|---|---|
| 1.0 | Initial version |
| 2.0 | Update for new protocol version 8 to clarify how LTE patients who have some data collected in a different database are summarized. Update to responder definition. |
| 3.0 | Update to:<br>1. Correct an omission from the protocol for the iPTH endpoint. iPTH where data in Period 3 data is collected but it was not shown as an endpoint<br>2. Clarify data handling for subject specific cases for DXA and iPTH<br>3. Add language to describe the estimands for albumin-corrected blood calcium and 24-hour urinary calcium excretion<br>4. Added an objective and endpoints regarding responder status for albumin-corrected blood calcium and 24-hour urinary calcium excretion |

# 3.0 Table of Contents

# 4.0 Purpose of the Analysis Plan

The Statistical Analysis Plan (SAP) pre-specifies the statistical analysis methods to support the completion of the clinical study report (CSR) for Study CLTX-305-201. The planned analyses identified in this SAP may be included in regulatory submissions, and/or future manuscripts. This plan may be updated as a result of an internal data review that will take place prior to database lock. Exploratory analyses, beyond what is defined in this SAP, may be performed to support the clinical development program. Any post-hoc or unplanned analyses that are performed for the CSR but not defined in this SAP, will be documented in the CSR, as will any changes from the planned analyses as stated in the study protocol.

# 5.0 Scope

The SAP outlines the following:

- Study Objectives
- Study Design
- Endpoints to be Analyzed and the Analysis sets
- Applicable Study Definitions
- Statistical Methods

# 6.0 Introduction

This SAP should be read in conjunction with the study protocol and case report form (CRF). Any further changes to the protocol or CRF may necessitate updates to the SAP.

Final approval of the SAP by the Sponsor and ICON statistician will occur prior to database lock.

Samples collected for research (FGF23 and mid-molecule parathyroid hormone [PTH]) will not be reported as part of the CSR.

## 6.1 Changes from Protocol

- Pharmacodynamics (PD) analysis set will not be used as it is expected to be the same as the Safety Analysis Set.
- Pharmacokinetics (PK) analysis set, clarify that all subjects with PK concentrations are included in the analysis.
- Even though non-intensive sampling is performed at Period 3 Week 24; PK parameters will be derived at that visit as there are sufficient planned sample collection times to assess the PK parameters.
- Added post-hoc inferential analysis.
- Even though blood Cyclic Adenosine Monophosphate (cAMP) is listed as a PD parameter in the protocol Tables E to K (see appendix Section 16.1.2); the central laboratory analyzing the samples has confirmed that cAMP is only an urine test.
- The endpoint for iPTH was updated to include Period 3 to correct the omission from the protocol

# 7.0 Study Objectives

This study is a single-site, open-label, dose-ranging study to evaluate the safety, tolerability and efficacy of CLTX-305 (encaleret) to maintain normalized albumin-corrected blood calcium (cCa) in subjects with hypocalcemia due to ADH1. The study consists of 2 cohorts, 3 periods and a Long-Term Extension (LTE) (Period 1, Period 2, Period 3 and Long-Term Extension [LTE]).

The **primary objectives** of this study are:

- Periods 1 and 2: Evaluate the safety and tolerability of single and multiple doses of CLTX-305 (encaleret) in subjects with ADH1.
- Period 3: Evaluate the safety, tolerability, and efficacy of CLTX-305 (encaleret) in subjects with ADH1 for 24 weeks.
- LTE: Evaluate the long-term (25 months or about 2 years) safety and tolerability of CLTX-305 (encaleret).

The **secondary objectives** of this study are:

Periods 1 and 2:

- Evaluate the effect of CLTX-305 (encaleret) to increase serum intact PTH (iPTH) levels after both single and multiple doses across a dose range in subjects with ADH1.

Periods 1, 2, and 3:

- Evaluate the PD effects of CLTX-305 on blood calcium concentrations.
- Evaluate the PD effects of CLTX-305 (encaleret) on associated measures of calcium. homeostasis including 1,25-$(OH)_2$ Vitamin D levels and urinary calcium excretion.
- Evaluate the PD effects of CLTX-305 (encaleret) on bone turnover markers including C-telopeptide (CTx) and procollagen type 1 N-propeptide (P1NP).
- Evaluate the PK of both single and multiple ascending doses of CLTX-305 (encaleret) in subjects with ADH1.

LTE:

- Obtain long-term data on efficacy to show durability of CLTX-305 (encaleret) on blood calcium concentrations.
- Evaluate the long-term PD effects of CLTX-305 (encaleret) on associated measures of mineral homeostasis including iPTH, 1,25-$(OH)_2$ Vitamin D levels and urinary calcium excretion.
- Evaluate the long-term effects of CLTX-305 on bone turnover markers including CTx and P1NP.

# 8.0 Study Design

This is a single-site, open-label, dose-ranging study and dose maintenance to evaluate the safety, tolerability and efficacy of CLTX-305 (encaleret) to maintain normalized cCa in subjects with hypocalcemia due to ADH1. The study consists of two cohorts and two inpatient periods of dose initiation, escalation and steady-state adjustment (Periods 1 and 2), an outpatient dose stabilization and maintenance period including National Institute of Health (NIH) clinical visits (Period 3), and a LTE period. See Figure 1.

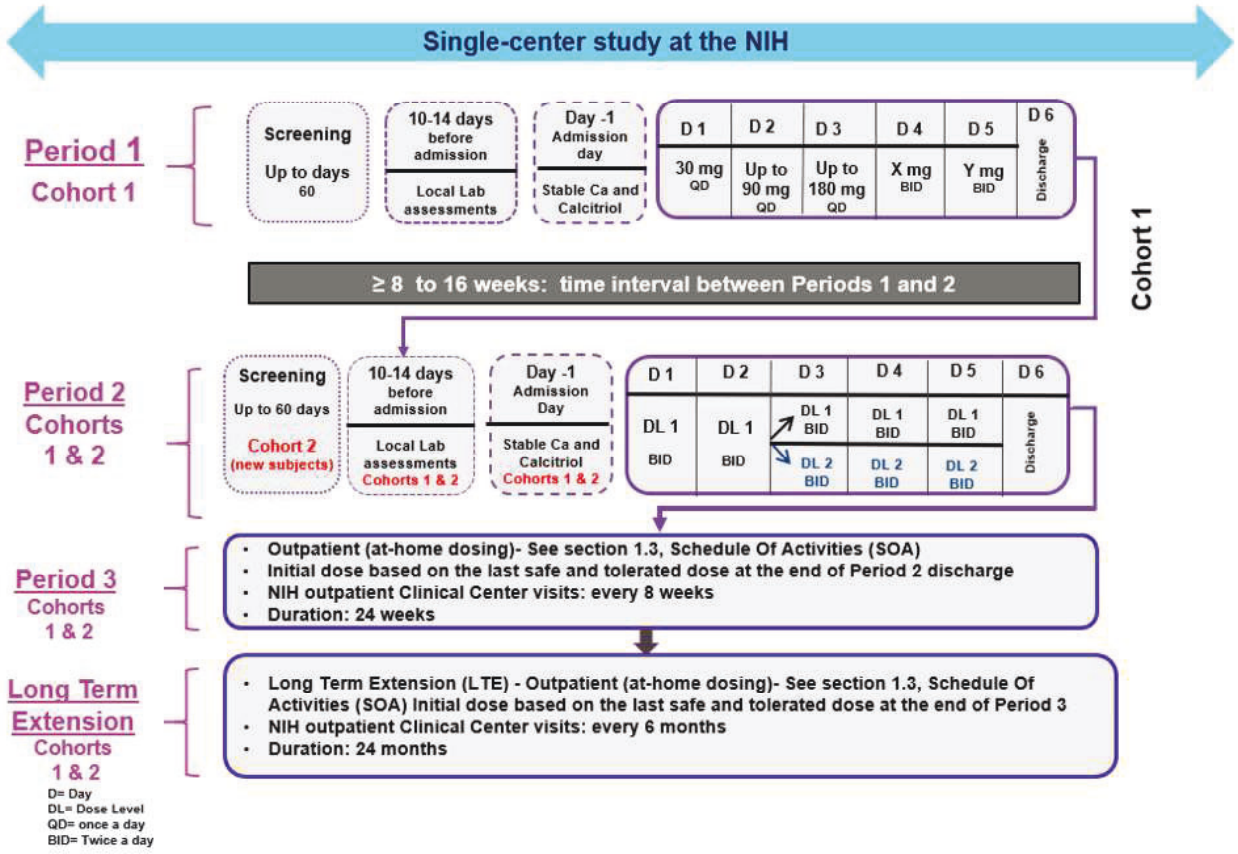
The LTE is designed to obtain additional safety data and evaluate durability of efficacy for CLTX-305 (encaleret). The LTE also allows for continued treatments of enrolled subjects with CLTX-305 (encaleret).

Two main cohorts are planned:

- **Cohort 1**: up to 8 subjects (minimum of 5) will initially be enrolled into Period 1 (inpatient), after which they may return to participate in Period 2 (inpatient, after at least an 8-week interval). Subjects who complete Period 2 will be eligible to enter Period 3 (outpatient) for up to 24 weeks of dosing with CLTX-305 (encaleret).
- **Cohort 2**: up to 10 additional subjects (minimum of 5) will enroll directly into Period 2 (inpatient). The initiation of Cohort 2 will be based on evaluation of accumulated data from Cohort 1. Cohort 2 subjects who complete Period 2 will be eligible to enter the outpatient Period 3 (outpatient) to receive up to 24 weeks of CLTX-305 (encaleret).

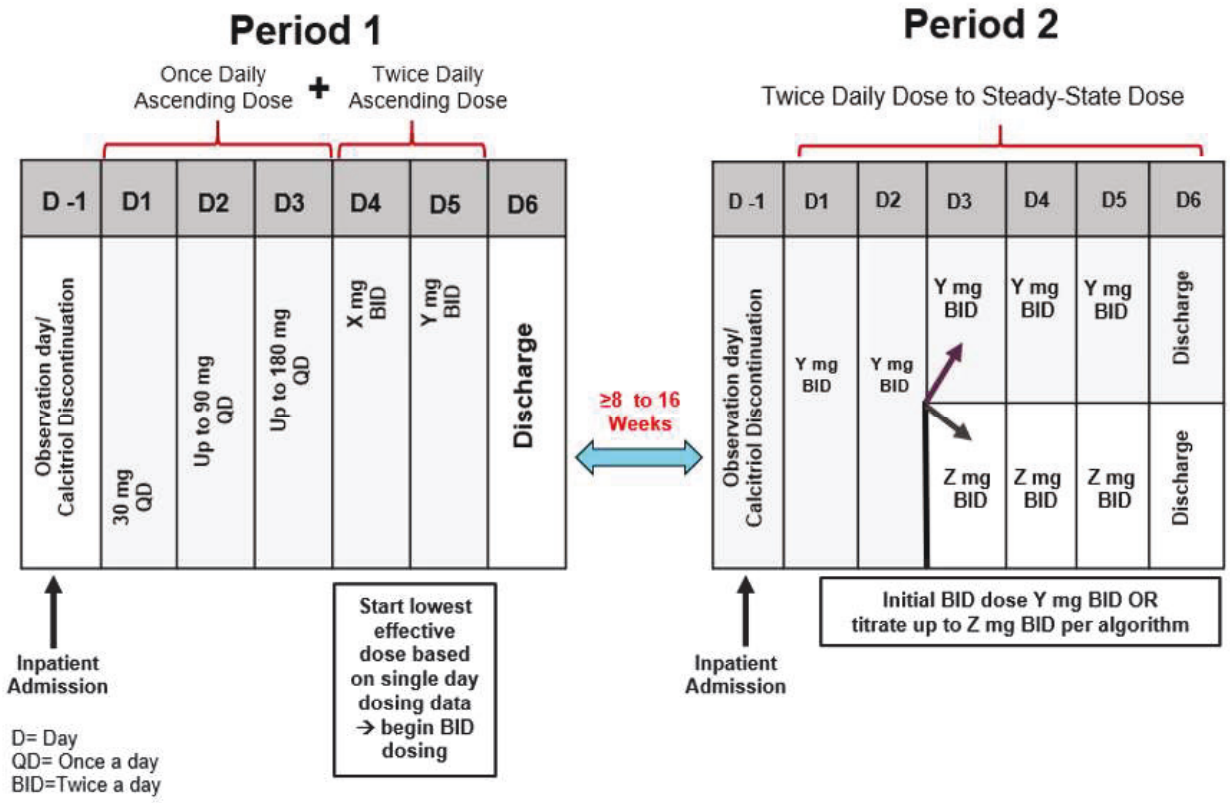
Subjects from both cohorts who complete Period 2 will be eligible to enter Period 3. Outpatient dosing with CLTX-305 (encaleret) will be determined from review of individual dose-response data from Periods 1 and/or 2 based on identifying well-tolerated twice daily (BID) doses with preliminary evidence for efficacy. During the initial outpatient period (Period 3), subjects will undergo additional individualized dose titration as necessary in response to safety data and the results of key efficacy measures. Subjects who complete Period 3 (up to Week 24) and who elect to continue in the LTE will receive CLTX-305 (encaleret) for an additional period of up to 25 months (approximately 2 years) or until they have access to CLTX-305 (encaleret) via prescription, whichever occurs first.

**Figure 1: Study Overview Schema**




Details for Periods 1 and 2 are illustrated below in Figure 2.

**Figure 2: Detailed Schematic for Period 1 and 2 (Single and Multiple Ascending Dose Testing)**




**Period 1** is an inpatient stay that consists of 5 dosing days during which subjects will undergo a once daily (QD) dose escalation for 3 days followed by 2 days of BID dosing at an individualized test dose of CLTX-305 (encaleret). Note: Tau will be 9 hours for the am dose and 15 hours for the pm dose.

**Period 2** is an inpatient stay that consists of 5 dosing days during which subjects will receive BID doses of CLTX-305 based on individual responses from Period 1 (for subjects who complete Period 1) or review of aggregate data from Period 1 (for subjects in Cohort 2 entering Period 2 without prior exposure to CLTX-305 [encaleret]). The initial dose, dose-level 1 (DL1) will be administered for 2 days (48 hours) with cCa monitoring. Participants will undergo encaleret dose up- or down-titration depending on cCa levels, with a potential increase in dose if cCa remains below the lower limit of normal and potential decrease in dose if cCa is greater than or equal to the upper limit of normal. A final test day (Day 5) will include frequent blood and urine sampling to collect 24-hour PK/PD profiles.

Dosing guidance for Period 1 and Period 2 are shown in Figure 3 and Figure 4.

