**Study Title: A Phase 3 Study of the Efficacy and Safety of Lixivaptan in Participants with Autosomal Dominant Polycystic Kidney Disease Consisting of a 1-year Double-blind, Placebo-controlled, Randomized Phase and 1-year Open-Label Phase: The ACTION Study**

**ClinicalTrials.gov ID: NCT04064346**

Statistical Analysis Plan Version 1.0, dated 24-Aug-2022

# Statistical Analysis Plan

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|---|---|
| Sponsor | Palladio Biosciences |
| Protocol Title: | A Phase 3 Study of the Efficacy and Safety of Lixivaptan in Participants with Autosomal Dominant Polycystic Kidney Disease Consisting of a 1-year Double-blind, Placebo-controlled, Randomized Phase and 1-year Open-Label Phase: The ACTION Study |
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# List of Abbreviations

| Abbreviation | Definition |
|---|---|
| ADPKD | autosomal dominant polycystic kidney disease |
| ADPKD-IS | ADPKD Impact Scale |
| ADPKD-PDS | ADPKD Pain and Discomfort Scale |
| ADPKD-UIS | ADPKD Urinary Impact Scale |
| AE | adverse event |
| AESI | adverse events of special interest |
| ALT | alanine aminotransferase |
| ANCOVA | analysis of covariance |
| ART | Anti-retroviral therapy |
| AST | aspartate aminotransferase |
| BHB | beta-hydroxybutyrate |
| BID | twice daily |
| BMI | body mass index |
| BSSR | blinded sample size re-estimation |
| CI | confidence interval |
| CKD | chronic kidney disease |
| CKD-EPI | chronic kidney disease epidemiology collaboration |

| Abbreviation | Definition |
|---|---|
| CKD-EPIcr_R | Chronic Kidney Disease – Epidemiology Collaboration equation (eGFR) using serum creatinine revised without the race variable |
| CKD-EPIcr-cys_R | Chronic Kidney Disease – Epidemiology Collaboration equation (eGFR) using serum creatinine and serum cystatin C without the race variable |
| CRF | case report form |
| CRO | contract research organization |
| CS | clinically significant |
| CT | computerized tomography |
| ECG | electrocardiogram |
| eCRF | electronic case report form |
| eGFR | estimated glomerular filtration rate |
| ET | early termination |
| GCP | good clinical practice |
| HBsAg | Positive test results for hepatitis B surface antigen |
| HCV | Positive test results for hepatitis C |
| HERC | hepatic events review committee |
| HIF-PH | hypoxia-inducible factor prolyl hydroxylase |
| HIV | human immunodeficiency virus |
| HgbA1c | glycosylated hemoglobin |

| Abbreviation | Definition |
|---|---|
| htLV | height-adjusted liver volume |
| htTKV | height-adjusted total kidney volume |
| ICF | informed consent form |
| IEC | independent ethics committee |
| IDMC | independent data monitoring committee |
| IRB | institutional review board |
| IRT | interactive response technology |
| ITT | intent-to-treat |
| IUD | intrauterine device |
| IUS | intrauterine hormone-releasing system |
| KDIGO | Kidney Disease Improving Global Outcomes |
| LOE | lack of efficacy |
| LTSS | lixivaptan treated safety set |
| LV | liver volume |
| MAR | missing at random |
| MCMC | Monte Carlo Markov Chain |
| MedDRA | Medical Dictionary for Regulatory Activities |
| MI | multiple imputation |
| mITT | modified intent-to-treat |

| Abbreviation | Definition |
|---|---|
| MMRM | mixed model for repeated measurements |
| MNAR | missing not at random |
| MRI | magnetic resource imaging |
| NCS | non-clinically significant |
| P2EAS | part 2 efficacy analysis set |
| P2SS | part 2 safety set |
| PCS | potentially clinically significant |
| PEAS | primary efficacy analysis set |
| PK | pharmacokinetics |
| PPAS | per-protocol efficacy analysis set |
| PRO | patient reported outcomes |
| QTcF | QT interval corrected for heart rate according to Fridericia's formula |
| SAE | serious adverse event |
| SAP | statistical analysis plan |
| SEAS | secondary efficacy analysis set |
| SD | standard deviation |
| SDAC | Statistical Data Analysis Center |
| SGLT2 | sodium-glucose cotransporter 2 |
| SOC | system organ class |

| Abbreviation | Definition |
|---|---|
| TEAE | treatment-emergent adverse event |
| TEAESI | treatment-emergent adverse event of special interest |
| TKV | total kidney volume |
| ULN | upper limit of normal |
| WHODD | World Health Organization Drug Dictionary |

# Table of Contents

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# 1. Introduction

This statistical analysis plan (SAP) provides a technical and detailed elaboration of the statistical analysis of efficacy and safety data for Palladio Biosciences protocol number PA-ADPKD-301 (A Phase 3 Study of the Efficacy and Safety of Lixivaptan in Participants with Autosomal Dominant Polycystic Kidney Disease Consisting of a 1-year Double-blind, Placebo-controlled, Randomized Phase and 1-year Open-Label Phase: The ACTION Study), version 3.0, dated 14-Feb-2022.

NOTE: Please see Section 12 for an explanation of the impact of early termination of this study on the actual output produced.

# 2. Study Objectives

## 2.1. Study Objectives of the Double-blind Phase (Part 1/Year 1)

### 2.1.1. Primary Objective

To demonstrate the efficacy of lixivaptan compared to placebo in the slowing of deterioration in kidney function in participants with ADPKD as demonstrated by the annualized change from baseline in estimated glomerular filtration rate (eGFR) determined from the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation without the race term (CKD-EPIcr_R).

### 2.1.2. Key Safety Objective

To compare the incidences of liver chemistry test elevations in participants randomized to lixivaptan with participants randomized to placebo.

### 2.1.3. Secondary Efficacy Objectives

- To compare the rate of change (slope) in on-treatment eGFR in participants treated with lixivaptan to participants treated with placebo;
- To assess the effect of lixivaptan on height-adjusted total kidney volume (TKV) as measured by magnetic resonance imaging (MRI) compared to placebo.

### 2.1.4. Secondary Safety Objective

To assess the non-hepatic safety and tolerability of lixivaptan.

### 2.1.5. Health Outcomes Related Objectives

- To evaluate medical resource utilization (e.g., medication use/changes; unplanned office visit; urgent care and emergency department usage; and hospitalizations) resulting from clinical events in participants randomized to lixivaptan compared with those on placebo

and assess which events are driving any observed differences in medical resource utilization between lixivaptan and placebo;

- To evaluate the change from baseline in domain scores of the ADPKD Impact Scale (ADPKD-IS), ADPKD Pain and Discomfort Scale (ADPKD-PDS), and ADPKD Urinary Impact Scale (ADPKD-UIS) in participants treated with lixivaptan compared with participants treated with placebo.

#### 2.1.6. Pharmacokinetics Objective

To characterize the PK profile of lixivaptan utilizing PopPK based on sparse plasma sampling.

#### 2.1.7. Exploratory Objectives

- To assess the effect of lixivaptan on height-adjusted liver volume (htLV) as measured by MRI compared to placebo;
- To evaluate the change from baseline in urine osmolality in participants treated with lixivaptan compared with participants treated with placebo;
- To evaluate the effect of lixivaptan on kidney function as assessed by eGFR using a novel serum creatinine and serum cystatin C-based CKD-EPI equation refit without the race variable (CKD-EPIcr-cys_R) compared with placebo.

### 2.2. Study Objectives of the Open-label Phase (Part 2/Year 2)

#### 2.2.1. Key Objective

To demonstrate the continued efficacy of lixivaptan in the slowing of deterioration in kidney function in participants randomized to lixivaptan in the double-blind phase (Part 1) as measured by the annualized change from baseline (Part 2) in eGFR as determined by the CKD-EPIcr_R equation at the end of the open-label phase (Part 2).

#### 2.2.2. Key Safety Objective

To assess the incidence of liver chemistry test abnormalities during the open-label phase.

#### 2.2.3. Secondary Objectives

- To assess the rate of change (slope) in on-treatment eGFR in Part 2 in participants treated with lixivaptan in Part 1 and Part 2;
- To assess the effect of lixivaptan on height-adjusted TKV (htTKV) as measured by MRI in Part 2 in participants treated with lixivaptan in Part 1 and Part 2.

#### 2.2.4. Secondary Safety Objective

To assess the non-hepatic safety and tolerability of lixivaptan.

### 2.2.5. Health Outcomes Related Objectives

- To evaluate medical resource utilization (e.g., medication use/changes; unplanned office visits; urgent care and emergency department usage; and hospitalizations) resulting from clinical events in participants on lixivaptan;
- To evaluate the change in domain scores of the ADPKD-IS, ADPKD-PDS, and ADPKD-UIS.

### 2.2.6. Pharmacokinetics Objective

To further characterize the PK profile of lixivaptan utilizing PopPK based on sparse plasma sampling.

### 2.2.7. Exploratory Objectives

- To assess the effect of lixivaptan on htLV as measured by MRI following 52 weeks of open-label treatment;
- To evaluate the change in urine osmolality;
- To evaluate the effect of lixivaptan on kidney function as assessed by eGFR using the CKD-EPIcr-cys_R equation following 52 weeks of open-label treatment.

## 3. Study Design

### 3.1. Overall Design

**Figure 1: Part 1 Study Schematic**


SB = Single-Blind, PBO = Placebo

**Figure 2: Part 2 Study Schematic**


## 3.2. Study Population

A total of 1350 participants with ADPKD who meet all inclusion criteria and none of the exclusion criteria (see below) and tolerate the study medications are planned to be randomized in the study at approximately 250 sites globally.

### 3.2.1. Inclusion Criteria

The following are requirements for entry into the study:

1. Male or female, between 18 and 60 years of age (inclusive) at the time of Screening (Visit 1a).
2. Diagnosis of ADPKD by modified Pei criteria:
   - For participants with family history of ADPKD, by ultrasound:
     - 18-39 years: ≥3 cysts, unilateral or bilateral;
     - 40-59 years: ≥2 cysts in each kidney;
     - 60 years: ≥4 cysts in each kidney; or
   - For participants with family history of ADPKD, by computerized tomography (CT) or MRI:
     - 18-40 years: ≥10 cysts in both kidneys; or
   - For participants without family history of ADPKD
     - a minimum of 10 cysts per kidney by any radiologic method and exclusion of other cystic kidney diseases (multiple simple kidney cysts, renal tubular acidosis, cystic dysplasia of the kidney, multicystic kidney, multilocular cysts of the kidney, medullary cystic kidney and acquired cystic disease of the kidney); or
     - genetic diagnosis of ADPKD.
3. At risk for rapid progression of ADPKD as based on the Mayo Clinic ADPKD Image Classification of 1C, 1D, or 1E based on age and height-adjusted TKV as determined by kidney MRI obtained during Screening, where class (class 1 [typical] versus class 2 [atypical]) and TKV are determined by a central imaging vendor.

4. eGFR ≥25 mL/min/1.73 m2 and ≤90 mL/min/1.73 $m^2$ based on the mean of 2 eGFR determinations (Visits 1a and 2 or Visits 1b and 2, if Visit 1b is required) calculated by the 2021 Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) eGFR creatinine equation refit without the race variable (CKD-EPIcr_R) from serum creatinine values obtained during Screening.
5. Appropriate control of hypertension for a minimum of 3 weeks including the use of an angiotensin converting enzyme inhibitor or angiotensin receptor blocker (unless not considered appropriate for the participant) as suggested by the 2021 Kidney Disease Improving Global Outcomes (KDIGO) "Clinical Practice Guideline for the Management of Blood Pressure in Chronic Kidney Disease," without the use of a diuretic.
6. Body mass index (BMI) between 18 and 40 kg/$m^2$ (inclusive) at the time of Screening.
7. Female participants must:

    a. not be pregnant, lactating, or breastfeeding.

    b. be either postmenopausal (defined as amenorrhea for ≥ 12 months), surgically sterile (defined as having undergone hysterectomy and/or bilateral oophorectomy) or, if of child-bearing potential, agree to practice acceptable methods of birth control or remain abstinent (only if this is the usual and preferred lifestyle of the participant) during the full duration of the trial and for 30 days after the last dose of study drug. Birth control methods that can be used during the study include the following:

    - hormonal contraceptives: combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (i.e., oral, intravaginal, transdermal); progestogen-only hormonal contraception (i.e., oral, injectable, implantable). Note: in women with severe polycystic liver disease, contraceptives containing estrogen (and hormone replacement therapy) may be involved in the development and growth of liver cysts and polycystic liver disease progression; the supplemental risk of initiating or continuing estrogen treatment, as well as potential alternative contraceptives for WOCBP will be discussed with the potential participant
    - intrauterine device (IUD), including progestin-containing intrauterine devices
    - intrauterine hormone-releasing system (IUS)
    - male sexual partner who has been vasectomized for at least 3 months prior to Screening and who has obtained a follow-up negative sperm count and is the sole sexual partner
    - bilateral tubal ligation
    - Essure® procedure (tubal occlusion)
    - male or female condom with spermicide (cream, spray, gel, suppository, or polymer film)

- diaphragm, cervical cap, or contraceptive sponge with spermicide (with or without male condom).

8. Male participants must agree to use an acceptable form of birth control or remain abstinent (only if this is the usual and preferred lifestyle of the participant) during the full duration of the trial and for 30 days after the last dose of study drug.
9. Have read, understood, and provided written informed consent after the nature of the study has been fully explained and must be willing to comply with protocol requirements and study-related procedures.

### 3.2.2. Exclusion Criteria

1. Advanced diabetes (e.g., glycosylated hemoglobin [HgbA1c] >7.5%, and/or glycosuria by dipstick, significant proteinuria [>300 mcg albumin/mg creatinine]), other significant kidney disease, kidney cancer, transplanted kidney, single kidney, kidney surgery within the past 6 months (including cyst drainage or fenestration) or acute kidney injury within 6 months prior to Screening.
2. Clinically significant incontinence, overactive bladder, or urinary retention (e.g., benign prostatic hyperplasia).
3. New York Heart Association Functional Class 3 or 4 heart failure or other significant cardiac or electrocardiogram (ECG) findings that could pose a safety risk to the participant.
4. History of infection with human immunodeficiency virus (HIV) unless the participant is clinically stable and doing well on a non-CYP interacting anti-retroviral therapy (ART) regimen and the participant has not required more than 2 changes in their ART regimen since treatment inception.
5. History of clinically significant drug or alcohol abuse in the 2 years prior to Screening Visit 1a.
6. Contraindications to or interference with MRI assessments (e.g., ferromagnetic metal prostheses, aneurysm clips, severe claustrophobia, or large abdominal/back tattoos). Investigator should seek MRI safety guidance from the local MRI facility.
7. Any malignancy within 5 years prior to Screening except for basal cell carcinoma successfully treated with local therapy or malignancies that are considered by the Investigator not to affect participant survival (after discussion with the medical monitor).
8. Medical history or findings that preclude safe participation in the trial or participants who are likely to be non-compliant with trial procedures in the opinion of the Investigator or medical monitor.
9. Clinically significant liver disease or impairment or alanine aminotransferase (ALT), aspartate aminotransferase (AST), or total bilirubin values >1.2 x ULN during Screening. Note: This criterion will preliminarily be reviewed at Visit 2 based on Visit 1a and Visit

1b results (if Visit 1b is required). The criterion must be re-evaluated no later than Visit 3 when results for Visit 2 are available.

