**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 a 1-year Open-label Phase: The ACTION Study**

**ClinicalTrials.gov ID: NCT04064346**

Clinical Study Protocol Version 3.0, dated 14-Feb-2022

Overview of Changes in the Conduct of the Study

Clinical Study Protocol Version 3.0, dated 14-Feb-2022, Summary of Changes

Clinical Study Protocol Version 2.0, dated 01-Feb-2022, Summary of Changes

# CLINICAL STUDY PROTOCOL

## 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 a 1-year Open-Label Phase: The ACTION Study

## Protocol Number: PA-ADPKD-301

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| **Sponsor:** | Palladio Biosciences, Inc.<br>5 Walnut Grove Drive<br>Suite 120<br>Horsham, PA 19044 |
| **Sponsor Contact:** | Neil Shusterman, MD FACP<br>Chief Medical Officer<br>Palladio Biosciences, Inc.<br>5 Walnut Grove Drive<br>Suite 120<br>Horsham, PA 19044 |
| **Regulatory Agency Identifying No.** | IND number 136,419; EudraCT number 2021-003062-12 |
| **Medical Monitor:** | Refer to the Study Contacts List in the Investigator Site File or Trial Master File |
| **Emergency Telephone Numbers** | **North America:** +1 510 722 8910<br>**South America:** +54 911 5479 8678<br>**Europe and Middle East Region:** +44 118 936 4096<br>**Australia, New Zealand:** +61 3921 39875 |
| **Version of Protocol:** | 3.0 |
| **Original Protocol Date:** | 30 JUN 2021 |
| **Protocol Version 2 Date:** | 01 FEB 2022 |
| **Protocol Version 3 Date:** | 14 FEB 2022 |

# CONFIDENTIAL

# PROTOCOL APPROVAL - SPONSOR SIGNATURE

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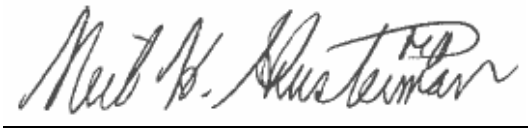
| **Protocol Title** | A Phase 3 Study of the Efficacy and Safety of Lixivaptan in Participants with Autosomal Dominant Polycystic Kidney Disease Consisting of a 1-year Double-blind, Placebo-controlled, Randomized Phase and a 1-year Open-label Phase: The ACTION Study |
| **Protocol Number** | PA-ADPKD-301 |
| **Protocol Version** | 3.0 |
| **Protocol Version Date** | 14 FEB 2022 |

Protocol accepted and approved by:

Neil H. Shusterman, MD FACP
Chief Medical Officer
Palladio Biosciences, Inc.
5 Walnut Grove Drive
Suite 120
Horsham, PA 19044



Signature

14Feb2022
Date

# DECLARATION OF INVESTIGATOR

I have read and understood all sections of the protocol titled "A Phase 3 Double-blind, Placebo-controlled, Randomized 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 a 1-year Open-label Phase: The ACTION Study".

I agree to supervise all aspects of the protocol and to conduct the clinical investigation in accordance with the protocol, the ICH harmonized tripartite guideline E6 (R2): Good Clinical Practices (GCP), including the Declaration of Helsinki, and all applicable government regulations. I will not make changes to the protocol before consulting with Palladio Biosciences or implement protocol changes without Institutional Review Board (IRB)/Ethics Committee (EC) approval except to eliminate an immediate risk to participants. I agree to administer study treatment only to participants under my personal supervision or the supervision of a Sub Investigator.

I will not supply the investigational drug to any person not authorized to receive it. Confidentiality will be protected. Participant identity will not be disclosed to third parties or appear in any study reports or publications.

I will not disclose information regarding this clinical investigation or publish results of the investigation without authorization from Palladio Biosciences.

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| Signature of Investigator | Date |

Printed Name of Investigator

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# PROTOCOL SYNOPSIS

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| **Protocol Number:** | PA-ADPKD-301 |
| **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 a 1-year Open-label Phase: The ACTION Study |
| **Sponsor:** | Palladio Biosciences, Inc.<br>5 Walnut Grove Drive<br>Suite 120<br>Horsham, PA 19044 |
| **Name of Active Ingredient:** | Lixivaptan |
| **Indication:** | Autosomal Dominant Polycystic Kidney Disease (ADPKD) |
| **Phase of Development:** | Phase 3 |
| **Number of Participants (Planned)** | 1350 randomized, Part 1 (estimated 1215 will continue into Part 2) |
| **Study Sites:** | Approximately 250 sites worldwide |
| **Study Duration** | 123 to 131 weeks (Part 1: 65 to 71 weeks; Part 2: 58 to 60 weeks) |
| **Principal/Lead Investigator** | Vicente E. Torres, MD, PhD |
| **Rationale:** | ADPKD is the most frequent inherited cause of end-stage kidney failure. Animal models have shown that vasopressin activity is necessary for the disease to manifest and progress. Studies of the vasopressin $V_2$ receptor antagonist tolvaptan have shown that it can slow the progression of kidney function deterioration in patients with ADPKD. However, serious drug-induced liver injury (DILI) occurs in a certain percentage of patients treated with tolvaptan, requiring diligent testing of liver chemistry in all patients and discontinuation of the drug when the test results are elevated in order to prevent serious outcomes.<br>The vasopressin $V_2$ receptor antagonist lixivaptan has also been shown to ameliorate polycystic disease manifestations in animal models of the disease. Evidence from quantitative systems toxicology modeling and simulation has demonstrated that lixivaptan does not have the same potential for liver injury as tolvaptan. In a patient with ADPKD who developed DILI while receiving tolvaptan, treatment with lixivaptan in an expanded access study was administered for 14 months without any alteration in liver chemistry tests. Thus, lixivaptan may represent a safer alternative to tolvaptan with similar efficacy.<br>In the ongoing lixivaptan study, PA-ADPKD-303, the hepatic and non-hepatic safety as well as the efficacy of lixivaptan is being evaluated in participants who permanently discontinued treatment with tolvaptan as a result of abnormal liver chemistries. |

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| | This Phase 3 trial will assess the efficacy and safety of lixivaptan in a broad population of adult participants with ADPKD. |
| **Objectives:** | Part 1 (Year 1):<br>The primary efficacy objective of Part 1 is:<br>• 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).<br>The key safety objective of Part 1 is:<br>• To compare the incidences of liver chemistry test elevations in participants randomized to lixivaptan with participants randomized to placebo.<br>The secondary efficacy objectives of Part 1 are:<br>• To compare the rate of change (slope) in on-treatment eGFR in participants treated with lixivaptan to participants treated with placebo.<br>• To assess the effect of lixivaptan on total kidney volume (TKV) as measured by magnetic resonance imaging (MRI) compared to placebo.<br>The secondary safety objective of Part 1 is:<br>• To assess the non-hepatic safety and tolerability of lixivaptan.<br>The health outcomes-related objectives of Part 1 are:<br>• 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.<br>• 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.<br>The population pharmacokinetics (PopPK) objective of Part 1 is:<br>• To characterize the pharmacokinetic (PK) profile of lixivaptan utilizing population PK (PopPK) based on sparse plasma sampling.<br>The exploratory objectives of Part 1 are:<br>• To assess the effect of lixivaptan on liver volume (LV) as measured by MRI compared to placebo.<br>• To evaluate the change from baseline in urine osmolality in participants treated with lixivaptan compared with participants treated with placebo. |

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| | • To evaluate the effect of lixivaptan on kidney function as assessed by eGFR using a novel serum creatinine and serum cystatin C-based Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation refit without race variable (CKD-EPIcr-cys_R) compared with placebo.<br><br>Part 2 (Year 2):<br>The key objective of Part 2 is:<br>• 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 at the end of the open-label phase (Part 2).<br>The key safety objective of Part 2 is:<br>• To assess the incidence of liver chemistry test abnormalities during the open-label phase.<br>The secondary objectives of Part 2 are:<br>• 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.<br>• To assess the effect of lixivaptan on TKV as measured by MRI in Part 2 in participants treated with lixivaptan in Part 1 and Part 2.<br>The secondary safety objective of Part 2 is:<br>• To assess the non-hepatic safety and tolerability of lixivaptan.<br>The health outcomes-related objectives of Part 2 are:<br>• 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.<br>• To evaluate the change in domain scores of the ADPKD-IS, ADPKD-PDS, and ADPKD-UIS.<br>The PopPK objective of Part 2 is:<br>• To further characterize the PK profile of lixivaptan utilizing PopPK based on sparse plasma sampling.<br>The exploratory objectives of Part 2 are:<br>• To assess the effect of lixivaptan on LV as measured by MRI following 52 weeks of open-label treatment.<br>• To evaluate the change in urine osmolality.<br>• 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. |
| **Study Population:** | Eligibility criteria are to be assessed at Visits 1a, 1b (if required) and 2. **Note**: On or prior to Visit 3, eligibility based on mean eGFR (Inclusion criterion #4) from Visit 1a and 2 or Visit 1b and 2 (if Visit 1b is required) and liver chemistry tests (Exclusion criterion #9) from Visit 2 must be re-confirmed.)<br>The following are requirements for entry into the study: |

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| | **Inclusion criteria:**<br>1. Male or female, between 18 and 60 years of age (inclusive) at the time of Screening (Visit 1a).<br>2. Diagnosis of ADPKD by modified Pei criteria:<br>• For participants with family history of ADPKD, by ultrasound:<br>• 18-39 years: ≥3 cysts, unilateral or bilateral;<br>• 40-59 years: ≥2 cysts in each kidney;<br>• 60 years: ≥4 cysts in each kidney; or<br>• For participants with family history of ADPKD, by computerized tomography (CT) or MRI:<br>• 18-40 years: ≥10 cysts in both kidneys; or<br>• For participants without family history of ADPKD<br>• 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<br>• genetic diagnosis of ADPKD.<br>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 total kidney volume (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.<br>4. eGFR ≥25 mL/min/1.73 $m^2$ 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. **Note**: This criterion will preliminarily be reviewed at Visit 2 based on Visit 1a or Visit 1b results (if Visit 1b is required). The criterion must be re-evaluated no later than Visit 3 when results for Visits 1a and 2 or Visits 1b and 2 are available to confirm that the participant remains eligible for participation.<br>5. Appropriate control of hypertension for a minimum of 3 weeks including the use of an angiotensin converting enzyme inhibitor or angiotensin receptor blocker at a stable dose (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.<br>6. Body mass index (BMI) between 18 and 40 kg/$m^2$ (inclusive) at the time of Screening. |

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| | 7. Female participants must:<br>a. not be pregnant, lactating, or breastfeeding.<br>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 (WOCBP), 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:<br>• 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<br>• intrauterine device (IUD), including progestin-containing intrauterine devices<br>• intrauterine hormone-releasing system (IUS)<br>• 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<br>• bilateral tubal ligation<br>• Essure® procedure (tubal occlusion)<br>• male or female condom with spermicide (cream, spray, gel, suppository, or polymer film)<br>• diaphragm, cervical cap, or contraceptive sponge with spermicide (with or without male condom).<br>8. Male participants must agree to use an acceptable form of birth control (see list above 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.<br>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. |

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| | **Exclusion criteria:**<br>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.<br>2. Clinically significant incontinence, overactive bladder, or urinary retention (e.g., benign prostatic hyperplasia).<br>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.<br>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.<br>5. History of clinically significant drug or alcohol abuse in the 2 years prior to Screening Visit 1a.<br>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.<br>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).<br>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.<br>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.<br>10. Requirement for ongoing diuretic use.<br>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.<br>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.<br>13. Prior use of a hypoxia-inducible factor prolyl hydroxylase (HIF-PH) inhibitor within the 2 months prior to Screening Visit 1a or expected |

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| | need for initiation of treatment with a HIF-PH inhibitor during the study.<br>14. Simvastatin at a total daily dose >10 mg or amlodipine at a total daily dose >5 mg.<br>15. Prior use of tolvaptan or lixivaptan within the 2 months prior to Screening Visit 1a.<br>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.<br>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.<br>18. Hypovolemia on physical examination at Screening.<br>19. Abnormal serum sodium concentration at Screening.<br>20. Positive test results for hepatitis B surface antigen (HBsAg).<br>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.<br>22. Known sensitivity or idiosyncratic reaction to lixivaptan and/or its excipients. |
| **Study Design:** | This is a Phase 3 trial consisting of a 2-arm, double-blind, placebo-controlled, randomized phase (Part 1) followed by a single-arm open-label phase (Part 2) to demonstrate the efficacy and safety of lixivaptan in participants with ADPKD. Part 1 of the trial is designed to demonstrate the efficacy of lixivaptan in slowing the decline in kidney function as measured by the difference in change from baseline of eGFR between the lixivaptan-treated and placebo-treated participants. Part 2 of the study is designed to provide confirmation of the durability of this effect. Additionally, both parts of the study will contribute to understanding the safety of lixivaptan, particularly any effects on liver chemistry tests. If agreeable to the participant and at the discretion of the Investigator, designated visits may be done remotely by a home healthcare clinician (HHC) (where available and locally approved by the Competent Authority (CA) and/or IRB/EC). Some remote visits may also include telehealth (e.g., telemedicine virtual visit, telephone or video call (without recording)) with the study site, as applicable. Remote visits may also be conducted by qualified site personnel who have been delegated the authority to carry out the procedures required at remote visits by the Investigator. Those visits are indicated in the Schedule of Procedures – Part 1 and Schedule of Procedures – Part 2.<br>**Part 1:** Approximately 2250 participants with ADPKD will be screened in order to randomize 1350 participants to lixivaptan or placebo in a 2:1 ratio in Part 1 of this study. After meeting entry criteria during a 3-to-5-week screening period (that may be extended up to 8 weeks to obtain baseline stability of medications), participants will enter a 1-week single-blind placebo |

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| | run-in period (participants will not know the identity of the study drug being administered) to obtain select baseline measurements followed by a 5 to 6 week single-blind dose titration period during which lixivaptan administered twice daily will be titrated to the highest tolerated dose. The minimum dose to enter the Double-blind, Randomized Treatment Period is 100 mg BID. Those participants successfully titrated and tolerating the study drug will then be randomized (2:1) to either continue lixivaptan or switch immediately to matching placebo in a double-blind manner. Randomization will be stratified for Chronic Kidney Disease (CKD) Stage and Mayo Clinic ADPKD Image Classification. Double-blind treatment will continue for 52 weeks after which study drug will be held, and final eGFR assessments will be obtained off-treatment during 3 follow-up visits starting on the 8$^{th}$ day after the last dose of double-blind study drug through the 28$^{th}$ day after the last dose of double-blind study drug.<br><br>**Part 2:** All participants (placebo and lixivaptan-treated) entering Part 1 will continue into Part 2 of the study and be treated with the active drug, lixivaptan, for an additional 52 weeks (following the Lixivaptan Re-titration Period) unless study drug was previously discontinued for a safety reason, or a participant withdraws consent. Assuming 90% of participants complete the Double-Blind Treatment Period (Part 1), approximately 1215 participants are projected to continue into Part 2. At the beginning of Part 2, all participants will start lixivaptan during the Lixivaptan Re-titration Period to re-establish the dose level that was tolerated during Part 1. The dose will be increased at weekly intervals until the dose level taken at the end of the Double-blind, Randomized Treatment Period in Part 1 is achieved. In order to maintain blinding of the treatment assignment from Part 1, re-titration during Part 2 will continue to be managed by the Interactive Response Technology (IRT) system.<br><br>Following re-titration, participants will continue on lixivaptan therapy for 52 weeks in the Maintenance Treatment Period of Part 2 and will be assessed at a study visit every 4 weeks. At the end of 52 weeks, study drug will be held, and final assessments will be obtained over 3 follow-up visits starting on the 8$^{th}$ day following the last dose of study drug through the 28$^{th}$ day after the last dose of study drug. |
| **Study Period Description and Estimated Duration:** | **Part 1:** The study schematic for Part 1 is depicted in Figure 1.<br><br>Screening Period (Visits 1a, 1b, and 2): After obtaining informed consent screening assessments will be performed at Visit 1a to determine initial participant eligibility. Participants will continue through Screening as follows:<br>• Participants with appropriate blood pressure control for a minimum of 3 weeks prior to enrollment (Visit 2) (as defined by the 2021 KDIGO guideline) including those on a stable regimen with an angiotensin-converting enzyme inhibitor (ACEi) or angiotensin II receptor blocker (ARB) treatment (unless not considered appropriate) and not receiving a diuretic will have a baseline MRI exam scheduled and will proceed to Visit 2, 3 to 5 weeks after Visit 1a, and after completion of the baseline MRI (allow approximately 1 week after completion of the MRI, however, a 3-week minimum between Visit 1a and Visit 2 is |

required). (Screening may be extended to a maximum of 8 weeks, in the event of extenuating circumstances. Prior approval from the medical monitor is required.)

- Participants who need to be discontinued from diuretic therapy and/or have their anti-hypertensive therapy optimized due to inadequate BP control (as defined by the 2021 KDIGO guideline) will return for Unscheduled visits for blood pressure monitoring following Visit 1a. Once their treatment and blood pressure have been stable for a minimum of 3 weeks, they will return for Visit 1b to have additional serum chemistry testing. The baseline MRI exam should be scheduled after completion of Visit 1a but may be performed before, at, or after Visit 1b (but prior to Visit 2) depending on scheduling. Upon attaining stability of antihypertensive therapy and completion of the baseline MRI, the participant will be scheduled for Visit 2 (approximately 1 week following the MRI and approximately 2 weeks following Visit 1b, whichever occurs later.) The total duration of the Screening Period may be extended up to 8 weeks, including the time to completion of Visit 2.

Participants who meet all of the inclusion criteria and none of the exclusion criteria and achieve stability of background anti-hypertensive medications (in the absence of diuretics) and appropriate blood pressure control (for a minimum of 3 weeks) will have Visit 2 end-of-screening assessments and eligibility determination (final eGFR eligibility will be delayed until Visit 2 serum creatinine results are obtained); fasting morning spot (i.e., discard first void and sample will be obtained from next void within 30 to 45 minutes) urine osmolality will also be measured. Participants who fail inclusion/exclusion criteria due to temporary or correctable reasons may be re-screened (up to 2 additional times after obtaining new informed consents) and after consultation with the medical monitor. At the successful completion of Visit 2, participants will be considered enrolled and will be provided with single-blind study drug (placebo) to start the Placebo Run-in Period. Participants will take 4 capsules of study drug twice daily (BID) approximately 10 hours apart. Throughout the study, participants will take 4 capsules BID to mask transitions from one study period to the next.

Placebo Run-in Period (Visit 3): During the 1-week Placebo Run-in Period, participants will receive single-blind placebo and take 4 capsules twice daily. At the end of the week, participants will attend Visit 3 at which time tolerability will be assessed to maintain participant blinding and final baseline measurements will be obtained. Single-blind lixivaptan at a dose of 50 mg BID will be dispensed to start the Lixivaptan Titration Period. At Visit 3, participants should take their first dose of study drug from the newly assigned (Visit 3) blister card in the clinic following completion of study visit procedures and in accordance with the sparse PK sampling schedule (0.5 hrs. to 1.5 hrs. post dose) detailed in the Schedule of Procedures – Part 1.

Lixivaptan Titration Period (Visits 4 to 9): During a period of 5 to 6 weeks for participants who complete the Placebo Run-in Period, the lixivaptan dose will be increased in a single-blind fashion according to the titration schedule in Table 1 to achieve the maximally tolerated dose up to the maximum dose allowed (Level 4 [200 mg BID]); as 4 capsules BID. The minimum dose to enter the Double-blind, Randomized Treatment Period is Level 2 (100 mg

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| | BID). Once the maximally tolerated dose has been achieved, tolerability will be confirmed over an additional week of dosing before the participant advances to randomization. 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. Further dose reductions are allowable as shown in the Lixivaptan Titration Period (Part 1) — Lixivaptan Titration Flowchart. Fasting morning spot (i.e., discard first void and sample will be obtained from next void within 30 to 45 minutes) urine osmolality will be measured at the last titration visit (Visit 9) and serum liver chemistry tests will be measured at Visits 5, 7, and 9.<br><br>Double-blind, Randomized Treatment Period (Visits 10 to 22): Following successful completion of titration, participants will be randomized to continue to receive lixivaptan at the dose achieved at the end of the Lixivaptan Titration Period or matching placebo in a double-blind fashion for 52 weeks as 4 capsules BID. Visits will be performed every 4 weeks (± 5 days) during this period. Assessments at each visit are detailed in the Schedule of Procedures – Part 1.<br><br>Follow-up Period I (Visits 23 to 25): Three visits will occur over a 28-day, study drug-free period following the last dose of study drug. Serum creatinine and serum cystatin C, to determine eGFR, for primary and exploratory analyses, respectively, will be drawn at 3 time points. The first visit (Visit 23) will occur on the 8$^{th}$ day (+ 3 days) after the last dose of study drug, and the last visit (Visit 25) will occur on the 28$^{th}$ day (± 3 days) after the last dose of study drug. Visit 24 must be scheduled to occur at least 24 hours after Visit 23 and at least 24 hours prior to Visit 25. At Visit 25, additional efficacy and safety assessments will be performed; a post-baseline MRI for assessment of TKV and LV is to be obtained between 25 days and 31 days post last dose of study drug and must be completed prior to or on the same day as all other Visit 25 study procedures. Following completion of these assessments, all participants (except those who discontinued due to an AE or who have withdrawn consent) will immediately continue into Part 2 at Visit 25.<br><br>**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 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. If study drug is interrupted for longer than 8 weeks, then study drug treatment will be permanently discontinued.<br><br>**Part 2:** The study schematic for Part 2 is depicted in Figure 2.<br><br>Lixivaptan Re-titration Period (Visits 26 to 29): Titration of lixivaptan will occur over a 2 to 4-week re-titration period and will start at a dose of 50 mg BID beginning at the conclusion of Visit 25. The dose will be increased at weekly intervals until the dose level from the end of the Double-blind, Randomized Treatment Period in Part 1 is achieved. For participants randomized to placebo in Part 1, lixivaptan dosing will be titrated to the final blinded inferred dose level (the dose level equal to the active dose level had the participant been randomized to the active arm) achieved during the |

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| | Double-Blind Treatment Period in accordance with Table 2. In order to maintain blinding of the treatment assignment from Part 1, the dose level assignment for Part 2 will continue to be managed by the IRT. The dose-titration schedule for this part of the study is described in Table 2.<br><br>Maintenance Treatment Period (Visits 30 to 42): Following completion of the Lixivaptan Re-titration Period, participants will continue to receive lixivaptan in the Maintenance Treatment Period for 52 weeks at the same dose achieved at the end of the Re-titration Period. Visits will be performed every 4 weeks (± 5 days) during this period. Assessments at each visit are detailed in the Schedule of Procedures – Part 2.<br><br>**Note**: In the event of tolerability issues at any time during the study including the Part 2 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 study drug is interrupted for longer than 8 weeks, then study drug treatment will be permanently discontinued.<br><br>Follow-up Period II (Visits 43 to 45): Three visits will occur over a 28-day, study drug-free period following the last dose of lixivaptan in the Maintenance Treatment Period. Serum creatinine and serum cystatin C, to determine eGFR for primary and exploratory analyses, respectively, will be drawn at 3 time points. The first visit (Visit 43) will occur on the 8$^{th}$ day (+ 3 days) after the last dose of lixivaptan, and the last visit (Visit 45) will occur on the 28$^{th}$ day (± 3 days) after the last dose of lixivaptan. Visit 44 must be scheduled to occur at least 24 hours after Visit 43 and at least 24 hours prior to Visit 45. At the final study visit, Visit 45, additional efficacy and safety assessments will be obtained; a post-baseline MRI for assessment of TKV and LV is to be obtained between 25 days and 31 days post last dose of study drug and must be completed prior to or on the same day as all other Visit 45 study procedures.<br><br>The total duration of participation in the study will be approximately 123 to 131 weeks (up to 71 weeks in Part 1 and up to 60 weeks in Part 2) depending on the length of Screening (3 to 8 weeks), Titration (5 to 6 weeks), and Re-titration (2 to 4 weeks). Note that the study may be interrupted at any time if safety issues identified by an Independent Data Monitoring Committee (IDMC) and sponsor potentially compromise the safety of the participants. |

**Study Drug, Dosage, and Route of Administration:**

The sponsor (Palladio Biosciences, Inc.) will supply lixivaptan 50 mg capsules and matching placebo for use during the study. Throughout the study, participants will take 4 capsules BID to mask transitions from one study period to the next. All doses will be self-administered by participants orally at home with the exception of Visit 3 and any other study visit with scheduled sparse PK sampling, where in-clinic dosing may be warranted.