**Figure 3: Dosing Guidance for Period 1**

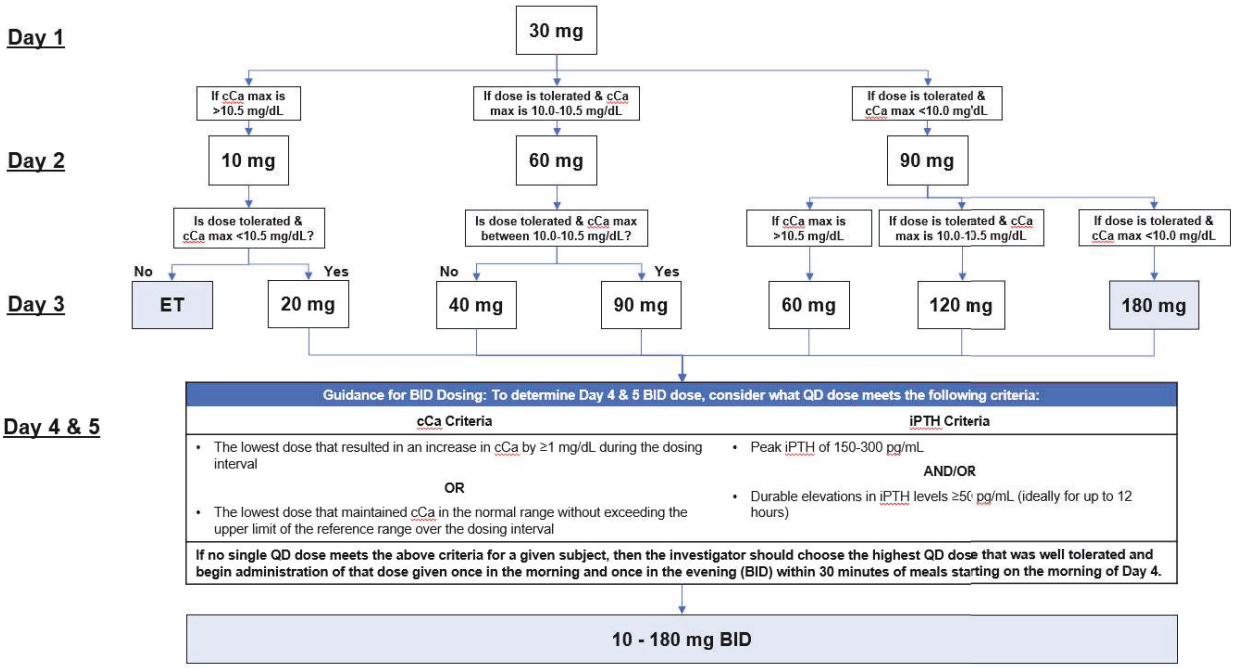
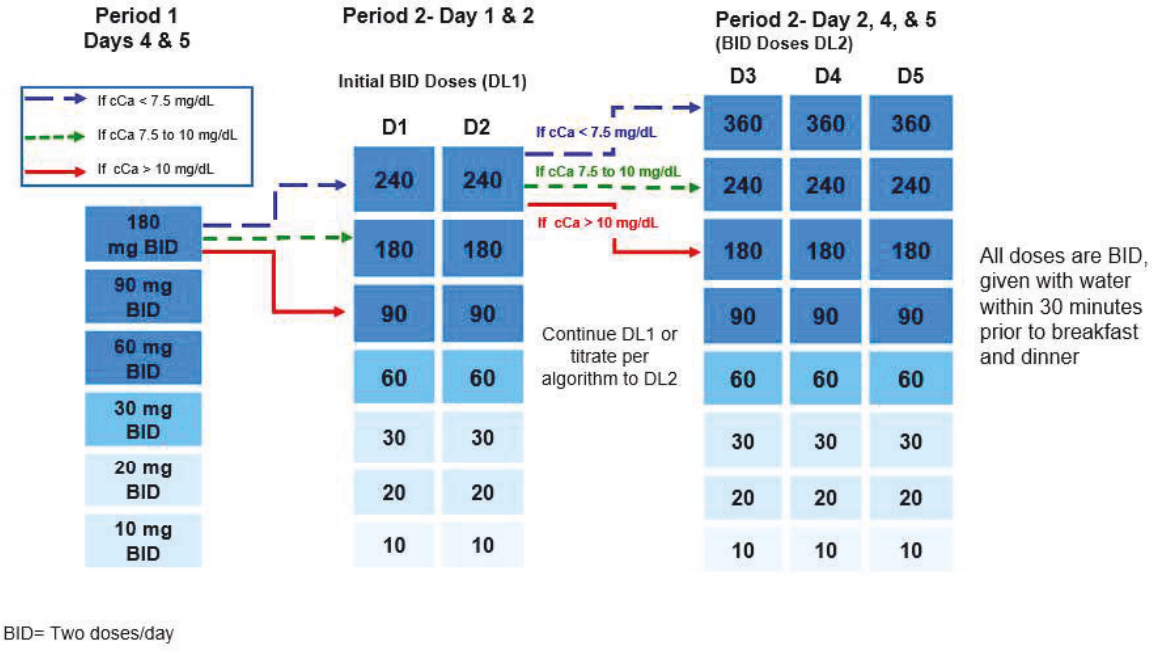



**Figure 4: Dosing Guidance for Period 2**

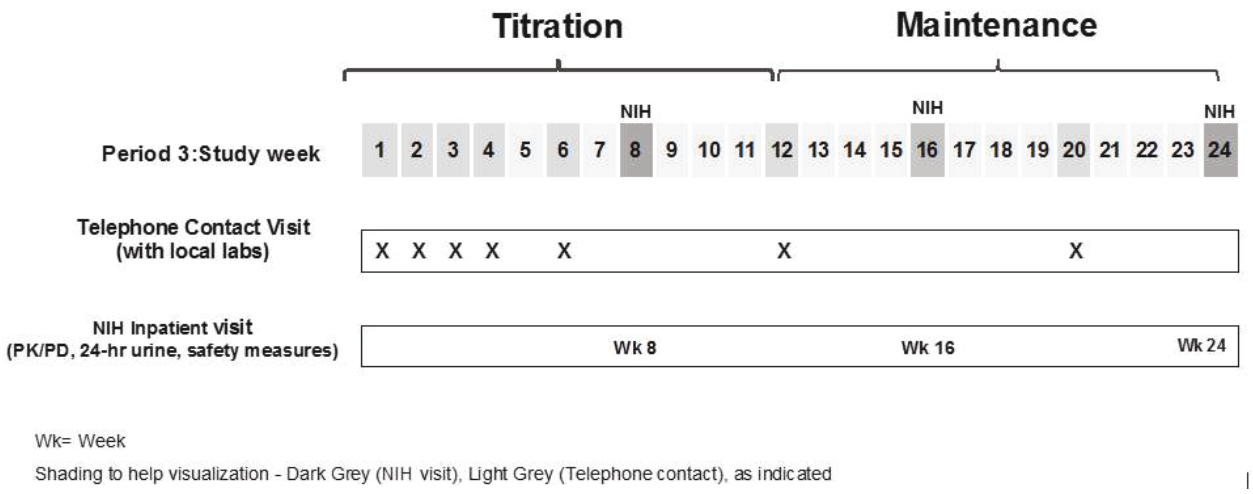



BID= Two doses/day

**Period 3** is an outpatient dosing period that consists of administration of CLTX-305 (encaleret) for up to 24 weeks. Subjects completing Period 2 will continue to self-administer CLTX-305 (encaleret) at an initial BID dose based on their tolerance and response to BID dosing during Period 2. Initial titration will be based on each subject's need for symptom control and ongoing monitoring of efficacy endpoints (primarily cCa and urine calcium excretion) with a goal to optimize cCa in the normal range

while minimizing hypercalciuria. Subjects will not take calcitriol but may take calcium supplementation as needed to ensure a minimum daily intake of 1000 mg. Participants may receive oral calcium supplements as directed by the investigative site if they are not able to achieve a minimum of 1000 mg per day of dietary calcium intake. Guidance on overall outpatient titration is discussed in Protocol Section 6.1.5 and in Protocol Section 8.2.3. The need for additional dose adjustments may depend on factors potentially related to the time course of changes in parathyroid gland function, intestinal calcium absorption, bone resorption and kidney function, all of which will be monitored. A schematic of Period 3 is shown in Figure 5.

**Figure 5: Detailed Schematic for Period 3**

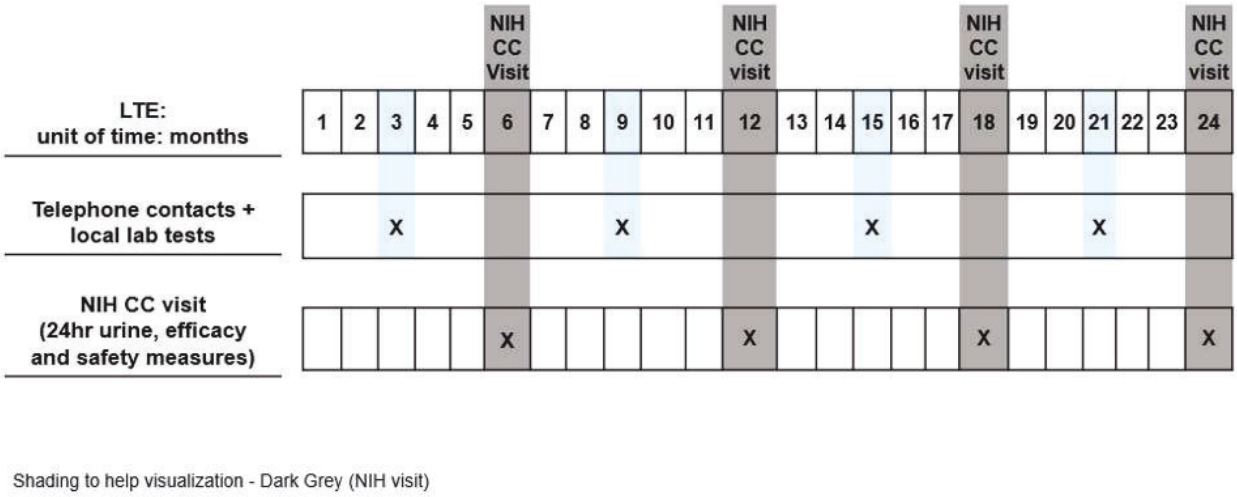



Wk= Week

Shading to help visualization - Dark Grey (NIH visit), Light Grey (Telephone contact), as indicated

**LTE** outpatient dosing with CLTX-305 (encaleret) will continue for up to 25 months (about 104 weeks) after completion of Week 24 visit in Period 3 or until they transition to the CLTX-305-302/CALIBRATE Phase 3 LTE, whichever occurs first. Subjects completing Period 3 will continue to receive clinical supplies of CLTX-305 at the same BID dose based on their tolerance and response to BID dosing during CLTX-305 dosing in Period 3. Subjects will not take calcitriol but may take calcium supplementations as needed to achieve a minimum daily intake of 1000 mg. A final follow-up safety visit in the LTE will occur 30 ± 7 days after the last dose of CLTX-305 (encaleret). A schematic of LTE is shown in Figure 6.

**Figure 6: Detailed Schematic for LTE**




## 8.1 Sample Size Considerations

The sample size of up to 8 subjects in Cohort 1 and up to 10 additional subjects in Cohort 2, is consistent with conventional first-in-human single and multiple ascending studies that generally enroll between 8-10 subjects per dose arm with escalation being based on safety and tolerability.

The sample size for the current study is not based on statistical testing of a formal powered hypothesis. The proposed clinical trial represents a re-purposing of CLTX-305, leveraging extensive exposure and safety data from the prior osteoporosis program, to conduct a modified single and multiple-ascending dose study in a new target population of subjects with ADH1. However, a recent publication of a different experimental calcilytic agent, NPSP795 (Roberts 2019), reported preliminary evidence for a dose-response on iPTH in subjects with ADH1 with a sample size of 5 subjects. The authors speculated that despite testing doses known to raise iPTH robustly in non-ADH1 subjects, larger doses would have been required to stimulate iPTH in ADH1 subjects sufficiently to also see elevations in cCa. The current protocol is designed to explore a broad potential dose range for safety and tolerability; however, the proposed sample size of approximately 16 subjects may be sufficient to confirm a definitive proof-of-concept on either iPTH and/or cCa if effective doses are achieved.

## 8.2 Randomization

This is an open-label non-randomized study and does not include treatment assignments.

# 9.0 Study Endpoints, Variables and Covariates

| Objectives | Endpoints |
|---|---|
| **Primary** | |
| Periods 1, 2, and 3:<br>• Evaluate the safety and tolerability of single and multiple doses of CLTX-305 (encaleret) in subjects with ADH1. | Periods 1, 2 and 3:<br>• Number of subjects with adverse events (AEs), serious AEs<br>• Absolute values and change from baseline in clinical safety (laboratory tests), vital signs, and electrocardiograms (ECGs).<br>• AEs and serious AEs (SAEs) – frequency count and percentages of subjects;<br>• Clinical safety laboratory tests, vital signs and ECGs – absolute levels and change from baseline. |
| Period 3:<br>• Evaluate the safety, tolerability and efficacy of CLTX-35 (encaleret) in subjects with ADH1 after 24 weeks. | Period 3:<br>• cCa after treatment with CLTX-305 for 24 weeks - absolute levels and change from baseline.<br>• Urinary calcium clearance (fractional excretion) after treatment with CLTX-305 for 24 weeks – absolute levels and change from baseline;<br>• Urinary calcium clearance (24-hour total excretion) after treatment with CLTX-305 for 24 weeks - absolute levels and change from baseline. |
| LTE:<br>• Evaluate the longer-term safety and tolerability of CLTX-305 (encaleret) in subjects with ADH1 from Period 3 for approximately 25 months (approximately 2 years). | LTE:<br>• AEs and SAEs – frequency count and percentages of subjects;<br>• Clinical safety laboratory tests and vital signs for approximately 25 months (approximately 2 years) – absolute levels and change from baseline. |
| **Secondary** | |
| Periods 1 and 2:<br>• Evaluate the effect of CLTX-305 (encaleret) to increase serum iPTH levels after both single and multiple doses across a dose range in subjects with ADH1. | Periods 1, 2, and 3:<br>• Absolute values and change from baseline in iPTH blood concentrations profiles (24-hours) over time after single and multiple doses of CLTX-305 (encaleret). |

| Objectives | Endpoints |
|---|---|
| **Secondary** | |
| Periods 1, 2, and 3:<br>• Evaluate the PD effects of CLTX-305 (encaleret) on blood calcium concentrations.<br>• Evaluate the PD effects of CLTX-305 (encaleret) on associated measures of calcium homeostasis including 1,25-$(OH)_2$ vitamin D levels and urinary calcium excretion.<br>• Evaluate the PD effects of CLTX-305 (encaleret) on bone turnover markers including CTx and P1NP. | Periods 1 and 2:<br>PD endpoints measured over time up to 24 weeks (final visit):<br>• Blood calcium - absolute levels and change from baseline in cCa;<br>• Urinary calcium clearance (fractional excretion) – absolute levels and change from baseline;<br>• Urinary calcium clearance (24-hour total excretion) - absolute levels and change from baseline.<br>Periods 1,2, and 3:<br>• Renal function (eGFR) - absolute levels and change from baseline;<br>• Serum levels of 1,25-(OH)2 Vitamin D -Absolute levels and change from baseline<br>• Blood samples for magnesium, phosphate, creatinine, cAMP - absolute levels and change from baseline;<br>• Time-intervals for urine samples for pH, creatinine and cAMP (time-intervals) - absolute levels and change from baseline;<br>• 24-hours urine samples for pH, magnesium excretion, potassium excretion, creatinine, phosphate excretion, sodium excretion and citrate – absolute levels and change form baseline;<br>• Fractional excretion for magnesium and tubular reabsorption of phosphate - absolute levels and change from baseline;<br>• Bone resorption markers (blood collagen cross-linked CTx) - absolute levels and change from baseline;<br>• Bone formation markers (blood P1NP) - absolute levels and change from baseline. |