10. Requirement for ongoing diuretic use.
11. Participants who are currently taking, or are expected to be taking, strong or moderate CYP3A4 or CYP2C8 inhibitors or inducers including regular use of grapefruit juice, Seville oranges, or St. John's wort. If applicable, there should be a 14-day washout of these treatments prior to Visit 2.
12. Prior use of a sodium-glucose cotransporter 2 (SGLT2) inhibitor (e.g., canagliflozin, dapagliflozin, empagliflozin, etc.) within the 2 months prior to Screening Visit 1a or expected need for initiation of treatment with a SGLT2 inhibitor during the study.
13. Prior use of a hypoxia-inducible factor prolyl hydroxylase (HIF-PH) inhibitor within the 2 months prior to Screening Visit 1a or expected need for initiation of treatment with a HIF-PH inhibitor during the study.
14. Simvastatin at a total daily dose >10 mg or amlodipine at a total daily dose >5 mg.
15. Prior use of tolvaptan or lixivaptan within the 2 months prior to Screening Visit 1a.
16. Prior use of conivaptan, somatostatin analogs (e.g., lanreotide, pasireotide, octreotide, etc.), metformin (except for diabetes), nicotinamide, bardoxolone, demeclocycline, mTOR kinase inhibitors (e.g., everolimus, sirolimus, etc.) or KetoCitra™ or any beta-hydroxybutyrate (BHB) containing supplements within the 2 months prior to Screening Visit 1a.
17. Participants who have taken any investigational drug or used an investigational device within 30 days, or 5 half-lives, whichever is longer, prior to Screening Visit 1a or plan to participate in an interventional trial during the study.
18. Hypovolemia on physical examination at Screening.
19. Abnormal serum sodium concentration at Screening.
20. Positive test results for hepatitis B surface antigen (HBsAg).
21. Positive test results for hepatitis C (HCV) antibody (Anti-HCV), with the exception of participants for whom the reflex HCV RNA titer test is negative.
22. Known sensitivity or idiosyncratic reaction to lixivaptan and/or its excipients.

### 3.3. Placebo Run-in/Lixivaptan Titration

Participants who meet the inclusion criteria and none of the exclusion criteria will be enrolled in the study and enter the Placebo Run-in Period. During the Placebo Run-in Period, participants will receive four capsules of single-blind placebo twice daily (BID) approximately 10 hours apart over one week of dosing. Throughout the study, participants will take 4 capsules BID to mask transitions from one study period to the next.

Participants who can tolerate the placebo will enter the Lixivaptan Titration Period. During the Lixivaptan Titration Period, single-blind lixivaptan will be started at Level 1 (50 mg BID) and

will be increased weekly through Levels 2 (100 mg BID), 3 (150mg BID), and 4 (200 mg BID) according to the dosing schedule in Table 1. At the 2 highest dose levels (Level 3 [150 mg BID] and Level 4 [200 mg BID]), there will be an opportunity to reduce the evening (PM) dose to Level 3a (150 mg AM/100 mg PM) or Level 4a (200 mg AM/150 mg PM) if aquaretic effects are problematic in certain participants.

**Table 1: Dosing Levels during the Titration Period**

| Dose Level | AM Dose | PM Dose |
|---|---|---|
| 1* | 50 mg | 50 mg |
| 2 | 100 mg | 100 mg |
| 3 | 150 mg | 150 mg |
| 3a** | 150 mg | 100 mg |
| 4 | 200 mg | 200 mg |
| 4a** | 200 mg | 150 mg |

*Dose Level 1 is for initiation of treatment.
**Participants having difficulty tolerating Dose Level 3 can drop back to 150 mg in the AM and 100 mg in the PM (Dose Level 3a: 150/100 mg).
***Participants having difficulty tolerating Dose Level 4 can drop back to 200 mg in the AM and 150 mg in the PM (Dose Level 4a: 200/150 mg).

Once the maximum tolerated dose is achieved during the Lixivaptan Titration Period, participants will generally stay on that dose for 1 additional week to confirm tolerability. As the maximum duration of the Lixivaptan Titration Period is 6 weeks, participants who require a dose reduction at Week 6 (Visit 9), as a result of emerging tolerability issues, will proceed to the double-blind period on the newly assigned (reduced) dose level without extension of the titration period. Participants who are unable to tolerate the minimum dose for entry into the Double-Blind, Randomized Treatment Period (100 mg BID/ Level 2) will be discontinued from the study.

### 3.4. Randomization

Once the maximum tolerated dose during the Lixivaptan Titration Period is at least 100 mg BID, the participant will be randomized in 2:1 lixivaptan to placebo ratio to either receive lixivaptan or placebo treatment during the Double-blind, Randomized Treatment Period stratified into 1 of 6 strata by the following stratification factors (determined at time of Screening):

1. CKD stage (CKD 2 vs. CKD 3/4)
2. Mayo Clinic ADPKD Image Classification (1C, ID, or IE)

### 3.5. Blinding

This is a double-blind study. Blinding was achieved by means of identical appearance for the capsules for lixivaptan and placebo. The placebo capsule does NOT contain the active ingredient (lixivaptan) but contains the same inactive ingredients as in the investigational

product. Throughout the study, participants will take 4 capsules BID to mask transitions from one study period to the next.

Treatment allocation after randomization will be blinded to all participants, investigators, site personnel, and sponsor employees and representatives except the Independent Data Monitoring Committee (IDMC) and its supporting individuals (e.g., study unblinded biostatistician) and Statistical Data Analysis Center (SDAC). The trial will be unblinded following the locking of the database in Part 1. In addition, all participants will be blinded to study drug (placebo or lixivaptan) during the Placebo Run-in Period and Lixivaptan Titration Period in Part 1.

To maintain study blinding, data that can potentially be used to identify the treatment allocation will NOT be shared with the participant, investigator, clinical sites, sponsor, and CRO until the locking of the database in Part 1 of the study. The set of such data are MRI volumetric calculations and other derived data (e.g., htTKV), urine osmolality data, and PK data after randomization.

### 3.6. Dose Administration

Participants who tolerate their optimized dose in the Lixivaptan Titration Period will then enter the Double-blind, Randomized Treatment Period during which time they will take the lixivaptan dose level achieved at the end of the Lixivaptan Titration Period if participants were randomized into the lixivaptan group or receive matching placebo capsules if participants were randomized into the placebo group. During the Double-blind, Randomized Treatment Period, the dose may be adjusted downward at the Investigator's discretion if needed to manage non-hepatic side effects. For these participants, the dose level should be increased back to the dose at the start of the Double-Blind, Randomized Treatment Period, once symptoms resolve. The Investigator may temporarily interrupt the study drug, if necessary, to manage acute intercurrent illness, tolerability issues, planned or unplanned surgical procedures or life situations, e.g., airplane travel, etc. In an effort to minimize missing data, wherever practicable, participants who experience a study drug interruption of 7 or more days will be scheduled to have 3 separate serum creatinine samples obtained (minimally 24 hrs. apart between 8 and 28 days after their last dose) for determination of eGFR. Participants who require a prolonged interruption (i.e., >4 weeks in duration) due to illness, including COVID-19, or other reasons may be able to restart the study drug when medically stable. Re-establishment is required in the event of a prolonged interruption. If study drug is interrupted for longer than 8 weeks, then study drug treatment will be permanently discontinued. Note: In the event of tolerability issues at any time during the study, dosing may be decreased or temporarily stopped. If dosing is resumed during the Double-Blind, Randomized Treatment Period at a dose level less than that achieved at Visit 9, attempts will be made every 4 to 8 weeks to re-establish the previously achieved dose level from Visit 9, if medically appropriate.

For the participants who completed Part 1 and continued to Part 2, the lixivaptan re-titration is required. During the Lixivaptan Re-titration Period, dosing will start at Level 1 (50 mg BID) for all participants in Part 2 and will be increased weekly until the dose level taken at the end of the Double-blind, Randomized Treatment Period is achieved, or, if they had received placebo during the Double-blind, Randomized Treatment Period, to the last inferred dose level (the dose level

equal to the active dose level had the participant been randomized to the active arm). Re-titration will be performed by the IRT, and the site, participant, and sponsor maintain a blinded status to the prior treatment assignment in Part 1. The dose level obtained at end of the Lixivaptan Re-Titration Period and equivalent to the dose level taken at the end of Double-blind, Randomized Treatment Period, will be continued for the remainder of Part 2. In the event of tolerability issues at any time during the study including either during the Lixivaptan Re-titration Period or during the Maintenance Treatment Period, dosing may be decreased or temporarily stopped. Attempts will be made every 4 to 8 weeks to re-establish the previously achieved dose level at the end of the Double-blind, Randomized Treatment Period of Part 1, if medically appropriate. If tolerability continues to be problematic, the participant may continue at the lower dose. If study drug is interrupted for longer than 8 weeks, then study drug treatment will be permanently discontinued.

## 3.7. Sample Size

**Part 1:**

Based on the results of the REPRISE trial with tolvaptan (Torres et al, 2017), it is assumed that the standard deviation for the primary efficacy endpoint, change from baseline to post-treatment follow-up in mean eGFR, is 6.20 mL per minute per 1.73 $m^2$. Assuming a between-treatment group difference of -1.40 mL per minute per 1.73 $m^2$ and a randomization ratio of 2:1 for lixivaptan to placebo, a sample size estimate of 1314 participants (438 placebo-treated participants and 876 lixivaptan-treated participants) is required to achieve 90% power at a significance level of 0.01. This estimate has been adjusted up to 1350 participants (450 placebo participants and 900 lixivaptan participants). It is estimated that approximately 2250 participants will need to be screened in order to randomize 1350 participants.

**Part 2:**

This is a convenience sample based on the number of participants who elect to continue into Part 2. It is estimated that approximately 1350 participants will be randomized in Part 1. All participants except those who discontinue due to an adverse event or withdraw consent will continue into Part 2. Approximately 90% (1215) of Part 1 participants are anticipated to continue into Part 2.

## 3.8. Schedule of Events

Please see the protocol for a detailed schedule of events.

# 4. Study Endpoints

## 4.1. Study Endpoints of the Double-blind Phase (Part 1/Year 1)

### 4.1.1. Efficacy Endpoints

#### 4.1.1.1. Primary Efficacy Endpoint

The primary efficacy endpoint of this study is the annualized change in eGFR calculated from the CKD-EPI equation (2021 CKD-EPIcr_R) for serum creatinine from baseline to the post-treatment follow-up, annualized by participant's elapsed duration as defined below. Baseline eGFR is defined as the mean of the 3 eGFR determinations obtained during the Screening and Placebo Run-in Periods i.e., the mean eGFR of Visit 1a or 1b, Visit 2, and Visit 3. The post-treatment follow-up eGFR is defined as the mean of 3 eGFR assessments obtained during the Follow-up Period I after Week 52 for on-treatment completers, or, for participants who discontinue study drug treatment prior to Week 52 [Visit 22], during the Follow-up Period 8 to 28 days following study drug treatment discontinuation. On-treatment completers are defined in Section 8.1. The participant's elapsed duration is defined as the number of years (number of days divided by 365.25) from the median of the collection dates of the baseline eGFR assessments to the median of the collection dates of the 3 eGFR assessments obtained during the post-treatment, follow-up period immediately following study drug discontinuation.

#### 4.1.1.2. Secondary Efficacy Endpoints

The secondary endpoints are:

- The annualized rate of change (slope) in eGFR, estimated from all on-treatment eGFRs, calculated from the CKD-EPI equation (2021 CKD-EPIcr_R) for serum creatinine, during the Double-Blind, Randomized Treatment Period in Part 1. An on-treatment eGFR is defined as any eGFR obtained while participants are on treatment (taking the study drug), i.e., any eGFR obtained within 24 hours after the latest dose.
- The annualized percent change in height-adjusted TKV (htTKV, mL/m) from baseline to post-treatment follow up during Follow-up Period I after Week 52 for on-treatment completers, or, for participants who discontinue study drug treatment prior to Week 52 [Visit 22], during the Follow-up Period 28 days following study drug treatment discontinuation. The htTKV is defined as the TKV value (mL) determined by MRI divided by the participant's baseline height (meters) measured at Screening. Baseline htTKV is the one obtained at Screening (Visit 1a).

#### 4.1.1.3. Exploratory Efficacy Endpoints

- The annualized percent change in height-adjusted LV (htLV, mL/m) from baseline to post-treatment follow up during Follow-up Period I after Week 52 for on-treatment completers, or, for participants who discontinue study drug treatment prior to Week 52 [Visit 22], during the Follow-up Period 28 days following study drug treatment discontinuation. The htLV is defined as the LV value (mL) determined by MRI divided

by the baseline participant's height (meters) observed at Screening. Baseline htLV is the one obtained at Screening (Visit 1a).

- Mean change from baseline in morning spot urine osmolality at Visits 15, 22, and 25; Baseline is defined as urine osmolality obtained at Screening (Visit 2).
- The annualized change in eGFR calculated from the 2021 CKD-EPIcr-cys_R equation from baseline to the post-treatment follow-up. The definitions of baseline eGFR, post-treatment follow-up eGFR, elapsed duration, and annualization are the same as the primary efficacy endpoint except for using the different eGFR equations.

### 4.1.2. Health Outcome Endpoints

#### 4.1.2.1. Medical Resource Utilization Endpoints

A detailed description of the analysis for the medical resource utilization and health economics assessments will be specified in a prospective health outcomes analysis plan.

#### 4.1.2.2. Patient Reported Health Outcomes

Endpoints for Patient Reported Health Outcomes consist of three patient questionnaires: ADPKD-IS, ADPKD-PDS, and ADPKD-UIS. Number of questions, domains and scoring for each questionnaire are listed in Table 2. Baseline score in each domain is defined as the observed score for that domain at Screening (Visit 2). If more than 50% of the items of a domain are missing, that domain score is missing; otherwise, the domain score is the mean of scores for all the non-missing items for that domain.