**Part 1:** During the Placebo Run-in Period, each participant will take 4 placebo capsules BID (twice daily, approximately 10 hours apart) for 1 week. During the Lixivaptan Titration Period, dosing will start at Level 1 (50 mg BID) and will be increased weekly minimally through Level 2 (100 mg BID) and maximally through Level 4 (200 mg BID) according to the dosing schedule shown in Table 1. At the 2 highest dose levels (Level 3 [150 mg BID] and Level 4 [200 mg BID]) where aquaretic effects may be problematic in certain participants, there will be an opportunity to reduce the evening (PM) dose to Level 3a (150 mg AM/100 mg PM) for those at Level 3 or Level 4a (200 mg AM/150 mg PM) for those at Level 4. Further dose reductions are allowable as shown in the Lixivaptan Titration Period (Part 1) — Lixivaptan Titration Flowchart. Throughout the study, participants will continue to take 4 capsules BID to mask transitions from one study period to the next.

**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 tolerability is achieved, participants will remain on that dose for 1 additional week to confirm tolerability (Lixivaptan Titration Period (Part 1) — Lixivaptan Titration Flowchart). As the maximum duration of the Lixivaptan Titration Period is 6 weeks, participants who require a dose reduction at Week 6 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.

Participants who tolerate their optimized dose will then enter the Double-blind, Randomized Treatment Period during which time they will be randomized 2:1 (lixivaptan: placebo) to continue at the lixivaptan dose level achieved at the end of the Lixivaptan Titration Period or receive matching placebo capsules. During the Double-blind, Randomized Treatment Period, the dose may be adjusted downward at the Investigator's discretion if needed

| | |
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| | 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 due to illness, including COVID-19, or other reasons may be able to restart the study drug when medically stable and following discussion with the medical monitor and sponsor. Re-titration 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.<br><br>**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 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.<br><br>**Part 2:** 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. This is shown in Table 2. That dose 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. |

<table>
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**Table 2. Dose Titration (Part 2) Based on Dose Level Achieved at End of Double-blind Randomized Treatment Period in Part 1 for Participants Assigned to Lixivaptan or Placebo in Part 1**

| Dose Level in Part 1* | Week 1 | Week 2 | Week 3 | Week 4 |
|---|---|---|---|---|
| Level 1<br>100mg BID or Placebo | 50mg BID | 100mg BID | | |
| Level 2<br>150mg BID or Placebo | 50mg BID | 100mg BID | 150mg BID | |
| Level 3a<br>150/100mg (AM/PM)<br>or Placebo | 50mg BID | 100mg BID | 150/100mg<br>(AM/PM) | |
| Level 4<br>200mg BID or Placebo | 50mg BID | 100mg BID | 150mg BID | 200mg BID |
| Level 4a<br>200/150mg (AM/PM)<br>or Placebo | 50mg BID | 100mg BID | 150mg BID | 200/150mg<br>(AM/PM) |

*Participants whose dose was reduced to Level 1 (50 mg BID lixivaptan or placebo) during the double-blind phase as a result of tolerability issues or treatment-emergent AEs can continue on that dose during the open-label phase. These participants will initiate dosing with lixivaptan at 50mg BID and remain on that dose for 2 weeks during the Re-titration Period and initiate the open-label phase at that dose. Attempts will be made every 4 to 8 weeks to re-establish dosing at Level 2 (100 mg BID) during the open-label phase.

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<tr><td>Study Assessments:</td><td>

**Efficacy:** eGFR based on serum creatinine, TKV by MRI

**Safety:** Liver chemistry tests (ALT, AST, total bilirubin, direct bilirubin, alkaline phosphatase), clinical laboratory parameters (hematology, non-hepatic clinical chemistry including serum sodium and urinalysis), 12-lead ECGs, vital signs, physical examinations, adverse events (AEs), and serious adverse events (SAEs)

**Pharmacokinetic:** Sparse plasma levels of lixivaptan for PopPK

**Health Outcomes:** Medical resource utilization (e.g., medication use/changes; unplanned office visits; urgent care and emergency department usage; and hospitalizations) resulting from clinical events during the study; ADPKD-IS; ADPKD-PDS; ADPKD-UIS

**Exploratory:** LV by MRI, urine osmolality, eGFR based on combined use of serum creatinine and cystatin C

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<tr><td>Criteria for Evaluation:</td><td>

**Part 1:**

**Primary Efficacy Endpoint:** Annualized change in eGFR, calculated from the 2021 CKD-EPI equation (CKD-EPIcr_R) for serum creatinine, from baseline (mean of 3 eGFR determinations obtained during Screening and Placebo Run-in Periods (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 or, for participants who discontinue treatment prior to Visit 22, during the Follow-up Period 8 to 28 days following study drug treatment discontinuation).

**Key Safety Endpoint:** Incidence of serum ALT levels >3 x ULN in participants randomized to lixivaptan compared to those randomized to placebo.

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| | **Part 2:**<br>**Key Comparison Endpoint:** Annualized change in eGFR from baseline (the mean of the 3 eGFR measurements obtained during Follow-up Period I) to the final post-treatment assessment in Part 2 (the mean of the 3 eGFR measurements obtained during Follow-up Period II).<br>**Key Safety Endpoint:** Incidence of serum ALT levels > 3 x ULN in participants exposed to lixivaptan in Part 2. |
| **Independent Data Monitoring Committee** | An independent data monitoring committee (IDMC) has been appointed for this study. |
| **Statistical Methods:** | **Sample Size:**<br>**Part 1:** Based on the results of the REPRISE trial with tolvaptan, 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 participants and 876 lixivaptan participants) is required to achieve 90% power at a significance level of 0.01. In order to compensate for possible dropouts, 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.<br><br>**Part 2:** This is a convenience sample based on the number of participants who 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 in Part 2. Approximately 90% (1215) of Part 1 participants are anticipated to continue in Part 2.<br>**Populations for Analysis:**<br>**Part 1:** The following populations will be used for analyzing the data from Part 1.<br>Treated Safety Set: The Treated Safety Set consists of all participants who received at least 1 dose of study drug in Part 1.<br>Randomized Safety Set: The Randomized Safety Set consists of all participants in the Treated Safety Set who are randomized and receive at least 1 dose of randomized study drug.<br>Primary Efficacy Analysis Set: The Primary Efficacy Analysis Set consists of all participants in the Randomized Safety Set who have both baseline and at least 1 valid assessment of eGFR in the Follow-up Period.<br>Secondary Efficacy Analysis Set: The Secondary Efficacy Analysis Set consists of all participants in the Randomized Safety Set who have both baseline and at least 1 on-treatment assessment of eGFR.<br>**Part 2:** The following populations will be used for analyzing the data from Part 2.<br>Long-term Safety Set: The Long-term Safety Set consists of all participants in the Randomized Safety Set of Part 1 who receive at least 1 dose of lixivaptan |

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| | during Part 2.<br>Long-term Full Analysis Set: All participants in the Long-term Safety Set who have at least 1 on-treatment measurement of eGFR in Part 2.<br>Long-term Efficacy Analysis Set: All participants in the Long-term Full Analysis Set who were randomized to lixivaptan in Part 1.<br><br>**Primary Efficacy Analysis:**<br>**Part 1:** Baseline eGFR is defined as the mean of the 3 eGFR assessments obtained during the Screening and single-blind, Placebo Run-in Periods. The primary efficacy analysis will utilize the change from this baseline eGFR to the post-treatment follow-up eGFR, annualized by each participant's treatment duration. The post-treatment follow-up eGFR is defined as the mean of 3 eGFR assessments obtained during the Follow-up Period I or the equivalent off-therapy eGFR determinations if a participant discontinues study drug earlier. The eGFR for the primary analysis will be based on serum creatinine using the CKD-EPIcr_R equation.<br>The estimand corresponding to the primary objective is the between-treatment group difference in the change from baseline in eGFR if all the participants in the Primary Efficacy Analysis Set (PEAS) had tolerated and adhered to their treatment for 12 months. Missing data due to randomized participants prematurely discontinuing from double-blind treatment will be handled by annualizing the changes from baseline as follows: each participant's change from baseline will be divided by the duration in days from the median of the baseline eGFR assessments to the median of the 3 eGFR assessments obtained during Follow-up Period I and then multiplied by 365.25 days.<br>The primary efficacy endpoint, the annualized change from baseline to post-treatment follow-up in mean eGFR, will be analyzed by means of an analysis of covariance model with fixed effects for treatment group and the randomization stratification factors and the baseline eGFR as a covariate.<br>The primary efficacy analysis will be performed at the 1% level of significance ($P<0.01$).<br><br>Sensitivity analyses of the primary efficacy analysis will include the use of the mean of eGFR values obtained pretreatment and the mean of eGFR values obtained off treatment after the end of the 52-week treatment period, regardless of adherence to randomized treatment and/or withdrawal for all randomized participants, i.e., Intent-To-Treat participants. Week 52 missing values will be imputed using multiple imputations (assuming missing at random). Bi-directional tipping point analyses will be included using a pattern-mixture model to impute missing data at Week 52 (assuming missing not at random); allowing varying assumptions about the missing outcomes on the two treatment arms.<br><br>**Key Comparison**<br>**Key Comparison Analysis**<br>**Part 2:** Baseline eGFR is defined as the mean of the 3 eGFR assessments obtained during Follow-up Period I.<br>Descriptive statistics will be presented for the mean of the annualized changes from this baseline to the mean of the 3 eGFR measurements during Follow-up |

| | |
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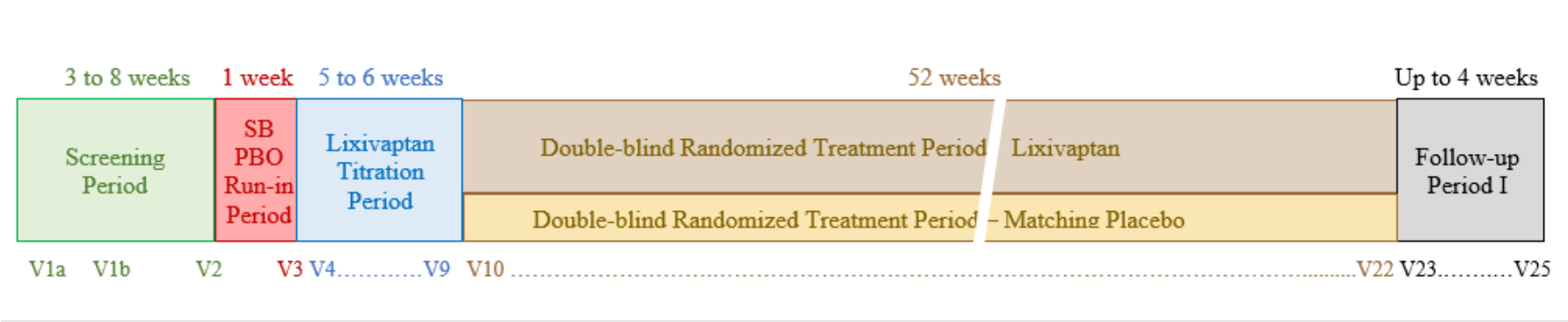
| | Period II for participants in the Long-term Efficacy Analysis Set. This will be compared to descriptive statistics for the corresponding annualized change in eGFR for Part 1 for the same analysis set.<br><br>**Safety**<br>**Key Safety – Liver Safety Analysis:**<br>**Part 1 and 2:** Incidence of serum ALT levels >3 x ULN will be summarized by treatment group in Part 1 and for all participants in Part 2.<br><br>**Additional Safety Analyses:**<br>**Part 1:** All safety analyses will be summarized for the following:<br>• Single-Blind Placebo Run-in Period for the Treated Safety Set<br>• Lixivaptan Titration Period for the Treated Safety Set<br>• The Randomized, Double-blind Treatment and Follow-up Periods for the Randomized Safety Set<br>Treatment-emergent adverse events, clinical laboratory data, 12-lead ECGs, and vital signs findings will be analyzed using appropriate descriptive statistics by treatment group. Potentially clinically significant results in clinical laboratory tests, 12-lead ECGs, and vital signs identified using prospectively defined criteria, including criteria on liver enzyme elevations, will also be summarized by treatment group.<br>**Part 2:** Safety analyses will be performed for the Long-term Safety Analysis Set based on TEAEs, clinical laboratory data, 12-lead ECGs, and vital signs findings using appropriate descriptive statistics overall and by treatment groups from Part 1. Potentially clinically significant results in clinical laboratory tests, 12-lead ECGs, and vital signs identified using prospectively defined criteria, including criteria on liver enzyme elevations, will also be summarized overall and by treatment group from Part 1. |

**Figure 1. Part 1 Study Schematic**




SB = Single-Blind, PBO = Placebo

**Figure 2. Part 2 Study Schematic**

**Figure 3. Lixivaptan Titration Period (Part 1) — Lixivaptan Titration Flowchart**

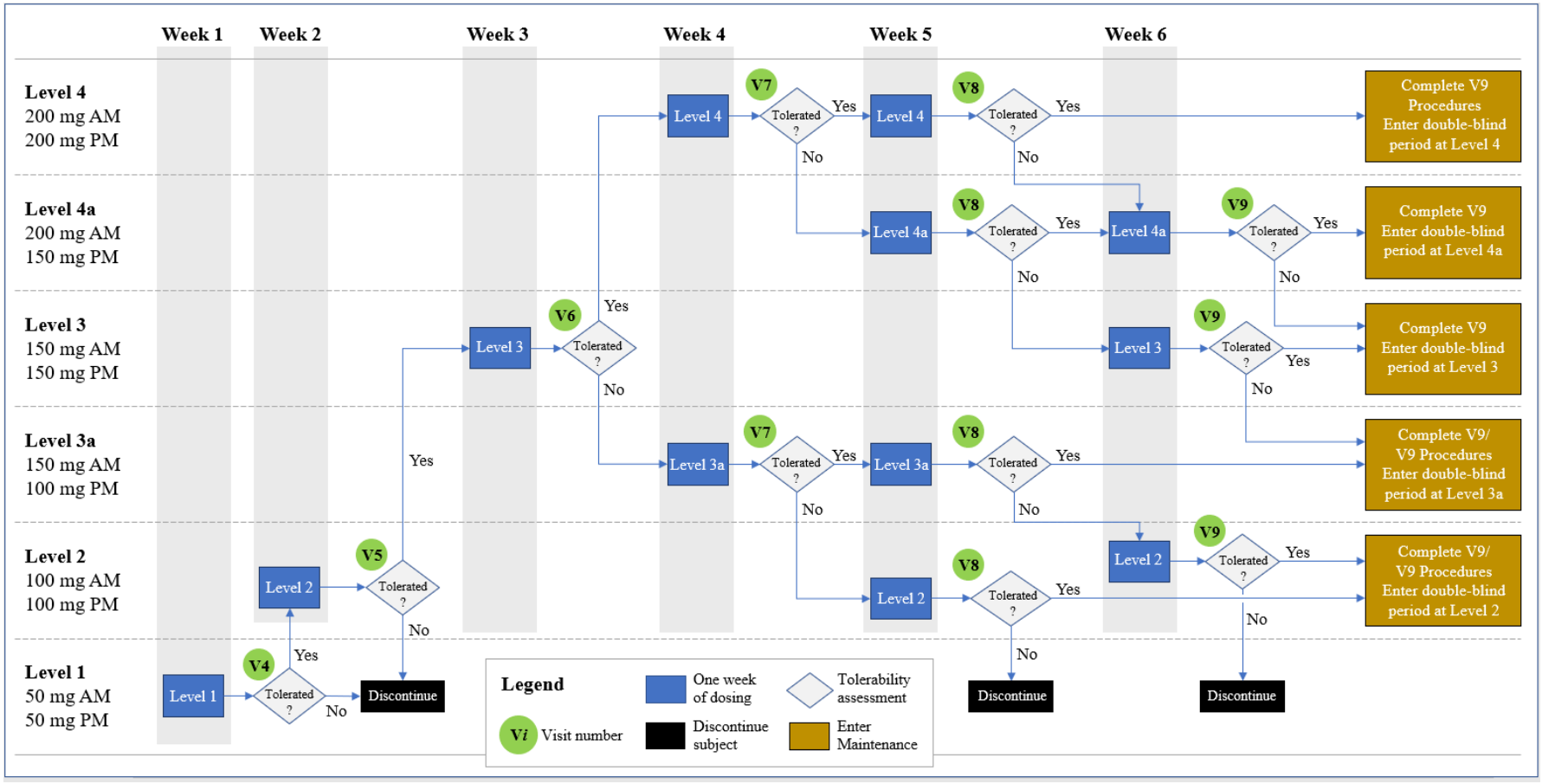


**Table 3. Schedule of Procedures – Part 1**

| | Screening Period | | | Single-Blind: Placebo Run-in Period | Single-Blind: Lixivaptan Titration Period[a] | | | | | | Double-blind Randomized Treatment Period | | Follow-up Period I[b] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Period Duration** | **3 to 8 weeks[c]** | | | **1 week** | **5 to 6 weeks** | | | | | | **52 weeks** | | **4 weeks** | | |
| **Visit Number** | **V1a** | **(V1b)** | **V2** | **V3** | **V4** | **V5** | **V6** | **V7** | **(V8)[d]** | **V9/ Last** | **V10-V21[e]** | **V22/ET[f]** | **V23** | **V24** | **V25** |
| **Visit Timing** | **V1b is utilized as described below[c]** | | **2-5 weeks after V1a; 1-5 weeks after V1b[c]** | **1 week ± 2 days after V2** | **Every week ± 2 days** | | | | | | **Every 4 weeks ± 5 days** | **Week 52 ± 5 days** | **8 + 3 days after last dose** | **≥ 24 hrs. after V23 and ≥ 24 hrs. before V25** | **28 ± 3 days after last dose** |
| **Informed consent** | X | | | | | | | | | | | | | | |
| **Inclusion/exclusion** | X | X | X | | | | | | | | | | | | |
| **Demographic information** | X | | | | | | | | | | | | | | |
| **Medical history** | X | | | | | | | | | | | | | | |
| **Vital signs[g]** | X | X | X | X | X | X | X | X | X | X | X | X | | | X |
| **Physical examination[h]** | X | | | | | | | | | | V15 | X | | | X |
| **Body weight/height[i]** | X | | | X | | | | | | X | | X | | | X |
| **Urine pregnancy test (WOCBP)[j]** | X | | | X | | | | | | X | V12, V15, V18 | X | | | X |
| **ECG** | X | | | | | | | | | X | V15 | X | | | X |
| **MRI[k]** | X | | | | | | | | | | | | | | X |
| **Chemistry blood samples[l]** | | | | | | | | | | | | | | | |
| **Chemistry** | X | X | X | | | | | | | X | V12, V15, V18 | X | | | X |
| **Serum sodium** | X | X | X | X | | X | | X | | X | X | X | | | X |
| **Liver chemistry** | X | X | X | X | | X | | X | | X | X | X | | | X |
| **Serum creatinine** | X | X | X | X | | | | | | X | X | X | X | X | X |
| **Serum cystatin C** | X | X | X | X | | | | | | | | | X | X | X |
| **Hematology** | X | | | X | | | | | | X | V15 | X | | | X |

| | Screening Period | | | Single-Blind: Placebo Run-in Period | Single-Blind: Lixivaptan Titration Period[a] | | | | | | Double-blind Randomized Treatment Period | | Follow-up Period I[b] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Period Duration** | **3 to 8 weeks[c]** | | | **1 week** | **5 to 6 weeks** | | | | | | **52 weeks** | | **4 weeks** | | |
| **Visit Number** | **V1a** | **(V1b)** | **V2** | **V3** | **V4** | **V5** | **V6** | **V7** | **(V8)[d]** | **V9/ Last** | **V10-V21[e]** | **V22/ET[f]** | **V23** | **V24** | **V25** |
| **Visit Timing** | **V1b is utilized as described below[c]** | | **2-5 weeks after V1a; 1-5 weeks after V1b[c]** | **1 week ± 2 days after V2** | **Every week ± 2 days** | | | | | | **Every 4 weeks ± 5 days** | **Week 52 ± 5 days** | **8 + 3 days after last dose** | **≥ 24 hrs. after V23 and ≥ 24 hrs. before V25** | **28 ± 3 days after last dose** |
| **Serology (HbsAg, Anti-HCV)[m]** | X | | | | | | | | | | | | | | |
| **PK plasma sample[n]** | | | X | X | | | | | | X | V12, V15, V18 | | | | |
| **Urinalysis** | X | | | | | | | | | X | | X | | | X |
| **Urine osmolality[o]** | | | X | | | | | | | X | V15 | X | | | X |
| **Study drug tolerability[p]** | | | | X | X | X | X | X | X | X | | | | | |
| **Randomization** | | | | | | | | | | X | | | | | |
| **Telephone contact[e]** | | | | | | | | | | | ←----------X----------→ | | | | |
| **Study drug dispensing** | | | X | X | X | X | X | X | X | X | X | | | | X[q] |
| **Study drug reconciliation and compliance** | | | | X | X | X | X | X | X | X | X | X | | | |
| **IRT entry** | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| **Medical resource utilization** | | | | X | X | X | X | X | X | X | X | X | X | X | X |
| **ADPKD-IS** | | | X | | | | | | | X | V12, V15, V18 | X | | | X |
| **ADPKD-PDS** | | | X | | | | | | | X | V12, V15, V18 | X | | | X |
| **ADPKD-UIS** | | | X | | X | X | X | X | X | X | V12, V15, V18 | X | | | X |
| **Adverse events/SAEs** | ←----------X----------→ | | | | | | | | | | | | | | |
| **Prior/concomitant medications** | ←----------X----------→ | | | | | | | | | | | | | | |

ADPKD = Autosomal Dominant Polycystic Kidney Disease; ADPKD-IS =ADPKD-Impact Scale; ADPKD-PDS = ADPKD-Pain and Discomfort Scale; ADPKD-UIS = ADPKD-Urinary Impact Scale; Anti-HCV = hepatitis C antibody; ET = Early termination visit; HbsAg = hepatitis B surface antigen; hrs. = hours; IRT = interactive response technology; Last = Last titration visit; PK = pharmacokinetic; V = Visit; WOCBP = women of childbearing potential; ( ) = Not all participants will need these visits

Visits 4 to 7, 10, 11, 13, 14, 16, 17, 19 to 21, 23 and 24 may be conducted in the clinic or remotely by a home healthcare clinician or qualified site personnel with the agreement of the participant and the discretion of the investigator, and where it is available and locally approved by the Competent Authority and/or Ethics Committee/Institutional Review Board. If conducted remotely, Visits 4 to 7 will require a telehealth visit (e.g., telemedicine virtual visit, telephone or video call (without recording)) with the Investigator 2 days prior to the scheduled in-home visit to assess tolerability. This will allow sufficient time for IRT study drug assignment and subsequent direct-to-participant drug dispensing prior to the scheduled in-home visit. All other remote visits may include a telehealth visit with the Investigator as needed.