| Objectives | Endpoints |
|---|---|
| **Secondary** | |
| • Evaluate the PK of both single and multiple ascending doses of CLTX-305 in subjects with ADH1 where intensive PK sampling was done.<br><br>Note: Intensive PK is planned during Period 1 (Day1, Day 2, Day 3 and Day 5) and Period 2 (Day 1 if CLTX dose is > 180 mg BID and Day 5 regardless of dose received). Even though non-intensive sampling is performed at Period 3 Week 24; PK parameters will be derived at that visit. | • PK parameters: maximum plasma concentration ($C_{max}$), time to maximum plasma concentration ($t_{max}$), apparent terminal half-life ($t_{½}$), area under the concentration-time curve (AUC) from time 0 to the last measurable time point ($AUC_{0-t}$), AUC from time 0 to 24 hours ($AUC_{0-24}$), AUC extrapolated to infinity ($AUC_{0-inf}$) following single-doses.<br>• Determination of the steady state PK parameters: $C_{max}$, trough concentration ($C_{trough}$), and AUC over the dosing interval $AUC_{0-tau.}$ |

| Objectives | Endpoints |
|---|---|
| **Secondary** | |
| LTE:<br>• Obtain long-term data on efficacy of CLTX-305 (encaleret) on blood calcium concentrations.<br>• Evaluate the long-term PD effects of CLTX-305 (encaleret) on associated measures of calcium homeostasis including, iPTH, 1.25-$(OH)_2$ Vitamin D levels and urinary calcium excretion.<br>• Evaluate the long-term PD effects of CLTX-305 (encaleret) on bone turnover markers including CTx and P1NP. | LTE:<br>Endpoints measured over time up to 24 months (final visit):<br>• Blood calcium - absolute levels and change from baseline in cCa;<br>• Urinary calcium clearance (fractional excretion) – absolute levels and change from baseline;<br>• Urinary calcium clearance (24-hour total excretion) - absolute levels and change from baseline;<br>• Blood levels of iPTH - absolute levels and change from baseline;<br>• Renal function (eGFR) - absolute levels and change from baseline;<br>• Serum levels of 1,25-$(OH)_2$ Vitamin D -Absolute levels and change from baseline<br>• Blood samples for magnesium, phosphate, creatinine - absolute levels and change from baseline;<br>• Time-intervals for urine samples for pH, creatinine and cAMP (time-intervals) - absolute levels and change from baseline;<br>• 24-hours urine samples for pH, magnesium excretion, potassium excretion, creatinine, phosphate excretion, sodium excretion and citrate – absolute levels and change form baseline;<br>• Fractional excretion for magnesium and tubular reabsorption of phosphate - absolute levels;<br>• Bone resorption markers (blood collagen cross-linked CTx) - absolute levels and change from baseline;<br>• Bone formation markers (blood P1NP) - absolute levels and change from baseline. |

# 10.0 Conventions and Derivations

## 10.1 Baseline

Baseline is defined as the last non-missing value before the first dose of study drug is administered to each subject. For subjects that are enrolled in either Period 1 and/or Period 2, baseline is the last non-missing value before the first dose of study drug in that period. For cohort 1 subjects, if there are no assessment performed prior to the first dose of Period 2, the baseline for Period 1 will be used as the baseline for Period 2. For Period 3 and LTE baseline is the same as the baseline for Period 2.

**Laboratory parameters**:

For the timed-intervals urine parameters, the baseline will be defined as follows:

- For the parameters collected as time-intervals (i.e. the results from samples collected at 15 minutes pre-dose, 0 to 4 hours, 4 to 8 hours, 8 to 13 hours, 13 to 17 hours and 17 to 24 hours), the baseline for Period 1 will be the 15 minutes pre-dose urine sample collected at Period 1 Day 1; the baseline for Period 2, Period 3 and LTE will be 15 minutes pre-dose urine sample collected at Period 2 Day 1.

- For the parameters collected on 0-24 hours, screening assessments will be considered as baseline (regardless of the laboratory analyzing the samples, local laboratories or NIH laboratory).

For chemistry parameters (serial sampling), the 15 minutes pre-dose samples on Day 1 from Period 1 will be the baseline for Period 1 and the 15 minutes pre-dose samples on Day 1 of Period 2 will be the baseline for Period 2. Screening values will be used for the count and percentage of subjects with a >50% decrease in 24-hr urine calcium excretion.

## 10.2 Change from Baseline

Change from baseline will be calculated as (value at post-baseline time point – value at baseline). Change from baseline will be calculated for subjects with both a baseline and post-baseline value as applicable. If a baseline or post-baseline value has not been recorded for a parameter, then change from baseline will not be calculated for that parameter. Subjects with missing change from baseline values will be excluded from descriptive statistics.

## 10.3 Derivations and Imputations

For laboratory data and for parameters that are continuous in nature but are reported as less than the lower limit of quantitation (LLOQ), lower than the limit of detection (LLOD) or above the upper limit of quantitation (ULOQ), the results will be imputed as follows:

- Results reported as '<xx' will be imputed as xx/2 (half of the numeric part).
- Results reported as '>xx' will be imputed as xx (numeric part).
- Results reported as '<LLOD' will be imputed as 0.

The following laboratory parameters will be derived:

- **Estimated Glomerular Filtration Rate (eGFR)** using CKD-EPI formula (mL/min/1.73m$^2$) = 141 x min (SCr/K, 1)$^{\alpha}$ x max(SCR/K, 1)$^{-1.209}$ x 0.993$^{Age}$ x 1.018 [if female] x 1.159 [if Black].

  Where

  SCr is the serum creatinine (mg/dL),

  K = 0.7 for female and 0.9 for males,

  α is -0.329 for female and -0.411 for males,

  Age (years) is the age at the sample collection date.
- **cCa (mg/dL)** = (0.8 * (4.0 - Albumin [g/dL])) + Serum Calcium (mg/dL).
- **Fractional Calcium Excretion** at pre-dose, 0-4h, 4-8h, 8-13h, 13-17h, 17-24h intervals will be derived as follow.

$$(\text{Fractional Calcium Excretion})_{a-b} = \frac{(\text{Urine Calcium})_{a-b} \times (\text{Serum Creatinine})_b}{(\text{Serum Calcium})_b \times (\text{Urine Creatinine})_{a-b}}$$

- **Fractional Magnesium Excretion** at pre-dose, 0-4h, 4-8h, 8-13h, 13-17h, 17-24h intervals will be derived as follow.

$$(\text{Fractional Magnesium Excretion})_{a-b} = \frac{[(\text{Urine Magnesium})_{a-b} \times (\text{Serum Creatinine})_b]}{0.7 \times [(\text{Serum Magnesium})_b \times (\text{Urine Creatinine})_{a-b}]} \times 100$$

- **Tubular Reabsorption of Phosphorus** at pre-dose, 0-4h, 4-8h, 8-13h, 13-17h, 17-24h intervals will be derived as follow.

$$(\text{Tubular Reabsorption of Phosphorus})_{a-b} = \left[1 - \frac{(\text{Urine Phosphorus})_{a-b} \times (\text{Serum Creatinine})_{b}}{(\text{Serum Phosphorus})_{b} \times (\text{Urine Creatinine})_{a-b}}\right] \times 100$$

Where

*a-b* is the interval considered (15 min pre-dose, 0-4h, 4-8h, 8-13h, 13-17h, 17-24h).

*b* is the end of the interval.

For Period 1 Day 5 and Period 2 Day 5, the 4, 8 and 15 hours post-evening dose blood sample results (i.e. serum results) will be used respectively, instead of the 13, 17 and 24 hours post-dose blood samples results.

For Period 1 Day 1 to Day 4 and Period 2 Day 1 to Day 4 and time-interval 17-24 hours, the 15 minutes pre-dose results from the following visit will be used (for example 15 minutes pre-dose Day 2 will be used to derive the 17-24 hours fractional excretion).

## 10.4 Study Day 1

**For Period 1 assessments**:

The day of the first dose of study drug in Period 1 is referred to as "Study Day 1".

**For Period 2, Period 3 and LTE assessments**:

The day of the first dose of study drug in Period 2 is referred to as "Study Day 1".

## 10.5 Study Day

For the considered period, Study Day is defined as the number of days from Study Day 1. If the day of interest is prior to Study Day 1, then Study Day is calculated as (Date of Interest – Date of Study Day 1). Therefore, the day prior to Study Day 1 is -1. If the day of interest is on or after Study Day 1, then Study Day is calculated as (Date of Interest – Date of Study Day 1) + 1.

## 10.6 Study / Treatment Completion

| Cohort | Analysis Period | Start of the Period | End of the Period |
|---|---|---|---|
| 1 | 1 | Earliest recorded medication | If the subject continued in Period 2: last dose in Period 1.<br>If the subject did not continue in Period 2: all medications are assigned to Period 1. |
| | 2 | Last dose date in Period 1 + 1 day | If the subject continued in Period 3: last dose in Period 2.<br>If the subject did not continue in Period 3: Date of completion/discontinuation of the study. |

| Cohort | Analysis Period | Start of the Period | End of the Period |
|---|---|---|---|
| | 3 | Last dose date in Period 2 + 1 day | If the subject continued in LTE: last dose in Period 3<br>If the subject did not continue in LTE: Date of completion/discontinuation of the study. |
| | LTE | Lase dose date in Period 3 + 1 day | Date of completion/discontinuation of the study. |
| 2 | 2 | Earliest recorded medication | If the subject continued in Period 3: last dose in Period 2.<br>If the subject did not continue in Period 3: Date of completion/discontinuation of the study. |
| | 3 | Last dose date in Period 2 + 1 day | If the subject continued in LTE: last dose in Period 3<br>If the subject did not continue in LTE: Date of completion/discontinuation of the study. |
| | LTE | Lase dose date in Period 3 + 1 day | Date of completion/discontinuation of the study. |

A subject is considered to have completed the study if the answer is "YES" to the question "Did the subject complete the Study?" on the End of Study eCRF page. A subject is considered to have completed a period if the answer is "YES" to the question "Did the subject complete the Treatment Phase?" on the End of Treatment eCRF page for that period.

## 10.7 Prior and Concomitant Medications

As there will be no interruption of study drug treatment between Period 2, Period 3 and LTE, those 3 periods will be considered only one period and referred to Period 2 in the below definitions.

Prior Medications – Period 1

For subjects in cohort 1, prior medications or supplements will be any medications or supplements with a start date before the start of treatment on Study Day 1.

Prior Medications – Period 2

For subjects in cohort 1, prior medications or supplements will be any medications or supplements with a start date after the end of treatment from Period 1 and before the start of treatment in Period 2.

For subjects in cohort 2, prior medications or supplements will be any medications or supplements with a start date before the start of treatment on Study Day 1.

Concomitant Medications – Period 1

Concomitant medications are defined as any medications ongoing at the Period 1 start of treatment (i.e. medications which started before the Period 1 start of treatment and are recorded as ongoing or

with a stop date after the Period 1 start of treatment) or with a start date on or after the Period 1 start of treatment, but on or prior to the Period 1 end of study treatment + 30 days

Concomitant Medications – Period 2

Concomitant medications are defined as any medications ongoing at the Period 2 start of treatment (i.e. medications which started before the Period 2 start of treatment and are recorded as ongoing or with a stop date after the Period 2 start of treatment) or with a start date on or after the Period 2 start of treatment, but on or prior to end of study treatment + 30 days.

Each medication is mapped to a Period based on their start date and as per the rules defined in Table 1. If the start date is partial, determination of the Period will be based on the imputed start date (see Section 10.11 for details). If the date is entirely missing, the medication will be assigned to Period 1 for subjects in Cohort 1 and to Period 2 for subjects in Cohort 2.

**Table 1: Mapping Rules of Medications to Each Period**

| Period | Prior | Concomitant |
|---|---|---|
| Period 1 | Medications with start date/time prior to Period 1 Day 1 | Medications with start date/time on or after Period 1 Day 1 and prior to the Period 2 Day 1 AM dose<br>Medications with start date/time prior to Period 1 Day 1 and end date/time on or after Period 1 Day 1/ongoing |
| Period 2/3 | Medications with start date/time prior to the Period 2 Day 1 AM dose | Medications with start date/time on or after the Period 2 Day 1 AM dose and prior to the first dose in the LTE. If the participant does not continue into the LTE, then all medications with a start date on or after the Period 2 Day 1 AM dose will be mapped to Period 2/3.<br>Medications with start date/time prior to the Period 2 Day 1 AM dose and end date/time on or after the Period 2 Day 1 AM dose/ongoing |
| LTE | Medications with start date/time prior to first dose in the LTE | Medications with start date/time on or after the first dose in the LTE.<br>Medications with start date/time prior to the first dose in the LTE and end date/time on or after the first dose in the LTE/ongoing |

## 10.8 Treatment-emergent Adverse Event (TEAE)

For subjects in Cohort 1, AEs will be considered treatment-emergent to Period 1 if they have missing or partial start dates for which it cannot be determined whether the AE started before or after the Period 1 or Period 2 first dose of study medication.

For subjects in Cohort 2, AEs will be considered treatment-emergent to Period 2 if they have missing or partial start dates for which it cannot be determined whether the AE started before or after the Period 2 first dose of study medication.

As there will be no interruption of study drug treatment between Period 2, Period 3 and LTE, those 3 periods will be considered only one period and referred to Period 2 in the below definitions.

Period 1

Treatment-emergent AEs (TEAEs) are defined as any AE(s) or SAE(s), regardless of relationship to study drug, that have an onset or worsening in severity on or after the first dose of study drug until 30 days after Period 1 last dose of treatment date.

Period 2

TEAEs are defined as any AE(s) or SAE(s), regardless of relationship to study drug, that have an onset or worsening in severity on or after the Period 2 first dose of study drug until 30 days after the last dose of treatment in the study.

Each AE is mapped to a Period based on their start date, using the rules defined in Table 2. If the start date is partial, determination of the Period will based on the imputed start date (see Section 10.11 for details). If the date is entirely missing, the AE will be assigned to Period 1 for subjects in Cohort 1 and to Period 2 for subjects in Cohort 2.