**Table 2: Domains and their Scoring of Three Patient Reported Health Outcomes**

| HRQoL PRO | Description | Scoring |
|---|---|---|
| ADPKD-Impact Scale (ADPKD-IS) | • 18-item participant administered questionnaire<br>• Scale of 1-5 with a two week recall period; 1 = not difficult at all/not bothered at all to 5 = extremely difficult/extremely bothered<br>• 3 conceptual domains: physical, emotion and fatigue | • Physical domain score = Items (1 + 2 + 3 + 4 + 5 + 15 + 16) /7<br>• Emotional domain score = Items (6 + 11 + 12 + 13)/4<br>• Fatigue domain score = Items (10 + 17 + 18)/3<br>• Items 7, 8, 9, and 14 are not included in the scoring |
| ADPKD-Pain and Discomfort Scale (ADPKD-PDS) | • 20-item participant administered questionnaire<br>• Scale of 1-5 with a one week recall period; 1 (none | • Dull pain severity score = Items (1 + 2 + 3)/3<br>• Sharp pain severity score = Items (4 + 5+ 6)/3 |

| | | |
|---|---|---|
| | or not at all) to 5 (extreme, constantly, or completely)<br>• Severity dimension consists of 4 severity scales: dull pain severity, sharp pain severity, discomfort severity, overall pain severity<br>• Interference dimension consists of 2 scales: dull pain interference, discomfort interference | • Discomfort severity score = Items (7 + 8 + 9)/3<br>• Overall pain severity score = (dull pain severity score + sharp pain severity score + discomfort severity score)/3<br>• Dull pain interference score = Items (10 + 11 + 12 + 13 + 14)/5<br>• Discomfort interference score = Items (16 + 17 + 18 + 19 + 20)/5<br>• Sharp pain interference score = Item 15 |
| ADPKD-Urinary Impact Scale (ADPKD-UIS) | • 11-item participant administered questionnaire<br>• Scale of 1-5 with a one week recall period; 1 (not difficult/bothered) to 5 (very difficult/bothered)<br>• 3 domains: frequency, urgency, and nocturia | • Frequency domain score = Items (1 + 3 + 6 + 8)/4<br>• Urgency domain score = Items (2 + 4 + 7 + 9)/4<br>• Nocturia domain score = Items (5 + 10 + 11)/3 |

Endpoints for Patient Reported Health Outcomes at Visits 12, 15, 18, 22, and 25 are as follows:

**ADPKD-IS:**

- Change from baseline in physical domain score
- Change from baseline in emotional domain score
- Change from baseline in fatigue domain score

**ADPKD-PDS:**

- Change from baseline in dull pain severity score
- Change from baseline in sharp pain severity score
- Change from baseline in discomfort severity score
- Change from baseline in overall pain severity score
- Change from baseline in dull pain interference score
- Change from baseline in discomfort interference score
- Change from baseline in sharp pain interference score

**ADPKD-UIS:**

- Change from baseline in frequency domain score
- Change from baseline in urgency domain score
- Change from baseline in nocturia domain score

In addition, the above endpoints will be calculated at the evaluation visits during Lixivaptan Titration Period. Those will be analyzed separately.

### 4.1.3. Pharmacokinetic Endpoints

The calculation of parameters for population PK analysis of plasma lixivaptan concentrations will be described in a separate PK analysis plan.

### 4.1.4. Safety Endpoints

#### 4.1.4.1. Key Safety Endpoint

The key liver safety endpoint is the incidence of participants with serum ALT levels >3 x ULN during the Double-Blind, Randomized Treatment Period in Part 1.

#### 4.1.4.2. Secondary Safety Endpoints

The safety and tolerability of lixivaptan assessed through evaluation of:

- Treatment-emergent adverse events (TEAEs); a TEAE is defined as any adverse event that either occurred after the first dose of the study drug (i.e., placebo during the Placebo Run-in Period) or occurred prior to the first dose of the study drug but worsened after the first dose of the study drug.
- Clinical laboratory findings (clinical chemistry including additional analyses of liver chemistry, hematology, and urinalysis);
- Vital signs;
- 12-lead electrocardiograms (ECG).

## 4.2. Study Endpoints of the Open-label Phase (Part 2/Year 2)

### 4.2.1. Key Endpoints

#### 4.2.1.1. Key Comparison Endpoint

The primary endpoint of Part 2 is the annualized change in eGFR calculated from the CKD-EPI equation (2021 CKD-EPIcr_R) for serum creatinine from baseline to the post-treatment follow-up in Part 2, annualized by participant's treatment elapsed duration in Part 2 as defined below. Baseline eGFR in Part 2 is defined as the mean of the 3 eGFR determinations obtained at 3 post-treatment follow-up visits (Visits 23, 24, and 25) after Week 52 in Part 1. The post-treatment follow-up eGFR of Part 2 is defined as the mean of 3 eGFR assessments obtained during the

Follow-up Period II after Week 104 for on-treatment completers or, for participants who discontinue study drug treatment prior to Week 104 [Visit 42], during the Follow-up Period 8 to 28 days following study drug treatment discontinuation in Part 2. The participant's treatment elapsed duration in Part 2 is defined as the number of years (number of days divided by 365.25) from the median of the collection dates of the baseline eGFR assessments to the median of the collection dates of the 3 eGFR assessments obtained during the post-treatment follow-up.

#### 4.2.1.2. Key Safety Endpoint

The key liver safety endpoint is incidence of serum ALT levels > 3 x ULN in participants exposed to lixivaptan in Part 2.

### 4.2.2. Other Endpoints

#### 4.2.2.1. Other Comparison Endpoints

The additional secondary endpoints are:

- The annualized rate of change (slope) in eGFR, estimated from all on-treatment eGFRs, calculated from the CKD-EPI equation (2021 CKD-EPIcr_R) for serum creatinine during the Maintenance Treatment Period in Part 2. An on-treatment eGFR is defined as any eGFR obtained while participants are on treatment (taking lixivaptan) in Part 2.
- The annualized percent change in htTKV from baseline (post-treatment follow-up after Week 52 in Part 1) to post-treatment follow-up during Follow-up Period II after Week 104 for on-treatment completers, or, for participants who discontinue study drug treatment prior to Week 104 [Visit 42], during the Follow-up Period 28 days following study drug treatment discontinuation in Part 2.

#### 4.2.2.2. Secondary Safety Endpoints

The safety and tolerability of lixivaptan assessed through evaluation of:

- Treatment-emergent adverse events (TEAEs);
- Clinical laboratory findings (clinical chemistry including additional analyses of liver chemistry tests), hematology, and urinalysis);
- Vital signs;
- 12-lead electrocardiograms (ECG).

#### 4.2.2.3. Medical Resource Utilization Endpoints

A detailed description of the analysis for the medical resource utilization and health economics assessments will be specified in a prospective health outcomes analysis plan.

#### 4.2.2.4. Endpoints for Patient Reported Health Outcomes

See Section 4.1.2.2 for the definitions for domains, how to calculate domain scores, and the definitions of endpoints for each patient report health outcome questionnaire. Baseline in Part 2 is defined as the observed domain score in the post-treatment follow-up visit (Visit 25) after Week 52 in Part 1.

### 4.2.3. Pharmacokinetic Endpoints

The calculation of parameters for population PK analysis of plasma lixivaptan concentrations will be described in a separate PK analysis plan.

#### 4.2.3.1. Exploratory Endpoints

- The annualized percent change in htLV determined by MRI from baseline (post-treatment follow-up visit [Visit 25] after Week 52 in Part 1) to post-treatment follow-up during Follow-up Period II after Week 104 for on-treatment completers, or, for participants who discontinue study drug treatment prior to Week 104 [Visit 42], during the Follow-up Period 28 days following study drug treatment discontinuation in Part 2;
- Mean change from baseline in morning spot urine osmolality in Part 2; Baseline is defined as urine osmolality obtained at 28 days post-treatment follow-up visit (Visit 25) after Week 52 in Part 1.
- The annualized change in eGFR calculated from the 2021 CKD-EPIcr-cys_R equation from baseline to the post-treatment follow-up. The definitions of baseline eGFR, post-treatment follow-up eGFR and annualization are the same as the key comparison endpoint in Part 2 (Section 4.2.1.1) except for using the different eGFR equations.

## 5. Analysis Populations

The following analysis populations are planned for this study:

**Part 1:**

- **Placebo Run-in Safety Set:** The Placebo Run-in Safety Set includes all participants who received at least one dose of placebo during Placebo Run-In Period in Part 1.
- **Lixivaptan Titration Safety Set:** The Lixivaptan Titration Safety Set includes all participants who received at least one dose of lixivaptan during Lixivaptan Titration Period in Part 1.
- **Randomized Safety Set:** The Randomized Safety Set includes all participants who received at least one dose of study drug after randomization.
- **Intent-to-Treat Analysis Set (ITT):** The ITT includes all participants who were randomized in Part 1.

- **Modified Intent-to-Treat Analysis Set (mITT):** The mITT includes all participants in the ITT who received at least one dose of study drug in Part 1. Note that the mITT Analysis Set consists of the same set of the participants in the Randomized Safety Set.
- **Primary Efficacy Analysis Set (PEAS):** The PEAS includes all participants in the mITT Analysis Set who have both baseline and at least one assessment of eGFR after the treatment is complete or discontinued in Part 1.
- **Secondary Efficacy Analysis Set (SEAS):** The SEAS includes all participants in the mITT Analysis Set who have both baseline and at least one on-treatment assessment of eGFR.
- **Per-protocol Efficacy Analysis Set (PPAS):** The PPAS includes all participants in the PEAS who do not have any protocol deviations that are deemed to potentially impact efficacy.

Assignment of participants to populations will be confirmed at a blinded data review meeting to be held before the study database is locked for Part 1.

**Part 2:**

- **Part 2 Safety Set (P2SS):** The Part 2 Safety Set includes all participants who receive at least one dose of lixivaptan in Part 2.
- **Part 2 Efficacy Analysis Set (P2EAS):** The Part 2 Efficacy Analysis Set includes all participants in the Part 2 Safety Set who have at least one assessment of off-treatment eGFR in Part 2.

**Parts 1 & 2 (Combined):**

- **Lixivaptan Treated Safety Set (LTSS):** The Lixivaptan Treated Safety Set is defined as all participants who received at least one dose of lixivaptan treatment after randomization during the entire study. This analysis population will be used to summarize safety data during the study for all randomized, lixivaptan-treated participants.

# 6. General Issues for Statistical Analysis

## 6.1. Statistical Definitions and Algorithms

### 6.1.1. Baseline

The last observation recorded before the first dose of lixivaptan during Lixivaptan Titration Period will be used as the baseline observation for all calculations of change from baseline unless otherwise specified.

### 6.1.2. Treatment Group and Cohort

For Part 1 summaries after randomization, the analysis results will be presented by two treatment groups: lixivaptan and Placebo.

Since all participants in Part 2 are treated with lixivaptan, the summaries for Part 2 will be

presented in the following treatment cohorts and overall (treatment cohorts combined):

- Part1/Part2 – Placebo/Lixivaptan: all participants in Part 2 who were randomized into Placebo treatment group in Part 1
- Part1/Part2 – Lixivaptan/Lixivaptan: all participants in Part 2 who were randomized into lixivaptan treatment group in Part 1

### 6.1.3. Multiple Comparisons

To control the family-wise type I error at the pre-specified level (≤ 0.01), the primary and secondary endpoints in Part 1 will be tested in hierarchical order:

- Treatment difference for the primary endpoint must be statistically significant (p-value ≤ 0.01)
- Treatment difference for annualized rate of change (slope) in on-treatment eGFRs is tested only if treatment difference for the primary endpoint is statistically significant (p-value ≤ 0.01)
- Treatment difference for annualized percent change from baseline in htTKV is tested only if treatment difference for annualized rate of change (slope) in on-treatment eGFRs is statistically significant (p-value ≤ 0.01)

Because the testing is sequential, the type I error rate of ≤ 0.01 is maintained. Failure at any stage in the sequence implies automatic failure at all subsequent stages. All p-values for each comparison without adjustments will be provided in the summary tables for informational purposes.

No adjustments will be made for multiple comparisons for other endpoints.

### 6.1.4. Handling of Dropouts or Missing Data

Assessments are planned, by protocol, to be collected from all randomized participants except those who 1) withdraw consent AND 2) do not consent to any collection of data. Every effort will be made to keep randomized participants in the study and offer them options for continued treatment and, at a minimum, continued study participation to the degree possible; strategies of these efforts are described in the study protocol Section 4.2.4, Section 4.2.7, and Appendix 5 (Section 13.5) in order to minimize missing data. While all possible efforts will be made to ensure that participants stay in the study and all data is collected as scheduled, the occurrence of missing data cannot be completely eliminated.

The handling of missing data is dependent upon the efficacy analyses (see Section 8 Efficacy Analysis for detail). The missing data for efficacy analyses are generally imputed by the following methods:

- Missing at random (MAR) – mixed-effects model for repeated measures (MMRM): a modeling approach (missing data are not imputed prior to the analysis)

- Missing at random (MAR) – multiple imputation (MI)
- Missing not at random (MNAR) – multiple imputation (MI) with assumptions of missing not at random. Treatment impact on efficacy endpoints for missing data varies independently on the two treatment arms bi-dimensionally. Pattern-mixture approach is applied to investigate the MNAR pattern based on dropout reasons. Specifically, MNAR in the following patterns of dropout reasons will be investigated as MNAR:
    - Lack of efficacy (LOE)
    - LOE and adverse events (AE)
    - All dropouts

#### 6.1.4.1. Multiple Imputation Methods

MI is a simulation-based approach where missing values are replaced using an appropriate stochastic model given the observed data and covariates, creating multiple completed data sets. These completed datasets (observed data and imputed data) are then analyzed using pre-specified analysis methods, and the different parameter estimates across the datasets are then combined to produce unique point estimates, standard errors, confidence intervals, and p-value taking into account the uncertainty of the imputation process.

The following 2 MI analysis models, one based on the standard MAR imputation approach and the other based on the MNAR imputation approach, will be used to examine robustness of the primary analysis results.