a. Lixivaptan Titration Period will last 5 to 6 weeks. At Visits 4 to 8 the lixivaptan dose will be titrated to achieve the highest tolerated dose. All participants will start at Level 1 (50 mg BID) at the end of V3 and will be titrated weekly through Level 2 (100 mg BID), Level 3 (150 mg BID), and Level 4 (200 mg BID), based on tolerability. The minimum dose to enter the Double-blind, Randomized Treatment Period is Level 2. If tolerability is limited at the 2 highest dose levels (Level 3 [150 mg BID] and Level 4 [200 mg BID]) by aquaretic effects, reduction of the evening dose is allowed: to Level 3a (150/100 mg [AM/PM]) or Level 4a (200/150 mg [AM/PM]), respectively. Further dose reductions are allowable as shown in the Lixivaptan Titration Period (Part 1) — Lixivaptan Titration Flowchart. **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 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. If study drug is interrupted for longer than 8 weeks, then study drug treatment will be permanently discontinued.
b. During the study drug-free Follow-up Period I, 3 visits will occur to obtain the 3 serum creatinine values for calculation of eGFR to determine the primary endpoint. The first visit (V23) will occur on the 8$^{th}$ day (+ 3 days) after the last dose of study drug and the last visit (V25) will occur on the 28$^{th}$ day (± 3 days) after the last dose of study drug. V24 must be scheduled to occur at least 24 hours after V23 and at least 24 hours prior to V25. At the final visit (V25), the listed additional safety and efficacy assessments will be completed. Participants who have not discontinued due to an AE or withdrawn consent will then continue into the Re-titration Period of Part 2 by resuming study drug dosing at Level 1 (50 mg BID).
c. At Visit 1a all indicated information will be collected to determine preliminary eligibility and the baseline MRI to determine TKV and LV will be scheduled.
   i) Participants with appropriate blood pressure control for a minimum of 3 weeks prior to enrollment (Visit 2) (as defined by the 2021 KDIGO guideline) including those on stable ACEi or ARB treatment (unless not considered appropriate) and not receiving a diuretic will proceed to V2 3 to 5 weeks after Visit 1a, and after completion of the baseline MRI (allow approximately 1 week between the completion of the MRI and Visit 2). (Screening may be extended to a maximum of 8 weeks, in the event of extenuating circumstances. Prior approval from the medical monitor is required.)
   ii) Participants who need to be discontinued from diuretic therapy and/or have their anti-hypertensive therapy optimized due to inadequate BP control (as defined by the 2021 KDIGO guideline) will return for Unscheduled visits for blood pressure monitoring following Visit 1a. Once their treatment and blood pressure have been stable for a minimum of 3 weeks, they will return for V1b to have additional serum chemistry testing. The baseline MRI exam may be performed before, at, or after Visit 1b, depending on scheduling. Upon attaining stability of antihypertensive therapy and completion of the baseline MRI, the participant will be scheduled for V2, approximately 1 week following the MRI and approximately 2 weeks following Visit 1b, whichever occurs later. The total duration of Screening, including completion of V2 should not exceed 8 weeks.
d. Participants who complete the Lixivaptan Titration Period in 5 weeks, including the additional week at the same dose to confirm tolerability, will skip V8 and instead complete the assessments scheduled for V9/Last Titration Visit. Participants who may be eligible to skip V8 (Lixivaptan Titration Period (Part 1) — Lixivaptan Titration Flowchart) should be provided with a collection kit for urine osmolality at V7 in the event that they will have V9 assessments performed at the next visit.
e. To encourage study compliance, during the Double-Blind Randomized Treatment Period, telephone contacts will be made 2 weeks (± 5 days) after every scheduled visit.. All telephone contacts with the participant will be recorded in the source document.

f. Participants who discontinue study drug or withdraw from the study at any time after the start of the Placebo Run-In Period should undergo V22/ET procedures within 7 days of the last dose of study drug. Randomized participants should additionally continue into Follow-up Period I for final assessments of safety and efficacy.
g. Vital signs after the participant has been sitting for 5 minutes include heart rate, blood pressure, respiratory rate, and temperature at V1a. At all subsequent visits, vital signs include sitting heart rate and sitting blood pressure. The same position should be used at each visit (Section 7.3.3).
h. A full physical examination will be performed at V1a. A brief physical examination will be performed at V15, V22 and V25. Additional physical examinations will be performed only for assessment of signs or symptoms reported by the participant that might require further evaluation.
i. Height will be measured at V1a. Weight will be measured at V1a, V3, V9/Last Titration, V22/ET, and V25. Weight may be measured at any other visit to assess hydration status, as necessary.
j. Routine pregnancy testing for WOCBP will be performed locally on urine. All positive urine results will be confirmed by a serum pregnancy test at the central lab.
k. Baseline MRI will be scheduled at Visit 1a for all participants in order to determine eligibility according to Mayo Clinic ADPKD Image Classification in time for Visit 2. The post-baseline MRI scheduled for Visit 25 must be completed between 25 days and 31 days post last dose of study drug and must be completed prior to or on the same day as all other Visit 25 study procedures. Volumetric calculations and other derived data from any post-baseline MRI will NOT be shared with the participant, the investigator, the clinical sites, the sponsor, or the contract research organization (CRO) because of the potential for unblinding.
l. Sampling for analysis of blood chemistry will follow the visit schedule specified in Table 3. Chemistry includes the following parameters: albumin; blood urea nitrogen (urea); calcium; chloride; carbon dioxide ($CO_2$); glucose; phosphorous; potassium; protein; sodium; uric acid. Serum sodium will be assessed at the time-points specified in Table 3 either as part of chemistry or liver chemistry testing. Liver chemistry includes alkaline phosphatase; ALT; AST; bilirubin (total and direct). Blood samples for serum creatinine and serum cystatin C will be collected for calculation of eGFR in accordance with Table 3. At Visits 1a, 1b (if required), 2, and 3 and again at Visits 23, 24, and 25, every effort should be made to draw blood samples for serum creatinine and serum cystatin C at approximately the same clock time for a given participant. Laboratory test results from samples collected as specified in Table 3 are to be reviewed and assessed.
m. Participants with positive test results for hepatitis C (HCV) antibody (Anti-HCV), will require a negative HCV RNA titer reflex test prior to enrollment. HCV sero-positive participants will undergo reflex testing at an Unscheduled Visit prior to Visit 2.
n. A single plasma specimen for sparse PK sampling will be obtained at the following visits and time-points: V2 (any time), V3 (0.5 to 1.5 hours post-dose), V9/Last Titration (any time post-dose), V12 (pre-dose), V15 (1 to 6 hours post-dose), and V18 (6 to 10 hours post-dose). Results from PK samples will NOT be shared with the participant, the investigator, the clinical sites, the sponsor, or the CRO because of the potential for unblinding.
o. Urine osmolality (analyzed centrally) will be collected as a fasting (≥8 hrs. except for water) AM trough (i.e., before the morning dose of study drug) urine specimen at V2, V9, V15, V22/ET, and V25. Participants will be provided with a collection kit for urine osmolality at the visit preceding urine osmolality assessments. Results from those visits occurring after randomization will NOT be shared with the participant, the investigator, the clinical sites, the sponsor, or the CRO because of the potential for unblinding.
p. At Visits 3 to 9, the following single tolerability question will be asked, “Would you take the study drug for the next 24 months?”
q. Participants continuing into Part 2 will be dispensed open-label lixivaptan (50mg BID) for initiation of the Lixivaptan Re-titration Period.

## Table 4. Schedule of Procedures – Part 2

| | Lixivaptan Re-titration Period[a] | | | | Maintenance Treatment Period | | Follow-up Period II[b] | | |
|---|---|---|---|---|---|---|---|---|---|
| **Period Duration** | 2 to 4 weeks | | | | 52 weeks | | 4 weeks | | |
| **Visit Number** | **V26** | **(V27)** | **(V28)** | **V29/ Last** | **V30 – V41** | **V42/ET[c]** | **V43** | **V44** | **V45** |
| **Visit Timing** | Every week ±2 days | | | | Every 4 weeks ± 5 days | Week 104 ± 5 days | 8 + 3 days after last dose | ≥ 24 hrs. after V23 and ≥ 24 hrs. before V25 | 28 ± 3 days after last dose |
| **Vital signs[d]** | X | X | X | X | X | X | | | X |
| **Physical examination[e]** | | | | | V35 | X | | | X |
| **Weight[f]** | | | | X | | X | | | X |
| **Urine pregnancy test (WOCBP)[g]** | | | | | V32, V35, V38 | X | | | X |
| **MRI[h]** | | | | | | | | | X |
| **Chemistry blood samples[i]** | | | | | | | | | |
| **Chemistry** | | | | X | V32, V35, V38 | X | | | X |
| **Serum sodium** | | | | X | X | X | | | X |
| **Liver chemistry** | | X | | X | X | X | | | X |
| **Serum creatinine** | | | | X | X | X | X | X | X |
| **Serum cystatin C** | | | | | | | X | X | X |
| **Hematology** | | | | X | V35 | X | | | X |
| **ECG** | | | | X | V35 | X | | | X |
| **PK plasma sample[j]** | | | | X | V32, V35, V38 | | | | |
| **Urinalysis** | | | | X | | X | | | X |
| **Urine osmolality[k]** | | | | X | V35 | X | | | X |
| **Telephone contact[l]** | | | | | ←----------X----------→ | | | | |
| **Study drug dispensing** | X | X | X | X | X | | | | |
| **Study drug reconciliation and compliance** | X | X | X | X | X | X | | | |
| **Medical resource utilization** | X | X | X | X | X | X | X | X | X |
| **ADPKD-IS** | | | | X | V32, V35, V38 | X | | | X |
| **ADPKD-PDS** | | | | X | V32, V35, V38 | X | | | X |

| | Lixivaptan Re-titration Period[a] | | | | Maintenance Treatment Period | | Follow-up Period II[b] | | |
|---|---|---|---|---|---|---|---|---|---|
| **Period Duration** | 2 to 4 weeks | | | | 52 weeks | | 4 weeks | | |
| **Visit Number** | **V26** | **(V27)** | **(V28)** | **V29/ Last** | **V30 – V41** | **V42/ET[c]** | **V43** | **V44** | **V45** |
| **Visit Timing** | Every week ±2 days | | | | Every 4 weeks ± 5 days | Week 104 ± 5 days | 8 + 3 days after last dose | ≥ 24 hrs. after V23 and ≥ 24 hrs. before V25 | 28 ± 3 days after last dose |
| **ADPKD-UIS** | X | X | X | X | V32, V35, V38 | X | | | X |
| **IRT Entry** | ←----------X----------→ | | | | | | | | |
| **Adverse events/SAEs** | ←----------X----------→ | | | | | | | | |
| **Concomitant medications** | ←----------X----------→ | | | | | | | | |

ADPKD = Autosomal Dominant Polycystic Kidney Disease; ADPKD-IS =ADPKD-Impact Scale; ADPKD-PDS = ADPKD-Pain and Discomfort Scale; ADPKD-UIS = ADPKD-Urinary Impact Scale; ET= Early termination visit; hrs. = hours; IRT = interactive response technology; Last = Last re-titration visit; PKD = Polycystic Kidney Disease; V = Visit; WOCBP = women of childbearing potential; ( ) = Not all participants will need these visits.
Visits 26 to 28, 30, 31, 33, 34, 36, 37, 39 to 41, 43, and 44 may be conducted in the clinic or remotely by a home healthcare clinician or qualified site personnel with the agreement of the participant and the discretion of the investigator, and where it is available and locally approved by the Competent Authority and/or Ethics Committee/Institutional Review Board. Remote visits may include a telehealth visit with the investigator as needed.

a. Lixivaptan will be resumed at V25 at a dose of 50 mg BID for 1 week, after which the dose will be increased weekly until the dose taken at the end of the Double-blind, Randomized Treatment Period of Part 1 (as determined by the IRT) is achieved (Table 2). Dosing will continue for the remainder of the study at this level. However, if there is difficulty with tolerability either during the Lixivaptan Re-titration Period or during the Maintenance Treatment Period, the dose may be decreased or temporarily stopped. Attempts will be made every 4 to 8 weeks to re-establish the previously achieved dose level from 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. Participants who complete the Lixivaptan Re-Titration Period in 2 weeks will skip V27 and V28 and those completing the re-titration period in 3 weeks will skip V28; instead, the assessments scheduled for V29/Last Titration Visit will be completed.
b. Follow-Up Period II will start at the completion of V42. During this study drug-free period, 3 visits will occur to obtain 3 serum creatinine values for calculation of eGFR to determine the key comparison endpoint. At the final follow-up visit (V45), the listed additional safety and efficacy assessments will be completed. The first visit will occur on the 8$^{th}$ day (+ 3 days) after the last dose of lixivaptan and the last visit will occur on the 28$^{th}$ day (± 3 days) after the last dose of lixivaptan. V44 must be scheduled to occur at least 24 hours after V43 and at least 24 hours prior to V45.
c. Participants who discontinue from the study should undergo V42/ET procedures within 7 days of the last dose of lixivaptan and subsequently continue into Follow-up Period II for final assessments of safety and efficacy.
d. Vital signs will include sitting heart rate and sitting blood pressure. The same position should be used at each visit (Section 7.3.3).
e. A brief physical examination will be performed at V35, V42/ET and V45. Additional physical examinations will be performed only for assessment of signs or symptoms reported by the participant that might require further evaluation.
f. Weight may be measured to assess hydration status as necessary at visits other than those indicated in Table 4.
g. Routine pregnancy testing for WOCBP will be performed locally on urine. All positive urine results will be confirmed by a serum pregnancy test at the central lab.

h. The post-baseline MRI scheduled for Visit 45 must be completed between 25 days and 31 days post last dose of study drug and must be completed prior to or on the same day as all other Visit 45 study procedures. Volumetric calculations and other derived data from any post-baseline MRI will NOT be shared with the participant, the investigator, the clinical sites, the sponsor, or the CRO because of the potential for unblinding.

i. Sampling for analysis of blood chemistry will follow the visit schedule specified in Table 4. Chemistry includes the following parameters: albumin; blood urea nitrogen (urea); calcium; chloride; carbon dioxide ($CO_2$); glucose; phosphorous; potassium; protein; sodium; uric acid. Serum sodium will be assessed at the time-points specified in Table 4 either as part of chemistry or liver chemistry testing. Liver chemistry includes alkaline phosphatase; ALT; AST; bilirubin (total and direct). Blood samples for serum creatinine and serum cystatin C will be collected for calculation of eGFR in accordance with Table 4. At Visits 43, 44, and 45 every effort should be made to draw blood samples for serum creatinine and serum cystatin C at approximately the same clock time as for Visits 23, 24, and 25 for a given participant. Laboratory test results from samples collected as specified in Table 4 are to be reviewed and assessed.

j. A single plasma specimen for sparse PK sampling will be obtained at the following visits and time-points: V29/Last Re-titration (1 to 6 hours post-dose), V32 (pre-dose), V35 (6 to 10 hours post-dose), and V38 (any time post-dose). Results from PK samples will NOT be shared with the participant, the investigator, the clinical sites, the sponsor, or the CRO because of the potential for unblinding.

k. Urine osmolality (analyzed centrally) will be collected as a fasting (≥8 hrs. except for water) AM trough (i.e., before the morning dose of study drug) urine specimen at V29, V35, V42, and V45. Participants should be provided with a collection kit for urine osmolality at the visit preceding urine osmolality assessments. Results from those visits occurring after randomization will NOT be shared with the participant, the investigator, the clinical sites, the sponsor, or the CRO because of the potential for unblinding.

l. During the Maintenance Treatment Period, participant follow-up via a telephone contact will be made 2 weeks (± 5 days) after every scheduled visit. All telephone contacts with the participant will be recorded in the source document.

## LIST OF ABBREVIATIONS

| Abbreviation | Definition |
|---|---|
| ACEi | angiotensin-converting enzyme inhibitor |
| 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 |
| ALT | alanine aminotransferase |
| ARB | angiotensin II receptor blocker |
| ART | anti-retroviral therapy |
| AST | aspartate aminotransferase |
| BID | twice per day |
| BMI | body mass index |
| BP | blood pressure |
| cAMP | cyclic adenosine 3',5'-monophosphate |
| CFR | Code of Federal Regulations |
| CKD | chronic kidney disease |
| CKD-EPI | Chronic Kidney Disease Epidemiology Collaboration |
| CKD-EPIcr_R | Chronic Kidney Disease Epidemiology Collaboration eGFR creatinine equation refit without the race variable |
| CKD-EPIcr-cys_R | Chronic Kidney Disease Epidemiology Collaboration eGFR creatinine and cystatin C equation refit without the race variable |
| CRA | clinical research associate |
| CRO | contract research organization |
| CS | clinically significant |
| CT | computerized tomography |
| DILI | drug induced liver injury |
| ECG | electrocardiogram |
| eCRF | electronic Case Report Form |
| EDC | electronic data capture |
| eDISH | evaluation of Drug-Induced Serious Hepatotoxicity |
| eGFR | estimated glomerular filtration rate |
| ET | early termination |
| FDA | Food and Drug Administration |

| Abbreviation | Definition |
|---|---|
| GCP | Good Clinical Practices |
| HbsAg | hepatitis B surface antigen |
| HCV | hepatitis C virus |
| HERC | Hepatic Events Review Committee |
| HHC | home healthcare clinician |
| HIF-PH | hypoxia-inducible factor prolyl hydroxylase |
| HIV | human immunodeficiency virus |
| HRQoL | health-related quality of life |
| IB | Investigator Brochure |
| ICF | Informed Consent Form |
| ICH | International Council for Harmonisation |
| IDMC | Independent Data Monitoring Committee |
| IND | Investigational New Drug |
| IRB | Institutional Review Board |
| KDIGO | The Kidney Disease: Improving Global Outcomes |
| LV | liver volume |
| MedDRA | Medical Dictionary for Regulatory Activities |
| MMRM | mixed-effects model for repeated measures |
| MRI | magnetic resonance imaging |
| NCS | not clinically significant |
| OTC | over-the-counter |
| PEAS | primary efficacy analysis set |
| PK | Pharmacokinetics |
| PKD | polycystic kidney disease |
| PT | preferred term |
| QTcF | QT interval corrected for heart rate according to Fridericia's formula |
| SAE | serious adverse event |
| SAP | statistical analysis plan |
| SD | standard deviation |
| SEAS | secondary efficacy analysis set |
| SGLT2 | sodium-glucose cotransporter 2 |
| SOC | system organ class |
| TEAE | treatment-emergent adverse event |
| TKV | total kidney volume |
| ULN | upper limit of normal |

| Abbreviation | Definition |
|---|---|
| $U_{osm}$ | urine osmolality |
| US | United States |

# 1 INTRODUCTION

## 1.1 Background

Lixivaptan (also known as VPA985, BIIB030, and CL 347,985) is a potent, non-peptide selective antagonist for the vasopressin $V_2$ receptor, which is expressed primarily in the collecting duct of the nephron (Chan et al, 1998). Lixivaptan antagonizes the effects of vasopressin that are mediated by the $V_2$ receptor, resulting in the pharmacologic effect of increased free water excretion, thus decreasing urine osmolality ($U_{osm}$), increasing urine flow, and increasing serum osmolality as well as restoring normal levels of intracellular cyclic adenosine 3',5'-monophosphate (cAMP) (Chebib, 2015).

The sponsor (Palladio Biosciences, Inc.) is currently developing lixivaptan for the treatment of autosomal dominant polycystic kidney disease (ADPKD). Lixivaptan had previously been under development for the treatment of symptomatic hypervolemic and euvolemic hyponatremia associated with heart failure and syndrome of inappropriate anti-diuretic hormone secretion.

Additional background information for lixivaptan can be found in the Lixivaptan Investigator's Brochure (IB).

## 1.2 Overview of ADPKD

**Definition**

ADPKD is a hereditary kidney disorder characterized by cyst formation and progressive enlargement in the kidney, liver, and other organs. It results from loss-of-function mutations in either of 2 genes (*pkd1* and *pkd2*), encoding transmembrane proteins polycystin 1 (PC1) and polycystin 2 (PC2), respectively.

In the kidney, loss-of-function mutations in *pkd1* or *pkd2* disrupt the normal differentiated phenotype of the renal tubular epithelium. These mutations lead to elevated intracellular levels of cAMP, which in turn results in increased cellular division and apoptosis, a loss of mitotic polarity, a disruption of the normal differentiated cellular phenotype, cyst formation, and fluid secretion into kidney cysts (Antignac et al, 2015). The progressive development and growth of numerous bilateral kidney cysts results in fibrosis, kidney architectural derangement, and destruction of normal kidney tissue. Disease progression is characterized by urine concentration defects, hypertension, acute and chronic pain, kidney stones, haematuria, cyst and urinary tract infections, and, most importantly, kidney function loss and kidney failure (Antignac et al, 2015, Chapman et al, 2015).

The most frequent extrarenal manifestation of ADPKD is polycystic liver disease, which is typically associated with increased kidney volume, older age, and female sex. Liver cysts are usually asymptomatic, and the liver chemistry is normal. However, in some cases the increased liver volume may lead to hepatomegaly as a result of the continuous cyst enlargement (Hogan et al, 2015). This may cause symptoms of extrinsic compression, such as abdominal pain, early satiety, and obstruction of the hepatic veins or bile duct. Moreover, liver cyst infections may cause fever, right upper abdominal pain, and/or possible elevated CA19.9 and alkaline phosphatase levels.

Epithelial cells derived from human ADPKD cysts generate elevated intracellular levels of cAMP in response to vasopressin, which in turn promotes cell proliferation and electrolyte secretion (Belibi et al, 2004). Increased fluid secretion into cysts is due to vasopressin-induced transepithelial secretion of chloride. Patients with ADPKD also have elevated plasma concentrations of vasopressin or an exaggerated vasopressin response to sodium challenge as compared to normal individuals (Torres, 2005). Pharmacological interventions aimed at inhibiting vasopressin signalling are, therefore, expected to restore normal cellular function and provide a disease-modifying effect on disease progression in ADPKD.

## 1.3 Overview of Available Therapies for ADPKD

Demonstration of the utility of vasopressin $V_2$ receptor antagonists for the treatment of ADPKD is provided by the experience with tolvaptan. Results from the TEMPO 3:4 Phase 3 trial showed that tolvaptan slowed the progression of kidney enlargement and delayed the worsening of kidney function (Torres et al, 2012). The study also established suppression of urine osmolality to <250 mOsm/kg as a predictive pharmacodynamic marker of clinical efficacy for a vasopressin $V_2$ antagonist for the treatment of ADPKD (Devuyst et al, 2017). Subsequently, the REPRISE Phase 3 study with tolvaptan demonstrated that the efficacy of vasopressin $V_2$ receptor antagonism is maintained in patients with later stage ADPKD (Torres et al, 2017).

The European Medicines Agency granted a positive opinion for tolvaptan (JINARC®) in 2015 to slow the progression of cyst development and failing kidney function in adult patients with ADPKD with normal to moderately-reduced kidney function who have rapidly progressing ADPKD. The Committee for Medicinal Products for Human Use recommended additional monitoring of the risk of liver damage with tolvaptan. Similar approvals were granted in Japan, Canada, and Australia/New Zealand.

In April 2018, tolvaptan (JYNARQUE®) was approved in the United States (US) to slow kidney function decline in adults at risk of rapidly progressing ADPKD. However, because of the risk of serious liver injury, Jynarque is only available through a restricted distribution program under a Risk Evaluation and Mitigation Strategy.

## 1.4 Rationale for Lixivaptan Therapy for ADPKD

Given that elevated kidney cAMP plays a pivotal role in the complex phenotypical manifestation of ADPKD (Belibi et al, 2004), and that vasopressin is the principal agonist pathway leading to the formation of cAMP in kidney tubule cells, it has been suggested that therapeutic interventions aimed at counterbalancing the effect of vasopressin and/or normalizing intracellular levels of cAMP may be effective in delaying disease progression in ADPKD (Torres, 2005). This hypothesis was confirmed by genetic experiments in mutated rats with no circulating serum vasopressin (Brattleboro rats). When these rats were crossed with rats harboring a PKD mutation, their offspring did not develop cysts (Wang et al, 2008), thus demonstrating that an intact vasopressin signaling pathway is a necessary requirement for the development of cystic disease. In addition, vasopressin receptor antagonists, including mozavaptan and tolvaptan, (Gattone et al, 2003 and Wang et al, 2005) have proved efficacious in normalizing kidney cAMP levels and correcting disease manifestations in preclinical rodent models of ADPKD. In ADPKD patients, elevated levels of circulating

copeptin, a marker for serum vasopressin concentration, are associated with faster disease progression (Boertien et al, 2012).

Further, definitive evidence in favor of the utility of vasopressin antagonism as a therapeutic approach for ADPKD is derived from tolvaptan, like lixivaptan a non-peptide vasopressin $V_2$ receptor antagonist in the drug class of vaptans. In Europe, tolvaptan (JINARC®) is approved to slow the progression of cyst development and kidney insufficiency of ADPKD in adults with CKD stage 1 to 3 at initiation of treatment with evidence of rapidly progressing disease. Approval was granted based on the findings from the pivotal Phase 3 trial TEMPO 3:4. The trial randomized 1445 patients with ADPKD with a total kidney volume of ≥750 mL and an estimated creatinine clearance of ≥60 mL/min to tolvaptan or placebo. Over a 3-year period, tolvaptan delayed the worsening of kidney function, a therapeutic effect that was subsequently found to have disease-modifying properties and slowed the progression of kidney enlargement (Torres et al, 2012 and 2017a). Importantly, the TEMPO 3:4 trial also demonstrated that, in patients receiving tolvaptan, there was a strong, inverse relationship between the magnitude of the aquaretic response, measured by decrease in urinary osmolality, and the loss of kidney function during the study (Devuyst et al, 2017). Because complete vasopressin suppression is ensured if urinary osmolality is lowered to <250 mOsm/kg, these findings strongly suggest that achieving urinary osmolality reduction below this target threshold can be considered a predictive biomarker of clinical efficacy of vasopressin antagonists for the treatment of ADPKD (Devuyst et al, 2017).