**Table 2: Mapping Rules of AE to Each Period**

| Cohort | Analysis Period | Start of the Period | End of the Period |
|---|---|---|---|
| 1 | 1* | Date of ICF | If the subject continued in Period 2: last dose in Period 1 + 30 days.<br>If the subject did not continue in Period 2: all AEs are assigned to Period 1. |
| | 2 | Date of Outpatient Admission Visit | If the subject continued in Period 3: last dose in Period 2.<br>If the subject did not continue in Period 3: Date of completion/discontinuation of the study |
| | 3 | Last dose date in Period 2 + 1 day | If the subject continued in LTE: last dose in Period 3.<br>If the subject did not continue in LTE: Date of completion/discontinuation of the study |
| | LTE | Last dose date in Period 3 + 1 day | Date of completion/discontinuation of the study |
| 2 | 2 | Date of ICF | If the subject continued in Period 3: last dose in Period 2.<br>If the subject did not continue in Period 3: all AEs are assigned to Period 2. |
| | 3 | Last dose date in Period 2 + 1 day | If the subject continued in LTE: last dose in Period 3.<br>If the subject did not continue in LTE: Date of completion/discontinuation of the study |
| | LTE | Last dose date in Period 3 + 1 day | Date of completion/discontinuation of the study |

* AEs that occurred between the last dose in Period 1 + 30 days and the date of ICF in Period 2, will be reported in a separate AE listing.

## 10.9 Adverse Events with Outcome of Death

Any AE with an outcome of "fatal" will be considered as AE with an outcome of death.

## 10.10 Treatment-related Adverse Events

Any AE with a relationship to study treatment of "Related" or missing will be considered a treatment-related AE as determined by the Investigator.

## 10.11 Imputation of AE and Concomitant Medication Start and Stop Dates

- Start Date:
    - If only 'day' is missing, and the month and year are not the same as the month of first dose, then the day will be imputed with 1 (first day of the month). Otherwise, if the month and year are the same as the dose date, the day will be imputed with the day of the first dose of treatment.
    - If 'day' and 'month' are missing, and 'year' is not missing, then the missing month and day will be imputed with the month and day of the first dose date (assuming same 'year').
    - If 'day' and 'month' are missing and 'year' is not missing and is not the same year as first dose date, then the day and month will be imputed with the 1st of January.
- Stop Date:
    - If only 'day' is missing, the day will be imputed with the last day of the month.
    - If 'day' and 'month' are missing, and 'year' is not missing, then the day and month will be imputed by the 31st of December (or by the date of study discontinuation or completion if earlier than the 31st of December and year is the same as the year of discontinuation).
    - If the stop date is completely missing and the AE/medication is not ongoing, it will be set to the date of study discontinuation or completion.

The imputed dates will be used to assess whether AEs should be considered as treatment-emergent and if medications should be included in the safety summaries as prior or concomitant medications. The original partial dates will be included in data listings.

The duration of AEs will be derived as the AE end date [(including imputation date for incomplete AE end date) – AE onset date (imputed date for incomplete AE onset date) + 1].

## 10.12 Analysis Visit Window

For initial screening the target day is -60 to -2 days before baseline. The visit will be mapped to screening if study day is -61 to -1 days. For Periods 1 and 2 once subjects are admitted to the inpatient facility, analysis will be performed for the day/time as scheduled.

For Period 3 and LTE, since the actual visit for a subject may not exactly coincide with the scheduled visit date, the actual visit date will be mapped to the study analysis visit as follows.

**Table 3: Period 3 Analysis Visit Windows**

| Analysis Visit | Target Day | Study Day | Interval (days) |
|---|---|---|---|
| Week 1 | 13 | 13 | 7 - 16 |
| Week 2 | 20 | 20 | 17 - 23 |
| Week 3 | 27 | 27 | 24 - 30 |
| Week 4 | 34 | 34 | 31 - 41 |
| Week 6 | 48 | 48 | 42 - 55 |
| Week 8 (NIH visit) | 62 | 62 | 56 - 76 |
| Week 12 | 90 | 90 | 77 - 104 |
| Week 16 (NIH visit) | 118 | 118 | 105 - 132 |
| Week 20 | 146 | 146 | 133 - 160 |
| Week 24 (NIH visit) | 174 | 174 | 161 - 188 |
| Follow-up* (2-3 days post last dose) | No analysis visit window applies | | |
| Follow-up* (7 days post last dose) | No analysis visit window applies | | |
| Follow-up* (30 days post last dose) | No analysis visit window applies | | |

* Follow-up visits are only performed if the subject does not continue in the LTE.

For the LTE, subjects will continue CLTX-305 (encaleret) per the dosing regimen established in the maintenance phase of Period 3. Telephone contacts along with outpatient laboratory assessments will be performed every 6 months starting at Month 3 with NIH inpatient visits occurring every 6 months starting at Month 6. The actual visit date will be mapped to the study analysis visit as follows.

**Table 4: LTE Analysis Visit Windows**

| Analysis Visit | Target Day | Study Day | Interval (days) |
|---|---|---|---|
| Month 3 | 264 | 264 | 189 - 309 |
| Month 6 | 354 | 354 | 310 - 399 |
| Month 9 | 444 | 444 | 400 - 489 |
| Month 12 | 534 | 534 | 490 - 579 |
| Month 15 | 624 | 624 | 580 - 669 |
| Month 18 | 714 | 714 | 670 - 759 |
| Month 21 | 804 | 804 | 760 - 849 |
| Month 24 | 894 | 894 | 850 - 939 |
| Follow-up (2-3 days post last dose) | No analysis visit window applies | | |
| Follow-up (7 days post last dose) | No analysis visit window applies | | |
| Follow-up (30 days post last dose) | No analysis visit window applies | | |

For assessments where more than one actual visit falls within the same defined window and it is not an inpatient NIH visit (Weeks 8, 16 and 24, Months 6, 12, 18 and 24), the visit closest to the target day with non-missing data will be considered for analysis. If two actual visit dates are at the same distance from the target day, the latest visit with non-missing data will be considered for analysis. If the scheduled visit is an inpatient NIH visit, the NIH inpatient visit will be considered for analysis.

All summaries and analyses will utilize analysis days/visits, where appropriate. Data listings will present actual day/visit names as recorded in the clinical database.

## 10.13 Period 2 – Special Considerations

Subject [redacted], temporarily discontinued from Period 2 Day 1 and re-started Period 2 on Day 1 after 6 months. TEAEs that occurred during this time will be included in table summaries. Otherwise, data from the incomplete Period 2 will not be included in the Tables and Figures, but will be included in data listings.

## 10.14 LTE Data

For administrative purposes, LTE data for participants may be housed in the eCRF for CLTX-305-201 and in the eCRF for CLTX-305-302/CALIBRATE. When the NIH site is activated in CLTX-305-302/CALIBRATE, the database for CLTX-305-201 will be cleaned and closed. LTE participants will be able to consent to the LTE portion of the CLTX-305-302/CALIBRATE study for the purpose of data entry to support the LTE summary of the CLTX-305-201 CSR. LTE data captured in the database of CLTX-305-201 and CLTX-305-302/CALIBRATE for these participants will be summarized in the CSR for CLTX-305-201. The CSR for CLTX-305-302/CALIBRATE will not include data from the subjects originating from CLTX-305-201.

A central lab will be used in CLTX-305-302/CALIBRATE. The lab data of the participants of CLTX-305-201 collected in the central lab and the lab data collected in CLTX-305-201 will be analyzed in the same outputs

## 10.15 Other Subject Data Considerations

DXA: DXA scans are performed throughout this study to assess bone mineral density over time. If a patient has surgical hardware, the DXA scan detects the density of the hardware and provides data that is falsely elevated at the anatomical site of the surgical hardware. In CLTX-305-201, two participants have surgical hardware that impacts the ability to interpret DXA data.

1. Subject [redacted] had a shoulder replacement during the course of the study, which does not allow for accurate interpretation of total body DXA data starting at Period 3 Week 24 and all remaining timepoints.
2. Subject [redacted] had a spinal fusion at the lumbar spine prior to study entry. As a result, DXA data for total body and lumbar spine are not able to be interpreted at Screening and all subsequent timepoints.

The DXA data for Subject [redacted] and [redacted] for all anatomical sites will be included in the listings, but the data from these subjects at the sites described above will be excluded from the tables since they do not reflect accurate assessments of bone mineral density.

iPTH: In this study, iPTH levels are analyzed via an electrochemiluminescence immunoassay. Interference with this assay can occur due to the presence of endogenous antibodies such as heterophile antibodies, anti-animal antibodies, or autoantibodies. The presence of interfering antibodies can lead to falsely high results. Subject [redacted] was found to have the intermittent presence of heterophile antibodies, which led to falsely elevated iPTH levels on some laboratory assessments. As a result, the iPTH levels for this patient were not included in the Tables but can be found in the Listings.

# 11.0 Analysis Sets

## 11.1 Safety Analysis Set

The safety analysis set is defined as all subjects who received at least one dose of study drug in the considered period.

The main objective of the study is to characterize the safety of subjects with ADH1 treated with CLTX-305 (encaleret). All analyses of the safety and efficacy (PD) data will be performed using the safety analysis set.

- For Period 1, unless otherwise specified, all descriptive statistics will be presented by dose level and by day (and timepoint where applicable).
- For Period 2, unless otherwise specified, all descriptive statistics will be presented by day (and timepoint where applicable).
- For Period 3 and LTE, all descriptive statistics will be presented by period (and timepoint where applicable).

## 11.2 PK Analysis Set

The PK analysis set is defined as all subjects who received at least one dose of study drug and who have at least one non-missing PK concentration results.

# 12.0 Reporting Analyses

Due to the open-label study design and quantitative laboratory analytes used to derive PK and PD endpoints, both Sponsor and investigator will be able to conduct joint periodic data reviews on an ongoing basis throughout the study.

Review of safety, tolerability and effects on iPTH and cCa will be conducted on Period 1 single-dose and multiple-dose (QD and BID) responses prior to commencement of Cohort 2.

Review of safety, tolerability and effects on selected PD endpoints will be conducted on all data collected by the time the last subjects has completed Period 2.

No formal interim analysis will be done.

A CSR will be written at the end of the Period 3. The Period 3 CSR will include all the data collected in Period 1, Period 2 and Period 3. An addendum to the CSR will be written to report the LTE data.

# 13.0 Statistical Methods

All data collected during this study will be displayed in data listings. Data listings will be sorted by period, cohort, and subject identifier. In addition, listings will include all relevant derived variables. The summary tables will be presented for by period, and by visit and timepoint when relevant.

Demographic and baseline summaries will be presented for each period; periods may be combined if the same subjects are included (and the data summarized is the same). Unless otherwise noted, categorical variables will be summarized using counts and percentages. Percentages will be rounded to one decimal place, except 100% will be displayed without any decimal places and percentages will not be displayed for zero counts.

Continuous variables will be summarized using the number of observations (n), mean, standard deviation (SD), median, minimum and maximum. The first and third quartile estimates (Q1 and Q3) will additionally be reported in descriptive analyses of continuous variables.

Summary statistics will be tabulated for each of the PK parameters by dose level and by study day separately for Period 1 and Period 2. Geometric means and coefficients of variation will be presented for $C_{max}$, $C_{trough}$, $AUC_{0\text{-}t}$, $AUC_{0\text{-}24}$, $AUC_{0\text{-}inf}$, $AUC_{0\text{-}tau}$, and $T_{1/2}$. Median, minimum and maximum will be presented for $T_{max}$. Actual times will be used for PK parameter reporting unless they are not available. In such cases nominal times will be used.

The mean, median, Q1 and Q3 values will be displayed with 1 decimal place, the minimum and maximum will be displayed with 0 or 1 decimal place depending on the data and the SD will be displayed with two additional decimal places. For fractional calcium excretion, the mean, median, minimum, maximum, Q1, Q3 and SD values will be displayed with 3 decimal places.

No formal statistical tests will be performed on efficacy variables; however, post-hoc inferential statistics will be carried out for specific parameters. Statistical hypothesis testing will be 2-tailed and carried out at the 5% level of significance.

All analyses will use SAS version 9.4 or higher.

This is a single site study. No subgroup analyses will be performed. No adjustments for multiplicity will be made.

## 13.1 Subject Disposition

The number and percentage of subjects treated in the study will be presented by Period. Disposition will be summarized for each period with the number and percentage of subjects who completed the treatment for each period, who withdrew from the treatment, withdrew from the study prematurely with a breakdown of the corresponding reasons for withdrawal.

The subject disposition summary will include:

- Number (%) of subjects in the safety analysis set,
- Number (%) of subjects who completed each period,
- Number (%) of subjects who prematurely discontinued from the study.

## 13.2 Important Protocol Deviations

It is the responsibility of the investigator to use continuous vigilance to identify and report deviations to the NIH Institutional Review Board (IRB) as per Policy 801. Deviations will be identified, listed, coded and summarized by the Sponsor/ designee (Clinical Research Organization [CRO]) and must be addressed in study source documents and reported to the Sponsor/designee CRO. The investigator is responsible for knowing and adhering to the reviewing IRB requirements. Additional details on the process and procedures of identifying and managing protocol deviations will be available in the Sponsor/designee (CRO) data monitoring plan.

Per CRO processes, important protocol deviations data will be entered into CRO system of record Predictivv Study Operations (PSO). The CRO study team and the Sponsor will conduct on-going reviews of the deviation data from PSO.

Additional listings to aid the Sponsor and CRO in the review of data and identification of possible deviations may be programmed if required. These additional listings will not form part of the Table, Figure and Listing (TFL) deliverable.

Important protocol deviations (IPD) are a subset of protocol deviations that might significantly affect the completeness, accuracy, and/or reliability of the study data or that might significantly affect a subject's rights, safety, or well-being. The sponsor and study team will review and categorize IPDs.

IPDs will be summarized by category and by Period and Overall for the Safety Analysis Set. A list of subjects with IPDs will be presented by Period.

### 13.2.1 NIH Definition of Protocol Deviation

A protocol deviation is any changes, divergence, or departure from the IRB-approved research protocol.

- Major deviations: Deviations from the IRB approved protocol that have, or may have the potential to, negatively impact the rights, welfare or safety of the subject, or to substantially negatively impact the scientific integrity or validity of the study.
- Minor deviations: Deviations that do not have the potential to negatively impact the rights, safety or welfare of subjects or others, or the scientific integrity or validity of the study.

## 13.3 Treatments

### 13.3.1 Extent of Study Drug Exposure

Exposure data will be summarized for Period 3 and LTE only using the Safety Analysis Set. The average daily dose for each period will be summarized for each period. The average daily dose will be derived as the sum of the dose levels times the number of days on each dose, divided by the number of days in the period considered. Additionally, the number of missed doses by period will be summarized.

### 13.3.2 Concomitant Medications

Medications received either prior to or concomitantly with study drug will be categorized by Anatomical Therapeutic Chemical Classification (ATC) and preferred name (PN) according to World Health Organization (WHO) Drug dictionary (version 2021MAR01 Global B3 or later). Prior and concomitant medications will be included in a subject data listing, and tabulated separately for Period 1 and for all subjects in the Safety Analysis Set. The prior and concomitant medication tables will be sorted by descending frequency in the medication class, followed by descending frequency PN.