##### 6.1.4.1.1 Standard MAR Imputation

The MAR imputation model will impute missing values using a regression-based multiple imputation model (Little et al, 1996). A by-treatment multiple regression model will be fit that includes the observed outcome at that visit as the dependent variable. The independent variables will generally include baseline value, sex, age, randomization stratification factors, and treatment duration of study drug unless otherwise specified. Using these regression models, a missing value for a participant will be imputed as a draw from the predictive distribution. This process will be repeated 50 times, resulting in 50 complete analysis data sets. The analysis has the following steps:

- Using Monte Carlo Markov Chain (MCMC) methodology from SAS PROC MI by treatment group to impute the missing data to obtain 50 complete analysis data sets
- Using the pre-specified analysis to analyze each set of the completed data along with the imputed data
- Obtaining the overall results using PROC MIANALYZE

##### 6.1.4.1.2 MNAR Imputation

The MNAR imputation of missing data and data analysis are implemented as following:

- Using a standard MAR-based multiple imputation approach (See Section 6.1.4.1.1) to impute missing data to obtain 50 complete analysis data sets
- The imputed data for missing reason matching the MNAR pattern will be adjusted by the following:
    - If the participant is randomized in the lixivaptan group, the imputed value will be reduced by k (e.g., 50%) times the treatment difference (obtained using the MAR approach)
    - If the participant is randomized in the placebo group, the imputed value will be increased by l (e.g., 100%) times the treatment difference (obtained using the MAR approach)
    - k and l are independently determined
- Using the pre-specified analysis to analyze each set of the completed data along with the imputed data
- Obtaining the overall results using PROC MIANALYZE

#### 6.1.4.1.3 Tipping Point Analysis

As a sensitivity analysis to the multiple imputation analysis as described above in Section 6.1.4.1.2, a tipping point analysis will be performed in order to determine the robustness of the primary efficacy analysis.

A tipping point is the inflection point at which the inference under the MNAR assumption changes substantially (i.e., the specific k and l in MNAR imputation to reverse the statistical significance).

Since the missing data with the MNAR pattern are imputed bi-dimensionally, the tipping point curve will be determined for each of MNAR patterns by the following:

- Let l = 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 125%, 150%, 175%, 200%, 250%, 300%, 350%, 400%, 450%, and 500%.

  Note that:

    - l = 0% (imputed missing in placebo as MAR)
    - l = 100% (imputed missing in placebo as lixivaptan)

- For each l, search a tipping point k by implementing k=0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 125%, 150%, 175%, 200%, 250%, 300%, 350%, 400%, 450%, and 500%. Report the analysis results at the tipping point when a tipping point is found; otherwise, report the analysis results for k=500% when a tipping point is not reached.

  Note that:

    - k = 0% (imputed missing in lixivaptan as MAR)
    - k = 100% (imputed missing in lixivaptan as placebo)

- The tipping point curve consists of all the paired points of k and l when the tipping point is found.

### 6.1.5. Analysis Visit

Data summarized by study visit will be based on the nominal visit as reported on the eCRF.

Data collected at unscheduled visits will only be included in by-visit summaries if the data is missing at a visit and the date of an unscheduled visit is within the visit window by protocol. All data will be included in participant listings.

Visit 22 is referred to as the Week 52 visit in Part 1.

Visit 42 is referred to as the Week 104 visit in Part 2.

Early Termination (ET) visit is defined as the last visit either during the Double-blind, Randomized Treatment Period in Part 1 or during the Maintenance Treatment Period in Part 2 for participants who early terminated from study or early discontinued from study drug during that period in Part 1 or Part 2.

Visit 22/ET is defined as either Visit 22 or the ET visit in Part 1. Similarly, Visit 42/ET is defined as either Visit 42 or the ET visit in Part 2.

### 6.1.6. Pooling of Sites

Analyses will be based on data pooled across investigative sites.

### 6.1.7. Data Adjustments/Handling/Conventions

All collected data will be presented in listings. Data not subject to analysis according to this plan will not appear in any tables or graphs but will be included only in the data listings.

All *P* values will be displayed to four decimal places and rounded using standard scientific notation (e.g., 0.XXXX). If a *P* value less than 0.0001 occurs it will be shown in tables as < 0.0001.

Adverse events in the clinical database will be coded using the MedDRA version 24.0.

A treatment related AE is any AE with a relationship to the study drug of possibly related, probably related, definitely related or missing relationship to the study drug.

If partial dates occur, the convention for replacing missing dates for the purpose of statistical analysis is as follows: if just day is missing then the day assigned is the first day of the month or the date of first dose (if in the same month), whichever is later; if just month is missing then the month assigned is the month of the first dose, unless that results in a date before the first dose in which case the month after the first dose is used; and if both month and day are missing then the

month assigned is the month of the first dose and the day assigned is either the first day of the month or the first dose date, whichever is later.

If partial times occur, the convention is as follows: if the missing time occurs on the day of the first dose and both the hour and minute are missing then the time assigned is the time of the first dose, otherwise if both the hour and minute are missing and the date is not the date of first dose the time assigned is 12:00; if the date is the same as the date of the first dose and only hour is missing the hour assigned is 12 or the hour of first dose, whichever is later, and if the date is the same as the date of first dose and only the minute is missing the minute assigned is 30 or the minute of first dose, whichever is later. Otherwise the hour assigned is 12 if the hour is missing and the date is not the same as the date of first dose and the minute assigned is 30 is the the date is not the same as the date of first dose.

## 7. Study Participants and Demographics

Summaries for disposition of participants and demographic and baseline characteristics will be presented by treatment group (treatment cohort and overall in Part 2).

### 7.1. Disposition of Participants and Withdrawals

Disposition of participants in Part 1 will include, but not limited to, the following:

- The number of participants who
    - Screened
        - Failed screening
        - Reasons failed screening
    - Placebo Run-in/Placebo Run-in Safety Set
        - Not completed
        - Reasons not completed
    - Lixivaptan Titration/Lixivaptan Titration Safety Set
        - Not completed
        - Reasons not completed
- The number and percentage (percentage is calculated based on all the randomized participants) of participants who
    - Randomized/ITT
        - Treated/mITT/Randomized Safety Set
        - Completers/PEAS
            - On-treatment completers

        - Off-treatment completers
    - At least one on-treatment eGFR assessment/SEAS
    - Discontinued from study drug early and reasons
    - Not completed in Part 1 and reasons
    - In PPAS

Disposition of participants in Part 2 will include, but not limited to, the following:

- The number and percentage (percentage is calculated based on all the continuing participants in Part 2) of participants who
    - Continued in Part 2
        - Treated/P2SS
        - At least 1 off-treatment eGFR/P2EAS
        - Discontinued from study drug early and reasons
        - Not completed in Part 2 and reasons

Note that Lixivaptan Treated Safety Set for Parts 1 and 2 combined consists of the participants who are randomized and treated (Randomized Safety Set) in lixivaptan treatment group in Part 1 and the participants who are treated (P2SS) in Part 1/Part 2 - Placebo/Lixivaptan cohort in Part 2.

## 7.2. Protocol Deviations

Protocol deviations will be tracked, recorded, and reviewed prior to database lock, following the Protocol Deviation Guidance Plan for this study.

Protocol deviations will be classified as “Important” or “Not Important”.

Important protocol deviations include:

- Enrollment of a participant that did not meet inclusion criteria of the study/Enrollment of a participant that met exclusion criteria of the study.
- Missed safety assessments during Screening that are required for the thorough vetting of study inclusion/exclusion criteria are considered important deviations. They will be documented through failure of the respective Inclusion or Exclusion Criterion.
- Informed Consent Form (ICF) not obtained, or obtained after initiation of respective study procedures, important deviation from GCP consenting procedure, such as wrong version used, missing signature(s) and/ or date(s), ICF signed prior to IRB/IEC approval, other important deviation from GCP consenting process
- Prohibited Medications - Use of a prohibited concomitant medication
- Dosing Adherence – defined as taking less than 80% of the protocol-specified amount of study drug across the study

- Randomization error
- Mis-stratification of a participant within IRT system
- Drug dispensation without IRT assignment, incorrect drug kit administration, dose mistakes over inappropriate titration/re-titration
- Used the study drug with incorrect storage (temperature excursions, improper transport, security, etc.) which compromises Investigational Product safety or chemical activity
- MRI examination of kidneys during the screening, in Follow Up Period I, in Follow Up Period II not done/done with deviation from imaging technique described in Imaging manual
- Serum creatinine blood sample from Screening, Placebo Run-In, Follow-up Period I (or for participants who discontinue treatment prior to Visit 22, from the Follow-up Period following study drug treatment discontinuation) and Follow-up Period II not collected/ not sent to Central Lab for evaluation/ non evaluable, laboratory manual requirements not followed, serum creatinine result missing for any other reason, age/ sex/ race data missing or incomplete
- Liver Chemistry laboratory sample not collected/ not performed as required per protocol
- Repeat Liver Chemistry tests not performed within 48 hours of knowledge that aminotransferase levels >3 x ULN or total bilirubin >2 x ULN

The following deviation codes will be used to categorize/summarize the important protocol deviation above:

- Inclusion Criteria
- Exclusion Criteria
- Study Drug
- Assessment – Safety
- Assessment – Efficacy
- Lab Endpoint Data
- Visit Window
- Informed Consent
- Prohibited Medication
- Other

Study participants will be excluded from the PPAS if they meet any of the following criteria:

- Participant who did not meet all inclusion and exclusion criteria
- Placebo randomized participant who received lixivaptan treatment

- Lixivaptan randomized subject who received placebo treatment only throughout study
- Any participant who received protocol prohibited medications that could affect efficacy
- Participant with overall study treatment compliance of less than 80%

The protocol deviations that impact the analysis populations will be recorded once identified and periodically reviewed by the study team. The final decision regarding inclusion and exclusion of participants from the analysis populations will be determined during a (blinded) data review meeting before any unblinding occurs or database freeze/lock, with input from the Clinical, Biostatistics, Data Management and Clinical Operations team members and approval from the Sponsor.

Additionally, inclusion and exclusion criteria not met and reasons for screen failures will be listed.

### 7.3. Demographics and Other Baseline Characteristics

Summary statistics for age, sex, race, ethnicity, height, weight, and BMI will be presented by treatment group.

For the continuous variables, the number of non-missing values and the mean, standard deviation, median, minimum, and maximum will be tabulated.

For the categorical variables, the counts and proportions of each value will be tabulated.

These analyses will be conducted for the Randomized Safety Set and PEAS (Part 1) and P2SS and P2EAS (Part 2).

The number and percent of participants with a family history of ADPKD, the counts and percentages of each method used to diagnose ADPKD, the counts and percentages of each Mayo Clinic ADPKD Classification, the counts and percentages of each CKD stage, and the time since diagnosis will be presented by treatment group for Part 1. The number and percent of participants prior treated with tolvaptan and associated safety information will be summarized by treatment group for Part 1. The number and percent of participants reporting various medical histories, grouped by MedDRA system organ class and preferred term (coded using MedDRA v.24), will be tabulated by treatment group. This analysis will be conducted for the Randomized Safety Set and PEAS (Part 1) and P2SS and P2EAS (Part 2).

In addition, the above summary will be presented for all the participants who entered in the Lixivaptan Titration Period (Lixivaptan Treated Safety Set).

### 7.4. Exposure and Compliance

Exposure and compliance will be presented for Randomized Safety Set during the Double-Blind, Randomized Treatment Period (Part 1) and P2SS during the Maintenance Treatment Period (Part 2).

The duration of exposure will be presented in days and calculated as the date of last dose in treatment period minus the date of first dose in treatment period, plus one. Duration of exposure

and total dose taken (mg) will be summarized using descriptive statistics by treatment group (treatment cohort in Part 2).

Compliance to study drug is calculated by dividing the total capsules taken by the total capsules the participants were scheduled to take during the treatment period:

$$\text{Treatment Compliance (\%)} = \frac{total\ Capsules\ Taken}{Total\ Capsules\ Scheduled} \text{ x } 100$$

Dosing compliance will be summarized using descriptive statistics by treatment group (treatment cohort in Part 2). The number and percentages of participants who are < 80% compliant and ≥ 80% compliant overall in the study will be summarized.

## 8. Efficacy Analysis

### 8.1. Definition of Completer and Non-completer

Part 1 completer and non-completer will be defined as the following:

- On-treatment completer: all the randomized participants who take double-blind study drug in accordance with the protocol through Visit 22 (Week 52), and complete some or all of their required trial visits/assessments through Visit 22 and have at least one follow-up serum creatinine assessment during off-treatment Follow-up Period I.
- Off-treatment completer: all the randomized participants who permanently discontinue double-blind study drug (or never begin double-blind treatment) and complete some or all of their required study visits/assessments through Visit 22 and have at least one follow-up serum creatinine assessment during Follow-up Period I.
- Non-completer: all the randomized participants who take the study drug (or never take the study drug) but do not complete the Visit 22/ET visit or complete Visit 22/ET visit and do not have at least 1 follow up serum creatinine assessment during Follow-up Period I.

Part 2 completer and non-completer will be defined as the following:

- On-treatment completer: all the randomized participants who enter Part 2 of the study, take open-label study drug in accordance with the protocol through Visit 42 (Week 104), and complete some or all of their required study visits/assessments through Visit 42 and have at least one follow-up serum creatinine assessment during Follow-up Period II.
- Off-treatment completer: all the randomized participants who enter Part 2 of the study, but permanently discontinue open-label study drug (or never begin open-label treatment) and complete some or all of their required study visits/assessments through Visit 42 and have at least one follow-up serum creatinine assessment during Follow-up Period II.
- Non-completer: all the randomized participants who enter Part 2 of the study, take the study drug (or never take the study drug) but do not complete the Visit 42/ET visit or

complete Visit 42/ET visit but do not have at least 1 follow up serum creatinine assessment during Follow-up Period II.

## 8.2. Primary Efficacy Analysis (Part 1)

The primary efficacy endpoint is the annualized change in eGFR (2021 CKD-EPIcr_R), with restriction as defined below, for serum creatinine from baseline (mean of 3 eGFR determinations obtained during Screening and Placebo Run-in Periods, i.e., Visits 1a (or 1b, if required), Visit 2, and Visit 3) to final assessment (mean of 3 eGFR determinations obtained during Follow-up Period I after Week 52 for on-treatment completers or, for participants who discontinue study drug treatment prior to Week 52 [Visit 22], 8 to 28 days following study drug treatment discontinuation).

Restriction refers to all annualized changes in eGFR of participants that discontinued study drug treatment that are greater (or less) than the maximum (or minimum) of the annualized eGFR change based on off-treatment serum creatinine assessments of all on-treatment completers regardless of treatment groups. For such participants, the annualized change in eGFR will assume the maximum (or minimum) value of all on-treatment completers to reduce the impact of the outliers created by the annualized eGFR change in participants that early discontinued study drug treatment.

The primary estimand for the primary efficacy endpoint is the between-treatment group difference in the change from baseline in off-treatment eGFR if all the participants in the PEAS had tolerated and adhered to their treatment for 52 weeks. Potential bias due to randomized participants prematurely discontinuing from double-blind treatment will be handled by annualizing the changes from baseline by multiplying each participant's change from baseline by 365.25 days divided by the duration from the median of the collection dates of the baseline eGFR assessments to the median of the collection dates of the 3 eGFR assessments made during the post-treatment, follow-up period immediately (8-28 days) following study drug discontinuation.