More recently, the results of REPRISE, a second pivotal Phase 3 trial with tolvaptan for the treatment of ADPKD, were published (Torres et al, 2017). This trial randomized 1370 patients with later stage ADPKD, which was defined as having a baseline estimated glomerular filtration rate (eGFR) of <65 mL/min for patients in the 18-55 age group or <45 mL/min for patients in the 56-65 age group, to tolvaptan or placebo. Over a 12-month period, tolvaptan delayed the eGFR decline, a surrogate for worsening kidney function, with a magnitude of effect that was comparable to the one seen in the TEMPO 3:4 study (35%; $p<0.0001$). Taken together, the results of these two studies demonstrate that the efficacy of vasopressin antagonism is maintained across multiple time periods and stages of disease.

The evidence supporting the potential utility of lixivaptan for the treatment of ADPKD is provided by experiments in animal models of polycystic kidney disease (PKD) and by the clinical effect of lixivaptan on urine osmolality, a pharmacodynamic biomarker of effective vasopressin antagonism that is correlated with efficacy in ADPKD, as discussed below.

**Lixivaptan ameliorates disease manifestations in animal models of PKD**

Lixivaptan was tested in rat and mouse animal models of PKD.

The PCK rat, an orthologous model of human PKD caused by a splicing mutation in the *pkhd1* gene, is one of the best-studied models in the field of PKD research because it is characterized by a phenotype that is highly reminiscent of the human disease. Compared to control animals, PCK rats treated with lixivaptan showed a marked protective effect on the development of kidney disease manifestations, including reduced cystic burden, reduced kidney volume increase, and delayed kidney function decline. These beneficial effects were accompanied by a reduction in kidney cAMP levels, as expected (Wang et al, 2019).

The Pkd1$^{RC/RC}$ mouse is a hypomorphic genetic model which closely mimics human ADPKD with slowly progressive PKD. Compared to control animals, Pkd1$^{RC/RC}$ mice treated with lixivaptan showed a significant reduction in kidney weight, cyst volume, and fibrosis volume (Di Mise et al, 2021). The magnitude of efficacy observed with lixivaptan in the RC/RC mouse model is comparable to historical experiments conducted with tolvaptan (Hopp et al, 2015).

Additional background information on the effect of lixivaptan in the PCK rat and Pkd1$^{RC/RC}$ mouse can be found in the IB.

**Lixivaptan causes sustained suppression of urine osmolality to levels that are associated with therapeutic efficacy in ADPKD**

The expectation about the efficacy of lixivaptan in ADKPD is supported by the observed clinical effects of lixivaptan on the pharmacodynamic biomarker of urinary osmolality. Clinical studies conducted with lixivaptan in healthy participants, ADPKD patients, and various other patient populations (patients with hypervolemic and euvolemic hyponatremia and patients with ESRD) demonstrated that treatment with lixivaptan readily suppresses urinary osmolality to levels below the target threshold of 250 mOsm/kg, irrespective of the specific patient population tested. Importantly, the magnitude of the effect observed with lixivaptan was comparable to the effect observed with tolvaptan in the same patient populations. In addition, like tolvaptan, treatment with lixivaptan was associated with a rapid-onset and fully reversible increase in serum creatinine. Taken together, these results suggest that treatment with lixivaptan can inhibit vasopressin receptor signaling to the extent necessary to observe clinical efficacy of a vasopressin antagonist for the treatment of ADPKD (Devuyst et al, 2017).

Additional background information on the effect of lixivaptan on urine osmolality can be found in the IB.

**Lixivaptan as an alternative to tolvaptan with potentially less risk of liver toxicity**

While tolvaptan has been approved in many territories to slow the progression of kidney function deterioration in patients with ADPKD, drug-induced liver injury (DILI) occurs in a certain percentage of patients taking tolvaptan. Acute liver failure requiring liver transplantation has also been reported (Endo et al, 2019). Patients on tolvaptan require frequent testing of liver chemistry and discontinuation of the drug when the test results are elevated in order to prevent serious outcomes.

DILI modeling using DILIsym$^{TM}$, a Quantitative Systems Pharmacology model of drug-induced liver injury, has allowed for the assessment of potential abnormal liver chemistry tests prior to conducting large clinical trials (Woodhead et al, 2017a). DILIsym integrates physiologically based pharmacokinetic (PBPK) and *in vitro* toxicity data of parent compound and its active metabolites. Specific *in vitro* data incorporated into the model includes drug induced bile acid transporter inhibition, mitochondrial dysfunction, and oxidative stress, all of which have been identified as putative cellular mechanisms for DILI.

DILIsym was employed to model tolvaptan-mediated liver injury and was able to successfully recapitulate the observed toxicity (Woodhead et al, 2017b). The frequency of

predicted ALT elevations following simulated 90/30 mg split daily dosing, was 7.9% compared with clinical observations of 4.4-5.6% in ADPKD trials. The simulations also demonstrated that the *in vivo* hepatic exposure to tolvaptan and its main metabolite, DM-4103, combined with alterations in bile acid disposition and inhibition of mitochondrial respiration were sufficient to account for the initiation of tolvaptan-mediated liver toxicity.

This same platform was used to evaluate the potential of lixivaptan to cause liver toxicity (Woodhead et al, 2020). *In vitro* data relating to reactive oxygen species formation, mitochondrial toxicity, and bile acid transporter inhibition for lixivaptan and its major metabolites (WAY-138451, WAY-141624, and WAY-138758) were collected in parallel with tolvaptan as a positive control. Using these data, lixivaptan and its metabolites were represented in DILIsym. Proposed ADPKD treatment dosing regimens were simulated and the predicted potential for liver enzyme elevations was compared to that previously determined for tolvaptan in DILIsym. Results showed that lixivaptan was not predicted to cause liver enzyme elevations in a simulated human population which included variability in toxicity susceptibility and pharmacokinetics, while tolvaptan was correctly predicted to cause rare liver enzyme elevations in a similar population (Table 5).

Additional information regarding the DILIsym evaluation of lixivaptan can be found in the IB.

**Table 5. DILIsym Simulations of Lixivaptan and Tolvaptan**

| Drug | Dose, Duration | Clinical ALT >3 x ULN | Simulated ALT >3 x ULN, n/N |
|---|---|---|---|
| Lixivaptan | 200/100 mg q AM/PM, 12 weeks | UNK* | 0/285 |
| Tolvaptan** | 90/30 mg q AM/PM, 24 weeks | 4.4% | 18/229 7.86% |

*UNK = Unknown; data to be acquired in Phase 3
**Source: Woodhead et al, 2017b

## 1.5 Summary of Nonclinical Studies

Experiments in rats and dogs, with or without pretreatment with vasopressin, and with or without free access to water, confirmed that lixivaptan is a potent vasopressin $V_2$ receptor antagonist. Selectivity for the $V_2$ receptor was demonstrated using cloned receptors and cell membranes isolated from kidney, platelet, and liver. Furthermore, using cell proliferation assays, lixivaptan had no agonist activity at human $V_{1a}$ or rat $V_{1b}$ ($V_3$) receptors.

Compared with conventional diuretics, lixivaptan increases urinary volume output about 3 to 4 times more than furosemide or hydrochlorothiazide at comparable doses and decreases urinary osmolality. Three identified human metabolites of lixivaptan (WAY-138451, WAY-138758, and WAY-141624) were found to be weakly active or inactive as vasopressin $V_2$ receptor antagonists as assessed by in vitro receptor binding and in vivo aquaretic studies in rats.

In addition to the pharmacology studies, lixivaptan was evaluated in a comprehensive toxicology program. Results showed the PK and metabolism are well characterized and there were no significant safety signals identified.

Additional information regarding nonclinical studies conducted with lixivaptan can be found in the IB.

## 1.6 Summary of Clinical Studies

Lixivaptan is a novel, highly selective, non-peptide, vasopressin $V_2$ receptor antagonist. It was previously developed for treating disease states associated with water retention, e.g., euvolemic and hypervolemic hyponatremia. Pharmacokinetic (PK), safety, tolerability, and efficacy data for lixivaptan are available from 36 clinical studies, including 22 Phase 1 studies in healthy and/or CKD participants, 10 Phase 2a studies, and 4 Phase 3 studies in participants with hyponatremia. More than 1600 participants received at least one dose of lixivaptan as part of the hyponatremia development program, including 867 who participated in Phase 2 and 3 studies. Lixivaptan was generally safe and well-tolerated in this patient population. The most common adverse events (AEs) were headaches, dizziness, thirst, orthostatic hypotension, and tachycardia events. Additional information regarding the clinical evaluation of lixivaptan in hyponatremia can be found in the IB.

Subsequent to the conclusion of the hyponatremia program, lixivaptan was tested in ADPKD patients in the open-label Phase 2 study, PA-102. This study was conducted to characterize the PK, safety, and pharmacodynamic profiles of lixivaptan following administration of twice per day (BID) oral doses of 50 and 200 mg for 7 days in participants with both ADPKD and CKD stage 1 (CKD1), stage 2 (CKD2), or stage 3 (CKD3). The dose of 200 mg BID successfully suppressed mean spot urine osmolality below 300 mOsm/kg (denoting dilute urine) throughout the 24-hour period on Day 7 in 100% of study participants with CKD2 and CKD3, indicating continuous vasopressin $V_2$ receptor inhibition. Lixivaptan was well tolerated by participants. Although a dose of 50 mg BID reduced $U_{osm}$ over part of the dosing interval, the duration was not long enough to ensure effective blockade of the vasopressin $V_2$ receptor throughout 24 hours. However, 50 mg BID is an appropriate starting dose to allow accommodation to the aquaretic effects of the drug. Lixivaptan was well tolerated by ADPKD participants; the most common AEs reported in 3 or more participants were dry mouth, headache, and nausea. No participant was discontinued because of an AE. Additional information regarding the results of this study can be found in the IB.

Study PA-103 was a Phase 2, open-label, repeat dose expanded access study designed to reduce the abdominal pain related to ADPKD in a single participant who had been incapacitated by ADPKD-related pain. The participant had been successfully treated for the pain with tolvaptan but was unable to continue with the drug because of DILI, which manifested as elevated ALT levels that occurred during 3 separate trials of repeated tolvaptan dosing following initial treatment and following 2 re-challenges after treatment discontinuations due to the elevations. The participant's nephrologist contacted Palladio and requested the use of lixivaptan in this participant. Lixivaptan doses up to 150 mg in the morning and 100 mg in the evening were allowed during the titration period and subsequently were allowed to increase to 200 mg BID in the Maintenance Treatment Period. Lixivaptan was well-tolerated by the participant in the study. Importantly, the participant completed 413 days of treatment with

lixivaptan without any evidence of liver injury. All liver chemistry tests were normal while the participant had been receiving lixivaptan. The participant's pain and quality of life modestly improved while on lixivaptan therapy, but because of continued discomfort the participant elected to discontinue lixivaptan in order to pursue more aggressive pain management treatments.

Study PA-ADPKD-303, which is currently ongoing, is a Phase 3, open-label, repeat dose study designed to assess hepatic safety, non-hepatic safety, and efficacy in participants who previously experienced abnormal liver chemistry test results while treated with tolvaptan. Participants who had to stop tolvaptan therapy due to abnormal liver chemistry test results do not currently have available therapeutic options. In this study, such participants are treated with lixivaptan to determine if it is a safer alternative. Up to 50 participants are being enrolled and treated. Evaluations include frequent testing of liver chemistry (every 1 week during the Titration Period and every 4 weeks during the Treatment Period), physical examinations, vital signs, safety labs (chemistry, hematology, urinalysis), urine osmolality determinations, and trough serum concentration of lixivaptan.

## 1.7 Study Rationale

This is a Phase 3, double-blind, placebo-controlled, randomized trial to demonstrate the efficacy and safety of lixivaptan compared with placebo in participants with ADPKD during a 52-week, double-blind treatment phase (Part 1). Participants who are not discontinued due to an adverse event or who have not withdrawn consent, will immediately continue into a 52-week open-label treatment phase (Part 2) with lixivaptan. This study is designed to demonstrate the efficacy of lixivaptan in slowing the decline in kidney function as measured by eGFR with confirmation of the durability of this effect in the open-label phase of the study. Additionally, the study will contribute to understanding the safety of lixivaptan in ADPKD patients, particularly any effects on liver chemistry tests.

## 1.8 Dose Rationale

The human equivalent exposure range of lixivaptan proposed for this study has been shown to be safe and tolerated in nonclinical animal studies and in previous clinical investigations conducted in a robust sample of healthy volunteers and participants with hyponatremia of various etiologies, as well as in Phase 2 investigations in participants with ADPKD.

The dose range selected for this study, 100 mg BID to 200 mg BID (with 50 mg BID as a starting dose), provides a minimal and maximal dose that has been shown to effectively reduce $U_{osm}$ throughout a 24-hour daily dosing cycle in patients with ADPKD and CKD stages 2-3. In a previous dose-ranging study, while 50 mg BID of lixivaptan was effective in reducing $U_{osm}$ over part of the dosing interval, the duration was not long enough to ensure effective blockade of the vasopressin $V_2$ receptor throughout 24 hours in a meaningful proportion of participants. In this study, the 50 mg BID dose will, therefore, be used as a starting dose to acclimate participants to vasopressin $V_2$ receptor blockade and assess tolerability. Throughout the study, doses will be titrated in individual patients based on tolerability or up to the maximum dose.

## 1.9 Benefit/Risk Assessment

Safety data gathered to date in participants with ADPKD are consistent with the robust safety experience from lixivaptan treatment in studies in earlier indications investigated for lixivaptan—congestive heart failure, liver cirrhosis with ascites, or syndrome of inappropriate antidiuretic hormone secretion. The most common (≥ 2%) adverse effects of lixivaptan are related to the exaggerated pharmacology of blockade of the renal concentrating mechanism. This results in urinary dilution with the production of increased amounts and frequency of urine output and, secondarily, the stimulation of thirst. No adverse effects on other body systems have been noted. Lixivaptan as a vasopressin V2 receptor antagonist is in the same pharmacological class as tolvaptan. DILI has been observed during the use of tolvaptan for ADPKD and JINARC/Jynarque® (brand names for tolvaptan) have boxed warnings for DILI in their Summary of Product Characteristics/US Prescribing Information. Although there have been no reports of DILI related to lixivaptan to-date, the potential risk of DILI during lixivaptan treatment exists; this protocol has been designed to frequently monitor the participants for liver test abnormalities, has established an IDMC for safety oversight and has established an independent Hepatic Event Review Committee to monitor and review any participant's signs, symptoms, and/or laboratory tests that meet hepatic event criteria, and described procedures to prevent liver toxicity. One of the two primary objectives of this study is to determine the risk of DILI as a result of lixivaptan treatment for ADPKD.

Additionally, pharmacodynamic data generated for vasopressin V2 receptor antagonists that assess the mechanism of action, primarily through the development program for tolvaptan, have been confirmed in Phase 2 studies with lixivaptan and support continued development of lixivaptan for this indication. As there additionally remains a large unmet medical need to address chronic treatment in patients with ADPKD, particularly those who have experienced liver chemistry abnormalities leading to discontinuation of treatment with tolvaptan, continued investigation of this drug for ADPKD is warranted. Based on the totality of evidence generated to date on lixivaptan, the benefit/risk assessment of lixivaptan remains favorable.

# 2 STUDY OBJECTIVES AND ENDPOINTS

## 2.1 Study Objectives

### 2.1.1 Objectives of the Double-blind Phase (Part 1/Year 1)

The primary efficacy objective of Part 1 is:

- 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).

The key safety objective of Part 1 is:

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

The secondary efficacy objectives of Part 1 are:

- 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 total kidney volume (TKV) as measured by magnetic resonance imaging (MRI) compared to placebo.

The secondary safety objective of Part 1 is:

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

The health outcomes-related objectives of Part 1 are:

- 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.

The population pharmacokinetics (PopPK) objective of Part 1 is:

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

The exploratory objectives of Part 1 are:

- To assess the effect of lixivaptan on liver volume (LV) 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 Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation refit without the race variable (CKD-EPIcr-cys_R) compared with placebo.

### 2.1.2 Objectives of the Open-label Phase (Part 2/Year 2)

The key objective of Part 2 is:

- 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 at the end of the open-label phase (Part 2).

The key safety objective of Part 2 is:

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

The secondary objectives of Part 2 are:

- 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 TKV as measured by MRI in Part 2 in participants treated with lixivaptan in Part 1 and Part 2.

The secondary safety objective of Part 2 is:

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

The health outcomes-related objectives of Part 2 are:

- 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.

The PopPK objective of Part 2 is:

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

The exploratory objectives of Part 2 are:

- To assess the effect of lixivaptan on LV 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.

## 2.2 Study Endpoints

### 2.2.1 Part 1/Year 1

#### 2.2.1.1 Primary Efficacy Endpoint

- The primary endpoint is the annualized change in eGFR calculated from the CKD-EPIcr_R equation for serum creatinine from baseline (mean of 3 eGFR determinations obtained during Screening and Placebo Run-in Periods (Visits 1a/1b (if required), Visit 2, and Visit 3) to final assessment (mean of 3 eGFR determinations obtained during Follow-up Period I or, for participants who discontinue treatment prior to Visit 22, during the Follow-up Period 8 to 28 days following study drug treatment discontinuation).

#### 2.2.1.2 Key Safety Endpoint

The key liver safety endpoint is:

- Incidence of serum ALT levels >3 x ULN in participants randomized to lixivaptan compared to those randomized to placebo.

#### 2.2.1.3 Secondary Efficacy Endpoints

The additional secondary endpoints are:

- The annualized rate of change (slope) from baseline in on-treatment eGFR, based on all on-treatment eGFR determinations during the Double-Blind, Randomized Treatment Period in Part 1, calculated from the CKD-EPI equation (CKD-EPIcr_R) for serum creatinine;
- The annualized change from baseline in TKV, determined by MRI, during Follow-up Period I.

#### 2.2.1.4 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 and serum sodium, hematology and urinalysis);
- Vital signs;
- 12-lead electrocardiograms (ECG).

#### 2.2.1.5 Health Outcome Endpoints

- Medical resource utilization (e.g., medication use/changes; unplanned office visits; urgent care and emergency department usage; and hospitalizations) resulting from clinical events.
- Patient Reported Outcomes: ADPKD-IS; ADPKD-PDS; ADPKD-UIS.

#### 2.2.1.6 Population Pharmacokinetics Endpoints

- Area under the plasma concentration-time curve during the dosing interval.
- Maximum plasma concentration.

#### 2.2.1.7 Exploratory Endpoints

- The annualized change from baseline in LV determined by MRI during Follow-up Period I.
- Mean change from baseline in morning spot urine osmolality.
- The annualized change in eGFR calculated from the CKD-EPIcr-cys_R equation from baseline to final assessment.

### 2.2.2 Part 2/Year 2

#### 2.2.2.1 Key Comparison Endpoint

- The primary endpoint of Part 2 is the annualized change in eGFR calculated from the CKD-EPI equation (CKD-EPIcr_R) for serum creatinine from baseline (mean of 3 eGFR determinations obtained during Follow-up Period I) to final assessment (mean of 3 eGFR determinations obtained during Follow-up Period II).

#### 2.2.2.2 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.

#### 2.2.2.3 Other Comparison Endpoints

The additional secondary endpoints are:

- The annualized rate of change (slope) from baseline (mean of 3 eGFR determinations obtained during Follow-up Period I) in on-treatment eGFR, based on all on-treatment eGFR determinations during the Maintenance Treatment Period in Part 2, calculated from the CKD-EPI equation (CKD-EPIcr_R) for serum creatinine;
- The annualized change in TKV determined by MRI.

#### 2.2.2.4 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, and serum sodium, hematology and urinalysis);
- Vital signs;
- 12-lead electrocardiograms (ECG).

#### 2.2.2.5 Health Outcome Endpoints

- Clinical events resulting in medical resource utilization;
- Patient Reported Outcomes: ADPKD-IS; ADPKD-PDS; ADPKD-UIS.

#### 2.2.2.6 Population Pharmacokinetics

- Area under the plasma concentration-time curve during the dosing interval.
- Maximum plasma concentration.

#### 2.2.2.7 Exploratory Endpoints

- The annualized change in LV determined by MRI;
- Mean change from baseline in morning spot urine osmolality;
- The annualized change in eGFR calculated from the CKD-EPIcr-cys_R equation from baseline to final assessment.

# 3 INVESTIGATIONAL PLAN

## 3.1 Study Design

### 3.1.1 Overview of Study Design

This is a global, Phase 3 trial consisting of a 2-arm, double-blind, placebo-controlled, randomized phase followed by a single-arm open-label phase to demonstrate the efficacy and safety of lixivaptan in participants with ADPKD. The randomized phase of the study (Part 1) is designed to demonstrate the efficacy and safety of lixivaptan in slowing the decline in kidney function as measured by eGFR and to demonstrate a difference between the lixivaptan-treated and placebo-treated participants. The open-label phase of the study (Part 2) is designed to provide confirmation of the durability of this effect. Importantly, this study will contribute to understanding the safety of lixivaptan, particularly any effects on liver chemistry tests.

Approximately 2250 participants with ADPKD will be screened in order to randomize 1350 participants to lixivaptan or placebo in a 2:1 ratio in Part 1 of the study. After meeting entry criteria during a 3- to 5-week Screening Period (that may be extended up to 8 weeks as described in Section 3.1.2), participants will enter a 1-week single-blind, Placebo Run-in period to obtain select baseline measurements. This will be followed by a 4 to 6 week single-blind dose titration period during which lixivaptan administered twice daily will be titrated to the highest tolerated dose (Lixivaptan Titration Period (Part 1) — Lixivaptan Titration Flowchart). The minimum dose to enter the Double-blind, Randomized Treatment Period is Level 2 (100 mg BID). Those participants successfully titrated and tolerating the drug will then be randomized (2:1) to either continue lixivaptan or switch immediately to matching placebo in a double-blind manner. Randomization will be stratified for CKD Stage and Mayo Clinic ADPKD Image Classification. Double-blind treatment will continue for 52 weeks after which study drug will be held, and final assessments obtained off-treatment over 3 visits starting on the 8$^{th}$ day after the last dose of double-blind study drug through the 28$^{th}$ day after the last dose of study drug.

Participants who have not discontinued due to an adverse event or withdrawn consent will continue into the 2- to 4-week Lixivaptan Re-titration Period to initiate Part 2 of the study. Dosing with lixivaptan 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. Lixivaptan treatment will continue for 52 weeks during the Maintenance Treatment Period after which study drug will be held, and final assessments obtained off-treatment over 3 visits starting on the 8$^{th}$ day after the last dose of double-blind study drug through the 28$^{th}$ day after the last dose of study drug.

The study schematic is depicted in Figure 1 and Figure 2.

### 3.1.2 Detailed Study Design

**Part 1:**

Screening Period (Visits 1a, 1b, 2): Participants who provide written informed consent will have medical history collected and undergo baseline testing over a 3 to up to 8-week Screening Period. The variable length of the Screening Period is intended to allow for discontinuation of diuretics or other disallowed medications (e.g., CYP3A4 inhibitors), antihypertensive medication optimization, stabilization of blood pressure and completion of the baseline MRI for Mayo Clinic ADPKD Image classification (Irazabal et al, 2015) by the central imaging vendor prior to Visit 2. For participants who do not require changes to background therapy, the Screening Period should be completed in 3 to 5 weeks.

Participants will continue through Screening as follows:

- Participants with appropriate blood pressure control for a minimum of 3 weeks prior to enrollment (Visit 2) (as defined by the 2021 KDIGO guideline) including those on stable ACEi or ARB treatment (unless not considered appropriate) and not receiving a diuretic will have a baseline MRI exam scheduled and will proceed to Visit 2, 3 to 5 weeks after Visit 1a, and after completion of the baseline MRI (allow approximately 1 week after completion of the MRI, however a 3-week minimum between Visit 1a and Visit 2 is required.) Screening may be extended to a maximum of 8 weeks, in the event of extenuating circumstances; prior approval from the medical monitor is required.
- Participants who need to be discontinued from diuretic therapy and/or have their anti-hypertensive therapy optimized due to inadequate BP control (as defined by the 2021 KDIGO guideline) will return for Unscheduled visits for blood pressure monitoring following Visit 1a. Once their treatment and blood pressure have been stable for a minimum of 3 weeks, they will return for Visit 1b to have additional serum chemistry testing. The baseline MRI examination should be scheduled after completion of Visit 1a but may be performed before, at, or after Visit 1b, dependent on scheduling availability. Upon attaining stability of antihypertensive therapy, completion of the baseline MRI, and following receipt of the Mayo Clinic ADPKD Classification from the central imaging vendor, the participant will complete Visit 2 (a minimum of 2 weeks after Visit 1b or approximately 1 week after completion of the MRI, whichever occurs later.) The total duration of the Screening Period may be extended up to 8 weeks, including the time to completion of Visit 2.