## 13.4 Demographic and Baseline Characteristics

Demographics and baseline characteristics will be summarized separately for Period 1 and for all subjects in the Safety Analysis Set.

Demographic characteristics will include age, sex, race, and ethnicity.

The baseline characteristics will include: weight, height, body mass index (BMI), DXA parameters as reported on CRF (BMD and bone mineral content), Calcium Sensing Receptor (CaSR) genetic

mutation and renal ultrasound status (normal/abnormal), and if abnormal presence of nephrolithiasis and/or nephrocalcinosis. All of the above information will be listed by subject.

Age will be calculated from date of birth to date of first dose in Period 1 (for Cohort 1 subjects) and Period 2 (for Cohort 2 subjects).

## 13.5 Medical History

Medical history will be mapped to preferred terms (PTs) and system organ classes (SOCs) using the Medical Dictionary for Regulatory Activities (MedDRA®) Dictionary (version 24.0 or later) and summarized by SOC and PT for all subjects in the Safety Analysis Set. Subjects may have more than one medical history term within a SOC and PT. These summaries will present the number and percentage of subjects having each PT. At each summary level subjects are counted once if they had one or more medical history term at that level. The table will be ordered by descending frequency of incidence of PT within each SOC.

All medical history data will be listed.

## 13.6 Safety Analyses

All safety analyses will be performed on the Safety Analysis Set.

### 13.6.1 **Adverse Events**

AE means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

TEAEs are those which first occur or increase in severity or relationship to study treatment after the first dose of study treatment and not more than 30 days after the last dose of study treatment. All adverse events which change in severity or relationship to study treatment are assigned a new start date and captured as a new record. For subjects in cohort 1, an AE that occurred more than 30 days after the Period 1 last dose of study treatment and before the Period 2 first are not treatment-emergent and are not mapped to a period. Those AEs will be listed separately.

As Period 1 is separate from the other periods, treatment emergence is defined separately for Period 1 and for the other periods; AEs will be mapped to each period for the reporting (see Section 10.8 for more details).

A summary of TEAEs, including the number of events reported, the number and percentage of subjects reporting at least one TEAE regardless of relatedness assessment, the number and percentage of subjects reporting at least one TEAE related to study drug, the number and percentage of subjects discontinuing due to a TEAE, the number and percentage of subjects with at least one serious adverse event, the number and percentage of subjects with at least one TEAE leading to discontinuation of study drug, the number and percentage of subjects reporting at least one TEAE of special interest (symptomatic/asymptomatic hypocalcemia), and the number and percentage of TEAE leading to death (seriousness death) will be presented by period (Period 1, Period 2 + Period 3 and LTE). The same summary will be repeated for AEs.

A breakdown of the number and percentage of subjects with at least one TEAE, categorized by SOC and PT coded according to the MedDRA dictionary, will be presented. Subjects are only counted once within each SOC or PT. The same summary will be repeated for all TEAE related to study drug and for TEAEs leading to discontinuation of study drug.

A summary of events reported, categorized by severity (Mild, Moderate and Severe), will also be provided. Subjects with multiple events within a particular SOC or PT will be counted under the category of their most severe event within that SOC or PT.

Adverse events of special interest will be presented separately by Periods. The number and percentage of subjects with hypocalcemia (symptomatic and/or asymptomatic) as well as number and percentage of subjects for each symptom for symptomatic hypocalcemia will be presented.

All adverse events (including non-treatment-emergent events) recorded on the CRF will be listed.

### 13.6.2 Deaths and Serious Adverse Events

Serious TEAEs and serious TEAEs related to study treatment will be summarized by SOC and PT for each Period and overall.

### 13.6.3 Laboratory Data

Safety Laboratory data (hematology and chemistry) will be presented using SI units and tabulated by dose, day and time-point (where applicable) for Period 1; by day and time-point (where applicable) for Period 2, and by analysis visit and time-point (where applicable) for Period 3 and by analysis visit for LTE.

Absolute values and change from baseline will be summarized. Creatine Kinase and Potassium will be summarized by time-points. The mean ± SD of creatine kinase concentrations and the mean ± SD of potassium concentrations at each visit and timepoint (where applicable) during each period will be graphically represented. In addition, spaghetti plots of the creatine kinase concentrations and potassium concentrations will be provided by visits and timepoints (where applicable) during each period.

Blood and urine samples for clinical laboratory tests will be collected at the times detailed in the Schedule of Activities in the protocol. At Screening (Period 1), the samples will be collected at the NIH Clinical Center. Additional testing during the Screening period or for Screening Period 2 (cohort 1 subjects) may be conducted at a local laboratory near the subject. All laboratory tests during the Treatment Periods 1 and 2 (days 1 to 5) and Weeks 8, 16 and 24 for Period 3 as well as Months 6, 12, 18 and 24 for LTE will be measured at the NIH Clinical Center. At other visits and per the schedule of events, local laboratory collection of blood and urine will be performed.

The clinical laboratory tests listed in Table 5 will be included in the safety summary tables. Creatine Kinase and Potassium will be summarized for all periods; the other parameters will be summarized for Period 3 and LTE only. Urinalysis results will be listed.

**Table 5: Safety Laboratory Parameters**

| | |
|---|---|
| **Hematology** | Hemoglobin, hematocrit, complete blood count including red cell count and indices, white blood cell count, platelet count, and differential. Coagulation tests (PT, PTT, INR). |
| **Blood Chemistry** | Electrolytes (sodium, potassium, chloride), bicarbonate, glucose, blood urea nitrogen, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, LDH, amylase, lipase, uric acid, creatine kinase, total protein, total bilirubin, 25-Hydroxy Vitamin D. |

### 13.6.4 Vital Signs

Observed values and changes from baseline in vital signs will be summarized for each vital sign parameter by period and visit.

The summaries will include pulse rate, systolic blood pressure (SBP), diastolic blood pressure (DBP), respiratory rate, temperature, and weight. Pulse rate, SBP and DPB will be summarized by position for Period 1 and Period 2. For Period 3 and LTE, baseline will be defined for SBP, DBP and Heart Rate as the Period 2 Baseline using the supine position. All vital signs will be listed

### 13.6.5 Electrocardiogram (ECG)

Absolute values and change from baseline for heart rate, PR interval, QRS duration, QT interval, QT interval corrected for heart rate based on Bazett's formula (QTcB), and QT interval corrected for heart rate based on Fridericia's formula (QTcF) will be tabulated by period and visit.

The number and percentage of subjects for each category of ECG evaluation (abnormal clinically significant, abnormal not clinically significant, normal and unevaluable) will be presented by visit for each period.

In addition, the number and percentage of subjects for each category below will be provided by period and visit.

QTcF interval prolongation:

- QTcF interval < 450 ms for male or < 460 ms for female,
- QTcF interval ≥ 450 ms for male or ≥ 460 ms for female.

Change from baseline in QTcF interval:

- QTcF interval increases from baseline 30-60 ms,
- QTcF interval increases from baseline >60 ms.

A listing of subjects with ECG assessments, including the clinical evaluation results, will be provided.

## 13.7 Efficacy/PD Analyses

Efficacy analyses using laboratory parameters will be presented in both Conventional and SI units when applicable for the given parameter.

### 13.7.1 Primary Analyses

The primary efficacy endpoint is the cCa concentrations and 24-hour urinary calcium excretion after treatment with CLTX-305 for up to 24 weeks in Period 3.

cCa concentrations absolute values and change from baseline (Period 2 baseline) will be presented by visits and timepoints during Period 3. The mean ± SD cCa concentration at each visit and timepoint during Period 3 will be graphically represented. In addition, spaghetti plots of the cCa concentration will be provided by visits and timepoints during Period 3.

The 24-hour urinary calcium excretion (total) absolute values and change from baseline (Period 2 screening if available, otherwise Period 1 screening) will be summarized by visits during Period 3. In addition, the urinary calcium clearance (fractional) absolute values and change from baseline (Period 2 Day 1, 15 min pre-dose) will be tabulated by visits and time-intervals during Period 3. Finally, the mean ± SD urinary calcium clearance (total and fractional) and associated plots will also be presented by visits and time-intervals (where relevant) during Period 3.

The estimand for the primary efficacy analysis of cCa consists of the following attributes:

- Population: all subjects who received at least one dose of study drug in Period 3 (safety analysis set).

- Variable: the subject's cCa values at baseline (defined in section 10.1) and average of all Period 3, Week 24 values
- Intercurrent event: no strategy was constructed before all subjects completed Period 3, Week 24. There were no early discontinuations. There were no prohibited concomitant medications taken during Week 24. All data are therefore included in the analysis.
- Population level summary: difference in average cCa at baseline and Period 3, Week 24

The estimand for the primary efficacy analysis of 24-hour urinary calcium excretion consists of the following attributes:

- Population: all subjects who received at least one dose of study drug in Period 3 (safety analysis set).
- Variable: 24-hour urinary calcium excretion of the subject at baseline and Period 3, Week 24
- Intercurrent event: no strategy was constructed before all subjects completed Period 3, Week 24. There were no early discontinuations. There were no prohibited concomitant medications taken during Week 24. All data are therefore included in the analysis.
- Population level summary: difference in 24-hour urinary calcium excretion at baseline and Period 3, Week 24

### 13.7.2 Secondary Analyses

The secondary efficacy endpoints at each visit and timepoints are presented in Table 6.

For each parameter in Table 6, absolute levels and change from baseline (where applicable) will be tabulated by visits, dose (for period 1 only) and timepoints (where relevant) and for each period separately. In addition, the mean ± SD and individual profiles for the absolute levels for selected parameters will be graphically presented by period, visits and timepoints (where relevant).

**Table 6: Efficacy/PD Parameters**

| Specimen | Parameter | Summary | | Plots |
|---|---|---|---|---|
| | | Absolute Value | Change from Baseline | |
| Blood | cCa levels | √ | √ | √ |
| | iPTH levels | √ | √ | √ |
| | 1,25-$(OH)_2$ Vitamin D levels | √ | √ | √ |
| | eGFR levels | √ | √ | √ |
| | Albumin levels | √ | √ | |
| | Magnesium levels | √ | √ | √ |
| | Phosphorus levels | √ | √ | √ |
| | Creatinine levels | √ | √ | √ |
| | CTx levels | √ | Period 1 only | √ |
| | P1NP levels | √ | Period 1 only | √ |
| Urine | Fractional urinary calcium excretion | √ | √ | √ |

| Specimen | Parameter | Summary | | Plots |
|---|---|---|---|---|
| | | Absolute Value | Change from Baseline | |
| | Total urinary calcium excretion | √ | √ | √ |
| | Fractional urinary excretion for Magnesium | √ | √ | √ |
| | Tubular reabsorption of phosphate | √ | √ | √ |
| | Total urinary excretion for Magnesium | √ | √ | |
| | Total urinary excretion for Phosphate | √ | √ | |
| | Total urinary excretion for Sodium | √ | √ | |
| | Total urinary excretion for Potassium | √ | √ | |
| | Total urinary excretion for Citrate | √ | | √ |
| | Time-intervals and 24-hours levels for cAMP* | √ | √** | |
| | Time-intervals for creatinine and pH | √ | √ | |
| | 24 hours creatinine levels | √ | √ | √ |
| | 24 hours pH levels | √ | √ | |

* Not collected during the LTE.
** For Period 1 only.

[redacted]

[redacted]

### 13.7.4 Post-hoc Inferential Analysis

For Period 2 and Period 3, a paired t-test to test whether the mean of baseline is equal to the mean of values at each visit will be carried out for the following parameters:

- cCa,
- iPTH,
- Blood Phosphorus,
- Blood Magnesium,
- Total Calcium Excretion.

If multiple samples are planned to be collected at a visit (serial sampling), average for that visit will be derived as follow.

- **Day 1**: average of the Day 1 post-dose samples and the 15 min pre-dose sample from Day 2;
- **Day 2**: average of the Day 2 post-dose samples and the 15 min pre-dose sample from Day 3;
- **Day 3**: average of the Day 3 post-dose samples and the 15 min pre-dose sample from Day 4;
- **Day 4**: average of the Day 4 post-dose samples and the 15 min pre-dose sample from Day 5;
- **Day 5**: average of the Day 5 post-morning and post-evening samples;
- **Week 8**: average of the Week 8 pre and post-dose samples;
- **Week 16**: average of the Week 16 pre and post-dose samples;
- **Week 24**: average of the Week 24 pre and post-dose samples.

## 13.8 PK Analyses

PK parameters will be determined for Period 1 and Period 2 on days where intensive sampling is performed; and on Period 3 Week 24 (am). See Protocol Section 1.3.1, and Table 15, Table ***16***, Table 17 and Table 18, for intensive sampling times for PK and laboratory parameters. PK parameters will not be calculated in those cases where non-intensive sampling was conducted (see Table 12, Table ***13*** and Table 14).

PK parameters to be determined after QD administration in Period 1, Days 1, 2 and 3 are: $C_{max}$, $t_{max}$, $t_{½}$, $AUC_{0\text{-}t}$, $AUC_{0\text{-}24}$, $AUC_{0\text{-}inf}$.

The steady state PK parameters ($C_{max}$, $t_{max}$, Trough concentration [$C_{min}$] and $AUC_{0\text{-}tau}$) will also be determined after BID administration in Period 1 Day 5, Period 2 Day 5 (i.e. when intensive sampling was conducted) and Period 3 Week 24 (after the am dose).

### 13.8.1 Handling of Missing Data for PK Analyses

While no data exclusions or imputations are planned, they may be made in the case of implausible or outlier data. Such cases will be adjudicated by a trained pharmacokineticist and reported with justification. In cases where imputations or exclusions may affect the conclusions or interpretation of the results significantly, an assessment of such impact may be conducted, at the discretion of the pharmacokineticist, by comparing the results with and without such actions. All imputations and exclusions need to be approved by the sponsor upon discussing the justification, and imputations and exclusions will be documented in the report along with the justification.

### 13.8.2 Plasma Concentrations

Serum levels with below quantification level (BQL) results at the beginning of the profile (i.e. before the first measurable serum levels) are given a value of zero. BQL at end of profile (eg 48 hr) should be replaced with 1/2 LLOQ= 1 ng/mL). BLQ values embedded between two measurable values will be imputed as zero. For serum levels Above Level of Quantification (ALQ) the upper limit of quantification will be used.

Descriptive statistics (n, mean, SD, coefficient of variation % (%CV), median, minimum, maximum, geometric mean, and geometric CV) of PK concentration will be presented by period, visit, dose and timepoint for Period 1 and by period, visit, and timepoint for Period 2 and Period 3. Data listings of individual subject concentrations by time point, visit and period will be provided (unscheduled results will not be included in the listing).