An analysis of covariance (ANCOVA) model with fixed effects for treatment group (0=placebo, 1=lixivaptan) and the randomization stratification factors (CKD stage [CKD2 versus CKD3/4] and Mayo Clinic ADPKD Image Classification [1C, 1D, or 1E]) and the baseline eGFR as a covariate.

The following SAS code will be used for the primary efficacy analysis:

```
PROC GLM ALPHA =0.01;
        CLASS TREATMENT CKD_STAGE MAYO_CLASS;
        MODEL ANNUALIZED_CHANGE = TREATMENT BASELINE CKD_STAGE MAYO_CLASS/SOLUTION
        SS3 CLPARM;
        LSMEANS TREATMENT/STDERR
        ESTIMATE "Treatment Effect: Lixivaptan - Placebo" TREATMENT -1 1;
RUN;
```

This analysis assumes that data are missing at random. The stratification by CKD stage at the time of randomization will be based on the baseline eGFR creatinine equation in effect at the

time of baseline visits. However, all efficacy analyses of change in eGFR will be based on the CKD-EPIcr_R equation.

The primary efficacy analysis will be performed on the PEAS at the 1% level of significance ($p<0.01$).

### 8.2.1. Sensitivity Analyses of the Primary Efficacy Endpoint

#### 8.2.1.1. Primary Sensitivity Analyses

Sensitivity analyses on the primary efficacy endpoint will be performed using the same ANCOVA model as the primary efficacy analysis with fixed effects for treatment group and the randomization stratification factors (CKD stage [CKD2 versus CKD3/4] and Mayo Clinic ADPKD Image Classification [1C, 1D, or 1E]) and the baseline eGFR as a covariate.

Two sensitivity analyses will be performed on the mITT:

- The annualized change in eGFR (2021 CKD-EPIcr_R) without restriction from baseline to off treatment assessment after Week 52 (mean of 3 eGFR values obtained during off-treatment follow-up visits after Week 52); missing data are handled by multiple imputation:
  - MAR (Section 6.1.4.1.1)
- The annualized change in eGFR (2021 CKD-EPIcr_R) with restriction from baseline to final assessment (mean of 3 eGFR values obtained during Follow-up Period I after Week 52 for on-treatment completers or, for participants who discontinue study drug treatment prior to Visit 22, during the Follow-up Period 8 to 28 days following study drug treatment discontinuation); missing data are handled by multiple imputation:
  - MAR (Section 6.1.4.1.1)
  - MNAR and tipping point analysis (Sections 6.1.4.1.2 & 6.1.4.1.3)

Two sensitivity analyses will be performed on the PEAS:

- The annualized change in off-treatment eGFR (2021 CKD-EPIcr_R) without restriction from baseline to final assessment (mean of 3 eGFR values obtained during Follow-up Period I after Week 52 for on-treatment completers or, for participants who discontinue study drug treatment prior to Visit 22, during the Follow-up Period 8 to 28 days following study drug treatment discontinuation)
- The annualized change in eGFR (2009 CKD-EPI) with restriction from baseline to final assessment (mean of 3 eGFR values obtained during Follow-up Period I after Week 52 for on-treatment completers or, for participants who discontinue study drug treatment prior to Visit 22, during the Follow-up Period 8 to 28 days following study drug treatment discontinuation)

One sensitivity analysis will be performed on the on-treatment completers of the PEAS :

- The annualized change in eGFR (2021 CKD-EPIcr_R) without restriction from baseline to final assessment (mean of 3 eGFR values obtained during Follow-up Period I after Week 52 for on-treatment completers or, for participants who discontinue study drug

treatment prior to Visit 22, during the Follow-up Period 8 to 28 days following study drug treatment discontinuation)

One sensitivity analysis will be performed on the PPAS:

- The annualized change in eGFR (2021 CKD-EPIcr_R) with restriction from baseline to final assessment (mean of 3 eGFR values obtained during Follow-up Period I after Week 52 for on-treatment completers or, for participants who discontinue study drug treatment prior to Visit 22, during the Follow-up Period 8 to 28 days following study drug treatment discontinuation)

#### 8.2.1.2. Secondary Sensitivity Analyses

Sensitivity on the annualized change in eGFR (2021 CKD-EPIcr_R) with restriction from baseline to final assessment (mean of 3 eGFR values obtained during Follow-up Period I after Week 52 for on-treatment completers or, for participants who discontinue study drug treatment prior to Visit 22, during the Follow-up Period 8 to 28 days following study drug treatment discontinuation) using a weighted ANCOVA will be performed on the PEAS. The weighted analysis is based on the reciprocal of the estimated variance of the "estimated slopes" for participants based on individual off-treatment eGFR values obtained during Follow-up Period I after Week 52 for on-treatment completers or, for participants who discontinue study drug treatment prior to Visit 22, during the Follow-up Period 8 to 28 days following study drug treatment discontinuation). The detailed algorithm to derive the weight is provided in an Appendix.

The weighted ANCOVA model will include fixed effects for treatment group and the randomization stratification factors (CKD stage [CKD2 versus CKD3/4] and Mayo Clinic ADPKD Image Classification [1C, 1D, or 1E]) and the baseline eGFR as a covariate.

#### 8.2.1.3. Exploratory Sensitivity Analysis

The annualized change from baseline in eGFR theoretically contains natural disease progression due to no treatment such as a period for placebo run-in or a period between the last treatment and serum creatinine assessment. To explore the hypothetical treatment effect, a sensitivity analysis will also be performed on the PEAS based on all the changes from baseline in off-treatment eGFRs (2021 CKD-EPIcr_R) that are assessed after 8 days off treatment after randomization in Part 1. The linear mixed effect model with effects of pre-randomization time, off-treatment time, on-treatment time, treatment-by-on-treatment time, randomization stratification factors (CKD stage [CKD2 versus CKD3/4] and Mayo Clinic ADPKD Image Classification [1C, 1D, or 1E]), and eGFR baseline will be used to fit the eGFR data. Intercept, pre-randomization time are both a fixed effect and a random effect. An unstructured variance-covariance matrix is used for modeling.

```
PROC MIXED EMPIRICAL;
        CLASS TREATMENT CKD_STAGE MAYO_CLASS USUBJID;
        MODEL CHANGE = BASELINE PRIOR-TIME OFF-TIME ON-TIME ON-TIME*TREATMENT
        CKD_STAGE MAYO_CLASS;
```

```
    RANDOM INTERCEPT PRIOR-TIME/TYPE=UN SUB= USUBJID;
    ESTIMATE "Annualized Change from Baseline in eGFR in Lixivaptan Group" BASELINE 1 ON-TIME
        1 ON-TIME*TREATMENT 0 1;
    ESTIMATE " Annualized Change from Baseline in eGFR in Placebo Group " BASELINE 1 ON-TIME 1
        ON-TIME*TREATMENT 1 0;
    ESTIMATE "Treatment Difference in Annualized Slope of On-Treatment eGFR" ON-
        TIME*TREATMENT -1 1;
RUN;
```

where:

- Treatment group (TREATMENT) is coded as 0=Placebo and 1=lixivaptan
- Total time (years) for each off-treatment eGFR = elapsed time from baseline [median date of 3 baseline eGFRs obtained during Screening and Placebo Run-In Periods (Visits1a [1b, if required], Visit 2, and Visit 3) to time of off-treatment eGFR (≥ 8 days off treatment). Total time is divided into three portions: pre-randomization time (PRIOR-TIME), on-treatment time (ON-TIME) and off-treatment time (OFF-TIME).
- Pre-randomization time (years) = elapsed time (years) from baseline to date of randomization
- On-treatment time (years) = time (years) on treatment after randomization, number of treated days with randomized treatment from randomization to date of eGFR assessment/365.25.
- Off-treatment time (years) = Total time (years) for each off-treatment eGFR - Pre-randomization time (years) - On-treatment time (years).

## 8.3. Secondary Efficacy Analysis (Part 1)

If the primary efficacy endpoint is statistically significant at the 1% level of significance, then the secondary efficacy endpoints will be tested at the 1% level of significance in sequential order on the SEAS:

1. Annualized rate of change (slope) in eGFR based on all on-treatment eGFR (2021 CKD-EPIcr_R) determinations during the Double-blind, Randomized Treatment Period
2. Annualized percent change from baseline in htTKV as measured by MRI

Because the testing is sequential, the type I error rate of ≤ 0.01 is maintained. Failure at any stage in the sequence implies automatic failure at all subsequent stages. If the primary efficacy endpoint is not significant at the 1% level of significance then all other p-values will be presented as exploratory only.

If the primary efficacy endpoint is significant at the 1% level of significance, the first secondary efficacy endpoint will be tested. If the first secondary efficacy endpoint is significant at the 1% level of significance, the second efficacy endpoint will be tested. If the first secondary efficacy endpoint is not significant at the 1% level of significance, the second secondary efficacy endpoint is presented for exploratory purposes only.

### 8.3.1. Annualized Rate of Change (Slope) in eGFR

The first secondary efficacy endpoint is annualized rate of change (slope) in eGFR (2021 CKD-EPIcr_R) at on-treatment timepoints using the SEAS. This annualized rate of change (slope) in eGFR on treatment will be estimated by utilizing a mixed-effects model for repeated measures (MMRM) on the observed changes in eGFR from baseline (mean of the 3 eGFR values obtained during the Screening and Placebo Run-in Periods) to all visits with on-treatment eGFR values during the Double-Blind, Randomized Treatment Period visits, i.e., Visit 10 (Week 4) to Visit 22 (Week 52), while on study drug treatment. Any eGFR values obtained 1 day after study drug treatment during the Double-Blind, Randomized Treatment Period are excluded from the analysis. The MMRM will include treatment group, visit, treatment group-by-visit, and randomization stratification factors (CKD stage [CKD2 versus CKD3/4] and Mayo Clinic ADPKD Image Classification [1C, 1D, or 1E]) as fixed effects, baseline eGFR as a covariate, and the baseline-by-visit interaction. An unstructured variance-covariance matrix is assumed for the repeated measures.

Analysis of MMRM will be applied to the change from baseline in eGFR among the 13 on-treatment visits: visit 10 (week 4), visit 11 (week 8),visit 12 (week 12), visit 13 (week 16), visit 14 (week 20), visit 15 (week 24), visit 16 (week 28), visit 17 (week 32), visit 18 (week 36), visit 19 (week 40), visit 20 (week 44), visit 21 (week 48) and visit 22 (week 52). The median of the 13 visits is the 7$^{th}$ visit, i.e., Visit 16 (Week 28).

To estimate the slope in eGFR on treatment, the contrast coefficients of the linear trend for visits will become -6, -5, -4, -3, -2, -1, 0, 1, 2, 3, 4, 5, 6. Under assumptions that the annualized slope and acute hemodynamic effect are constant over time, this contrast can be used to estimate the slope (note that the acute hemodynamic effect is eliminated by the contrast). The resulted estimate corresponding to those coefficients will be slope (per week) multiplied by 728 (-6*4 -5*8 -4*12 -3*16 -2*20 -1*24 +0*28 +1*32 +2*36 +3*40 +4*44 +5*48 +6*52=728) or annualized slope multiplied by 14 (728/52=14).

Thus, the coefficients of the linear trend contrast of these 13 visits to estimate the annualized slope are -6/14, -5/14, -4/14, -3/14, -2/14, -1/14, 0/14, 1/14, 2/14, 3/14, 4/14, 5/14, 6/14.

Example SAS code is shown below:

```
PROC MIXED EMPIRICAL;
        CLASS TREATMENT VISIT CKD_STAGE MAYO_CLASS USUBJID;
        MODEL CHANGE = TREATMENT BASELINE VISIT VISIT*TREATMENT BASELINE*VISIT CKD_STAGE
        MAYO_CLASS /SOLUTION;
        REPEATED VISIT/TYPE=UN SUB= USUBJID;
        ESTIMATE “Lixivaptan Annualized Slope" VISIT -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6
            VISIT*TREATMENT 0 0 0 0 0 0 0 0 0 0 0 0 0 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6/DIVISOR=14;
        ESTIMATE “Placebo Annualized Slope" VISIT -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6
            VISIT*TREATMENT -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 0 0 0 0 0 0 0 0 0 0 0 0 0 /DIVISOR=14;
        ESTIMATE “Treatment Difference in Annualized Slope"
        VISIT*TREATMENT 6 5 4 3 2 1 0 -1 -2 -3 -4 -5 -6  -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6/DIVISOR=14;
RUN;
```

A linear contrast of the treatment slope differences for visits will be used for this analysis.

#### 8.3.1.1. Sensitivity Analysis of Annualized Rate of Change (Slope) in eGFR

Two sensitivity analyses will be performed on the annualized rate of change (slope) in eGFR (2021 CKD-EPIcr_R) using the PPAS and SEAS.

The sensitivity analysis using the PPAS is the same as the primary analysis for the annualized rate of change (slope) in eGFR on treatment (Section 8.3.1) but using PPAS.

The sensitivity analysis using the SEAS will use the change from baseline in eGFR values assessed at the end of lixivaptan titration, on-treatment eGFR values assessed during the Double-blind, Randomized Treatment visits and off-treatment eGFR values assessed during the Follow-up Period I visits. Note that on-treatment eGFR values obtained 1 days after the latest dose and off-treatment eGFR values obtained prior to 8 days after the last dose will be excluded in the analysis. An acute hemodynamic effect will be considered for eGFR at the end of lixivaptan titration for all the participants and for all eGFRs on treatment for the lixivaptan participants. All other eGFRs will not be considered for the hemodynamic effect. The acute hemodynamic effect indicator/flag (ACUHEMO) will be included in the model (1=Yes) or (0=No).

The linear mixed effect model with effects of time (as a continuous variable in years), treatment, time-by-treatment interaction, acute hemodynamic effect, baseline (of the primary endpoint), and randomization stratification factors (CKD stage [CKD2 versus CKD3/4] and Mayo Clinic ADPKD Image Classification [1C, 1D, or 1E]) will be used to fit the GFR estimates, in which the intercept and time are both a fixed effect and a random effect. An unstructured variance-covariance matrix is assumed for the modeling.

Example SAS code is shown below:

```
PROC MIXED EMPIRICAL;
        CLASS TREATMENT ACUHEMO CKD_STAGE MAYO_CLASS USUBJID;
        MODEL CHG_eGFR = TREATMENT ACUHEMO BASELINE TIME TIME*TREATMENT CKD_STAGE
        MAYO_CLASS /SOLUTION;
        RANDOM INTERCEPT TIME/TYPE=UN SUB= USUBJID;
        ESTIMATE "Annualized Slope of On-Treatment eGFR" TIME*TREATMENT -1 1;
RUN;
```

The estimate of the treatment group by visit interaction will be used for this analysis.