Participants are eligible for enrollment if they are between the ages of 18 to 60 years (inclusive), have a diagnosis of ADPKD, have an eGFR of 25 to 90 mL/min/1.73 $m^2$ as calculated using the 2021 CKD-EPIcr_R equation Appendix 1 (Section 13.1); (note that the final determination of eligibility will be made during the Placebo Run-in Period based on the mean of the eGFR values obtained at Visits 1a and 2 or 1b and 2, if Visit 1b is required), have a Mayo Clinic ADPKD classification of 1C, 1D, or 1E as determined by the central imaging vendor's assessment of the baseline MRI, have appropriately controlled blood pressure without the use of a diuretic, and meet all other study entry criteria (Section 4.1). Participants who were enrolled based on the 2009 CKD-EPI equation (referenced in

Appendix 1 (Section 13.1) will not be discontinued from the study should their baseline eGFR calculated subsequently with the CKD-EPIcr_R equation, yield a mean eGFR value outside of the range of 25 to 90 mL/min/1.73 $m^2$. The stratification by CKD stage (Section 8.4.1) will be based on the baseline eGFR creatinine equation in effect at the time of randomization. However, all efficacy analyses of change in eGFR will be based on the CKD-EPIcr_R equation. Participants who fail inclusion/exclusion criteria due to temporary or correctable reasons may be re-screened (up to 2 additional times after obtaining new informed consents) and after consultation with the medical monitor. **Note**: Discontinuation of effective treatment for the sole purpose of making a participant eligible for participation in the study is prohibited.

At the successful completion of Visit 2, participants will be considered enrolled and will be assigned single-blind study drug (placebo) via the IRT to start the Placebo Run-in Period. Participants will take 4 capsules of study drug twice daily (BID) approximately 10 hours apart. All dosing throughout the study will be 4 capsules BID.

Placebo Run-in Period (Visit 3): During the 1-week Placebo Run-in Period, participants will receive single-blind placebo and take 4 capsules twice daily. At the end of the 1-week Placebo Run-in Period, participants will attend Visit 3 at which time tolerability will be assessed to maintain participant blinding and final baseline measurements will be obtained. At this visit and those during the Lixivaptan Titration Period (Visits 4 to 9), tolerability will be assessed by asking the participant a single question "Would you take the study drug for the next 24 months?" Participants reporting tolerability issues will be discontinued from the study and complete Visit 22/Early Termination (ET) procedures. All other participants will be assigned single-blind lixivaptan via IRT at a dose of 50 mg BID to start the Lixivaptan Titration Period. At Visit 3, participants should take their first dose of study drug from the newly assigned (Visit 3) blister card in the clinic following completion of study visit procedures and in accordance with the sparse PK sampling schedule for this visit (0.5 hrs. to 1.5 hrs. post dose).

Lixivaptan Titration Period (Visits 4 to 9): During a period of 5 to 6 weeks for participants who complete the Placebo Run-in Period, the lixivaptan dose initiated at Visit 3 will be increased in a single-blind fashion according to the titration schedule in Lixivaptan Titration Period (Part 1) — Lixivaptan Titration Flowchart to achieve the maximally tolerated dose up to the maximum dose allowed (Level 4 [200 mg BID]). 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. Further dose reductions are allowable as shown in the Lixivaptan Titration Period (Part 1) — Lixivaptan Titration Flowchart. However, the minimum dose to enter the Double-blind, Randomized Treatment Period is Level 2 (100 mg BID).

Tolerability will be assessed at each titration visit (Visit 4 to Visit 9, as applicable). If the participant confirms the dose can be tolerated, the participant will be titrated to the next dose level (i.e., the dose will be increased). This assessment of tolerability will continue at weekly intervals until either the maximum dose level is reached (Level 4 [200 mg BID]) or until the participant confirms the dose cannot be tolerated for a 24-month period. If the dose cannot

be tolerated, it will be reduced by one dose level per titration visit as described in the Lixivaptan Titration Period (Part 1) — Lixivaptan Titration Flowchart.

Once the maximally tolerated dose has been achieved, tolerability will be confirmed over an additional week of dosing before the participant advances to randomization. As the maximum duration of the Lixivaptan Titration Period is 6 weeks, participants who require a dose reduction at Week 6 as a result of emerging tolerability issues will proceed to the Double-blind, Randomized Treatment Period on the newly assigned (reduced) dose level without extension of the titration period. Serum liver chemistry tests will be performed at Visits 5, 7, and 9 and fasting morning urine osmolality, collected prior to the morning dose of study drug, will be measured at the last titration visit (Visit 9).

Double-blind, Randomized Treatment Period (Visits 10 to 22): Following successful completion of titration, participants will be randomized 2:1 (lixivaptan: placebo) to continue to receive lixivaptan at the dose achieved at the end of the Lixivaptan Titration Period or to matching placebo in a double-blind fashion for 52 weeks. Study visits will be scheduled every 4 weeks during this period. Assessments at each visit are detailed in the Schedule of Procedures – Part 1.

Follow-up Period I (Visits 23 to 25): Three visits will occur over a 28-day, study drug-free period following the last dose of study drug. Blood samples will be drawn at 3 time points for serum creatinine and serum cystatin C, to determine eGFR for primary and exploratory analyses, respectively. The first visit (Visit 23) will occur on the 8$^{th}$ day (+ 3 days) after the last dose of study drug, and the last visit (Visit 25) will occur on the 28$^{th}$ day (± 3 days) after the last dose of study drug. Visit 24 must be scheduled to occur at least 24 hours after Visit 23 and at least 24 hours prior to Visit 25. At Visit 25, additional efficacy and safety assessments will be performed; a post-baseline MRI for assessment of TKV and LV is to be obtained between 25 days and 31 days post last dose of study drug and must be completed prior to or on the same day as all other Visit 25 study procedures. Following completion of these assessments, all participants (except those who discontinued due to an AE or who have withdrawn consent) will immediately continue into Part 2 at Visit 25.

**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 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. If study drug is interrupted for longer than 8 weeks, then study drug treatment will be permanently discontinued.

**Part 2:**

Lixivaptan Re-Titration Period (Visits 26 to 29): For all participants, titration of lixivaptan will occur over a 2 to 4-week re-titration period and will start at a dose of 50 mg BID beginning at the conclusion of Visit 25. The dose will be increased at weekly intervals until the dose level from the end of the Double-blind, Randomized Treatment Period in Part 1 is achieved. In order to maintain blinding of the treatment assignment from Part 1, the dose level assignment for Part 2 will continue to be managed by the IRT. The dose-titration schedule for this part of the study is described in Table 2. In the event of tolerability issues,

dosing may be decreased. **Note**: during the Re-titration Period of Part 2, all participants are re-titrated with lixivaptan to the last actual dose level if they had received lixivaptan during the Double-blind, Randomized Treatment Period, 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. If no adjustment is made to the inferred dose during the Double-Blind, Randomized Treatment Period, the last inferred dose for a participant randomized to placebo will be equal to the maximal tolerated lixivaptan dose established during the Titration Period in Part 1.

Maintenance Treatment Period (Visits 30 to 42): Following completion of the Lixivaptan Re-titration Period, participants will continue to receive lixivaptan in the Maintenance Treatment Period for 52 weeks at the same dose achieved at the end of the Re-titration Period in Part 2. If the participant's dose was decreased during the Lixivaptan Re-Titration Period, attempts will be made every 4 to 8 weeks to re-establish the previously achieved dose level (actual or inferred) at the end of the Double-blind, Randomized Treatment Period of Part 1. Visits will be performed every 4 weeks during this period. Assessments at each visit are detailed in the Schedule of Procedures – Part 2.

**Note**: In the event of tolerability issues at any time during the study including the Part 2 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 study drug is interrupted for longer than 8 weeks, then study drug treatment will be permanently discontinued.

Follow-up Period II (Visits 43 to 45): Three visits will occur over a 28-day, study drug-free period following the last dose of lixivaptan in the Maintenance Treatment Period. Blood samples will be drawn at 3 time points for serum creatinine and serum cystatin C, to determine eGFR for primary and exploratory analyses, respectively. The first visit (Visit 43) will occur on the 8$^{th}$ day (+ 3 days) after the last dose of lixivaptan, and the last visit (Visit 45) will occur on the 28$^{th}$ day (± 3 days) after the last dose of lixivaptan. Visit 44 must be scheduled to occur at least 24 hours after Visit 43 and at least 24 hours prior to Visit 45. At the final study visit, Visit 45, additional efficacy and safety assessments will be obtained; a post-baseline MRI for assessment of TKV and LV is to be obtained between 25 days and 31 days post last dose of study drug and must be completed prior to or on the same day as all other Visit 45 study procedures.

The maximum duration of participation in this study is 131 weeks (up to 71 weeks in Part 1 and up to 60 weeks in Part 2) depending on the length of Screening (3 to 8 weeks), Titration (5 to 6 weeks) and Re-titration (2 to 4 weeks). Note that the study may be interrupted at any time if safety issues identified by an IDMC and sponsor potentially compromise the safety of the participants.

## 3.2 Rationale for Study Design

A placebo-controlled, double-blind assessment of lixivaptan is the preferred study design for demonstrating the efficacy and safety of the drug in the treatment of patients with ADPKD. This type of design was used both in the TEMPO 3:4 and REPRISE studies to assess the efficacy and safety of tolvaptan. Furthermore, following the double-blind phase of the study with a 52-week open label treatment phase allows for the demonstration of the durability of effect of lixivaptan as well as assessment of long-term safety. The inclusion/exclusion criteria in Section 4.1 were chosen to recruit an appropriate number of participants representative of the ADPKD population with declining kidney function who are most likely to benefit from vasopressin $V_2$ receptor blockade in order to achieve the study's primary objective as presented in Section 2.1.1.

Treatment with lixivaptan is associated with transient and reversible increases in serum creatinine related to acute kidney hemodynamic effects. In addition, based on clinical evidence with the related vasopressin antagonist tolvaptan (Torres et al, 2012), lixivaptan may be associated with transient and reversible decreases in TKV. In order to eliminate these confounding influences on these endpoints, assessment of serum creatinine for the primary endpoint (change in eGFR) and the secondary endpoint of annualized change in TKV will occur at baseline before the start of study drug and shortly after completion of study drug (8 to 28 days following the last dose).

The sample size of 1350 participants in total, randomized 2:1, lixivaptan to placebo, will provide adequate power to detect a clinically meaningful change in the primary endpoint of eGFR over 1 year of treatment. A blinded sample size re-estimation (BSSR) to estimate the variance corresponding to the primary efficacy estimand is planned after 20% of the total randomized participants have completed Part 1 of the study or before screening for the study has ended (Section 8.1).

# 4 PARTICIPANT SELECTION AND WITHDRAWAL CRITERIA

## 4.1 Selection of Study Population

Approximately 2250 participants will be screened in order to randomize 1350 participants at approximately 250 sites globally. Participants will be enrolled only if they meet all of the inclusion criteria and none of the exclusion criteria. Eligibility criteria are to be assessed at Visits 1a, 1b (if required) and 2. **Note**: On or prior to Visit 3, eligibility based on mean eGFR (Inclusion criterion #4) from Visit 1a and 2 or Visit 1b and 2 (if Visit 1b is required) and liver chemistry tests (Exclusion criterion #9) from Visit 2 must be re-confirmed.)

Deviations from the inclusion and exclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability, or participant safety. Therefore, adherence to the criteria as specified in the protocol is essential. Prospective approval of protocol deviations to eligibility criteria, also known as protocol waivers or exemptions, is not permitted.

### 4.1.1 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 total kidney volume (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 $m^2$ 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 (Appendix 1 (Section 13.1)) **Note**: This criterion will preliminarily be reviewed at Visit 2 based on Visit 1a or Visit 1b results (if Visit 1b is required). The criterion must be re-evaluated no later than Visit 3 when results for Visits 1a and 2 or Visits 1b and 2 are available to confirm that the participant remains eligible for participation.

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 (WOCBP), 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 (see list above) 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.

### 4.1.2 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 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.

## 4.2 Completion and Withdrawal of Participants from the Study

### 4.2.1 Definition of Completed Participants

For the purposes of this study, participants who are randomized, 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 Follow-up Period I will be defined as "Part 1 On-Treatment Completers."

Participants who are randomized, but 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 will be defined as "Part 1 Off-Treatment Completers."

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 will be defined as "Part 2 On-Treatment Completers."

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 will be defined as "Part 2 Off-Treatment Completers."

The end of the study is defined as the last participant's last visit.

### 4.2.2 Screening and Placebo Run-in/Lixivaptan Titration Failures

Participants may withdraw from the study at any time and for any reason without prejudice to their future medical care by the Investigator or at the study site.
During the Screening Period, participants who withdraw their consent or fail to meet all of the entry criteria to participate in the study will be designated as "Screen failures." These participants will not complete an ET visit (Visit 22). Screen failures will be recorded as such on the electronic Case Report Form (eCRF). Screen failures who do not meet study entry requirements may be rescreened at a later date (up to two times) after consultation with the medical monitor.

Participants who discontinue or are discontinued after screening but before randomization (i.e., during the Placebo Run-in Period or Lixivaptan Titration Period) will be considered "Placebo Run-in/Lixivaptan Titration failures."

Placebo Run-in/Lixivaptan Titration failures will complete an ET visit (Visit 22) upon withdrawal from the trial and within 7 days of the last dose of study drug. The ET visit assessments for Part 1 are delineated in the Schedule of Procedures – Part 1. Placebo Run-in/Lixivaptan Titration Failures will not participate in Follow-up Period I, however, Placebo Run-in/Lixivaptan Titration failures with ongoing AEs will continue to be followed according to the instructions in Section 7.3.8.7.

### 4.2.3 Treatment Interruption

In a study of this duration, it is expected that some participants may have one or more treatment interruptions during the Double-blind, Randomized Treatment Period of Part 1 or the Lixivaptan Re-Titration or Maintenance Treatment Periods of Part 2. If a participant's study drug must be interrupted for medical or surgical reasons, liver chemistry test abnormalities, tolerability issues, temporary use of a prohibited concomitant medication, life situations, e.g., airplane travel, etc... or other reasons, the interruption should be recorded as a "Treatment Interruption" on the eCRF. The participant's study drug should be resumed as early as the situation allows, e.g., airplane travel ends or recovery from surgery allows re-initiation/re-establishment of study drug treatment. The maximum duration of an interruption is 8 weeks; if interruption is greater than 8 weeks, study drug will be permanently discontinued.

The participant should be instructed to immediately inform the Investigator of any missed doses reaching or expected to reach 2 days or more so that the Investigator can continue to monitor the participant's treatment and prepare for a possible extended study drug

interruption. The Investigator and/or site staff will notify the CRA and/or medical monitor of a potential interruption within 24 hours.

If a study drug interruption is expected to last 7 or more consecutive days, the participant will be scheduled to have 3 separate serum creatinine and serum cystatin C samples obtained (minimally 24 hrs. apart) beginning on Day 8 after the last dose of study drug for determination of eGFR in the event the study drug interruption leads to permanent discontinuation of treatment. In such cases, the 3rd sample will be collected up to Day 28 (± 3 days) after the last dose of study drug. If, in the Investigator's opinion, the treatment interruption is expected to be prolonged (i.e., >4 weeks in duration) or permanent, then a postbaseline MRI will also be obtained.

Treatment may still be restarted during or after these assessments are completed; any remaining serum creatinine assessments and the post-baseline MRI will not need to be completed if treatment is restarted during this period. If treatment is restarted, and the participant resumes study visits to Week 52 (completion of Visit 22 if in Part 1) or Week 104 (completion of Visit 42 if in Part 2), and has at least 1 scheduled Follow-up Period assessment for Part 1 or Part 2, respectively, then the eGFR assessments and MRI obtained during the period of study drug interruption will be considered as unscheduled and will not be included in the primary efficacy analysis for eGFR or the secondary efficacy analysis related to TKV. This procedure is designed to ensure that data for the primary and secondary endpoints are collected from all participants.

Participants with a study drug interruption 4 weeks or less in duration should resume study treatment at their last dispensed dose (or at a lower dose, if medically appropriate) without the need for stepwise dose re-establishment.

Participants with a prolonged study drug interruption (i.e., greater than 4 weeks in duration) equal to or less than 8 weeks), who are re-initiating treatment are required to re-establish their last tolerated dose over a 2-week interval. Re-initiation of treatment will begin with study drug at Level 2 for one week and will then be increased to the highest level the participant was receiving prior to the study drug interruption during the second week as described in Table 6 (see pharmacy manual for further details related to study drug dispensing following a prolonged drug interruption.) The dose level assigned during Week 2 may be lowered based on Investigator discretion and clinical need. The IRT system will be used to manage re-establishment of dose.

**Table 6. Dose Re-establishment Following a Prolonged (> 4-week) Study Drug Interruption**

| Last tolerated study drug dose level/ dose* | Week 1 | Week 2** |
|---|---|---|
| Level 2/ 100mg BID | 100mg BID | 100mg BID |
| Level 3/ 150mg BID | 100mg BID | 150mg BID |
| Level 3a/ 150/100mg (AM/PM) | 100mg BID | 150/100mg (AM/PM) |
| Level 4/ 200mg BID | 100mg BID | 200mg BID |
| Level 4a/ 200/150mg (AM/PM) | 100mg BID | 200/150mg (AM/PM) |

*Participants whose last tolerated dose level was 50mg BID prior to the prolonged treatment interruption, will re-initiate study drug dosing at Level 1/ 50mg BID.
**A lower dose level may be assigned at Week 2 if clinically indicated.

Participants with a prolonged study drug interruption of greater than 8 weeks will not be allowed to re-initiate and/or re-establish study drug treatment i.e., study drug treatment will be permanently discontinued. These participants should be encouraged to continue their participation in the study following the procedures described in Section 4.2.7 and Appendix 5 (Section 13.5).

### 4.2.4 Treatment Discontinuation

Participants may elect to stop treatment, (i.e., stop taking their study drug) permanently before the end of the study for various reasons. This is separate and distinct from study discontinuation and/or withdrawal (Section 4.2.5) although the reason(s) for study drug discontinuation may be identical and/or similar to reason(s) for study discontinuation. Every effort should 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 as described in Section 4.2.7 and Appendix 5 (Section 13.5) in order to minimize missing data. Participants who permanently discontinue study drug will have the reason for study drug discontinuation (e.g., adverse event) recorded on the eCRF. They will have an ET visit (Visit 22 if in Part 1 or Visit 42 if in Part 2), which should be scheduled within 7 days of the participant's last dose of study drug, to collect data listed in the Schedule of Procedures – Part 1 and Schedule of Procedures – Part 2. Randomized participants who permanently discontinue treatment will immediately proceed into the 4-week Follow-up Period and will complete the 3 visits in the Follow-up Period between Day 8 and Day 28 following the last dose of study drug. The first two visits, Visits 23 and 24 in Part 1 or Visits 43 and 44 in Part 2 (performed no less than 24 hours apart), will be for the collection of serum creatinine and serum cystatin C to determine eGFR, and the third visit, Visit 25 (Part 1) or Visit 45 (Part 2) (performed on Day 28 ± 3 days, and no less than 24 hours from Visit 24 (Part 1) or Visit 44 (Part 2)) will have additional safety and efficacy measurements (including the post-baseline

MRI) in addition to serum creatinine and serum cystatin C eGFR measurements (unless the participant withdraws consent for further data collection).

Following completion of the ET visit and Follow-up Period procedures in Part 1 or Part 2, participants who have permanently discontinued study treatment should be encouraged to remain in the study, in an effort to minimize missing data, and to continue to complete all protocol visits and assessments up to and including their originally scheduled Visit 45 (end of Follow-up Period II). Changes to or initiation of new medications during this time-period should continue to be recorded on the eCRF.

### 4.2.5 Reasons for Treatment/Study Discontinuation

Participants who permanently discontinue study treatment before the end of the study, should be encouraged to continue in the study through the last study visit. They may discontinue or may be discontinued from study drug or from the study for any of the following reasons.
**Note:** Participants who discontinue from the study following randomization are not eligible to re-enroll.

1. Noncompliance with the protocol (protocol deviation);
2. Noncompliance with study drug;
3. Lack of efficacy (including need for transplantation or dialysis);
4. A serious or intolerable AE that, in the Investigator's opinion, requires discontinuation of study drug or permanent withdrawal from the study, including but not limited to laboratory safety assessments that reveal clinically significant (CS) hematological or biochemical changes from the baseline values, other than changes that are expected for vasopressin antagonists, and symptoms or an intercurrent illness that justifies withdrawal. Mild elevations in serum creatinine or serum sodium are known pharmacodynamic effects of vasopressin antagonists like lixivaptan and are transient and reversible. Therefore, participants should not be withdrawn due to mildly elevated serum creatinine or serum sodium and should be discussed with the medical monitor, if necessary;
5. Death;
6. Lost to follow-up (detailed procedures to prevent such loss are discussed further);
7. Withdrawal of consent (partial or complete);
8. Investigator or sponsor decides to discontinue the participant's participation in the study;
9. Pregnancy;
10. Site terminated by sponsor;
11. Study terminated by sponsor;
12. Other (e.g., trial burden, development of contraindication to use of study drug).

Upon occurrence of an SAE or intolerable AE, the Investigator will confer with the medical monitor and sponsor. If a participant is discontinued from study drug because of an AE, the event will be followed until it is resolved or stable as determined by the Investigator.

Additionally, the procedures described in Section 4.2.4 (Treatment Discontinuation) should be followed. Any participant may withdraw his or her consent at any time. Participants' safety will be closely monitored throughout the study, and the study will be conducted following Good Clinical Practices (GCP).

### 4.2.6 Study Termination

The study may be stopped at a study site at any time by the site investigator or the sponsor. The sponsor may stop the study for any reason with appropriate notification, including inability to enroll a sufficient number of participants to meet study goals, an untoward safety signal as recommended by the IDMC and sponsor, or discontinuation of development of lixivaptan for ADPKD. The study may also be stopped by a regulatory authority.

### 4.2.7 Methods to Prevent Loss to Follow Up

The Investigator must make every attempt to contact participants who fail to return for scheduled visits to prevent participants from being "lost to follow-up" and follow-up with participants who have withdrawn from the study at any time and for any reason. Appendix 6 (Section 13.6) specifies the steps expected to be taken to prevent loss to follow-up. All attempts to follow-up with participants in accordance with Section (13.6), must be documented in the source document. Only after all measures to mitigate loss to follow-up have been exhausted should participants be identified as "lost to follow-up" in the eCRF.

### 4.2.8 Withdrawal of Consent

Participants are free to withdraw from the study or study drug at any time. Participation in the study may be stopped at any time at the discretion of the Investigator or at the request of the sponsor. Participants should be followed for all protocol-specified evaluations and assessments, even after premature discontinuation of study drug, unless there is written withdrawal of consent or other written documentation by the Investigator confirming the participants' verbal intent to completely withdraw from the trial. Declining follow up by all of the methods listed below will constitute a withdrawal of consent (these methods of follow up will also be noted in the trial ICF).

- Participation in all study visits and/or procedures specified in the protocol (whether in-clinic, by telephone, or by an in-home visit).
- Participation in a subset of protocol-specified visits and/or procedures (by a frequency schedule and method as agreed by participant and Investigator).
- Contact of the participant by trial personnel, even if only by telephone, to assess current medical condition and obtain necessary medical or laboratory reports relevant to the trial's objectives.
- Access to medical information from alternative sources (e.g., hospital/clinic medical records, referring doctor's notes, public records, dialysis, transplantation or vital registries, social media sources).

Withdrawal of consent is a critical trial event and should be handled with a high degree of importance and care. Reasons for participants' intended withdrawal must be understood, documented and managed to protect the rights of participants and the integrity of the trial. Participants may initially want to interrupt or discontinue study drug, but that is not the same as complete withdrawal of consent for further participation. A participant may, for example, consider that further trial participation is a burden. The Investigator should then follow the procedures outlined in Appendix 5 (Section 13.5) to determine if the participant can continue participation in the trial if modifications to his/her treatment and/or schedule of procedures can be accommodated, including the potential to continue in the study at a lower dose level. The scientific value of collecting data and information even after study drug is discontinued should be discussed with a participant who is at risk of withdrawing consent. Participants who withdraw their permission for all of the follow-up procedures listed above are considered to have completely withdrawn their consent to participate in the study.