In addition, the mean plasma concentrations and individual subject plasma concentrations will be presented in both the linear and semi-log scales by period, visit and timepoint.

### 13.8.3 Pharmacokinetics (PK) Data Analysis Methods

PK parameter estimates for CLTX-305 (encaleret) in plasma will be calculated by ICON using standard noncompartmental methods of analysis found in WinNonlin Phoenix version 8.1 or higher (Certara, Princeton, NJ, USA).

For calculation of AUC, BQL values on the leading edge of the profile (i.e., before $t_{max}$) are set equal to zero in the dataset loaded into WinNonlin (WNL) for PK analysis. BQL values after $t_{max}$ are set to missing. BLQ values embedded between two measurable values will be treated as missing. Actual times will be used in the calculation of PK parameters. If actual times are missing, then nominal times will be used.

Descriptive statistics (n, mean, SD, %CV, median, minimum, maximum, geometric mean, and geometric CV) of PK parameters will be presented for each study dose, visit and period.

PK of CLTX-305 (encaleret) will be assessed by measuring the PK parameters included in Table 7.

**Table 7: PK Parameters**

| Parameter | Description | PK Analysis Notes | SAS Programming Notes |
|---|---|---|---|
| $C_{max}$ | Maximum plasma concentration. Observed peak analyte concentration obtained directly from the experimental data without interpolation, expressed in concentration units | single and multiple doses | Cmax from WNL |
| $t_{max}$ | Time to reach Cmax in plasma. The time to reach maximum concentration is obtained directly from the experimental data without interpolation and expressed in time units. | single doses and multiple doses | Tmax from WNL |

| Parameter | Description | PK Analysis Notes | SAS Programming Notes |
|---|---|---|---|
| $t_{1/2}$ | Terminal elimination phase half-life expressed in time units.<br><br>$t_{1/2}$, will be calculated as ln2/Lambda z, where Lz is as defined below. | Single dose<br><br>If there is no valid terminal phase and terminal elimination rate constant (Lambda z), $t_{1/2}$ cannot be calculated | HL_Lambda_z from WNL<br><br>Flag to List only if: AUC_%_obs >20%<br>OR<br><br>Rsq_< 0.8 |
| Lambda z | First-order rate constant associated with the terminal (log-linear)<br>portion of the curve. Estimated by linear regression of time vs. log concentration. | Single dose | Lambda_z from WNL<br><br>Flag to list only |
| $AUC_{0-t}$ | Area under the concentration-time curve from time 0 (dosing) to the time of the last quantifiable concentration<br>AUC(0-T) is expressed in concentration*time units. | Single dose<br><br>Calculated by the linear up/log down method in WNL. | AUClast from WNL |
| $AUC_{0-24}$ | Area under the concentration-time curve from time 0 (dosing) to 24 hours<br>observed, without interpolation or extrapolation, AUC(0-24) is expressed in concentration*time units. | Single dose<br><br>Calculated by the linear up/log down method in WNL. | AUC0-24 from WNL |

| Parameter | Description | PK Analysis Notes | SAS Programming Notes |
|---|---|---|---|
| $AUC_{0\text{-}inf}$ | Area under the concentration-time curve from time 0 (dosing) extrapolated to infinity, estimated by summing AUC(0-T) and the extrapolated area, AUC%Extrap, computed by the quotient of the last quantifiable concentration (Clast) and Lz.<br>AUC(INF) = AUC(0-T) + Clast/Lz.<br>AUC(INF) is expressed in concentration*time units. | Single dose<br><br>Calculated by the linear up/log down method in WNL<br><br>If there is no valid terminal phase and terminal elimination rate constant (Lambda z), AUC(0-inf) cannot be calculated | AUCINF_obs from WNL<br><br>Flag to List only if: AUC_%_obs >20%<br><br>OR<br><br>Rsq_< 0.8 |
| AUC%Extrap | Percentage of AUC(INF) due to extrapolation from the last quantifiable concentration observed to infinity.<br><br>AUC%Extrap = [AUC(INF)-AUClast]/ AUC(INF) *100 | Single dose | AUC_%Extrap_obs from WNL<br><br>Flag to List only |
| Rsq | Goodness of fit statistic for the terminal elimination phase. | Single dose | Rsq_from WNL<br><br>Flag to List only |
| $AUC_{0\text{-}tau}$ | Area under the concentration-time curve from time 0 to one dosing period tau, tau = 9 hours for the am dose and 15 hours for the pm dose<br><br>AUC(0-tau) is expressed in concentration*time units. | Multiple dose only<br><br>Calculated by the linear up/log down method in WNL. | AUC_TAU from WNL |
| $C_{trough}$ | Trough concentration | Multiple dose only | Cmin from WNL |

# 14.0 References

Roberts, Mary Scott; Gafni, Rachel I.; Brillante, Beth; Guthrie, Lori C.; Streit, Jamie; Gash, David et al. (2019): Treatment of Autosomal Dominant Hypocalcemia Type 1 with the Calcilytic NPSP795 (SHP635). In *Journal of bone and mineral research: the official journal of the American Society for Bone and Mineral Research*. DOI: 10.1002/jbmr.3747.

# 15.0 Glossary of Abbreviations

| **Glossary of Abbreviations:** | |
|---|---|
| ADH1 | Autosomal Dominant Hypocalcemia Type 1 |
| AE | Adverse event |
| ALQ | Above level of quantification |
| ATC | Anatomic Therapeutic Classification |
| AUC | Area under the curve |
| $AUC_{0\text{-}24}$ | AUC from time 0 to 24 hours |
| $AUC_{0\text{-}inf}$ | AUC extrapolated to infinity |
| $AUC_{0\text{-}t}$ | AUC from time 0 to the last measurable time point |
| $AUC_{0\text{-}tau}$ | AUC over the dosing interval |
| BID | Twice daily |
| BMD | Bone mineral density |
| BMI | Body mass index |
| BQL | Below quantification level |
| CaSR | Calcium Sensing Receptor |
| cCa | Albumin-corrected blood calcium |
| $C_{max}$ | Maximum plasma concentration |
| $C_{min}$ | Trough concentration |
| Cr | Creatinine |
| CRF | Case Report Form |
| CRO | Clinical Research Organization |
| CSR | Clinical Study Report |
| $C_{trough}$ | Trough concentration |
| CTx | C-telopeptide |
| CV | Coefficient of variation |
| DL1 | Dose-level 1 |
| DL2 | Dose-level 2 |

| | |
|---|---|
| DBP | Diastolic blood pressure |
| DXA | Dual-energy x-ray absorptiometry |
| ECG | Electrocardiogram |
| IPD | Important Protocol Deviations |
| iPTH | Intact PTH |
| IRB | Institutional Review Board |
| K | Potassium |
| LLOD | Lower limit of detection |
| LLOQ | Lower limit of quantification |
| LTE | Long-Term Extension |
| MedDRA | Medical Dictionary for Regulatory Activities |
| Mg | Magnesium |
| NIH | National Institute of Health |
| P1NP | Procollagen type 1 N-propeptide |
| PD | Pharmacodynamics |
| PK | Pharmacokinetics |
| PN | Preferred name |
| $PO_4$ | Phosphate |
| PSO | Predictivv Study Operations |
| PT | Preferred term |
| PTH | Parathyroid hormone |
| Q1 | 1st quartile |
| Q3 | 3rd quartile |
| QD | Once daily |
| $QT_cF$ | QT interval corrected for changes in the heart rate based on Bazett's formula |
| $QT_cF$ | QT interval corrected for changes in the heart rate based on Fridericia's formula |
| SAP | Statistical Analysis Plan |
| SAE | Serious Adverse Event |
| SD | Standard deviation |
| SOC | System organ class |
| SBP | Systolic blood pressure |
| $t_{½}$ | Apparent terminal half-life |
| TFL | Table, Figure and Listing |

| | |
|---|---|
| $t_{max}$ | Time to maximum plasma concentration |
| TEAE | Treatment-emergent Adverse Event |
| ULOQ | Upper limit of quantitation |
| WHO | World Health Organization |

# 16.0 Appendix

### 16.1.1 Schedule of Event

**Table 8: Period 1 Schedule of Activities**

| Period 1 | Screening[1] | Interval labs[12] | NIH CC inpatient Period 1 | | | | | | Discharge/ EoT/ET[2] | FU Lab[3] | FU Post last dose |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Admission | QD Dosing | | | BID Dosing | | | | |
| Days | -60 to -2 | -14 to-10 | -1 | 1 | 2 | 3 | 4 | 5 | 6 | 7–8 | 9-11<br>30 ± 7 |
| Informed Consent | X | | | | | | | | | | |
| Admission to clinic | X | | X | | | | | | | | |
| Demographics, Medical history[4] | X | | | | | | | | | | |
| Eligibility assessment | X | | | | | | | | | | |
| Safety Lab Tests[5] | X | | X | | | | | | | | |
| Height[6] and weight | X | | X | | | | | X | X | | |
| Vital signs[7] | X | | X | X | X | X | X | X | X | | |
| Physical examination | X | | X | | | | | | X | | |
| Renal Ultrasound, DXA Scan | X | | | | | | | | | | |
| FSH level test (postmenopausal women) | X | | | | | | | | | | |
| Blood β-HCG pregnancy test (WOCBP only) | X | | X | | | | | X | | | |
| ECG, 12-lead | X | | X | | | | | X | | | |
| AEs and SAEs, Prior/Concomitant meds[8] | X | | X | ←---------- ---→ | | | | | | | |
| Study drug administration[9] | | | | X | X | X | X | X | | | |
| PK/PD blood sample collection | | | | Tables E, H, I, L | | | | | | X | |
| 24 hr. Urine[10] | X | X | | | | | | | | | |
| Outpatient laboratory testing | | X[11] | | | | | | | | X[3] | |
| Discharge[13] | | | | | | | | | X | | |
| Telephone Contact[14, 15] | | | | | | | | | | | X |

Abbreviations: Adverse Event = AE; Albumin = Alb; Alanine aminotransferase = ALT Blood urea nitrogen = BUN; Aspartate aminotransferase = AST; Alkaline Phosphatase = Alk Phos; Electrocardiogram = ECG; BID = Twice daily; Blood Pressure = BP; Calcium = Ca; Calcium Sensing Receptor = CASR; Chloride = Cl; Concomitant Medication = Con. Meds; Creatinine = Cr; Dual-energy X-ray Absorptiometry = DXA; Early Termination = (ET); End of Treatment = EoT; Follow-up = FU; Follicle-Stimulating Hormone = FSH; Glucose = Glu; Heart Rate = HR; Hematocrit = Hct; Hemoglobin = Hgb; Lactate Dehydrogenase = LDH; Intact Parathyroid Hormone = iPTH; Magnesium = Mg; National Institute of Health Clinical Center = NIH CC; Parathyroid Hormone = PTH; Phosphate = $PO_4$; Phosphorus = P; Potassium = K; Pharmacodynamic = PD; Pharmacokinetic = PK;

Prothrombin Time/ Prothrombin Time = PT/INR; Red Blood Cell Count = RBC; Thromboplastin Time = PTT; Serious Adverse Event = SAE; QD = Once daily; Total Bilirubin = Tbili; Triglycerides = TGL; White Blood Count = WBC; Women Of Child Bearing Potential = WOCBP

1. Screening Visit at NIH Clinical Center.
2. If participant discontinues from the study early (ET) or withdraws from taking the CLTX-305 (encaleret) (EoT), perform related assessments, resume prior medication regimen, arrange FU labs within 1-2 days, and a FU call within 30 ± 7 days after last dose of the CLTX-305 (encaleret).
3. Outpatient laboratory testing includes blood Cr, Ca, Alb, Mg, $PO_4$, iPTH, and PK sample collection on Day 7 or 8 after discharge from NIH CC on Day 6.
4. Including CaSR mutational analysis (if not documented).
5. Safety Labs – Chemistry (Na, K, Cl, bicarbonate, Glu, BUN, Cr, Alb, total protein, Ca, Mg, $PO_4$, iPTH, 25-OH Vitamin D, cholesterol, TG, AST, ALT, Tbili, Alk phos, LDH, amylase, lipase, uric acid), Hematology (RBC, Hgb, Hct, RBC indices, WBC, differential), Coagulation (PT/PTT/INR), urinalysis. HIV, viral hepatitis panel to be done at screening visit only.
6. Height measured at screening only.
7. Vital signs include supine or sitting blood pressure (BP) by automated cuff, heart rate (HR), and respiratory rate, to be collected q8 hours during inpatient days. Orthostatic BP and HR will be assessed once at screening and once daily during Period 1.
8. Discontinue calcitriol on Day -1 during the admission.
9. On Days 1, 2, & 3 participants receive CLTX-305 (encaleret) per the dosing algorithm. On Day 4 & 5 participants receive BID doses based on Days 1-3.
10. 24-hour urine at the screening visit and 10-14 days prior to Day 1 dosing (Ca, Mg, $PO_4$, Cr, Na, K, Citrate, pH).
11. Outpatient laboratory testing on day -14-10 includes: blood Cr, calcium, albumin, magnesium, phosphate, 25-OH Vitamin D.
12. Interval lab tests at outpatient laboratory will be done in those for whom the screening visit is >21 days prior to Period 1.
13. After the last dose of CLTX-305 (encaleret), participants resume prior outpatient conventional treatment regimen prior to discharge.
14. Telephone contact: should occur on days 9 to 11 or once calcium results are available. PI/study staff to review results with participant.
15. Telephone contact: 30 ± 7 days after last dose of CLTX-305 (encaleret) in Period 1 to review AEs/Con meds

## Table 9: Period 2 Schedule of Activities

| Period 2 | Screening[1] | Interval Labs [11] | NIH CC inpatient Period 2 | | | | | | Discharge/EoT/ET[2] | FU Post last dose |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Admission | BID Dosing | | | | | | |
| Days | -60 to -2 | -14 to -10 | -1 | 1 | 2 | 3 | 4 | 5 | 6 | |
| Informed Consent | X | | | | | | | | | |
| Admission to clinic | X | | X | | | | | | | |
| Demographics, Medical history[3] | X | | | | | | | | | |
| Eligibility assessment | X | | | | | | | | | |
| Safety Lab Tests[4] | X | | X | | | | | | | |
| Height[5] and weight | X | | X | | | | | X | X | |
| Vital signs[6] | X | | X | X | X | X | X | X | X | |
| Physical examination | X | | X | | | | | | X | |
| Renal Ultrasound, DXA Scan | X | | | | | | | | | |
| FSH (postmenopausal women) | X | | | | | | | | | |
| Blood β-HCG pregnancy test (WOCBP only) | X | | X | | | | | X | | |
| ECG, 12-lead | X | | X | X[14] | | X[14] | | X[14] | | |
| AEs and SAEs, Prior/Concomitant meds[7] | X | | X | ←---------- | | | | | | ----------→ |
| Study drug administration[8] | | | | X | X | X | X | X | | |
| PK/PD blood sample collection | | | | Tables F, G, J, K | | | | | | |
| 24 hr. Urine[9] | X | X | | | | | | | | |
| Outpatient laboratory testing | | X[10] | | | | | | | X[2] | |
| Dispense study drug for Period 3, Discharge[12] | | | | | | | | | X | |
| Telephone Contact[13] | | | | | | | | | | X |

Abbreviations: Adverse Event = AE; Albumin = Alb; Alanine aminotransferase = ALT; Blood urea nitrogen = BUN; Aspartate aminotransferase = AST; Alkaline Phosphatase = Alk Phos; Electrocardiogram = ECG; BID = Twice daily; Blood Pressure = BP; Calcium = Ca; Calcium Sensing Receptor = CaSR; Chloride = Cl; Concomitant medication = Con. meds; Creatinine = Cr ; Dual-energy X-ray Absorptiometry = DXA; Early Termination = (ET); End of Treatment = EoT; Follow-up = FU; Follicle-Stimulating Hormone = FSH; Glucose = Glu; Heart Rate = HR; Hematocrit = Hct; Hemoglobin = Hgb; Lactate Dehydrogenase = LDH; Intact Parathyroid Hormone = iPTH; Magnesium = Mg; National Institute of Health Clinical Center = NIH CC; Parathyroid Hormone = PTH; Phosphate = $PO_4$; Phosphorus = P; Potassium = K; Pharmacodynamic = PD; Pharmacokinetic = PK; Prothrombin Time/ Prothrombin Time = PT/INR; Red Blood Cell Count = RBC; Thromboplastin Time = PTT; Serious Adverse Event = SAE; QD = Once daily; Total Bilirubin = Tbili; Triglycerides = TGL; White Blood Count = WBC; Women Of Child Bearing Potential = WOCBP

1 Screening Visit at NIH CC for Cohort 2 participants. Participants may complete the Screening Visit as outpatients or be housed overnight at NIH CC. Cohort 1 participants do not require re-screening assessments. Participants who complete the screening visit midweek have the option to stay overnight at NIH CC during the intervening days prior to the start of Period 2, Day -1.