### 8.3.2. Annualized Percent Change from Baseline in htTKV

The 2nd secondary efficacy endpoint is the annualized percent change in htTKV from baseline to 28 days after the last dose of the study drug, i.e., Visit 25 during the Follow-up Period I after Week 52 for on-treatment completers or, for participants who discontinue study drug treatment prior to Week 52 [Visit 22], during the Follow-up Period 28 days following study drug treatment discontinuation. Baseline htTKV is the one obtained at Screening (Visit 1a).

Missing data will be handled by multiple imputation using an ANCOVA with factors of treatment, stratification factors and sex with age, time on treatment (date of last dose – date of first dose + 1), and baseline as covariates.

In order to reduce the variability in the time of post-baseline MRI acquisition, elapsed time from baseline to post-baseline MRI will be treated as a continuous variable, expressed as years from date of baseline MRI acquisition to date of off-treatment MRI acquisition visit (date of MRI visit – date of Baseline MRI + 1,). In addition, in order to reduce heterogeneity in variance and achieve linearity over time, log10 transformation will be applied to the htTKV data.

An ANCOVA will be used to analyze the change from baseline in log-transformed htTKV data on the PEAS to estimate annualized percent change in htTKV by time. The model will include fixed effects for the randomization stratification factors (CKD stage [CKD2 versus CKD3/4] and Mayo Clinic ADPKD Image Classification [1C, 1D, or 1E]) with baseline htTKV, and treatment group-by-time interaction as a covariate.

Example SAS code is shown below (treatment group is coded as 0=placebo and 1=lixivaptan):

```
PROC GLM ALPHA=0.01;
        CLASS TREATMENT CKD_STAGE MAYO_CLASS;
        MODEL CHG_LOGTKV = LOG_BASETKV TIME*TREATMENT CKD_STAGE MAYO_CLASS /
        SOLUTION SS3 CLPARM;
        ESTIMATE "Annualized Change from Baseline for Lixivaptan (Log-scale)" INTERCEPT 1
            LOG_BASETKV mean TIME*TREATMENT 0 1 CKD_STAGE 0.5 0.5 MAYO_CLASS 0.3333 0.3333
            0.3333;
        ESTIMATE "Annualized Change from Baseline for Placebo (Log-scale)" INTERCEPT 1
            LOG_BASETKV mean TIME*TREATMENT 1 0 CKD_STAGE 0.5 0.5 MAYO_CLASS 0.3333 0.3333
            0.3333;
        ESTIMATE "Treatment Difference in Annualized Change from Baseline (Log-scale)"
            TIME*TREATMENT -1 1;
RUN;
```

Where “mean” is the overall mean of log-transformed baseline htTKV values.

A significance level of 0.01 (two-sided) will be used to declare statistical significance of treatment effect at the final analysis. Antilog (with a base of 10) of the treatment effect and 99% confidence intervals (99% CIs) derived from the model (in a log10 scale) provide a ratio of geometric means of the relative change (ratio) from baseline in htTKV (i.e., 100% plus annualized percent change).

#### 8.3.2.1. Sensitivity Analysis of Annualized Percent Change from Baseline in htTKV

Two sensitivity analyses will be performed on the annualized percent change from baseline in htTKV using the PPAS and SEAS.

The sensitivity analysis using the PPAS is the same as the primary analysis for the annualized percent change from baseline in htTKV (Section 8.3.2) but using PPAS.

The sensitivity analysis will be performed for the annualized percent change from baseline to off-treatment follow-up Visit 25 after Week 52 in htTKV using the SEAS. Missing data will be

handled by multiple imputation using an ANCOVA with factors of treatment, stratification factors and sex with age, time on treatment (date of last dose – date of first dose + 1), and baseline as covariates. An ANCOVA model with fixed effects of treatment (0=placebo, 1=lixivaptan) and randomization stratification factors, and baseline, (elapsed) time, treatment-by-time as a covariate will be used to fit the log10-transformed htTKV data.

## 8.4. Subgroup Efficacy Analysis (Part 1)

Descriptive statistics will be presented for the primary and secondary efficacy analyses for the following subgroups:

- Age (Tertiles)
- Sex (Male, Female)
- Race (White, non-White)
- Region (US, non-US)
- Baseline eGFR (≤median, >median)
- CKD stage (CKD2, CKD3/4)
- Mayo Clinic ADPKD Image Classification (1C, 1D, and 1E)
- Baseline htTKV (≤median, >median)

## 8.5. Health Outcomes Analyses (Part 1)

The endpoints from ADPKD-Impact Scale (ADPKD-IS), ADPKD-Pain and Impact Scale (ADPKD-PDS), ADPKD-Urinary Impact Scale (ADPKD-UIS) Patient Reported Outcomes (PROs) will be evaluated separately by domain (see Section 4.1.2.2) for the PEAS. Missing data are not imputed prior to the analysis.

An analysis utilizing a MMRM will be performed on the changes in each domain score from baseline (visit 2) to all Double-Blind, Randomized Treatment Period visits up to Follow up Period I (i.e., Visits 12, 15, 18, 22 and 25). The MMRM will include treatment group, visit, treatment group-by-visit, and randomization stratification factors (CKD stage [CKD2 versus CKD3/4] and Mayo Clinic ADPKD Image Classification [1C, 1D, or 1E]) as fixed effects, baseline as a covariate, and the baseline-by-visit interaction. An unstructured variance-covariance matrix is assumed for the repeated measures. The treatment difference in change from baseline at each post-baseline visit will be produced from the analysis.

Example SAS code is shown below:

```
PROC MIXED EMPIRICAL ALPHA=0.01;
        CLASS TREATMENT VISIT CKD_STAGE MAYO_CLASS USUBJID;
        MODEL CHANGE = TREATMENT BASELINE VISIT VISIT*TREATMENT BASELINE*VISIT CKD_STAGE
        MAYO_CLASS /SOLUTION CL;
        REPEATED VISIT/TYPE=UN SUB= USUBJID;
        LSMEANS VISIT*TREATMENT/ALPHA=0.01 PDIFF CL;
        ESTIMATE  'Lixivaptan – Placebo Change from Baseline at Visit 12'
                TREATMENT -1 1 VISIT*TREATMENT -1 0 0 0 0 1 0 0 0 0;
```

```
        ESTIMATE  'Lixivaptan – Placebo Change from Baseline at Visit 15'
                  TREATMENT -1 1 VISIT*TREATMENT 0 -1 0 0 0 0 1 0 0 0;
        …
RUN;
```

In addition, descriptive summary (n, mean, standard deviation, median, minimum, and maximum) will be used to summarize the change from baseline in each domain score at the Lixivaptan Titration Period visits (Visits 4-9) for all the participants in the Lixivaptan Treated Safety Set.

## 8.6. Exploratory Efficacy Analyses (Part 1)

### 8.6.1. Annualized Change in eGFR (2021 CKD-EPIcr-cys_R)

This analysis is based on the annualized change in eGFR (2021 CKD-EPIcr-cys_R) with restriction (defined in Section 8.2 Primary Efficacy Analysis except that in this analysis the eGFR values will be calculated from 2021 CKD-EPIcr-cys_R) on the PEAS. An ANCOVA model with fixed effects for treatment group (0=placebo, 1=lixivaptan) and the randomization stratification factors (CKD stage [CKD2 versus CKD3/4] and Mayo Clinic ADPKD Image Classification [1C, 1D, or 1E]) and the baseline eGFR as a covariate.

SAS code from the primary efficacy endpoint analysis can be used (Section 8.2).

This analysis assumes that data are missing at random.

### 8.6.2. Annualized Percent Change in Height-adjusted Liver Volume (htLV)

This analysis is based on the annualized percent change in htLV from baseline to 28 days after the last dose of the study drug, i.e., Visit 25 during the Follow-up Period I after Week 52 for on-treatment completers or, for participants who discontinue study drug treatment prior to Week 52 [Visit 22], during the Follow-up Period 28 days following study drug treatment discontinuation. Baseline htLV is the one obtained at Screening (Visit 1a). Missing data will not be imputed for this analysis.

In order to reduce the variability in the time of post-baseline MRI acquisition, elapsed time from baseline to post-baseline MRI will be treated as a continuous variable, expressed as years from date of Baseline MRI acquisition to date of MRI acquisition visit (date of MRI visit – date of Baseline MRI + 1). In addition, in order to reduce heterogeneity in variance and achieve linearity over time, log10 transformation will be applied to the htLV data.

An ANCOVA will be used to analyze the log-transformed htLV data on the PEAS to estimate annualized percent change in htLV by time. The model will include fixed effects for treatment group (0=placebo, 1=lixivaptan), the randomization stratification factors (CKD stage [CKD2 versus CKD3/4] and Mayo Clinic ADPKD Image Classification [1C, 1D, or 1E]), baseline htLV, (elapsed) time, and treatment group-by-time interaction as a covariate.

Example SAS code in Section 8.3.2 for the htTKV analysis can be used to analyze the htLV when the log-transformed htTKV data are replaced by the log-transformed htLV data.

A significance level of 0.01 (two-sided) will be used to declare statistical significance of treatment effect at the final analysis. Antilog (with a base of 10) of the treatment effect and 99% confidence intervals (99% CIs) derived from the model (in a log10 scale) provide a ratio of geometric means of the relative change (ratio) from baseline in htLV (i.e., 100% plus annualized percent change).

### 8.6.3. Urine Osmolality

Descriptive statistics for the change from baseline (Visit 2) in morning spot urine osmolality will be presented by treatment group for the PEAS. The number (%) of participants who show suppressed urine osmolality in each treatment group, defined by 2 levels of spot urine osmolality ≤250 mOsm/kg and ≤300 mOsm/kg will be summarized descriptively at each time point.

## 8.7. Key Comparison Endpoint Analysis (Part 2)

All the eGFR assessments for this analysis are calculated from the 2021 CKD-EPI equation (2021 CKD-EPIcr_R).

Descriptive statistics will be presented by treatment cohort for the key comparison analysis of annualized change in eGFR from baseline (mean of 3 eGFR values obtained at off-treatment visits after Week 52 during Follow-up Period I) to final assessment (mean of 3 eGFR values obtained during Follow-up Period II after Week 104 for on-treatment completers or, for participants who discontinue study drug treatment prior to Week 104 [Visit 42], 8 to 28 days following study drug treatment discontinuation) for the P2EAS.

To assess durability and continuation of treatment effect observed in Part 1, the mean annualized change from baseline by treatment cohort for Part 2 will be presented along with Part 1 results for the participants in that treatment cohort.

In addition, descriptive statistics for the annualized change from baseline in the eGFR to final assessment will be presented for all the participants in P2EAS (overall).

## 8.8. Sensitivity Analysis of the Key Comparison Analysis (Part 2)

All the eGFR assessments for this analysis are calculated from the 2021 CKD-EPI equation (2021 CKD-EPIcr_R).

Descriptive statistics will be performed for all available annualized changes from baseline (the mean of the 3 off-treatment eGFR values obtained after Week 52 in Part 1) to the mean of the 3 off-treatment eGFRs after Week 104 for the P2EAS. Descriptive statistics will be presented by treatment cohort and overall along with Part 1 results for the same participants in each treatment cohort.

## 8.9. Other Comparison Analyses (Part 2)

### 8.9.1. Annualized Rate of Change (Slope) in eGFR

All the eGFR assessments for this analysis are calculated from the 2021 CKD-EPI equation (2021 CKD-EPIcr_R).

All the observed changes from baseline (mean of the 3 eGFR values obtained at off-treatment visits after Week 52 in Part 1) to all visits with on-treatment eGFR values during the Maintenance Treatment Period visits, i.e., Visit 30 (Week 56) up to Visit 42 (Week 104), while on study drug treatment in the P2EAS will be utilized for determining the annualized rate of change (slope) in eGFR. Any eGFR values obtained after 1 or more days off of study drug treatment during the Maintenance Treatment Period are excluded from the analysis.

To determine the annualized rate of change (slope) in eGFR, the average of observed changes from baseline at each visit by treatment cohort and overall will be calculated. The annualized rate of change (slope) in the eGFR will be calculated by:

The annualized rate of change (slope) in eGFR = (-6 x mean change at Visit 30 -5 x mean change at Visit 31 -4 x mean change at Visit 32 -3 x mean change at Visit 33 -2 x mean change at Visit 34 -1 x mean change at Visit 35 -0 x mean change at Visit 36 +1 x mean change at Visit 37 +2 x mean change at Visit 38 +3 x mean change at Visit 39 + 4 x mean change at Visit 40 + 5 x mean change at Visit 41 + 6 x mean change at Visit 42)/14.

The annualized rate of change (slope) from baseline in eGFR in Part 2 will be presented by treatment cohort and overall for participants in the P2EAS along with the annualized rate of change (slope) from baseline at Part 1 for the participants in each treatment cohort.

### 8.9.2. Annualized Percent Change from Baseline in htTKV

The annualized percent change from baseline in htTKV is estimated by the annualized change in log10-transformed htTKV, annualized by the elapsed time (years) between two dates of MRI taken, from baseline to 28 days after the last dose of the study drug, i.e., Visit 45 during the Follow-up Period II after Week 104 for on-treatment completers or, for participants who discontinue study drug treatment prior to Week 104 [Visit 42], during the Follow-up Period 28 days following study drug treatment discontinuation. Baseline htTKV is the one obtained at off treatment follow-up Visit 25 after Week 52 in Part 1. The annualized change from baseline in log10 transformed htTKV will be summarized by treatment cohort for all participants in the P2EAS. Anti-log of the mean annualized change from baseline in log10 transformed htTKV will provide an estimate of the geometric mean of the related growth of htTKV to baseline htTKV (i.e., 100% plus annualized percent change).

The annualized percent change from baseline in htTKV in Part 2 will be presented by treatment cohort and overall for participants in the P2EAS along with the annualized percent change from baseline at Part 1 for the participants in each treatment cohort.

The above comparison will also be performed by replacing the log10-transformed htTKV value at 28 days after the last dose of the study drug by the log10-transformed htTKV value at 28 days after Week 104.

## 8.10. Health Outcome Analyses (Part 2)

The descriptive statistics for change from baseline in each domain score of three patient reported health questionnaires are presented at each evaluated visit in Part 2 by treatment cohort and overall for all the participants in the P2EAS. Baseline score is defined as the domain score from Follow-up period I (Visit 25) after Week 52 in Part 1. In order to compare the results from Part 2 to those in Part 1, the descriptive statistics for change from baseline in each domain score of three patient reported health questionnaires are presented at each corresponding visit at Part 1 by treatment cohort.

## 8.11. Exploratory Comparison Analyses (Part 2)

### 8.11.1. Annualized Percent Change in htLV

The derivation of the annualized percent change from baseline in htLV in Part 2 and presentation of the analysis results will be the same as the analysis of htTKV described in Section 8.9.2 except that the htTKV data will be replaced by the htLV data.