### 4.2.9 Replacements

Participants who are randomized and subsequently withdraw from the study (either Part 1 or Part 2) will not be replaced.

# 5 STUDY DRUG AND CONCOMITANT MEDICATION

## 5.1 Study drug

### 5.1.1 Identity of the Investigational Product

The investigational product, lixivaptan capsule, is formulated as a white, banded, hard gelatin capsule containing 50 mg of lixivaptan and the inactive ingredients listed in Table 7.

**Table 7. Investigational Product**

| | Lixivaptan |
|---|---|
| **Strength** | 50 mg |
| **Formulation** | Capsules |
| **Inactive ingredients** | Polyethylene glycol 400, NF/EP; Polyethylene glycol 1000, NF/EP; Povidone (K-17), USP/EP; Polysorbate 80, NF/EP; Butylated hydroxytoluene NF/EP; Butylated hydroxyanisole NF/EP |
| **Manufacturer** | PMRS, Inc.<br>202 Precision Road<br>Horsham, PA 19044 – USA |
| **Packager** | PCI Pharma Services<br>4545 Assembly Drive<br>Rockford, IL 61109 – USA |

### 5.1.2 Identity of Placebo

The placebo capsule is identical in size and appearance to the lixivaptan capsule. The placebo capsule does NOT contain the active ingredient (lixivaptan) but contains the same *inactive* ingredients as in the investigational product.

### 5.1.3 Route of Administration

The study drug (lixivaptan or placebo capsule) is intended to be taken orally, i.e., to be swallowed whole with water. The study drug can be taken with or without food. The capsule should NOT be opened.

### 5.1.4 Study Drug Packaging, Labeling, and Storage

Study drug will be packaged by the sponsor according to all local legal requirements and labeled in accordance with applicable regulatory requirements. It will be packaged in blister cards designed to dispense four capsules BID throughout the study.

All investigational drug supplies should be stored in a secure, locked area, under the responsibility of the Investigator or other authorized individual. Study drug should be stored in accordance with the specifications detailed in the study pharmacy manual. Participants should be instructed to store study drug in its original packaging and in accordance with study drug labelling at all times until ready to take.

For study visits that are conducted remotely, study drug may be dispensed via a secure and traceable delivery method (where permissible by local, statutory, federal/country law) and after approval by the sponsor or sponsor designee. In such cases, participants will be provided with written instructions to securely store the study drug in its original packaging under the conditions described above and not to open the package until the home healthcare clinician (HHC)/qualified site personnel arrives. Alternatively, where permissible and approved by the sponsor or sponsor designee, qualified study staff conducting remote visits may dispense study drug directly to participants at the time of the remote visit.

### 5.1.5 Accountability

The Investigator will maintain accurate records of study drug receipt and disposition. In addition, accurate records will be kept regarding when and how much study drug is dispensed, used, and returned by each participant in the study. Reasons for departure from the expected dispensing regimen will also be recorded. At the completion of the study, to satisfy regulatory requirements regarding drug accountability, all unused study drugs will be reconciled and returned to the sponsor or designee or destroyed according to applicable regulations and site's applicable Standard Operating Procedures.

## 5.2 Dose Administered

During all treatment periods, study drug will be administered BID, with the PM dose administered approximately 10 hours after the AM dose. Study drug will be packaged in blister cards designed to dispense four capsules BID throughout the study to reduce the awareness of transitions during the study. Depending on the study period, treatment arm to which the participant is randomized, and current dose level, the set of 4 capsules may be all placebo, all active or a combination of active and placebo. Study drug will be assigned via the IRT system throughout the study. During the Placebo Run-in Period, participants will receive four capsules of single-blind placebo BID over one week of dosing. 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. Two additional dose levels (Levels 3a and 4a) are provided that reduce the evening (PM) dose by 50 mg (from 150 mg to 100 mg and from 200 mg to 150 mg) if aquaretic effects are limiting participants' tolerability at either the 150 mg BID or 200 mg BID dose levels, respectively (**Note**: further dose reductions are allowable as shown in the Lixivaptan Titration Period (Part 1) — Lixivaptan Titration Flowchart.)

Tolerability will be assessed by asking the participant a single question "Would you take the study drug for the next 24 months?" If the participant confirms the dose can be tolerated, the participant will be titrated to the next dose level (i.e., the dose will be increased). This assessment of tolerability will continue at weekly intervals until either the maximum dose level is reached (Level 4 [200 mg BID]) or until the participant confirms that the dose cannot be tolerated for a 24-month period. In that case, the dose will be reduced by one dose level (assigned by the IRT system) as depicted in the Lixivaptan Titration Period (Part 1) — Lixivaptan Titration Flowchart. **Note**: Assessment of tolerability will begin at Visit 3, the end of the Placebo Run-in Period.

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, 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.

At the start of the Double-blind Randomized Treatment Period participants will be randomized to either continue at the lixivaptan dose level achieved at the end of the Lixivaptan Titration Period or receive matching placebo capsules. 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 and if medically advisable. Attempts will be made every 4 to 8 weeks to reinstate the dose achieved at Visit 9. 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. The participant's study drug should be resumed as early as the situation allows, e.g., airplane travel ends or recovery from surgery allows re-initiation/re-establishment of study drug treatment. The maximum duration of an interruption is 8 weeks; if interruption is greater than 8 weeks, study drug treatment will be permanently discontinued (Section 4.2.3).

At the completion of Follow-up Period I (Visit 25), participants will be dispensed lixivaptan at a dose of 50 mg BID to initiate the Lixivaptan Re-titration Period of study Part 2. Dosing will be increased weekly until the dose level taken at the end of the Double-blind, Randomized Treatment Period is achieved (2 to 4 weeks). This is shown in Table 2. That dose will be continued into the Maintenance Treatment Period for the remainder of Part 2 unless tolerability issues arise either during the Lixivaptan Re-titration Period or during the Maintenance Treatment Period, in which case the dose may be lowered or temporarily stopped. Attempts will be made every 4 to 8 weeks to re-establish the previously achieved dose level from 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.

## 5.3 Method of Assigning Participants to Treatment Groups

Each investigator will be assigned a unique 5-digit site number (XXXXX) where the first 2 digits represent the country, and the last 3 digits are unique for a site. This site number will be concatenated with a leading protocol number (301) and a following 3-digit participant number (YYY) to assure that each participant will be uniquely identified in the clinical database. Thus, a prototypical participant number in this study will look like 301-XXXXX-YYY. As participants are screened, the next participant qualified chronologically at a site will be assigned the next number in ascending numerical order. The participant identification number assigned at Screening will be used for that participant throughout the study. Participants who screen fail and are subsequently eligible to re-screen, will retain their original identification number.

All treatment periods will use active or placebo study drug that is identical in appearance. During the single-blind, Placebo Run-in and Lixivaptan Titration Periods, participants should not be told whether they are receiving active or placebo medication although site personnel will be aware.

At the randomization visit (Visit 9), participants will be randomized to receive either lixivaptan capsules or placebo capsules during the Double-blind Randomized Treatment Period. Throughout the study, and at all subsequent visits or contacts with the participant, the participant will be reminded of the importance of their commitment to continue participation in the study, however, the participant will not be told that this day is the point at which randomization to long-term therapy occurs. Randomization will occur through an IRT system that will be programmed for the stratification factors and the randomization ratio (2:1 lixivaptan to placebo). After randomization, treatment allocation will be blinded to all participants, investigators, site personnel, and sponsor employees and representatives.

## 5.4 Treatment Compliance

Dispensing of study drug and reconciliation will be performed at each visit during the Placebo Run-in and Lixivaptan Titration Periods in Part 1 and during the Lixivaptan Re-titration Period in Part 2. Dispensing and reconciliation will be performed monthly during the Double-blind Randomized Treatment Period. Participant compliance will be monitored by capsule counts as study drug is returned. For visits that are conducted remotely, direct-to-participant drug dispensing will be utilized (where applicable) prior to the scheduled in-home visit with the HHC/qualified site personnel. Participants should be instructed not to open the study drug shipment until the contents can be verified by the HHC/qualified site personnel. Compliance will be assessed by the HHC/qualified site personnel during these visits.

The dates of all study drug dosing, including interruptions, missed doses or overdose, must be recorded in the source document and study drug accountability forms. Compliance is defined as a minimum of 80% dosing compliance during any study interval. If the participant is not ≥ 80% compliant with the prescribed study drug doses during the study overall, it will be noted as a protocol deviation. The participant should be re-educated regarding the correct study drug doses to be administered whenever compliance is < 80%. However, if the Investigator, medical monitor, and sponsor have agreed to a study drug interruption (Section 4.2.3), then this period of non-compliance will not be considered a protocol deviation.

## 5.5 Prior and Concomitant Therapy

Participants will be allowed to take their chronic medications unless excluded by the protocol (Section 5.5.1.2) and provided that their chronic medication therapy meets the conditions outlined in Section 5.5.1.1, including remaining constant throughout the duration of the study.

Use of all prior medications within 2 months prior to study drug administration will be recorded in the participant's eCRF. The minimum requirement is that drug name and the dates of administration are recorded. This will include all prescription drugs, herbal products, vitamins, minerals, and over-the-counter (OTC) medications. Any changes in prior or concomitant medications will also be recorded in the participant's eCRF.

Any concomitant medication deemed necessary for the well-being of the participant during the study may be given at the discretion of the Investigator after consideration of the clinical situation (Section 5.5). **Note**: in female participants with severe polycystic liver disease, contraceptives (and hormone replacement therapy) containing estrogen 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. The Investigator is responsible for ensuring that details regarding concomitant medication use are recorded in the eCRF. For each concomitant medication administered the following details will be documented and recorded in the participant's eCRF: name of medication, dose administered, date and time of administration, and reason for medication use.

Upon entering the study, each participant will be instructed on the importance of not taking any medication (including OTC medications) without consulting the Investigator.

#### 5.5.1.1 Permitted Therapy

Treatment considered necessary for the participant's welfare may be given at the discretion of the Investigator. Allowed medications include those typically prescribed to treat CKD and its complications such as hypertension; these include ARBs (e.g., valsartan, candesartan, telmisartan, irbesartan) and ACE inhibitors (e.g., enalapril, lisinopril). While optimization of these medications is allowed during the Screening Period, it is expected that the dose will remain stable for the duration of the study. Any dose adjustments in antihypertensive medications must be discussed with the medical monitor.

Acetaminophen (paracetamol), at doses ≤1 g per day, is permitted for use any time during the study in participants who usually take acetaminophen to control episodic pain. Because of their effect on kidney function, use of non-steroidal anti-inflammatory drugs (NSAIDs) should be minimized.

Non-sedating antihistamines (other than loratadine) and decongestants may be permitted on an as-needed basis. Low-dose aspirin (up to 150 mg per day) is permitted.

Vaccines, including the COVID-19 vaccine and/or vaccine booster are permitted during study participation.

Chronic use of other concomitant medications that are required to treat a medical condition may be permitted unless otherwise prohibited (Sections 4.1.2 and 5.5.1.2). All medication treatment dosing regimens should be stable for 1 month prior to screening, allowing for minor adjustments per the standard of care to treat medical conditions and avoiding, whenever possible, adjustments that will significantly impact serum creatinine levels. The stability of treatment regimens of certain drugs, such as insulin and warfarin, should be based on the standard of care rather than on a stable dose.

#### 5.5.1.2 Prohibited Therapy

The following medications are prohibited throughout the study:

- bardoxolone;
- conivaptan;
- demeclocycline;

- diuretics (must be discontinued during Screening when anti-hypertensive medications are being adjusted) except for a short course (up to 4 weeks at a time) of loop diuretic therapy to manage a temporary fluid retention condition;
- HIF-PH inhibitors;
- metformin (for ADPKD);
- mTOR kinase inhibitors (e.g., everolimus, sirolimus, etc.);
- nicotinamide (for ADPKD);
- SGLT2 inhibitors (e.g., canagliflozin, dapagliflozin, empagliflozin, etc.);
- somatostatin analogs (e.g., lanreotide, pasireotide, octreotide, etc.);
- tolvaptan;
- strong or moderate CYP3A4 or CYP2C8 inducers, including barbiturates, bosentan, carbamazepine, efavirenz, enzalutamide, etravirine, modafinil, mitotane, nevirapine, oxcarbazepine, phenytoin, pioglitazone, rifabutin, rifampin, and St. John's wort;
- strong or moderate CYP3A4 or CYP2C8 inhibitors, including aprepitant, boceprevir, clarithromycin, chloramphenicol (not eye drops), cimetidine, ciprofloxacin, clopidogrel, clotrimazole (if used orally), cobicistat and cobicistat-containing products, crizotinib, cyclosporine, danazol, deferasirox, diltiazem, dronedarone, erythromycin, fluconazole, fluvoxamine, gemfibrozil, HIV protease inhibitors, imatinib, isoniazid, itraconazole, josamycin, ketoconazole, nefazodone, Paxlovid™ (nirmatrelvir co-packaged with ritonavir), posaconazole, voriconazole, quinupristin/dalfopristin, remdesivir, ritonavir and ritonavir-containing products, telaprevir, teriflunomide, tofisopam, troleandomycin, verapamil, and voriconazole;
- any investigational drug or device;
- KetoCitra™ or any beta-hydroxybutyrate (BHB) containing supplements;
- medications, supplements, and herbal preparations that may interfere with the accurate measurement of serum creatinine including cimetidine, trimethoprim, pyrimethamine, phenacemide, and aspirin (aspirin dose above 150 mg per day);
- vasopressin or drugs with vasopressin activity.

## 5.6 Diet, Fluid, and Activity Control

Restriction of excess dietary sodium and cooked meat protein may prove beneficial to participants with a history of, or predisposition for, hypertension or kidney disease in general. In the absence of alternate regional practices, dietary salt should be restricted to <5g/day and dietary cooked meat protein to <1 g/kg/day. In addition, the amount of meat protein should remain stable from day-to-day, and an increased consumption of meat should not occur the day before scheduled serum creatinine measurements.

Participants will refrain from consumption of grapefruit or Seville oranges (or their juices) from 7 days prior to the first dose of study drug until after the completion of the study.

Increased fluid intake is encouraged in participants with ADPKD. Given the potential for dehydration with lixivaptan treatment, all participants should be instructed to ingest fluids in anticipation of, or at the first sign of, thirst in order to avoid excessive thirst or dehydration. At the initiation of the Placebo Run-In Period, all participants should receive the recommendation to ingest at least 3-4 quarts (or liters as an approximation of quarts) of fluid (including in solid, semi-solid, and liquid foods) per day, unless otherwise directed by the

Investigator. This recommendation should start at the beginning of the Placebo Run-In Period and continue through the end of the study (Part 1 and Part 2). Additionally, participants should ingest 1 to 2 cups (approximately 250-500 mL) of water before bedtime regardless of perceived thirst and replenish fluids overnight with each episode of nocturia. Dehydration can be monitored by participant self-assessment of symptoms such as lightheadedness or dizziness.

Daily activity including exercise should remain stable during the study and participants should avoid heavy lifting or intense physical exertion on the day before scheduled serum creatinine measurements.

Consumption of alcohol should be limited to 1 alcoholic drink equivalent per day in women and 2 alcoholic drink equivalents per day in men. An alcoholic drink equivalent is 12 ounces (360 ml) of regular beer, or 5 ounces (150 ml) of wine, or 1.5 ounces (45 ml) of distilled spirits.

# 6 TIMING OF STUDY PROCEDURES

The timing of study procedures is presented in the Schedule of Procedures – Part 1 and Schedule of Procedures – Part 2. As detailed in the Schedule of Procedures for each part of the study, certain visits must occur in the clinic, while other visits may occur in the clinic or may be conducted remotely by an HHC/qualified site personnel (with the agreement of the participant and the discretion of the investigator and where available and locally approved by the CA and/or IRB/EC). **Note**: During the Lixivaptan Titration Period (Part 1), designated visits that are conducted remotely also require a telehealth visit (e.g., telemedicine virtual visit, telephone or video call (without recording)) with the Investigator as described in Section 6.3.1.

For a given participant, every attempt will be made to draw blood samples for eGFR determinations at approximately the same clock time during Visits 1a/1b, 2, and 3 and Visits 23, 24, and 25, and again at Visits 43, 44, and 45.

In response to local, regional, or national restrictions resulting from the ongoing SARS-CoV-2 (COVID-19) pandemic or in the event of other public health crises that may limit or interfere with the conduct of this study protocol, modifications to the study plan may be permitted. In such cases, impacted in-person study visits may be conducted remotely by an HHC/qualified site personnel and/or via a telehealth visit with the Investigator and/or study assessments may be deferred following approval from the medical monitor and sponsor. In such cases, the source documents and eCRFs must clearly denote the study visits/assessments impacted by the public health crisis.

## 6.1 Screening Period

The Screening Period will last from 3 to 8 weeks. Screening for participants who do not require adjustments to medications should be completed in 3 to 5 weeks. Longer screening is available for participants who require modification of medical care for this trial or where scheduling baseline tests is difficult, as examples. Visit 1a will be used to collect historical information to determine (i) whether the participant is preliminarily eligible; and (ii) whether additional time is needed to modify background medical treatment, e.g., discontinuation of diuretics or intensification of anti-hypertensive therapy.

If longer screening is required for modification of medical care, then Visit 1b should be delayed until those modifications are completed, and blood pressure and treatment have been stable for a minimum of 3 weeks. At that point Visit 1b may occur. **Note:** All laboratory results from Visit 1b should be available prior to the completion of Visit 2. The baseline MRI, which may be completed before, at, or after Visit 1b, is to be completed approximately 1 week prior to Visit 2 to allow for receipt of the Mayo Clinic ADPKD Image Classification from the central imaging vendor (**Note**: Visit 2 should be scheduled approximately 1 week after the baseline MRI and approximately 2 weeks after completion of Visit 1b, whichever occurs later). All assessments needed for evaluation of eligibility and for establishing baseline values during the Screening Period must be completed within 8 weeks.

Participants who do not require modification to medical treatment should be scheduled for the baseline MRI and should proceed to Visit 2 in 3 to 5 weeks following Visit 1a. **Note:** Visit 2 should be scheduled approximately 1 week following completion of the MRI to allow

for receipt of the Mayo Clinic ADPKD Image Classification from the central imaging vendor. Participants must have adequate BP control for a minimum of 3 weeks prior to enrollment (Visit 2). The Screening Period may be extended up to 8 weeks for these participants under extenuating circumstances and with prior approval from the medical monitor.

### 6.1.1 Visit 1a

Visit 1a will be conducted at the clinical site. Participants will undergo informed consent and after signing the informed consent form, the following screening procedures will be performed at Visit 1a:

- Collect and review demographic information.
- Collect and review medical history information, including smoking and alcohol history.
- Collect and review prior and concomitant medication information with particular emphasis on collection of use and dosing of any protocol-excluded medications (Section 4.1.2 and 5.5.1.2).
- Perform a complete physical examination including assessment for hypovolemia.
- Collect body weight and height measurements; BMI will be auto-calculated by the EDC system.
- Check vital signs (includes sitting blood pressure, heart rate, respiratory rate, and temperature).
- Perform a 12-lead ECG.
- Collect blood samples for:
    - chemistry, including serum sodium;
    - liver chemistry;
    - serum creatinine and serum cystatin C for eGFR determinations. (**Note**: For a given participant, every attempt will be made to draw blood samples for eGFR determinations at approximately the same clock time during Visits 1a/1b, 2, and 3 and Visits 23, 24, and 25, and again at Visits 43, 44, and 45);
    - hematology;
    - serology: HbsAg and Anti-HCV (**Note**: A positive anti-HCV test requires a negative HCV RNA titer reflex test prior to enrollment. HCV sero-positive participants should undergo reflex testing at an Unscheduled Visit prior to Visit 2).
- Collect urine for:
    - urinalysis;
    - local urine pregnancy test (**Note**: all positive tests will be confirmed by a serum pregnancy test at the central laboratory).
- Review and assess AEs and SAEs.
- Review eligibility criteria (Section 4.1.1 and 4.1.2).
- Contact the IRT to assign the participant an identification number as described in Section 5.3.

- If no medication changes are necessary:
    - schedule baseline MRI to determine eligibility according to Mayo Clinic ADPKD Image Classification criteria;
    - schedule Visit 2 approximately 1 week after the scheduled MRI (to allow for receipt of the Mayo Clinic ADPKD Image Classification from the central imaging vendor). Visit 2 should be 3 to 5 weeks after Visit 1a.
    - provide participant with a urine collection container and written instructions to produce a first morning, fasting (≥8 hours, except for water) urine specimen to be brought in at Visit 2 for assessment of urine osmolality.
- If medication changes are necessary (i.e., discontinuation of diuretics or revision of anti-hypertensive medications), participants should return for Unscheduled visits for blood pressure monitoring per medical standard of care. The participant should then be observed on stable medications for a minimum of 3 weeks and then:
    - schedule Visit 1b;
    - schedule baseline MRI to determine eligibility according to Mayo Clinic ADPKD Image Classification criteria (may be completed before, at, or after Visit 1b, but prior to Visit 2).

### 6.1.2 Visit 1b

This visit only applies to those participants who require modification of medical treatment.

The following procedures will be performed at Visit 1b:

- Collect and review new concomitant medication information.
- Check vital signs.
- Review and assess AEs and SAEs.
- Review eligibility (Section 4.1.1 and 4.1.2).
- Collect blood samples for the following laboratory testing:
    - chemistry, including serum sodium;
    - liver chemistry;
    - serum creatinine and serum cystatin C for eGFR determinations. **Note**: Every attempt will be made to draw blood samples for eGFR determinations at approximately the same clock time during Visits 1a/1b, 2, and 3 and Visits 23, 24, and 25, and again at Visits 43, 44, and 45, for a given participant.
- Provide participant with a urine collection container and written instructions to produce a first morning, fasting (≥8 hours, except for water) urine specimen to be brought in at Visit 2 for assessment of urine osmolality.
- Contact the IRT to register the participant visit.

- Schedule Visit 2 approximately 1 week after completion of the baseline MRI to allow for receipt of the Mayo Clinic ADPKD Image Classification from the central imaging vendor and approximately 2 weeks following Visit 1b (whichever occurs later).

### 6.1.3 Visit 2

The following procedures will be performed at Visit 2:

- Process urine specimen collected by participant as a fasting AM urine specimen the morning of Visit 2 for determination of urine osmolality.
- Collect and review new concomitant medication information.
- Check vital signs.
- Review and assess AEs and SAEs.
- Review all eligibility (Section 4.1.1 and 4.1.2) including prior laboratory results, ECG results, and Mayo Clinic ADPKD Image Classification from central imaging vendor. **Note**: the eGFR criterion (Inclusion #4) will be preliminarily assessed on the basis of the single measurement from Visit 1a or 1b (if Visit 1b was required). Mean eGFR will be reviewed again no later than Visit 3 after the results of the Visit 2 eGFR measurement are available. Additionally, eligibility based on liver chemistry tests (Exclusion #9) must also be re-confirmed by Visit 3, following receipt of laboratory results from Visit 2.
    - If the participant is not eligible to participate, record the participant as a Screen Failure and contact the IRT to discontinue the participant from the study and complete the appropriate eCRFs.
    - If the participant is eligible to participate:
        - Collect blood samples for the following laboratory testing:
            - chemistry, including serum sodium;
            - liver chemistry;
            - serum creatinine and serum cystatin C for eGFR determinations. **Note**: Every attempt will be made to draw blood samples for eGFR determinations at approximately the same clock time during Visits 1a/1b, 2, and 3 and Visits 23, 24, and 25, and again at Visits 43, 44, and 45, for a given participant;
            - sparse PK sampling (blood draw can occur at any time during this visit).
        - Administer the following health-outcomes scales to participant (**Note**: responses should be reviewed prior to completion of the visit in order to clarify any ambiguous or missing responses):
            - ADPKD-IS
            - ADPKD-PDS

- ADPKD-UIS

- Contact the IRT to obtain a study drug kit number and dispense study drug to initiate the single-blind, Placebo Run-in Period – the participant is blinded. Instruct the participant to take 4 capsules twice a day daily approximately 10 hours apart.
- Remind the participant to increase fluid intake to prevent dehydration
- Schedule the participant to return for Visit 3 (to occur in 1 week ± 2 days). Provide the participant with an appointment reminder card and any written instructions.