2 If participant discontinues from the study early (ET) or withdraws from taking CLTX-305 (encaleret) (EoT), perform related assessments, resume prior medication regimen, arrange FU labs within 1-2 days. Participants instructed to obtain outpatient laboratory testing (See Footnote 10) approximately 1-2 days after discharge from NIH CC. PI/Site staff should contact the participant within 3-5 days to review test results and provide guidance regarding clinical management as they transition to prior clinical care providers. Optionally, participants may stay in the NIH CC overnight to complete assessments.

[3] Including CaSR mutational analysis (if not documented).

[4] Safety Labs – Chemistry (Na, K, Cl, bicarbonate, Glu, BUN, Cr, alb, total protein, Ca, Mg, $PO_4$, iPTH, 25-OH Vitamin D, cholesterol, TG, AST, ALT, Tbili, Alk phos, LDH, amylase, lipase, uric acid), Hematology (RBC, Hgb, Hct, RBC indices, WBC, diff), Coagulation (PT/PTT/INR), urinalysis. HIV, viral hepatitis panel, and iron panel to be done at screening visit only unless repeat measures are clinically indicated.

[5] Height measured at screening only.

[6] Vital signs include supine or sitting blood pressure (BP) by automated cuff, heart rate (HR), and respiratory rate, to be collected q8 hours during inpatient days. Orthostatic BP and HR will be assessed once at screening and once daily during Period 2.

[7] Discontinue calcitriol on Day -1 during the admission.

[8] Initial dose (Days 1 & 2) of CLTX-305 (encaleret) BID based on results from Period 1. If calcium has not increased into the normal range, then the CLTX-305 (encaleret) dose will be increased for Days 3-5. Participants will undergo frequent PK/PD sampling over 24h on Day 5.

[9] 24-hour urine at the screening visit 14 to 10 days prior to Day 1 dosing (Ca, Mg, $PO_4$, Cr, Na, K, Citrate, pH)

[10] For cohorts 1 and 2: outpatient laboratory testing on days -14 to -10 before admission include: blood Cr, Ca, Alb, Mg, $PO_4$, 25-OH Vitamin D, Hematology (RBC, Hgb, Hct, RBC indices, WBC)

[11] Interval lab tests at outpatient laboratory will be done in those for whom the screening visit is >21 days prior to Period 2.

[12] After the last dose of CLTX-305 (encaleret), for participants continuing to Period 3, dispense supply of CLTX-305 (encaleret) before discharge.

[13] ET and EoT - a final FU call should occur within 30 ±7 days from the date of the last dose of CLTX-305 (encaleret) in Period 2.

[14] ECG assessments: Day 1, 3 and 5 each at 3 hrs ± 30 min post dose (AM)

## Table 10: Period 3 Schedule of Activities

| Period 3 - After discharge in Period 2 (Day 6) | | | | | | | | FU outpatient labs[2] 2 to 3 ± 2days | FU outpatient labs[2] 7 ± 2 days | FU- after last dose 30 ± 7 days | Un-scheduled visit[3] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Study Dosing Period | | | | | | | | | | | |
| Weeks | 1, 2, 3, 4 | 6 | NIH CC 8 | 12 | NIH CC 16 | 20 | NIH CC EoT/ET[1] 24 | | | | |
| Days (windows) | ± 2 | ± 5 | ± 5 | ± 5 | ± 5 | ± 5 | ± 5 | | | | |
| Telephone Contact (NIH PI/Study Staff) | X | X | | X | | X | X | X | X | X | X |
| AEs and SAEs, Con meds | X | X | X | X | X | X | X | | | X | X |
| Review compliance[4]/Titration | X | X | X | X | X | X | X | | | | |
| Outpatient laboratory[5] | X | X | | X | | X | | X | X | X | |
| NIH CC visit | | | X | | X | | X | | | | |
| Renal ultrasound, DXA scan | | | | | | | X | | | | |
| Dispense study drug | | | X | | X | | X[15] | | | | |
| Height[6] and weight | | | X | | X | | X | | | | |
| Vital signs[7] | | | X | | X | | X | | | | |
| Physical examination[8] | | | X | | X | | X | | | | |
| Safety Laboratory [9] | | | X | | X | | X | | | | |
| Blood β-HCG pregnancy test (WOCBP only) | | | X | | X | | X | | | | |
| ECG, 12-lead | | | X | | X | | X | | | | |
| Study drug administration[10] | | | X | | X | | X | | | | |
| PD blood collection[11] | | | X | | X | | X | | | | |
| PK blood collection[12] | | | | | | | X | | | | |
| Timed Interval & 24-hour Urine[13,14] | | | X | | X | X[14] | X | | | X[14] | |

Abbreviations: Adverse Event = AE; Albumin = Alb; Alanine aminotransferase = ALT; Blood urea nitrogen = BUN; Aspartate aminotransferase = AST; Alkaline Phosphatase = Alk Phos; Electrocardiogram = ECG; BID = Twice daily; Blood Pressure = BP; Calcium = Ca; Calcium Sensing Receptor = CaSR; Chloride = Cl; Concomitant medication = Con. meds; Creatinine = Cr; C-telopeptide = CTX; Dual-energy X-ray Absorptiometry = DXA; Early Termination = (ET); End of Treatment = EoT; Follow-up = FU; Follicle-Stimulating Hormone = FSH; Glucose = Glu; Heart Rate = HR; Hematocrit = Hct; Hemoglobin = Hgb; Lactate Dehydrogenase = LDH; Intact Parathyroid Hormone = iPTH; Magnesium = Mg; National Institute of Health Clinical Center = NIH CC; Parathyroid Hormone = PTH; Phosphate = $PO_4$; Phosphorus = P; Potassium = K; Pharmacodynamic = PD; Pharmacokinetic = PK; Procollagen type 1 N-propeptide = P1NP; Prothrombin Time/ Prothrombin Time = PT/INR; Red Blood Cell Count = RBC; Thromboplastin Time = PTT; Serious Adverse Event = SAE; QD = Once daily; Total Bilirubin = Tbili; Triglycerides = TGL; White Blood Count = WBC; Women Of Child Bearing Potential = WOCBP

[1] If participant discontinues from the study early (ET) or withdraws from taking CLTX-305 (encaleret) (EoT), before completing Period 3 per protocol, participant will be asked to return to the NIH CC as soon as possible for the ET/EoT visit assessments and return the unused CLTX-305 (encaleret). Participant should revert to their outpatient regimen of oral calcium and active vitamin D. Participants will go through FU activities as stated in table and footnote 2, and then will have a safety FU call 30 day ± 7 days.

[2] Participants who withdraw from taking CLTX-305 (encaleret) (EoT) before completing Period 3 per protocol or who elect not to continue into the LTE will obtain the following outpatient laboratory assessments after the last dose of CLTX-305 (encaleret) and after re-starting their prior regimen of oral calcium and active vitamin D: blood Cr, Ca, Alb, Mg, PO4. Follow-up (FU) call should occur within 3-5 days to review the lab results and receive guidance on clinical management of their ADH1 as they transition to their prior clinical care providers.

[3] Unscheduled visits: For laboratory evaluation following dose adjustment or assessment or follow-up of an AE, in which clinically indicated procedures will be performed.

[4] Document current CLTX-305 (encaleret) dose and calcium dosing regimen, any modifications, and reason for modifications.

[5] Outpatient laboratory testing: Blood samples for clinical laboratory panels (Na, Cl, bicarbonate, K, BUN, Cr and including Alb, Ca, Mg, $PO_4$, iPTH, CTX and P1NP).

[6] Height measured only at ET visit.

[7] Vital signs include supine or sitting blood pressure (BP) by automated cuff, heart rate (HR), and respiratory rate.

[8] Focused Physical exams: weight measurement at all visits.

[9] Safety Labs collected at the NIH CC visits– Chemistry (Na, K, Cl, bicarbonate, Glu, BUN, Cr, Alb, total protein, Ca, Mg, $PO_4$, 25-OH Vitamin D, Cholesterol, TG, AST, ALT, Tbili, Alk phos, LDH, amylase, lipase, uric acid), Hematology (RBC, Hgb, Hct, RBC indices, WBC, diff), Coagulation (PT/PTT/INR).

[10] CLTX-305 (encaleret) administered and recorded during the NIH CC visit/in-house PK/PD collection days.

[11] See Table E: Non-intensive Sampling Days for PD measures and timepoints and Table L: Research Sample Collection.

[12] See Table E: Use Non-intensive Sampling Days schedule for PK timepoints.

[13] See Table E: see time intervals for urine collections for calculation of fractional excretion rates and 24-hour total excretion of urine Ca, Mg, $PO_4$, Cr, cAMP, citrate, Na, K, pH during the NIH CC visits

[14] Outpatient 24-hr urine Ca, Mg, $PO_4$, Cr, Na, K, citrate, pH at 20 weeks. Participants who withdraw from taking CLTX-305 (encaleret) (EoT) before completing Period 3 per protocol or chose not to continue into the LTE will obtain 24-hr urine at 30 ± 7 days follow-up.

[15] Week 24 drug dispensing is only for participants who continue into the LTE; If a participant is not immediately continuing into the LTE, no drug will be dispensed.

## Table 11: LTE Schedule of Activities

| LTE - After last day in week 24 in Period 3 | | | | | | | | | | FU outpatient labs[2] 2 to 3 ± 2 days | FU outpatient labs[2] 7 ± 2 days | FU- after last dose[11] 30 ± 7 days | Un-scheduled visit[3] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Study Dosing Period** | | | | | | | | | | | | | |
| **Months** | **NIH CC** | | **NIH CC** | | **NIH CC** | | **NIH CC** | | **NIH CC EoT/ET[1]** | | | | |
| | **0[12]** | **3** | **6** | **9** | **12** | **15** | **18** | **21** | **24** | | | | |
| **Days (windows)** | | **± 7** | **± 7** | **± 7** | **± 7** | **± 7** | **± 7** | **± 7** | **± 30** | | | | |
| Reconsent[12] | X | | | | | | | | | | | | |
| Telephone Contact (NIH PI/Study Staff) | | X | | X | | X | | X | | X | X | X | X |
| AEs and SAEs, Con meds | X | ←---------- | | | | | | | ----------→ | X | X | X | X |
| Review compliance[4]/dose adjustment as needed | | X | X | X | X | X | X | X | X | | | | |
| Outpatient laboratory[5] | | X | | X | | X | | X | | X | X | X | |
| NIH CC visit | | | X | | X | | X | | X | | | | |
| Renal ultrasound, DXA scan | | | | | X | | | | X | | | | |
| Dispense study drug | X | | X | | X | | X | | | | | | |
| Height[6] and weight | | | X | | X | | X | | X | | | | |
| Vital signs[7] | | | X | | X | | X | | X | | | | |
| Physical examination | | | X | | X | | X | | X | | | | |
| Safety and Drug Monitoring Laboratory[8] | X[12] | | X | | X | | X | | X | | | | |
| Blood β-HCG pregnancy test (WOCBP only) | | | X | | X | | X | | X | | | | |
| ECG, 12-lead | | | X | | X | | X | | X | | | | |
| Study drug administration[9] | X | ←---------- | | | | | | | ----------→ | | | | |
| 24-hour Urine[10] | | X | X | X | X | X | X | X | X | | | X[11] | |

Abbreviations: Adverse Event = AE; Albumin = Alb; Alanine aminotransferase = ALT; Blood urea nitrogen = BUN; Aspartate aminotransferase = AST; Alkaline Phosphatase = Alk Phos; Electrocardiogram = ECG; BID = Twice daily; Blood Pressure = BP; Calcium = Ca; Calcium Sensing Receptor = CaSR; Chloride = Cl; Concomitant medication = Con. meds; Creatine Kinase = CK; Creatinine = Cr; C-telopeptide = CTX; Dual-energy X-ray Absorptiometry = DXA; Early Termination = (ET); End of Treatment = EoT; Follow-up = FU; Follicle-Stimulating Hormone = FSH; Glucose = Glu; Heart Rate = HR; Hematocrit = Hct; Hemoglobin = Hgb; Lactate Dehydrogenase = LDH; Intact Parathyroid Hormone = iPTH; Magnesium = Mg; National Institute of Health Clinical Center = NIH CC; Parathyroid Hormone = PTH; Phosphate = $PO_4$; Phosphorus = P; Potassium = K; Pharmacodynamic = PD; Pharmacokinetic = PK; Procollagen type 1 N-propeptide = P1NP; Prothrombin Time/ Prothrombin Time = PT/INR; Red Blood Cell

Count = RBC; Thromboplastin Time = PTT; Serious Adverse Event = SAE; QD = Once daily; Total Bilirubin = Tbili; Triglycerides = TGL; White Blood Count = WBC; Women Of Child Bearing Potential = WOCBP

[1] If participant discontinues from the study early (ET) or withdraws from taking CLTX-305 (encaleret) (EoT), before completing LTE per protocol, participant will be asked to return to the NIH CC within 30 days following the last dose for the ET/EoT visit assessments and return the unused CLTX-305 (encaleret). Participant should resume oral calcium and active vitamin D under the supervision of the study team while the encaleret is being safely discontinued. Follow-up labs should beperformed per this Schedule of Assessments.