### 8.11.2. Urine Osmolality

Descriptive statistics for the change from baseline (Visit 2) in morning spot urine osmolality will be presented for the participants in the P2EAS by treatment cohort and overall at each scheduled time point. The number (%) of participants who show suppressed urine osmolality by treatment cohort and overall, defined by two levels of spot urine osmolality ≤250 mOsm/kg and ≤300 mOsm/kg will be summarized descriptively at each time point.

### 8.11.3. Annualized Change from Baseline in eGFR (2021 CKD-EPIcr-cys_R)

The analysis of the annualized change in eGFR analysis will be the same as described in Section 8.7, except that the eGFR values used will be determined using the 2021 CKD-EPIcr-cys_R equation for serum creatinine and cystatin C.

## 8.12. Additional Analyses (Part 2)

Additional exploratory analyses may be conducted to address any issues caused by potentially different treatment/exposure patterns between Part 1 and Part 2 for the participants in the P2EAS.

# 9. Safety and Tolerability Analysis

Safety will be evaluated from reported AEs, changes in clinical laboratory values, changes in

vital signs, and electrocardiogram (ECG results).

A Safety Analyses will be performed for the following:

- Single-Blind Placebo Run-in Period for the Placebo Run-in Safety Set (Part 1)
- Lixivaptan Titration Period for the Lixivaptan Treated Safety Set (Part 1)
- Double-blind, Randomized Treatment and Follow-up I Periods for the Randomized Safety Set (Part 1)
- Maintenance Treatment and Follow-up II Periods for the P2SS (Part 2)
- Double-blind, Randomized Treatment and Follow-up I Periods and Maintenance Treatment and Follow-up II Periods for the LTSS (Part 1 and Part 2 combined)

The tolerability will be summarized by:

- Incidence of participants with the maximum dose level taken by the final tolerated dose level determined for the double-blind treatment period during Lixivaptan Titration Period
- Count and percentage of participants up-titrated, down-titrated, remaining unchanged, or discontinued by the dose levels by visit (or time after titration initiation) during Lixivaptan Titration Period
- Count and percentage of participants by dose level by Visit (or time after randomization) during the double-blind treatment period in Part 1 by treatment group
- Count and percentage of participants by dose level by Visit (or time after initiation of maintenance period) during the maintenance period in Part 2 by treatment group

## 9.1. Key Liver Safety Analysis

The incidence of participants who develop serum ALT levels >3 x ULN will be summarized by treatment group for Part 1 and by treatment cohort for Part 2. For Part 1, Fisher's exact test will be used to test if the incidence rate in the lixivaptan group is different from that in the placebo group.

In addition, an eDISH plot will be presented for Part 1.

## 9.2. Adverse Events

An AE is defined as any untoward medical occurrence in a participant enrolled into this study regardless of its causal relationship to study drug.

A treatment-emergent AE (TEAE) is defined as any event not present before exposure to study drug or any event already present that worsens in either intensity or frequency after exposure to study drug.

A serious adverse event (SAE) is defined as any event that:

- results in death,
- is immediately life threatening,

- requires in-participant hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity, or
- is a congenital anomaly/birth defect.
- Important medical events that may not result in death, be life threatening, or require hospitalization may be considered SAEs when, based upon appropriate medical judgment, they may jeopardize the participant or may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Hospitalization does not include admissions for treatment of a pre-existing condition, including surgery, not associated with a new AE; respite care; social reasons; administrative reasons; skilled nursing care; rehabilitation; hospice; or same-day surgery.

All AEs, TEAEs, and SAEs will be coded using the MedDRA dictionary (v24.0 March 2021).

The causality to treatment will be grouped into three categories for the summaries: not related (reported as either "not related" or "unlikely related"), possibly related (reported as "possible related"), and related (reported as either "probably related", "definitely related", or missing causality)

For Part 1 and Part 2 the number and percent of participants reporting TEAEs, grouped by MedDRA system organ class and preferred term, will be tabulated by treatment group (treatment cohort for Part 2) and causality to treatment (total, not related, possibly related, or related). In the case of multiple occurrences of the same TEAE within the same participant, the most related one will be counted for the participant. This summary will be repeated for the common TEAEs defined as the incidence rate of ≥ 3% at the lixivaptan group in Part 1.

The number and percent of participants reporting TEAEs, grouped by MedDRA system organ class and preferred term, will be tabulated by severity and treatment group (treatment cohort for Part 2) for all the TEAEs. In the case of multiple occurrences of the same TEAE within the same participant, the most severe one will be counted.

Adverse event summaries, grouping by MedDRA system organ class and preferred term, will be repeated by severity and treatment group (treatment cohort for Part 2) for treatment related TEAEs including possibly related TEAEs.

An overall summary of TEAEs will be presented by treatment group. The overall summary will include the total number and percent of participants reporting:

- Any TEAEs
- Any treatment-related TEAE
- Any severe TEAE
- Any severe treatment-related TEAE
- Any serious TEAE

- Any serious treatment-related TEAE
- Any TEAE of special interest (TEAESI)
- Any TEAE leading to permanent study drug discontinuation
- TEAEs resulting in death

In the AE data listings, all AEs will be displayed to include whether the adverse events are SAEs, AESIs, date of AEs, relationship to study drug, severity, and action taken. AEs that are not treatment-emergent will be flagged.

### 9.2.1. Adverse Events Leading to Study Drug Discontinuation

For Parts 1 and 2, a summary of incidence rates (frequencies and percentages) of the participants with TEAEs leading to study drug discontinuation, by treatment group (treatment cohort for Part 2), SOC, and preferred term will be prepared. No inferential statistical tests will be performed.

A data listing of AEs leading to study drug discontinuation will also be provided, displaying details of the event(s) captured on the CRF.

### 9.2.2. Deaths and Serious Adverse Events

Any deaths that occur during the study will be listed.

Serious adverse events will be listed and also tabulated by system organ class and preferred term and presented by treatment group (treatment cohort for Part 2).

### 9.2.3. Other Significant Adverse Events

Adverse events of special interest (AESI) will be as collected on the CRF as a special interest event involving liver.

## 9.3. Clinical Laboratory Evaluations

For Part 1, descriptive statistics (n, mean, standard deviation [SD], median, minimum, and maximum) for clinical laboratory data and changes from baseline at each post-baseline time point will be presented by treatment group (treatment cohort for Part 2).

The baseline value is the last value observed prior to first administration of study drug and any information obtained after first administration of study drug is regarded as post-baseline information. The change-from-baseline variables will be calculated as the post-baseline value minus the value at baseline. Change from baseline on continuous data will be summarized using descriptive statistics at each post-baseline time point by treatment group. For categorical data, change from baseline will be summarized using frequency and proportion at each post-baseline timepoint by treatment group (treatment cohort for Part 2).

For all continuous clinical laboratory variables, a shift table comparing the baseline value (normal, low, and high) to the last observation on treatment will be presented by treatment group (treatment cohort for Part 2).

For urinalysis, a shift table (using number of participants with results of negative, trace, or positive) comparing the baseline value to the maximum value will be presented by treatment group (treatment cohort for Part 2).

For Part 2, descriptive statistics (n, mean, standard deviation [SD], median, minimum, and maximum) for clinical laboratory data and changes from baseline (last value observed during Follow-up Period I in Part 1) at each post-baseline time point will be presented for all participants for each scheduled time point.

For all continuous clinical laboratory variables in Part 2, a shift table comparing the baseline (last value observed during Follow-up Period I in Part 1) value (normal, low, and high) to the last observation on treatment will be presented for all participants.

For urinalysis in Part 2, a shift table comparing the baseline (last value observed during Follow-up Period I in Part 1) value to the maximum value will be presented for all participants (using number of participants with results of negative, trace, or positive).

Potentially clinically significant results of clinical laboratory evaluations identified using prospectively defined criteria (Table 3) will also be summarized descriptively by treatment group (treatment cohort for Part 2).

**Table 3: Potentially Clinically Important Criteria (PCS): Laboratory**

| Lab Test Category | PCS Criteria |
|---|---|
| Chemistry | Sodium: <132 mmol/L |
| | Sodium: >149 mmol/L |
| | Potassium:<2.5 mmol/L |
| | Potassium: >6.5 mmol/L |
| | Calcium: <1.62 mmol/L |
| | Calcium:>3.49 mmol/L |
| | Uric acid:>715 µmol/L |
| | Albumin: <25 g/L |
| | eGFR: Decrease from baseline of ≥25% |
| | Glucose: ≤2.5 mmol/L |
| | Glucose:≥14.0 mmol/L |
| Hematology | Leukocytes: ≤ 2.5 x 109/L |
| | Leukocytes: ≥ 15.0 x 109/L |
| | Neutrophils: ≤ 1.5 x 109/L |
| | Platelets: ≤ 50.0 x 109/L |
| | Hemoglobin: Decrease from baseline of ≥ 20 g/L or ≤ 80 g/L (absolute value) |
| | Hematocrit: Decrease from baseline of ≥ 10% (absolute change) or ≤ 24% (absolute value) |
| Urinalysis | Glucose: ≥2+ |
| | RBC/HPF: >10 |
| | Protein: >2+ |
| | Ketones: > 2 + |
| | Leukocytes: >10 |

For Part 1 and Part 2, individual data listings of clinical laboratory results will be presented for each participant. Values that meet laboratory PCS will be flagged in the clinical laboratory listings (see Table 3).

### 9.3.1. Special Liver Events

For Part 1 and 2, summarizes using incidence (rates) for participants with special liver events will be presented for all participants by treatment group (treatment cohort for Part 2) for the following:

- ALT > 5 x, 10 x, or 20 x ULN
- AST > 3 x, 5 x, 10 x, or 20 x ULN
- ALT or AST > 3 x, 5 x, 10 x, or 20 x ULN
- Total Bilirubin > 2 x ULN
- Alkaline phosphatase >1.5 x ULN
- Hy's Law (ALT > 3 x ULN and Total Bilirubin > 2 x ULN)
- Elevation of aminotransferase in temporal association with nausea, vomiting, anorexia, abdominal pain, or fatigue
- Possible liver-related deaths and liver-related treatment discontinuations

A time to first event analysis will also be presented using Kaplan-Meier product-limit estimates by treatment group for ALT > 3 x, AST > 3 x, ALT or AST > 3 x, and Total Bilirubin > 2 x ULN.

## 9.4. Vital Signs

Descriptive summaries of actual values and changes from baseline (last value observed prior to the first dose of study drug) will be calculated by treatment group (treatment cohort for Part 2) for systolic blood pressure, diastolic blood pressure, heart rate, respiratory rate, and oral body temperature at each post-baseline timepoint.

In addition, potentially clinically significant results of vital signs identified using prospectively defined criteria (Table 4) will also be summarized descriptively.

**Table 4: Potentially Clinically Important Criteria (PCS): Vital Signs**

| |
|---|
| Heart Rate Decrease of ≥20 beats/min or ≤ 40 beats/min |
| Heart Rate Increase of ≥20 beats/min or ≥ 120 beats/min |
| Diastolic Blood Pressure Decrease of ≥ 20 mmHg or ≤ 50 mmHg |
| Diastolic Blood Pressure Increase of ≥ 20 mmHg or ≥ 105 mmHg |
| Systolic Blood Pressure Decrease of ≥ 25 mmHg or ≤ 90 mmHg |
| Systolic Blood Pressure Increase of ≥ 25 mmHg or ≥ 180 mmHg |
| Weight Decrease of ≥ 10% |
| Weight Increase of ≥ 10% |

Individual data listings of vital sign results will be presented for each participant. Values that meet vital sign PCS will be flagged in the listings (see Table 4).

## 9.5. Electrocardiograms

For Part 1 and Part 2, observed values and changes from baseline (last value observed prior to the first dose of study drug) at each post-baseline timepoint for continuous ECG parameters including heart rate, ventricular rate, PR interval, QRS duration, QT interval (uncorrected), and QTcF interval will be summarized by treatment group (treatment cohort for Part 2) using descriptive statistics (n, mean, SD, median, minimum, and maximum).

Overall evaluation of ECG will be summarized using frequency counts and percentage of participants as normal or abnormal, and the relevance of the abnormality will be summarized by CS or NCS. QTcF will be summarized using frequency and percentage for the following categories:

Participants with the highest QTcF post-baseline value:

- < 450 msec
- 450-480 msec
- ≥ 480 msec

Participants with the highest QTcF change value:

- < 30 msec
- 30-60 msec
- > 60 msec

Potentially clinically important criteria (Sponsor defined):

- ≥ 450 msec

In addition, a summary shift table comparing baseline interpretation (normal, abnormal - NCS, abnormal - CS) to the Investigator interpretation at each time point will also be presented.

ECG results will be listed by treatment group (treatment cohort for Part 2) for all participants. Additionally, a listing will be provided for Investigator-identified ECG abnormalities.

## 9.6. Lixivaptan Treatment Safety Analysis

The lixivaptan treatment safety will summarize the following safety information occurred post the first dose of lixivaptan treatment after randomization during the study based on the LTSS by treatment cohort and overall:

- Incidence of special liver events (ALT > 3 x, AST > 3 x, ALT or AST > 3 x, and Total Bilirubin > 2 x ULN)
- Kaplan-Meier product-limit curve for special liver events
- Summaries of TEAEs (see Section 9.2)

- Overall summary of TEAEs
- TEAEs by preferred term and causality
- TEAEs by preferred term and severity
- Treatment-related TEAEs by preferred term and severity

### 9.7. Body Height, Weight and BMI

Body height measured at Screening in Part 1 will be used to calculate the BMI at each time point in Part 1 and Part 2.

For Part 1, changes in body weight and BMI from baseline (last value observed prior to the first dose of study drug) to end of the Double-blind, Randomized Period and to end of Follow-up Period I (last value observed) will be summarized by treatment group using descriptive statistics (n, mean, SD, median, minimum, and maximum).

For Part 2, changes in body weight and BMI from baseline (last value observed during Follow-up Period I in Part 1) to end of Maintenance Treatment Period and to end of Follow-up Period II (last value observed) will be summarized for all participants using descriptive statistics (n, mean, SD, median, minimum, and maximum).

For Part 1 and Part 2, the change from baseline is defined as the post-baseline value minus the baseline value. There will not be any imputation for missing values. Body height, weight and BMI data will be listed individually for all participants.

### 9.8. Concomitant Medication

For Part 1 and 2, prior and concomitant medications will be summarized descriptively by treatment group (treatment cohort for Part 2) using counts and percentages.

Prior medications will be presented separately from concomitant medications. Medications that started before first dose of study drug will be considered prior medications whether or not they were stopped before first dose of study drug. Any medications continuing or starting after study drug will be considered to be concomitant. If a medication starts before study drug and continues after study drug it will be considered both prior and concomitant.