## 6.2 Placebo Run-in Period and Visit 3

During the single-blind, Placebo Run-in Period, participants will self-administer 4 capsules containing placebo twice daily for 1 week. The afternoon dose will be taken approximately 10 hours after the morning dose. Visit 3 will occur at the end of the Placebo Run-in Period (± 2 days). The following procedures will be performed at Visit 3:

- Review and assess AEs and SAEs.
- Collect and review new concomitant medication information.
- Collect and review any acetaminophen use information.
- Review and assess laboratory test results.
- Review the mean eGFR (based on serum creatinine) from Visits 1a and 2 or Visits 1b and 2, if Visit 1b was required. If less than 25 ml/min/1.73 $m^2$ or greater than 90 ml/min/1.73 $m^2$, complete procedures for ET visit (Visit 22) and contact the IRT to discontinue the participant from the study and complete early termination procedures (Section 6.4.2). Participant will not participate in the Follow-up Period.
- Review ALT, AST, and total bilirubin from Visit 2. If ALT, AST, or total bilirubin >1.2 x ULN complete procedures for ET visit (Visit 22) and contact the IRT to discontinue the participant from the study and complete early termination procedures (Section 6.4.2). Participant will not participate in the Follow-up Period.
- Perform study drug reconciliation and review study drug compliance.
- Review tolerability of dosing.
    - If the participant reports the study drug was not tolerated (in response to the question, “Would you take the study drug for the next 24 months?”), contact the IRT to discontinue the participant from the study and complete early termination procedures (Section 6.4.2). Participant will not participate in the Follow-up Period.

For participants who remain eligible to continue in the study:

- Collect medical resource utilization data since the last visit (i.e., medication use or changes; unplanned office visits; urgent care or emergency department usage and hospitalizations) based on any AEs/SAEs reported.

- Collect body weight measurement.
- Check vital signs.
- Perform a urine pregnancy test for WOCBP.
- Collect blood samples for the following laboratory testing:
    - serum sodium
    - liver chemistry;
    - serum creatinine and serum cystatin C for eGFR determinations. **Note**: Every attempt will be made to draw blood samples for eGFR determinations at approximately the same clock time during Visits 1a/1b, 2, and 3 and Visits 23, 24, and 25, and again at Visits 43, 44, and 45, for a given participant;
    - hematology.
- Contact the IRT to obtain a study drug kit number and dispense study drug to initiate the single-blind, Lixivaptan Titration Period – the participant is blinded.
    - Administer the first dose from the newly assigned study drug kit in the clinic.
    - Collect a blood sample for sparse PK (blood draw to occur 0.5 hrs to 1.5 hrs post dose).
    - Instruct the participant to take 4 capsules twice a day daily approximately 10 hours apart.
    - Remind the participant to increase fluid intake to prevent dehydration
    - Schedule the participant for Visit 4 (to occur in 1 week ± 2 days). Provide the participant with an appointment reminder card and any written instructions.

## 6.3 Lixivaptan Titration Period

The Lixivaptan Titration Period will last from 5 to 6 weeks. Participants will self-administer lixivaptan at home (following the initial dose in the clinic). All participants will start at Level 1 (50 mg BID) and will be increased weekly through Level 2 (100 mg BID), Level 3 (150 mg BID), and Level 4 (200 mg BID), as necessary, according to the schedule described in Table 1 until either the maximum dose level (Level 4/200 mg BID) or maximum tolerated dose is achieved. Two additional dose levels (Levels 3a and 4a) are provided that reduce the evening (PM) dose by 50 mg (from 150 mg to 100 mg and from 200 mg to 150 mg) if aquaretic effects are limiting participants' tolerability at either the 150 mg BID or 200 mg BID dose levels, respectively. Further dose reductions are allowable as shown in the Lixivaptan Titration Period (Part 1) — Lixivaptan Titration Flowchart. However, the minimum dose to enter the Double-blind, Randomized Treatment Period is Level 2 (100 mg BID). Once the maximum dose or maximum tolerated dose is achieved, 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 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 will then proceed to Week 9/Last Titration Visit.

### 6.3.1 Visits 4 to 8 (Lixivaptan Titration Period)

Visits 4, 5, 6, 7, and 8 (Visit 8 only if needed) will occur weekly ± 2 days for titration. Titration visits 4 to 7 may either be conducted in the clinic or remotely by an HHC/qualified site personnel (with the agreement of the participant and the discretion of the investigator and where available and locally approved by the CA and/or IRB/EC). **Note**: If conducted remotely, Visits 4 to 7 will require a telehealth visit (e.g., telemedicine virtual visit, telephone or video call (without recording)) with the Investigator 2 days prior to the scheduled in-home visit to assess tolerability. This will allow sufficient time for IRT study drug assignment and subsequent direct-to-participant drug dispensing prior to the scheduled in-home visit. Some participants may complete the Lixivaptan Titration Period in 5 weeks, depending on their highest tolerated dose. These participants will skip Visit 8 assessments and will instead complete the assessments for Visit 9/Last Titration Visit. For participants requiring Visit 8, Visit 8 will be conducted in the clinic. The following procedures will be performed at each visit unless otherwise noted:

- Review and assess AEs and SAEs.
- Collect and review new concomitant medication information.
- Collect and review any acetaminophen use information.
- Review and assess laboratory test results (from Visit 4 and Visit 7 blood collection).
- Collect medical resource utilization data since the last visit (i.e., medication use or changes; unplanned office visits; urgent care or emergency department usage and hospitalizations) based on any AEs/SAEs reported.
- Check vital signs.
- Perform study drug reconciliation and review study drug compliance.
- Review tolerability of dosing.
    - At Visit 4 (only): If the participant reports the study drug was not tolerated (in response to the question, “Would you take the study drug for the next 24 months?”), contact the IRT to discontinue the participant from the study.
    - At Visits 5 to 8: If the participant reports the study drug was not tolerated (in response to the question, “Would you take the study drug for the next 24 months?”), reduce the dose by 1 level (via IRT) in accordance with the Lixivaptan Titration Period (Part 1) — Lixivaptan Titration Flowchart.
    - If the participant reports the study drug was tolerated, proceed with titration to the next dose level (via IRT) based on the Lixivaptan Titration Period (Part 1) — Lixivaptan Titration Flowchart and these general principles (**Note**: all study drug assignments will continue to be managed by the IRT system):
        - Level 1 is an introductory dose to allow participants to become accustomed to the aquaretic effects of the study drug.

  - The goal of titration is to continue to escalate the dose to achieve either the maximum dose (Level 4 [200 mg BID]) or the highest tolerated dose.
  - In those participants who are titrated up to Levels 3 and 4 but have limited aquaretic tolerability at these dose levels, the reduced dose Levels 3a and 4a are available, respectively, which lower the evening dose by 50 mg. Further dose reductions are allowable as shown in the Lixivaptan Titration Period (Part 1) — Lixivaptan Titration Flowchart. This is meant to assist with aquaretic tolerability, and, in particular, nocturia.
  - As shown in the flowchart, most dose levels incorporate a second week of dosing at the same level to assure tolerability. However, as the Lixivaptan Titration Period is designed to be a maximum of 6 weeks in duration, participants will stay on the final tolerable dose for only 1 week if a dose adjustment (i.e., dose decrease) is required at Week 6 (Visit 9/Last Titration). Those participants will immediately proceed to the Double-Blind, Randomized Treatment Period on the newly decreased dose.

- At Visit 4, document CKD Stage based on mean eGFR calculated from Visits 1a, 2, and 3 or Visits 1b, 2, and 3 (if Visit 1b was required).
  - CKD Stage 2: eGFR 60 to 90 ml/min/1.73 $m^2$
  - CKD Stage 3: eGFR 30 to 59 ml/min/1.73 $m^2$
  - CKD Stage 4: eGFR 15 to 29 ml/min/1.73 $m^2$
- Administer the following health-outcomes scale to participant (**Note**: responses should be reviewed prior to completion of the visit in order to clarify any ambiguous or missing responses):
  - ADPKD-UIS
- Collect blood samples for the following laboratory testing:
  - Liver chemistry and serum sodium (Visits 5 and 7 only) (**Note**: For participants exhibiting an increase in liver chemistry tests during the Lixivaptan Titration Period (>1.2 x ULN), follow the instructions in Section 7.3.7.1.2 (Liver Test Abnormalities during the Placebo Run-in and/or Lixivaptan Titration Period).
- At Visit 7 (if participant is initiating his/her second week to confirm tolerability and may not require Visit 8 *or* at Visit 8 for all other participants), provide participant with a urine collection container and written instructions to produce a first morning, fasting (≥8 hour except for water) urine specimen to be brought in at Visit 9 for assessment of urine osmolality.
- Contact the IRT to obtain a study drug kit number and dispense study drug. Instruct the participant to take 4 capsules twice a day daily approximately 10 hours apart.

- Remind the participant to increase fluid intake to prevent dehydration
- Schedule the participant for the next visit in the Lixivaptan Titration Period (to occur in 1 week ± 2 days). Provide the participant with an appointment reminder card and any written instructions.

Participants who do not tolerate the study drug during the titration period and have no further adjustment step available in accordance with the Lixivaptan Titration Period (Part 1) — Lixivaptan Titration Flowchart will terminate from the study. Participants who terminate from the study during the titration period will undergo Visit 22/ET procedures within 7 days of the last dose of study drug.

### 6.3.2 Visit 9/Last Titration Visit

Visit 9/Last Titration Visit is intended to confirm that the participant tolerates lixivaptan at the dose determined during the Lixivaptan Titration Period, to collect additional data, and to randomize the participant to study drug for the Double-blind Randomized Treatment Period. The following procedures will be performed at Visit 9/Last Titration Visit:

- Process urine specimen collected by participant as a fasting AM urine specimen the morning of Visit 9 for determination of urine osmolality.
- Review and assess AEs and SAEs.
- Collect and review new concomitant medication information.
- Collect and review any acetaminophen use information.
- Review and assess laboratory test results.
- Collect medical resource utilization data since the last visit (i.e., medication use or changes; unplanned office visits; urgent care or emergency department usage and hospitalizations) based on any AEs/SAEs reported.
- Perform study drug reconciliation and review study drug compliance.
- Review tolerability of dosing.
- Collect body weight measurement.
- Check vital signs.
- Collect urine for:
    - urinalysis;
    - local urine pregnancy test (**Note**: all positive tests will be confirmed by a serum pregnancy test at the central laboratory).
- Perform a 12-lead ECG.
- Collect blood samples for the following laboratory testing:
    - chemistry, including serum sodium;

  - liver chemistry;
  - serum creatinine for eGFR determination;
  - hematology;
  - sparse PK sampling (blood draw can occur at any time post-dose).
- Administer the following health-outcomes scales to participants (**Note**: responses should be reviewed prior to completion of the visit in order to clarify any ambiguous or missing responses):
  - ADPKD-IS
  - ADPKD-PDS
  - ADPKD-UIS
- Contact the IRT to randomize the participant and to obtain 4 study drug kit numbers (4-week supply). **Note**: Mayo Clinic ADPKD Image Classification as assessed by the central imaging vendor (prior to Visit 2) and CKD Stage will be used by the IRT System for stratification purposes. Dispense study drug to initiate the Double-blind, Randomized Treatment Period. Instruct the participant to take 4 capsules twice a day daily approximately 10 hours apart.
- Remind the participant to increase fluid intake to prevent dehydration
- Schedule the participant for the next visit (to occur in 4 weeks ± 5 days). Provide the participant with an appointment reminder card and any written instructions.

**Note**: During the Double-Blind Randomized Treatment Periodsites will contact participants by telephone 2 weeks (± 5 days) after every scheduled visit to ascertain continued tolerability and adherence to study drug treatment. Telephone contacts will be recorded in the source document.

## 6.4 Double-blind, Randomized Treatment Period

The Double-blind Randomized Treatment Period will last 52 weeks, and visits will occur every 4 weeks ± 5 days (Visit 10 to Visit 22).

### 6.4.1 Visits 10 to 21

Visits 10 to 21 will occur every 4 weeks ± 5 days. Visits 10, 11, 13, 14, 16, 17, 19, 20 and 21 may be conducted remotely by an HHC/qualified site personnel (with the agreement of the participant and the discretion of the investigator and where available and locally approved by the CA and/or IRB/EC). Visits 12, 15, and 18 must be conducted in the clinic. The following procedures will be performed at each visit unless otherwise noted:

- Provide participant with a urine collection container and written instructions to produce a first morning, fasting (≥8 hours, except for water) urine specimen to be brought in at Visit 15 for assessment of urine osmolality.

- Process urine specimen collected by participant as a fasting AM urine specimen the morning of Visit 15 only for determination of urine osmolality.
- Review and assess AEs and SAEs.
- Collect and review new concomitant medication information.
- Collect and review any acetaminophen use information.
- Review and assess laboratory test results.
- Collect medical resource utilization data since the last visit (i.e., medication use or changes; unplanned office visits; urgent care or emergency department usage and hospitalizations) based on any AEs/SAEs reported.
- Perform study drug reconciliation and review study drug compliance.
- Check vital signs.
- Perform a urine pregnancy test for WOCBP (Visit 12, Visit 15, and Visit 18 only).
- Perform a brief physical examination (Visit 15 only or if indicated by signs or symptoms).
- Perform a 12-lead ECG (Visit 15 only).
- Collect blood samples for the following laboratory testing:
    - chemistry (Visit 12, Visit 15, and Visit 18 only);
    - serum sodium (all visits); assessed as part of chemistry at Visits 12, 15 and 18 and as part of liver chemistry at all other visits;
    - liver chemistry;
    - serum creatinine for eGFR determination;
    - hematology (Visit 15 only);
    - sparse PK sampling (Visit 12 (pre-dose), Visit 15 (1 to 6 hrs post-dose), and Visit 18 (6 to 10 hrs post-dose) only and in accordance with sparse PK sampling schedule (Schedule of Procedures – Part 1).
- Administer the following health-outcomes scales to participants at Visits 12, 15, and Visit 18 only. (**Note**: responses should be reviewed prior to completion of the visit in order to clarify any ambiguous or missing responses):
    - ADPKD-IS
    - ADPKD-PDS
    - ADPKD-UIS
- Contact the IRT to obtain 4 study drug kit numbers (4-week supply). Dispense double-blind study drug. Instruct the participant to take 4 capsules twice a day daily approximately 10 hours apart.

- At Visit 21, provide participant with a urine collection container and written instructions to produce a first morning, fasting (≥8 hours, except for water) urine specimen to be brought in the morning of Visit 22 for assessment of urine osmolality.
- Remind the participant to increase fluid intake to prevent dehydration
- Schedule the participant for the next visit (to occur in 4 weeks ± 5 days). Provide the participant with an appointment reminder card and any written instructions.

### 6.4.2 Visit 22/Early Termination

Visit 22 is the end of the double-blind period and will also serve as the Early Termination visit. For participants completing the Double-blind, Randomized Treatment Period, the visit will occur at Week 52 ± 3 days. For participants prematurely terminating from study treatment (any time after the start of the Placebo Run-in Period), the ET visit must be conducted within 7 days of the last dose of study drug; ***randomized participants will subsequently be required to complete Follow-up Period I.*** Visit 22/ET must be conducted in the clinic. The following procedures will be performed at Visit 22/ET:

- Process urine specimen collected by participant as a fasting AM urine specimen the morning of Visit 22 for determination of urine osmolality.
- Review and assess AEs and SAEs.
- Collect and review new concomitant medication information.
- Collect and review any acetaminophen use information.
- Review and assess laboratory test results.
- Collect medical resource utilization data since the last visit (i.e., medication use or changes; unplanned office visits; urgent care or emergency department usage and hospitalizations) based on any AEs/SAEs reported.
- Perform study drug reconciliation and review study drug compliance.
- Check vital signs.
- Collect body weight measurement.
- Collect urine for:
    - urinalysis;
    - local urine pregnancy test (**Note**: all positive tests will be confirmed by a serum pregnancy test at the central laboratory).
- Perform a brief physical examination.
- Perform a 12-lead ECG.
- Collect blood samples for the following laboratory testing:
    - chemistry, including serum sodium;
    - liver chemistry;

  - serum creatinine for eGFR determination;
  - hematology.

- Administer the following health-outcomes scales to participants (**Note**: responses should be reviewed prior to completion of the visit in order to clarify any ambiguous or missing responses):
  - ADPKD-IS
  - ADPKD-PDS
  - ADPKD-UIS
- Contact the IRT to register completion of or early termination from the double-blind period.
- **Randomized Participants:** Schedule the post-baseline MRI for assessment of TKV and LV to occur between 25 days and 31 days post last dose of study drug and must be completed prior to or on the same day as all other Visit 25 study procedures.
- **Randomized Participants:** Schedule the next visit (to occur 8 + 3 days after the last dose). During Follow-up Period I, every attempt will be made to draw blood samples for serum creatinine and serum cystatin C at approximately the same clock time as for Visits 1a, 2, and 3 or Visits 1b, 2 and 3, for a given participant. Provide the participant with an appointment reminder card for the next visit as well as for the post-baseline MRI and provide any written instructions that might be required.

## 6.5 Follow-up Period I

The Follow-up Period will consist of 3 visits and will last up to 28 (± 3 days).

### 6.5.1 Visit 23 and Visit 24

Visit 23 will occur no earlier than 8 (+ 3) days after the last dose of double-blind study drug and Visit 24 will occur at least 24 hours after Visit 23 and at least 24 hours prior to Visit 25. Visits 23 and 24 may be conducted remotely by an HHC/qualified site personnel (with the agreement of the participant and the discretion of the investigator and where available and locally approved by the CA and/or IRB/EC). The following procedures will be performed at each visit:

- Review and assess AEs and SAEs.
- Collect and review new concomitant medication information.
- Collect and review any acetaminophen use information.
- Review and assess laboratory test results (at Visit 23).
- Collect medical resource utilization data since the last visit (i.e., medication use or changes; unplanned office visits; urgent care or emergency department usage and hospitalizations) based on any AEs/SAEs reported.
- Collect a blood sample for:

  - serum creatinine and serum cystatin C for eGFR determination. **Note**: Every attempt will be made to draw blood samples for eGFR determination at approximately the same clock time during Visits 1a/1b, 2, and 3 and Visits 23, 24, and 25, and again at Visits 43, 44, and 45, for a given participant.

- Contact the IRT to register completion of the visit.
- At Visit 23, schedule the participant for the second follow-up visit (Visit 24) to occur at least 24 hours from Visit 23.
- At Visit 24, provide participant with a urine collection container and written instructions to produce a first morning, fasting (≥8 hours, except for water) urine specimen to be brought in at the next visit for assessment of urine osmolality.
- At Visit 24, schedule the participant to return for the final follow-up visit (Visit 25) (to occur 28 ± 3 days after the last dose of double-blind study drug, and minimally 24 hours after Visit 24). (**Note**: The post-baseline MRI must be completed between 25 days and 31 days post last dose of study drug and must be completed prior to or on the same day as all other Visit 25 study procedures.)

### 6.5.2 Visit 25

Visit 25 is the last visit of Part 1 and initiates Part 2 of the study. It will occur 28 ± 3 days after the last dose of double- blind study drug. The following procedures will be performed at Visit 25:

- Process urine specimen collected by participant as a fasting AM urine specimen the morning of Visit 25 for determination of urine osmolality.
- Confirm the post-baseline MRI for assessment of TKV and LV has been completed.
- Review and assess AEs and SAEs.
- Collect and review new concomitant medication information.
- Collect and review any acetaminophen use information.
- Collect medical resource utilization data since the last visit (i.e., medication use or changes; unplanned office visits; urgent care or emergency department usage and hospitalizations) based on any AEs/SAEs reported.
- Check vital signs.
- Collect body weight measurement.
- Collect urine for:
  - urinalysis;
  - local urine pregnancy test (**Note**: all positive tests will be confirmed by a serum pregnancy test at the central laboratory).
- Perform a brief physical examination.
- Perform a 12-lead ECG.

- Collect blood samples for the following laboratory testing:
    - chemistry, including serum sodium;
    - liver chemistry;
    - serum creatinine and serum cystatin C for eGFR determinations. **Note**: Every attempt will be made to draw blood samples for eGFR determinations at approximately the same clock time during Visits 1a/1b, 2, and 3 and Visits 23, 24, and 25, and again at Visits 43, 44, and 45, for a given participant;
    - hematology.
- Administer the following health-outcomes scales to participants (**Note**: responses should be reviewed prior to completion of the visit in order to clarify any ambiguous or missing responses):
    - ADPKD-IS
    - ADPKD-PDS
    - ADPKD-UIS
- Contact the IRT to register completion of the visit and to obtain 1 study drug kit number to initiate the Lixivaptan Re-Titration Period (Part 2). Dispense open-label study drug. Instruct the participant to take 4 capsules twice a day daily approximately 10 hours apart.
- Remind the participant to increase fluid intake to prevent dehydration
- Schedule the participant for the next visit (to occur in 1 week ± 2 days). Provide the participant with an appointment reminder card and any written instructions.

## 6.6 Lixivaptan Re-titration Period – Part 2

During the 2-to-4-week Lixivaptan Re-titration Period, all participants in Part 2 will be re-titrated to the dose level last taken during the Double-blind, Randomized Treatment Period in Part 1 (i.e., the last actual dose level if they had received lixivaptan during the Double-blind, Randomized Treatment Period or, if they received placebo during the Double-blind, Randomized Treatment Period, the last inferred dose level (the dose level equal to the active dose level had the participant been randomized to the active arm)) in accordance with Table 2. This will be performed by the IRT and the site, participant, and sponsor maintain a blinded status. For example, if no adjustment is made to the inferred dose during the Double-Blind, Randomized Treatment Period, the last inferred dose for a participant randomized to placebo will be equal to the maximal tolerated lixivaptan dose established during the Titration Period in Part 1.

All participants will initiate re-titration at a lixivaptan dose of 50 mg BID (at Visit 25). Visits 26 to 29 will occur weekly ± 2 days as needed to achieve the last dose level.

### 6.6.1 Visits 26 to 28

Re-titration visits (26 to 28) may be performed remotely by an HHC/qualified site personnel (with the agreement of the participant and the discretion of the investigator and where available and locally approved by the CA and/or IRB/EC) or in the clinic. Remote visits may include a telehealth visit (e.g., telemedicine virtual visit, telephone or video call (without recording)) with the investigator as needed. Participants who complete re-titration in 2 weeks will skip Visit 27 and Visit 28 and those completing the re-titration period in 3 weeks will skip Visit 28; instead, the assessments scheduled for Visit 29/Last Titration Visit will be completed. The following procedures will be performed at each visit unless otherwise noted:

- Review and assess AEs and SAEs.
- Collect and review new concomitant medication information.
- Collect and review any acetaminophen use information.
- Review and assess laboratory test results (from Visit 27 if Visit 28 is conducted)
- Collect medical resource utilization data since the last visit (i.e., medication use or changes; unplanned office visits; urgent care or emergency department usage and hospitalizations) based on any AEs/SAEs reported.
- Perform study drug reconciliation and review study drug compliance.
- Check vital signs.
- Administer the following health-outcomes scale to participant (**Note**: responses should be reviewed prior to completion of the visit in order to clarify any ambiguous or missing responses):
    - ADPKD-UIS
- At Visit 27, collect a blood specimen for liver chemistry.
- Contact the IRT to obtain 1 study drug kit number. Dispense study drug. Instruct the participant to take 4 capsules twice a day daily approximately 10 hours apart.
- At Visit 28 (or earlier if the participant will be completing titration in 2 or 3 weeks), provide participant with a urine collection container and written instructions to produce a first morning, fasting (≥8 hours, except for water) urine specimen to be brought in at Visit 29 for assessment of urine osmolality.
- Remind the participant to increase fluid intake to prevent dehydration
- Schedule the participant for the next visit (to occur in 1 week ± 2 days). Provide the participant with an appointment reminder card and any written instructions.

### 6.6.2 Visit 29/Last Re-titration Visit

The following procedures will be performed at Visit 29:

- Process urine specimen collected by participant as a fasting AM urine specimen the morning of Visit 29 for determination of urine osmolality.

- Review and assess AEs and SAEs.
- Collect and review new concomitant medication information.
- Collect and review any acetaminophen use information.
- Review and assess laboratory test results (from Visit 27 if Visit 28 is not conducted)
- Collect medical resource utilization data since the last visit (i.e., medication use or changes; unplanned office visits; urgent care or emergency department usage and hospitalizations) based on any AEs/SAEs reported.
- Perform study drug reconciliation and review study drug compliance.
- Check vital signs.
- Collect body weight measurement.
- Collect urine for urinalysis.
- Perform a 12-lead ECG.
- Collect blood samples for the following laboratory testing:
    - chemistry, including serum sodium;
    - liver chemistry;
    - serum creatinine for eGFR determination;
    - hematology;
    - sparse PK sampling (1 to 6 hrs post-dose).
- Administer the following health-outcomes scales to participants (**Note**: responses should be reviewed prior to completion of the visit in order to clarify any ambiguous or missing responses):
    - ADPKD-IS
    - ADPKD-PDS
    - ADPKD-UIS
- Contact the IRT to obtain 4 study drug kit numbers (4-week supply). Dispense open-label study drug. Instruct the participant to take 4 capsules twice a day daily approximately 10 hours apart.
- Remind the participant to increase fluid intake to prevent dehydration
- Schedule the participant for the next visit (to occur in 4 weeks ± 5 days). Provide the participant with an appointment reminder card and any written instructions.