[2] 2 Participants who withdraw from taking CLTX-305 (encaleret) (EoT) before transitioning to the CLTX-305-302/CALIBRATE LTE per protocol will obtain the following outpatient laboratory assessments after the last dose of CLTX-305 (encaleret) and after re-starting their prior regimen of oral calcium and active vitamin D: blood Cr, Ca, Alb, Mg, iPTH, PO4. Follow-up (FU) call should occur within 3-5 days to review the lab results and receive guidance on clinical management of their ADH1 as participants transition to prior standard of care.

[3] Unscheduled visits: For laboratory evaluation following dose adjustment or assessment or follow-up of an AE, in which clinically indicated procedures will be performed

[4] Document current CLTX-305 (encaleret) dose and calcium dosing regimen, any modifications, and reason for modifications

[5] Outpatient laboratory testing: Blood samples for clinical laboratory panels (Na, Cl, bicarbonate, K, BUN, Cr and including Alb, Ca, Mg, $PO_4$, iPTH, CTX, and P1NP).

[6] Height measured only at month 24/final visit or ET visit

[7] Vital signs include supine or sitting blood pressure (BP) by automated cuff, heart rate (HR), and respiratory rate.

[8] Safety and Drug Monitoring Labs collected at the NIH CC visits approximately 4 hours after the morning encaleret dose – Chemistry (Na, K, Cl, bicarbonate, Glu, BUN, Cr, Alb, total protein, Ca, Mg, PO4, iPTH, 25-OH Vitamin D, CTX, P1NP, Cholesterol, TG, AST, ALT, Tbili, Alk phos, LDH, amylase, lipase, uric acid, CK), Hematology (RBC, Hgb, Hct, RBC indices, WBC, diff), Coagulation (PT/PTT/INR), Research labs (FGF23 and mid-molecule PTH). HIV, viral hepatitis panel should be repeated within 3 months prior to transition to CLTX-305-302/CALIBRATE.

[9] CLTX-305 (encaleret) administered and recorded during the NIH CC visit/in-house days.

[10] Outpatient 24-hr urine collection for Ca, Mg, PO4, Cr, Na, K, citrate, pH

[11] A final follow-up safety telephone call and outpatient labs will occur 30 ± 7 days after the last dose of CLTX-305 (encaleret).

[12] If a participant is not able to enter the LTE immediately following Period 3, then participant will be reconsented to and may restart CLTX-305 (encaleret) at the previous therapeutic dose determined in Period 3. Safety and Drug Monitoring Laboratory tests should be done while participant is at the NIH CC.

[13] When participant transitions to CLTX-305-302/CALIBRATE, participant will be asked to return to the NIH CC to complete the ET/EoT visit assessments, return the unused CLTX-305 (encaleret), and complete the screening assessments for the CLTX-305-302/CALIBRATE Phase 3 study. Follow-up labs will not be needed.

### 16.1.2 PD sampling

**Table 12: Non-intensive BID Sampling Days**

**Period 1: Day 4 (BID Dosing) and Period 3: PK/PD sampling days**

| | **Timepoint ± 20 min. (relative to AM dose)** |
|---|---|
| **Blood Assays** | |
| PK[1] samples | -15 min, +30 min, 2, 4, 8, 11, 13 hrs |
| Intact PTH | -15 min, +30 min*, 2, 4, 8, 11, 13, 17 hrs |
| Serum bone markers: CTX, P1NP | -15 min, 13 hrs |
| 1, 25-$(OH)_2$ Vitamin D, cAMP | -15 min, 4, 8, 13, 17, 24 hrs[2] |
| Ca, $PO_4$, Mg, Cr, albumin | -15 min, +30 min*, 2, 4, 8, 11, 13, 17, 24 hrs[2] |
| K, CK | -15 min, 13 hrs |
| **Urine Assays:** | |
| **Timed Interval Urine Collections:** Ca, Mg, $PO_4$, Cr, cAMP, pH | -15 min 0-4 h, 4-8 h, 8-13 h, 13-17 h, 17-24 h |
| **24-Hour urine:** Ca, Mg, $PO_4$, Cr, Na, K, Citrate, pH | 0, 24 hrs (Start collection after first morning void and end 24 hours later) |

Abbreviations: Ca = Calcium; CK = Creatine Kinase; Cr = Creatinine; CTX = C-telopeptide; K = Potassium; Mg = Magnesium; Na = Sodium; P1NP = Procollagen type 1 N-propeptide; PK = Pharmacokinetics; $PO_4$ =Phosphate; PTH = Parathyroid hormone.

[1] PK in Period 3 only collected at 24 weeks;

[2] 24 hour timepoint only collected in Period 3

* +30 min timepoint only collected in Period 3 at 24 weeks

**Table 13: Non-intensive BID Sampling Days - (if CLTX-305 (encaleret) dose is ≤ 180 mg BID)**

**Period 2: Days 1, 2, 3 & 4**

| | **Timepoint ± 20 min. (relative to AM dose)** |
|---|---|
| **Blood Assays** | |
| PK samples | -15 min, 2, 4 hrs |
| Intact PTH | -15 min, +30 min, 2, 4, 8, 11, 13, 17 hrs |
| 1, 25-$(OH)_2$ Vitamin D | -15 min, 4, 8 hrs |
| Ca, $PO_4$, Mg, Cr, albumin | -15 min, +30 min, 2, 4, 8, 11, 13, 17 hrs |
| K, CK | -15 min, 13 hrs |
| **Urine Assays:** | |
| **Timed Interval Urine Collections:** Ca, Mg, $PO_4$, Cr, pH | -15 min[1], 0-4 h, 4-8 h, 8-13 h, 13-17 h, 17-24 h |
| **24-Hour urine:** Ca, Mg, $PO_4$, Cr, Na, K, Citrate, pH | 0, 24 hrs (Start collection after first morning void and end 24 hours later) |

Abbreviations: Ca = Calcium; CK = Creatine Kinase; Cr = Creatinine; K = Potassium; Mg = Magnesium; Na = Sodium; PK = Pharmacokinetics; $PO_4$ =Phosphate; PTH = Parathyroid hormone.

[1] -15 min urine is done on Period 2 (Day 1) only

**Table 14: Non-intensive BID Sampling Days - (if CLTX-305 (encaleret) dose is > 180 mg BID)**

**Period 2: Days 2, 3 & 4**

| | **Timepoint ± 20 min. (relative to AM dose)** |
|---|---|
| **Blood Assays** | |
| PK samples | -15 min, +30 min, 2, 4, 8, 11, 13 hrs |
| Intact PTH | -15 min, +30 min, 2, 4, 8, 11, 13, 17 hrs |
| 1, 25-$(OH)_2$ Vitamin D | -15 min, 4, 8, |
| Ca, $PO_4$, Mg, Cr, albumin | -15 min, +30 min, 2, 4, 8, 11, 13, 17 hrs |
| K, CK | -15 min, 13 hrs |
| **Urine Assays:** | |
| **Timed Interval Urine Collections:** Ca, Mg, P, Cr, pH | 0-4 h, 4-8 h, 8-13 h, 13-17 h, 17-24 h |
| **24-Hour urine:** Ca, Mg, $PO_4$, Cr, Na, K, Citrate, pH | 0, 24 hrs (Start collection after first morning void and end 24 hours later) |

Abbreviations: Ca = Calcium; CK = Creatine Kinase; Cr = Creatinine; K = Potassium; Mg = Magnesium; Na = Sodium; PK = Pharmacokinetics; $PO_4$ =Phosphate; PTH = Parathyroid hormone.

## Table 15: Intensive Sampling Days for QD dosing

### Period 1: Days 1, 2, 3

| | Timepoint ± 10 min (relative to AM dose) |
|---|---|
| **Blood Assays:** | |
| PK samples | -15 min, +30 min, 1, 1.5, 2, 3, 4, 6, 8, 13 hrs |
| Intact PTH | -15 min, +30 min, 1, 1.5, 2, 3, 4, 6, 8, 13, 17 hrs |
| Serum bone markers: CTX, P1NP | -15 min, 13 hrs |
| 1, 25-$(OH)_2$ Vitamin D, cAMP | -15 min, 4, 8, 13, 17 hrs |
| Ca, $PO_4$, Mg, Cr, albumin | -15 min, +30 min, 1, 2, 3, 4, 6, 8, 13, 17 hrs |
| K, CK | -15 min, 13 hrs |
| **Urine Assays** | |
| **Timed Interval Urine Collections:** Ca, Mg, $PO_4$, Cr, cAMP, pH | -15 min[1], 0-4 h, 4-8h, 8-13h, 13-17h, 17-24 h |
| **24-Hour urine:** Ca, Mg, $PO_4$, Cr, Citrate, Na, K, pH | 0, 24 hrs (Start collection after first morning void and end 24 hours later) |

Abbreviations: Ca = Calcium; CK = Creatine Kinase; Cr = Creatinine; CTX = C-telopeptide; K = Potassium; Mg = Magnesium; Na = Sodium; P1NP = Procollagen type 1 N-propeptide; PK = Pharmacokinetics; $PO_4$ =Phosphate; PTH = Parathyroid hormone.

[1] -15 min urine is done on Period 1 (Day 1) only

**Table 16: Intensive Sampling Days for BID dosing**

**Period 1: Day 5**

| | **Timepoints ± 10 min (relative to AM & PM doses)** |
|---|---|
| **Blood Assays** | |
| PK samples | TRT AM Dose: -15 min, +30 min, 1, 1.5, 2, 3, 4, 6, 8 hrs |
| | TRT PM Dose: +30 min, 1, 1.5, 2, 3, 4, 6, 15 hrs |
| Intact PTH | TRT AM Dose: -15 min, +30 min, 1, 1.5, 2, 3, 4, 6, 8 hrs |
| | TRT PM Dose: +30 min, 1, 1.5, 2, 3, 4, 6, 8, 15 hrs |
| Serum bone markers: CTX, P1NP | TRT AM Dose: -15 min, 8 hrs |
| 1, 25-$(OH)_2$ Vitamin D, cAMP | TRT AM Dose: -15 min, 4, 8 hrs |
| | TRT PM Dose: 4, 8, 15 hrs |
| Ca, P, Mg, Cr, albumin | TRT AM Dose: -15 min, +30 min, 1, 1.5, 2, 3, 4, 6, 8 hrs |
| | TRT PM Dose: +30 min, 1, 1.5, 2, 3, 4, 6, 8, 15 hrs |
| K, CK | TRT AM Dose: -15 min, 8 hrs |
| **Urine Assays** | |
| **Timed Interval Urine Collections:** Ca, Mg, $PO_4$, Cr, cAMP, pH | 0-4 h, 4-8h, 8-13h, 13-17h, 17-24 h |
| **24-Hour urine:** Ca, Mg, $PO_4$, Cr, Citrate, Na, K, pH | 0-24 hrs (Start collection after first morning void and end 24 hours later) |

Abbreviation: TRT = Time Relative To; Ca = Calcium; CK = Creatine Kinase; Cr = Creatinine; CTX = C-telopeptide; K = Potassium; Mg = Magnesium; Na = Sodium; P1NP = Procollagen type 1 N-propeptide; PK = Pharmacokinetics; $PO_4$ =Phosphate; PTH = Parathyroid hormone.

**Table 17: Intensive Sampling Days for BID dosing - (if CLTX-305 (encaleret) dose is > 180 mg BID)**

**Period 2: Day 5**

| | **Timepoints ± 10 min (relative to AM & PM doses)** |
|---|---|
| **Blood Assays** | |
| PK samples | TRT AM Dose: -15 min, +30 min, 1.5, 2, 4, 6, 8 hrs |
| | TRT PM Dose: +30 min, 1.5, 2, 4, 6, 15 hrs |
| Intact PTH | TRT AM Dose: -15 min, +30 min, 1.5, 2, 4, 6, 8 hrs |
| | TRT PM Dose: +30 min, 1.5, 2, 4, 6, 8, 15 hrs |
| Ca, $PO_4$, Mg, Cr, albumin | TRT AM Dose: -15 min, +30 min, 1.5, 2, 4, 6, 8 hrs |
| | TRT PM Dose: +30 min, 1.5, 2, 4, 6, 8, 15 hrs |
| K, CK | TRT AM Dose: -15 min, 8 hrs |
| **Urine Assays** | |
| **Timed Interval Urine Collections:** Ca, Mg, $PO_4$, Cr, pH | 0-4 h, 4-8h, 8-13h, 13-17h, 17-24 h |
| **24-Hour urine:** Ca, Mg, $PO_4$, Cr, Citrate, Na, K, pH | 0-24 h (Start collection after first morning void and end 24 hours later) |

Abbreviation: TRT = Time Relative To; Ca = Calcium; CK = Creatine Kinase; Cr = Creatinine; K = Potassium; Mg = Magnesium; Na = Sodium; PK = Pharmacokinetics; $PO_4$ =Phosphate; PTH = Parathyroid hormone.

**Table 18: Intensive Sampling Days for BID dosing- (if CLTX-305 (encaleret) dose is ≤ 180 mg BID)**

**Period 2: Day 5**

| | **Timepoints ± 10 min (relative to AM & PM doses)** |
|---|---|
| **Blood Assays** | |
| PK samples | TRT AM Dose: -15 min, 1.5, 4 h |
| | TRT PM Dose: +30 min, 1.5, 4, 15 h |
| Intact PTH | TRT AM Dose: -15 min, 1.5, 4, 8 hrs |
| | TRT PM Dose: +30 min, 1.5, 4, 8, 15 hrs. |
| Serum bone markers: CTX, P1NP | TRT AM Dose: -15 min 8 hrs. |
| 1, 25-$(OH)_2$ Vitamin D, cAMP | TRT AM Dose: -15 min 4, 8 hrs. |
| | TRT PM Dose: 4, 8, 15 hrs. |
| Ca, $PO_4$, Mg, Cr, albumin | TRT AM Dose: -15 min, 1.5, 4, 8 hrs. |
| | TRT PM Dose: +30 min, 1.5, 4, 8, 15 hrs. |
| K, CK | TRT AM Dose: -15 min, 8 hrs. |
| **Urine Assays** | |
| **Timed Interval Urine Collections:** Ca, Mg, $PO_4$, Cr, cAMP, pH | 0-4 h, 4-8h, 8-13h, 13-17h, 17-24 h |
| **24-Hour urine:** Ca, Mg, $PO_4$, Cr, Citrate, Na, K, pH | 0-24 h (Start collection after first morning void and end 24 hours later) |

Abbreviation: TRT = Time Relative To; Ca = Calcium; CK = Creatine Kinase; Cr = Creatinine; CTX = C-telopeptide; K = Potassium; Mg = Magnesium; Na = Sodium; P1NP = Procollagen type 1 N-propeptide; PK = Pharmacokinetics; $PO_4$ =Phosphate; PTH = Parathyroid hormone.