Medications will be coded using World Health Organization Drug Dictionary (WHODD) v. Global B3 March 2021.

### 9.9. Pharmacokinetic Analysis

The PK analysis is not included in this SAP.

## 10. Derived Variables

- Change from baseline = value at current time point – value at baseline.

- Annualized change in eGFR from baseline to post-treatment follow up = (Post-treatment follow-up eGFR – Baseline) / Duration (in years), where Duration (in years) = (median of post-treatment follow-up assessment dates – median of baseline eGFR assessment dates + 1) /365.25
- htTKV (mL/m) = TKV(mL)/participant's height (m).
- htLV = LV(mL)/participant's height (m).
- 2009 CKD-EPI equation:

  eGFR (mL/min/1.73 $m^2$) = $141 \times min\left(\frac{Scr}{k}, 1\right)^{\alpha} \times max\left(\frac{Scr}{k}, 1\right)^{-1.209} \times 0.993^{age} \times 1.018$ [if female]$\times$ 1.159 [if black]

  where Scr is serum creatinine (mg/dL), κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr or 1, age = years.
- 2021 CKD-EPIcr_R equation:

  eGFR (mL/min/1.73 $m^2$) = $142 \times min\left(\frac{Scr}{k}, 1\right)^{\alpha} \times max\left(\frac{Scr}{k}, 1\right)^{-1.200} \times 0.9938^{age} \times 1.012$ [if female]

  where Scr is serum creatinine (mg/dL), κ is 0.7 for females and 0.9 for males, α is -0.241 for females and -0.302 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ or 1, age = years. (Delgado et al, 2021)
- 2021 CKD-EPIcr-cys_R equation:

  eGFR (mL/min/1.73 $m^2$) = $135 \times min\left(\frac{Scr}{k}, 1\right)^{\alpha} \times max\left(\frac{Scr}{k}, 1\right)^{-0.544} \times min\left(\frac{Scys}{0.8}, 1\right)^{-0.323} \times max\left(\frac{Scys}{0.8}, 1\right)^{-0.778} \times 0.9961^{age} \times 0.963$ [if female]

  where Scr is serum creatinine (mg/dL), Scys is serum cystatin C (mg/L), $k$ = 0.7 (female) or 0.9 (male), $\alpha$ = -0.219 (female) or -0.144 (male), min indicates the minimum of Scr/κ or 1 OR Scys/0.8 or 1, max indicates the maximum of Scr/κ or 1 OR Scys/0.8 or 1, age = years. (Delgado et al, 2021)
- Age for the calculation of eGFR: Since date of birth is not collected in the study and to avoid the calculation error due to the whole number of age, relative age used for the calculation of eGFR is defined as follows:

  Age (years) = Age at Screening + (date of blood sample collection for serum creatinine – date of Inform Consent +1)/365.25, rounded to three decimals.

  For all efficacy analyses, the eGFR values will be calculated and rounded to 1 decimal based on equations and age defined above for analysis. However, the calculated eGFRs for efficacy analyses will not impact the determination of the randomization stratification factors.

## 11. Interim Analysis and Data Monitoring

Data analyses are planned to be conducted for Part 1 after the completion of Part 1 and unblinding of study after completion of Part 1. No interim analysis is planned for the efficacy analysis.

### 11.1. Independent Data Monitoring Committee (IDMC)

In order to ensure the safety of participants, the sponsor will use an IDMC. An IDMC charter was written to describe the IDMC objectives, schedule for data reviews, and general responsibilities in respect to the study. The IDMC will meet on a regular basis as specified in the Charter. In addition to the pre-determined data review meetings, the IDMC may meet on an *ad hoc* basis in the face of an emerging safety issue. The Committee will have access to unblinded data and will make recommendations to the sponsor without revealing participant treatment assignment. No efficacy assessments will be performed by the IDMC, therefore, no adjustment of type I error is required for the final data analysis.

### 11.2. Hepatic Events Review Committee (HERC)

In order to adequately characterize the liver safety profile of lixivaptan, additional testing and data collection will be required for any participant who develops clinically significant liver chemistry test abnormalities and/or signs or symptoms of liver dysfunction during the study. The HERC will independently and in a treatment-blinded manner expertly judge attribution to study drug as detailed in the HERC Charter and will communicate in writing with the sponsor and the IDMC. The IDMC reviews data at a study-level and, as such, its recommendations will not be made known to the HERC so as not to bias the HERC when adjudicating participant cases. These two committees are independent and will have a one-way line of communication from HERC to IDMC. The results of the HERC review and adjudication will be incorporated into the reporting of the safety results of the trial.

## 12. Statistical Analysis and Reporting

Data processing, tabulation of descriptive statistics, calculation of inferential statistics, and graphical representations will primarily use SAS (release 9.4 or higher). If the use of other software is warranted, the final statistical methodology report will detail what software, including version, was used for what purposes.

Continuous (quantitative) variable summaries will include the number of participants (n) with non-missing values, mean (geometric mean for log-transformed data), standard deviation (SD), median, minimum, and maximum.

Categorical (qualitative) variable summaries will include the frequency and percentage of participants who are in the particular category or each possible value. In general, the denominator for the percentage calculation will be based upon the total number of participants

with non-missing data in the study population for the treatment groups, unless otherwise specified.

The minimum and maximum will be reported with the same degree of precision (i.e., the same number of decimal places) as the observed data. Measures of location (mean and median) will be reported to 1 degree of precision more than the observed data and measures of spread (SD) will be reported to 2 degrees of precision more than the observed data.

Percentages will be presented to 1 decimal place, unless otherwise specified.

This study has been early terminated by the Sponsor. Due to only 5 participants being randomized into double-blinded, randomized treatment period in Part 1 and all being prematurely terminated in Part 1 of the study, no statistical analyses will be performed and only a limited set of summary tables will be generated along with the study data listings to facilitate the creation of a synoptic clinical study report.

## 13. Tables, Listings, and Figures

All listings, tables, and figures will have

- A header showing the sponsor company name, protocol, and page number
- A footer showing the file name and path, the source of the data (ADaM dataset or listing number) and date/time created.

The layouts of tables, listings, and figures are presented in SAP Mock Tables, Listings and Figures. These layouts incorporate all the appropriate table titles, table numbers, and footnotes.

## 14. Mock Tables, Listings, and Figures

The table, listing, and figure shells will be provided in a separate document.

## 15. Changes from Protocol

Any analyses that deviated from the study protocol are documented below.

| Text in Protocol | Change in SAP | Justification |
|---|---|---|
| None | Placebo Run-in Safety Set, Lixivaptan Titration Safety Set, ITT, mITT, PPAS, Lixivaptan Treated Safety Set are added in Section 5 Analysis Population | Those analyses population will be needed to summarize the efficacy or safety data |
| Analysis safety and efficacy sets for Part 2 in Section 5 Analysis Populations | Re-define as two analysis sets: P2SS for safety and P2EAS efficacy, but summary in Part 2 will be presented by treatment cohort | The revised analysis populations are more appropriate for safety and efficacy analyses in Part 2. |
| LV for analysis | htLV for analysis | To reduce the variance of LV and normalize to participant's height |
| Annualized change from baseline in htTKV (htLV) | Annualized percent change from baseline in htTKV (htLV) | Anti-log of annualized change from baseline based on the log-transformed htTKV (htLV) implies the annualized ratio of htTKV (htLV) at the evaluated timepoint to baseline htTKV (htLV). This ratio minus one as expressed as percentage will represent annualized percent change from baseline in htTKV (htLV), but not annualized change from baseline in htTKV (htLV). |
| None | Lixivaptan treated safety summaries have been added in Section 9.6 | To add overall safety after randomization during the entire study for all lixivaptan-treated participants during double-bind phase and/or open-label phase. |
| Blind sample size re-estimation (BSSR) | BSSR is removed | BSSR is not needed for the study due to the following:<br>• Sample size increased due to the increased power from 85% to 90%<br>• BSSR may not be feasible according to the currently projected enrollment |

## 16. Revision History

The non-editorial changes made to the statistical analysis plan (SAP) will be recorded in the table below. All changes require a new signature page be completed.

| Description of the Change/Reason for Change | Document Version Number Before Change | Date of Change |
|---|---|---|
| Original | N/A | N/A |
| | | |

## 17. References

ASA. (2018) Ethical Guidelines for Statistical Practice. Prepared by the Committee on Professional Ethics, April 2018. http://www.amstat.org/about/ethicalguidelines.cfm

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RSS. (2014) The Royal Statistical Society: Code of Conduct, 2014. https://rss.org.uk/about/policy-and-guidelines/code-of-conduct/.

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Little R., Yau L. 1996. Intent-to-Treat Analysis for Longitudinal Studies with Drop-Outs, *Biometrics*. 52. 1324-1333.

Torres VE, Chapman AB, Devuyst O, Gansevoort RT, Perrone RD, Koch G, Ouyang J, McQuade RD, Blais JD, Czerwiec FS, Sergeyeva O. 2017. Tolvaptan in later-stage autosomal dominant polycystic kidney disease. N Engl J Med. 377:1930-1942.

# Appendix: Method to Derive the Weight for the Weighted ANCOVA Analysis for the Primary Efficacy Endpoint

The following method is proposed to derive the weight for the weighted ANCOVA analysis for the primary efficacy endpoint if the number of eGFR observations is kept at 3 in the pre-treatment baseline period. In order to derive the weight used in the weighted analysis, the following model is considered:

$$y_{i,0,k} = \alpha_i + e_{i,0,k} \qquad \text{where } k = 1, 2, K_{i,0} \qquad (1)$$

$$y_{i,j,k} = \alpha_i + \delta_{i,j} + e_{i,j,k} \qquad \text{where } k = 1, 2, K_{i,j} \qquad (2)$$

where $K_{i,0}$ is the number of eGFR observations during the pre-treatment baseline period for subject $i$, and $K_{i,j}$ is the number of eGFR observations during the post-treatment follow-up period for subject $i$, with visit $j$ as the visit 22 (Week 52) for completers or mapped regular visits for early dropouts. $\alpha_i$ is a random variable for the "real" eGFR baseline of subject $i$, and this variable will be cancelled out for change from baseline. $\delta_{i,j}$ is a random variable for change from pre-treatment baseline for subject $i$ to visit $j$. These $\delta_{i,j}$s are normally distributed, with means being $\delta_{P,j}$ for placebo subjects and $\delta_{L,j}$ for lixivaptan subjects, and variance $\sigma_{\delta,j}^2$. These $\delta_{i,j}$s are supposed to be independent from subject to subject, and each subject has only one post-baseline visit $j$ in the primary analysis. In addition, $\alpha_i$s are assumed iid normally distributed, $e_{i,j,k}$ are assumed iid $N(0, \sigma^2)$, and all these random variables are mutually independent. Their average over the $K_{i,0}$ observations at baseline and the $K_{i,j}$ observations at post-treatment follow-up will be:

$$\bar{y}_{i,0} = \alpha_i + \bar{e}_{i,0}, \qquad \text{where } \bar{e}_{i,0} \sim N(0, \sigma^2/K_{i,0}) \qquad (3)$$

$$\bar{y}_{i,j} = {}_{\alpha i} + \delta_{i,j} + \bar{e}_{i,j}, \qquad \text{where } \bar{e}_{i,j} \sim N(0, \sigma^2/K_{i,j}) \qquad (4)$$

the distribution of their difference is:

$$\bar{y}_{i,j} - \bar{y}_{i,0} = \delta_{i,j} + \bar{e}_{i,j} - \bar{e}_{i,0} \sim N(.,\sigma_{\delta,j}^2 + \sigma^2(1/K_{i,0} + 1/K_{i,j})), \qquad (5)$$

where the mean of the normal distribution is $\delta_{P,j}$ for placebo subjects and $\delta_{L,j}$ for lixivaptan subjects.

In order to estimate the variance components given in (5), a further assumption of all $\sigma_{\delta,j}^2$'s are equal, ie, $\sigma_{\delta,j}^2 = \sigma_\delta^2$ is made, since there may not be enough subjects withdraw to stabilized the estimate of $\sigma_{\delta,j}^2$ at some visits. In addition, it is assumed all subjects get 3 eGFR observations at baseline. This assumption is reasonable, since usually subjects follow protocol schedules more strictly at the beginning of the trial and could simplify the estimation of the variance components. Then, a formula of change from baseline can be written similar to (5) for the estimation of the variance components:

$$y_{i,j,k} - \bar{y}_{i,0} = \delta_{i,j} + ei_{,j,k} - \bar{e}_{i,0} \sim N(.,\sigma_\delta^2 + \sigma^2(1 + 1/3)), \qquad (6)$$

A mixed model with fixed effect factors of treatment nested within visit, replication (for the repeated observations at the post-treatment follow-up in eGFR) will be applied to change from baseline (as the average of the 3 pre-treatment eGFR observations) in eGFR observed at each replication. In this mixed model, replications at the post-treatment follow-up are considered as the repeated measurements, with a compound symmetric variance matrix structure. In this

estimated variance-covariance matrix, the diagonal elements are the estimate of $\sigma_\delta^2 + \sigma^2(1 + 1/3)$, and the off-diagonal elements are the estimate of $\sigma_\delta^2 + \sigma^2(1/3)$. Solving these two equations will get the estimates of $\sigma_\delta^2$ and $\sigma^2$. With these variance component estimates, the variance given in formula (5) is estimated for each subject. Dividing the estimated variance given in (5) by the subject's trial duration will provide an estimated variance for the subject's annualized change in eGFR. The inverse of this estimated variance will be the weight of the subject used in the primary analysis.

SAS code for the estimation of variance component

```
PROC MIXED;
        CLASS USUBJID VISIT TREATMENT REPLICATION;
        MODEL CHANGE = TREATMENT(VISIT) REPLICATION;
        REPEATED REPLICATION/TYPE=CS SUB=USUBJID;
RUN;
```

In this estimation of variance components, it is assumed the post-treatment follow-up eGFR observations of early withdrew subjects are mapped into scheduled visits. Since the 4-week scheduled visits in this protocol, for a subject early withdrew the study drug, compared to the subject's last scheduled on-treatment visit, if the first post-treatment follow-up eGFR is observed less or equal to 24 days (= 14 + 7 + 3) after the last scheduled on treatment visit, then the subject's post-treatment follow-up eGFR observations will be mapped to the subject's last scheduled on-treatment visit; otherwise, if the first posttreatment follow-up eGFR is observed less or equal to 52 days (= 28 + 14 + 7 + 3) after the last scheduled on treatment visit, then the subject's post-treatment follow-up eGFR observations will be mapped to the visit after the subject's last scheduled on-treatment visit; etc.