## 6.7 Maintenance Treatment Period

The Maintenance Treatment Period will last 52 weeks, and visits will occur every 4 weeks ± 5 days (Visit 30 to Visit 42).

Sites will contact participants by telephone 2 weeks (± 5 days) after every scheduled visit during the Maintenance Treatment Period to ascertain continued tolerability and adherence to study drug treatment. Telephone contacts will be recorded in the source document.

### 6.7.1 Visits 30 to 41

Visits 30 to 41 will occur every 4 weeks ± 5 days. Visits 30, 31, 33, 34, 36, 37, 39, 40 and 41 may be conducted remotely by an HHC/qualified site personnel (with the agreement of the participant and the discretion of the investigator and where available and locally approved by the CA and/or IRB/EC). Remote visits may include a telehealth visit (e.g., telemedicine virtual visit, telephone or video call (without recording)) with the investigator as needed. Visits 32, 35, and 38 must be conducted in the clinic. The following procedures will be performed at each visit unless otherwise noted:

- Review and assess AEs and SAEs.
- Collect and review new concomitant medication information.
- Collect and review any acetaminophen use information.
- Review and assess laboratory test results.
- Collect medical resource utilization data since the last visit (i.e., medication use or changes; unplanned office visits; urgent care or emergency department usage and hospitalizations) based on any AEs/SAEs reported.
- Perform study drug reconciliation and review study drug compliance.
- Check vital signs.
- Perform a urine pregnancy test for WOCBP (Visit 32, Visit 35, and Visit 38 only).
- Perform a brief physical examination (Visit 35 only).
- Perform a 12-lead ECG (Visit 35 only).
- Collect blood samples for the following laboratory testing:
    - Chemistry (Visit 32, Visit 35, and Visit 38 only);
    - serum sodium (all visits); assessed as part of chemistry at Visits 32, 35 and 38 and as part of liver chemistry at other visits;
    - liver chemistry;
    - serum creatinine for eGFR determination;
    - hematology (Visit 35 only);
    - sparse PK sampling (Visit 32 (pre-dose), Visit 35 (6 to 10 hrs post-dose), and Visit 38 (any time post-dose) only and in accordance with sparse PK sampling schedule in the Schedule of Procedures – Part 2).

- Administer the following health-outcomes scales to participants at Visit 32, Visit 35 and Visit 38 only. (**Note**: responses should be reviewed prior to completion of the visit in order to clarify any ambiguous or missing responses):
    - ADPKD-IS
    - ADPKD-PDS
    - ADPKD-UIS
- Contact the IRT to obtain 4 study drug kit numbers (4-week supply). Dispense open-label study drug. Instruct the participant to take 4 capsules twice a day daily approximately 10 hours apart.
- At Visit 34 and Visit 41, provide participant with a urine collection container and written instructions to produce a first morning, fasting (≥8 hours, except for water) urine specimen to be brought in at the next scheduled visit, Visit 35 and Visit 42, respectively, for assessment of urine osmolality.
- At Visit 35, process urine specimen collected by participant as a fasting AM urine specimen the morning of Visit 35 for determination of urine osmolality.
- Remind the participant to increase fluid intake to prevent dehydration.
- Schedule the participant for the next visit (to occur in 4 weeks ± 5 days). Provide the participant with an appointment reminder card and any written instructions.

### 6.7.2 Visit 42/Early Termination (Part 2)

Visit 42 is the end of the Maintenance Treatment Period and will also serve as the Early Termination visit for Part 2 of the study. For participants completing the Maintenance Treatment Period, the visit will occur at Week 104 ± 3 days. For participants prematurely terminating from open-label study treatment, the ET visit must be conducted within 7 days of the last dose of study drug and will be followed by completion of Follow-up Period II. Visit 42/ET must be conducted in the clinic. The following procedures will be performed at Visit 42/ET:

- Process urine specimen collected by participant as a fasting AM urine specimen the morning of Visit 42 for determination of urine osmolality.
- Review and assess AEs and SAEs.
- Collect and review new concomitant medication information.
- Collect and review any acetaminophen use information.
- Review and assess laboratory test results.
- Collect medical resource utilization data since the last visit (i.e., medication use or changes; unplanned office visits; urgent care or emergency department usage and hospitalizations) based on any AEs/SAEs reported.
- Perform study drug reconciliation and review study drug compliance.
- Check vital signs.

- Collect body weight measurement.
- Collect urine for:
  - urinalysis;
  - local urine pregnancy test (**Note**: all positive tests will be confirmed by a serum pregnancy test at the central laboratory).
- Perform a brief physical examination.
- Perform a 12-lead ECG.
- Collect blood samples for the following laboratory testing:
  - Chemistry, including serum sodium;
  - liver chemistry;
  - serum creatinine for eGFR determination;
  - hematology.
- Administer the following health-outcomes scales to participants (**Note**: responses should be reviewed prior to completion of the visit in order to clarify any ambiguous or missing responses):
  - ADPKD-IS
  - ADPKD-PDS
  - ADPKD-UIS
- Contact the IRT to register completion of the Maintenance Treatment Period.
- Schedule participant for the post-baseline MRI for assessment of TKV and LV to occur between 25 days and 31 days after the last dose of study drug; the MRI must be completed prior to or on the same day as all other Visit 45 study procedures.
- Schedule the participant for the next visit (to occur 8 + 3 days after the last dose). During Follow-up Period II, every attempt will be made to draw blood samples for serum creatinine at approximately the same clock time as for Visits 23, 24, and 25, for a given participant. Provide the participant with an appointment reminder card for the next visit as well as for the post-baseline MRI and provide any written instructions that might be required.

## 6.8 Follow-up Period II

The Follow-up Period will consist of 3 visits and will last up to 28 (± 3 days).

### 6.8.1 Visit 43 and Visit 44

Visit 43 will occur no earlier than 8 (+ 3) days after the last dose of double-blind study drug and Visit 44 will occur at least 24 hours after Visit 43 and at least 24 hours prior to Visit 45. Visits 43 and 44 may be conducted remotely by an HHC/qualified site personnel (with the agreement of the participant and the discretion of the investigator and where available and locally approved by the CA and/or IRB/EC). Remote visits may include a telehealth visit

(e.g., telemedicine virtual visit, telephone or video call (without recording)) with the investigator as needed. The following procedures will be performed at each visit:

- Review and assess AEs and SAEs.
- Collect and review new concomitant medication information.
- Collect and review any acetaminophen use information.
- Review and assess laboratory test results (Visit 43).
- Collect medical resource utilization data since the last visit (i.e., medication use or changes; unplanned office visits; urgent care or emergency department usage and hospitalizations) based on any AEs/SAEs reported.
- Collect a blood sample for:
    - serum creatinine and serum cystatin C for eGFR determination. **Note**: Every attempt will be made to draw blood samples for eGFR determination at approximately the same clock time during Visits 1a/1b, 2, and 3 and Visits 23, 24, and 25, and again at Visits 43, 44, and 45, for a given participant.
- Contact the IRT to register completion of the visit.
- At Visit 43, schedule the participant to return for the second follow-up visit (Visit 44) (to occur at least 24 hours from Visit 43).
- At Visit 44, provide participant with a urine collection container and written instructions to produce a first morning, fasting (≥8 hours, except for water) urine specimen to be brought in at Visit 45 for assessment of urine osmolality.
- At Visit 44, schedule the participant to return for the final follow-up visit (Visit 45) (to occur 25 to 31 days after the last dose of study drug and minimally 24 hours after Visit 44). Provide the participant with an appointment reminder card for the next visit and provide any written instructions that might be required. **Note:** the post-baseline MRI must be completed between 25 days and 31 days post last dose of study drug and must be completed prior to or on the same day as all other Visit 45 study procedures.

### 6.8.2 Visit 45

Visit 45 is the last visit in this study and will occur 28 ± 3 days after the last dose of open-label study drug. The following procedures will be performed at Visit 45:

- Process urine specimen collected by participant as a fasting AM urine specimen the morning of Visit 45 for determination of urine osmolality.
- Confirm the post-baseline MRI for assessment of TKV and LV has been completed.
- Review and assess AEs and SAEs.
- Collect and review new concomitant medication information.
- Collect and review any acetaminophen use information.

- Review and assess laboratory test results (after the visit).
- Collect medical resource utilization data since the last visit (i.e., medication use or changes; unplanned office visits; urgent care or emergency department usage and hospitalizations) based on any AEs/SAEs reported.
- Check vital signs.
- Collect body weight measurement.
- Collect urine for:
    - urinalysis;
    - local urine pregnancy test (**Note**: all positive tests will be confirmed by a serum pregnancy test at the central laboratory).
- Perform a brief physical examination.
- Perform a 12-lead ECG.
- Collect blood samples for the following laboratory testing:
    - chemistry, including serum sodium;
    - liver chemistry;
    - serum creatinine and serum cystatin C for eGFR determinations. **Note**: Every attempt will be made to draw blood samples for eGFR determinations at approximately the same clock time during Visits 1a/1b, 2, and 3 and Visits 23, 24, and 25, and again at Visits 43, 44, and 45, for a given participant;
    - hematology.
- Administer the following health-outcomes scales to participants (**Note:** responses should be reviewed prior to completion of the visit in order to clarify any ambiguous or missing responses):
    - ADPKD-IS
    - ADPKD-PDS
    - ADPKD-UIS
- Contact the IRT to register completion of the study.

## 6.9 Early Termination Procedures

Participants who terminate from the study during Screening will not have follow up assessments.

Participants who terminate from the study during Part 1 at any time after the start of the Placebo Run-in Period will undergo Visit 22/ET procedures within 7 days of the last dose of study drug. Randomized participants will additionally complete Follow-up Period I for final assessments of safety and efficacy. Participants who terminate from the study at any time during Part 2 will undergo Visit 42/ET procedures within 7 days of the last dose of study

drug and additionally complete Follow-up Period II for final assessments of safety and efficacy.

**Note**: the intent of this study is that all randomized participants complete all visits and procedures regardless of whether study drug is continued or not. Detailed information about accommodating participants who may not be able or want to continue in the study is available as follows:

- Treatment Discontinuation – Section 4.2.4
- Methods to Prevent Loss to Follow Up – Section 4.2.7
- Withdrawal of Consent – Section 4.2.8
- Minimization of Missing Data: Study and Study Treatment Modification – Appendix 5 (Section 13.5)

# 7 DESCRIPTION OF STUDY ASSESSMENTS AND PROCEDURES

Before performing any study procedures, all potential participants will sign an ICF. Participants will have the opportunity to have any questions answered before signing the ICF. The Investigator must address all questions raised by the participant.

The study assessments to be performed at each visit and timepoint are specified in the study Schedule of Procedures – Part 1 and Schedule of Procedures – Part 2.

## 7.1 Efficacy Assessments

### 7.1.1 eGFR Determination

The eGFR values for the primary analysis will be calculated according to the 2021 CKD-EPI formula (CKD-EPIcr_R) (Appendix 1 (Section 13.1)) from the serum creatinine concentrations taken during the Screening Period and at the visits specified in the Schedule of Procedures – Part 1 and Schedule of Procedures – Part 2 of the study. During the Screening Period, serum creatinine assessments obtained at Visits 1a, 2, and 3 or Visits 1b, 2, and 3 (if Visit 1b is required) will be used to determine the mean eGFR for analysis purposes. The averaged value will be used as the eGFR baseline. For Part 2, the serum creatinine assessments obtained during Follow-up Period I (Visit 23, Visit 24, and Visit 25) will be used as the eGFR baseline. Every attempt will be made to draw blood samples for eGFR determination at approximately the same clock time during Visits 1a/1b, 2, and 3 and Visits 23, 24, and 25, and again at Visits 43, 44, and 45 for a given participant.

As an exploratory endpoint, eGFR will also be assessed by the measurement of serum creatinine and serum cystatin C using a novel CKD-EPI equation (CKD-EPIcr-cys_R) (Appendix 1 (Section 13.1)). Along with serum creatinine concentrations, serum cystatin C will be obtained at Visits 1a, 2 and 3 or Visits 1b, 2 and 3 and during Follow-up Period 1 (Visits 23, 24, and 25) and during Follow-up Period II (Visits 43, 44 and 45). The mean of the 3 eGFR measurements calculated from the CKD-EPIcr-cys equation during each period will be used for the baseline and follow-up period for analyses of the exploratory eGFR endpoint. To reduce potential decision errors, eGFR calculated from the CKD-EPIcr-cys_R equation will not be shared with sites as all eligibility and treatment decisions will be based on eGFR calculated from the CKD-EPIcr_R equation.

Alteration in the metabolism of creatinine based on changes in meat intake and exercise may impact accuracy of serum creatinine and determination of eGFR. Measures to reduce variability are provided in Section 5.6.

### 7.1.2 MRI Assessment

MRI scans will be obtained for 2 purposes:

1) to qualify each participant's participation during Screening based on the Mayo Clinic ADPKD Image Classification (prognostic criteria) (Irazabal et al, 2015);
2) to investigate lixivaptan's pharmacodynamic effect on TKV and LV.

Participants will undergo a standardized abdominal MRI assessment without use of intravenous contrast agent for the determination of TKV (combined kidney volume of both kidneys) and LV at 3 timepoints: Screening; Follow-up Period I (Visit 25); and Follow-up Period II (Visit 45).

The MRI acquisition protocol will be detailed in the study imaging manual. MRI images will be sent to a blinded central imaging vendor for a) quality control; b) determination of Mayo Clinic ADPKD Image Classification (Class 1 [typical] or Class 2 [atypical] at Screening only); and c) measurement of TKV and LV.

Participants with ferro-magnetic prostheses, aneurysm clips, severe claustrophobia, or other contraindications or exclusions interfering with MRI measurements will not be enrolled in the study. Investigator should seek MRI safety guidance from the local MRI facility.

To maintain study blinding, volumetric calculations and other derived data from MRI imaging obtained after randomization (Visit 9) will not be shared with the participant, the investigator, the clinical sites, the sponsor, or the CRO.

### 7.1.3 Urine Osmolality

Fasting (≥ 8 hrs, except for water) spot first AM urine collections will be obtained at the timepoints specified in the Schedule of Procedures – Part 1 and Schedule of Procedures – Part 2 of the study. Participants will be provided a urine collection container in advance of each visit where $U_{osm}$ is determined. They will be asked to completely empty their bladders upon arising in the morning and then provide a spot urine specimen within the next 30-45 minutes, prior to taking the morning dose of study drug. They will bring that specimen to their clinic visit. All $U_{osm}$ assessments in the study will conducted using this method.

All samples will be sent to the central laboratory for analysis. To maintain study blinding, results of $U_{osm}$ assessments obtained after randomization (Visit 9) will not be shared with the participant, the investigator, the clinical sites, the sponsor, or the CRO.

## 7.2 Health Outcomes Assessments

### 7.2.1 Medical Resource Utilization

Medical resource utilization will be evaluated throughout Part 1 and Part 2 of the study. Beginning at Visit 3, participants reporting clinical events as either AEs or SAEs should be questioned on whether any of the clinical events resulted in medication use or medication changes (e.g., change in dose; change in medication); an unplanned office visit, an urgent care visit and/or an emergency department visit or in-patient hospitalization in order to diagnose, treat or manage the clinical event). Data collected will be entered in the appropriate eCRF.

### 7.2.2 ADPKD- Impact Scale

The ADPKD-IS (Oberdhan et al, 2017), a participant self-administered questionnaire consisting of 14 items, measures the impact of ADPKD on a patient's health-related quality of life (HRQoL) using 3 conceptual domains: physical, emotional, and fatigue plus 4 additional questions. Each item is measured on a scale of 1 (not difficult/bothered at all) to 5 (extremely difficult/bothered) with a recall period of 14 days. Each domain is scored by summing the scores of all items in the domain and dividing the total number by the number of items in that domain. Each domain score is measured on a scale of 1–5, from 1, not difficult/bothered at all, to 5, extremely difficult/bothered, with a recall period of 14 days. The ADPKD-IS will be administered throughout Part 1 and Part 2 of the study at the time-points specified in the Schedule of Procedures – Part 1 and Schedule of Procedures – Part 2. In this study, participants will directly record their responses into an electronic data capture device (i.e., tablet). During remote visits, participants will record their responses on a paper copy, which will later be transcribed into the database by site personnel.

### 7.2.3 ADPKD- Pain and Discomfort Scale

ADPKD-PDS (Oberdhan et al, 2015), a participant self-administered questionnaire consisting of 20 items, measures the impact of three distinct types of ADPKD-related pain: dull kidney pain, sharp kidney pain, and fullness/discomfort. Each item is measured on a 5-point scale from 1 (no pain/discomfort) to 5 (extreme pain/discomfort). The questionnaire has a recall period of 7 days. The ADPKD-PDS will be administered throughout Part 1 and Part 2 of the study at the time-points specified in the Schedule of Procedures – Part 1 and Schedule of Procedures – Part 2. In this study, participants will directly record their responses into an electronic data capture device (i.e., tablet). During remote visits, participants will record their responses on a paper copy, which will later be transcribed into the database by site personnel.

### 7.2.4 ADPKD- Urinary Impact Scale

The ADPKD-UIS (Oberdhan et al, 2013), a participant self-administered questionnaire, measures the burden of urinary concerns in ADPKD using 11 items that assess 3 domains: daytime urinary frequency, daytime urinary urgency, and nocturia. Each item is measured on a scale from 1 (not difficult/bothered) to 5 (very difficult/bothered). ADPKD-UIS measures each domain by summing the scores for all items in the domain and dividing it by the number of items in that domain. The questionnaire has a recall period of 7 days. The ADPKD-UIS will be administered throughout Part 1 and Part 2 of the study at the time-points specified in the Schedule of Procedures – Part 1 and Schedule of Procedures – Part 2. In this study, participants will directly record their responses into an electronic data capture device (i.e., tablet). During remote visits, participants will record their responses on a paper copy, which will later be transcribed into the database by site personnel.

## 7.3 Safety and Tolerability Assessments

The safety and tolerability of lixivaptan will be assessed by evaluation of AEs, SAEs, physical examinations, vital sign measurements, ECGs, and clinical laboratory parameters (hematology, clinical chemistry including liver chemistry tests, and urinalysis). Additional safety assessments may be performed as needed at the discretion of the Investigator. Safety assessments will be performed throughout the study as described in the Schedule of Procedures – Part 1 and Schedule of Procedures – Part 2. Assessment windows are presented in Table 3 and Table 4.

### 7.3.1 Body Height, Weight, and BMI

Body height (inches or centimeters) and weight (pounds or kilograms) will be measured at Screening Visit 1a to the nearest tenth, and BMI will be calculated automatically by the eCRF functionality (BMI [$kg/m^2$] = body weight [kg] / height$^2$ [$m^2$]). Body weight will be subsequently measured as described in the Schedule of Procedures – Part 1 and Schedule of Procedures – Part 2. It is preferable to use the same scale at each visit where body weight is measured, and measurements of height and weight will be without the participant wearing shoes.

### 7.3.2 Physical Examinations and Medical History

A complete physical examination will be performed during Screening Visit 1a. Brief physical examinations will subsequently be performed at the timepoints specified in the study Schedule of Procedures – Part 1 and Schedule of Procedures – Part 2.

A complete physical examination will include, at a minimum, height and weight, general appearance, and assessment of the following systems: skin, head, ears, eyes, and throat, respiratory system, cardiovascular system, gastrointestinal system, neurologic system, blood and lymphatic systems, and the musculoskeletal system. The physical examination may be performed by the Investigator, a sub-investigator who is a medical doctor, a qualified nurse practitioner or a physician's assistant in accordance with the site's current practice and local requirements, as applicable.

Brief physical examinations include, at a minimum, weight, and assessment of the following systems: chest and heart auscultation, abdominal examination, neurologic condition (i.e., alert and oriented x3; not including cranial nerves), and edema and hydration assessment. The brief physical examination may be performed by the same individuals who can perform a complete physical examination.

Physical examination findings noted during Screening Visit 1a will become part of medical history. New physical examination findings observed after initiation of study drug will be classified as being in 1 of 3 categories: normal, abnormal but not clinically significant (NCS), or abnormal and clinically significant (CS). All CS findings will be reported as AEs.

Physical examinations may be performed at various unscheduled time points if deemed necessary by the Investigator. Symptom-directed physical examinations will be performed as appropriate for participants experiencing AEs.

Medical history, smoking and alcohol history, and demographic data will be recorded at Visit 1a as specified in the Schedule of Procedures – Part 1.

### 7.3.3 Vital Sign Measurements

Vital sign measurements will include systolic blood pressure, diastolic blood pressure, and pulse rate and will be performed at the timepoints specified in the Schedule of Procedures – Part 1 and Schedule of Procedures – Part 2. Respiratory rate and body temperature will be measured once at Screening Visit 1a.

Participants will remain at rest in a seated position for a minimum of 5 minutes before vital sign measurements are obtained. For all participants, blood pressure and pulse rate will be measured once at each specified time using an automated sphygmomanometer. Blood pressure measurements will be taken with the appropriate cuff size and in the same arm (whenever possible) for the duration of the study. The arm should be positioned level with the heart and the blood pressure cuff applied directly to the skin (not over clothing). A confirmatory repeat vital sign measurement may be performed at the discretion of the Investigator. Although sitting is the standard position for measurement of vital signs, a site that uses a different standard of care, e.g., semi-recumbent, may utilize that position as long as all measurements at that site are done in the same position. Other positions to obtain vital signs may be used after discussion with the medical monitor. Any confirmed, clinically significant vital sign measurements should be recorded as medical history at Screening Visit 1a and as an AE thereafter. If other procedures are scheduled at the same timepoint, vital signs should be obtained first, before an ECG and/or blood draw.

### 7.3.4 12-Lead ECGs

Standard 12-lead ECGs will be performed at the timepoints specified in the study Schedule of Procedures – Part 1 and Schedule of Procedures – Part 2.

ECG intervals will be measured using an ECG machine that automatically calculates the HR and measures PR, QRS, QT and QTcF. The same ECG machine should be used for the same participant throughout the study.

ECGs will be obtained with the participant remaining in a supine or semi-recumbent position following 5 minutes of rest. All ECGs throughout the study for a given participant should be measured in the same position, i.e., either all in a supine position or all in a semi-recumbent position. If other procedures are scheduled at the same timepoint, the ECG should be obtained after vital sign measurements and/or before the scheduled blood draw.

ECGs will be transmitted electronically to the central ECG laboratory for analysis according to the instructions provided by the laboratory to be read by a cardiologist. The ECGs will be classified as being in one of three categories: normal, abnormal but not clinically significant (NCS), or abnormal and CS. All CS findings should be reported as medical history at Screening Visit 1a and as AEs thereafter.

Additional ECGs may be performed if deemed medically appropriate.

### 7.3.5 Clinical Laboratory Tests

Blood and urine specimens will be collected for clinical laboratory determinations (hematology, clinical chemistry including serum sodium, liver chemistry and serum creatinine and serum cystatin C, serology, and urinalysis) by the central laboratory and local urine pregnancy tests (WOCBP only) at the timepoints specified in the study Schedule of Procedures – Part 1 and Schedule of Procedures – Part 2. A confirmatory assessment through the central laboratory should be obtained as soon as possible for any CS abnormal laboratory parameter if deemed necessary by the Investigator. All CS findings should be reported as medical history at Screening Visit 1a and as AEs thereafter. Repeat testing for abnormal liver chemistry tests is described in Section 7.3.7.1. Any other repeat laboratory testing for values that are not CS should first be discussed with the medical monitor before such tests are ordered. All positive urine pregnancy testing results will be confirmed by a serum pregnancy test at the central laboratory.

With the exception of participants' at-home collection of urine specimen for measurement of urine osmolality by the central laboratory, laboratory tests required by the protocol do not require overnight fasting. Mild elevations in serum creatinine or serum sodium are known pharmacodynamic effects of vasopressin antagonists like lixivaptan, therefore, confirmatory assessments for serum creatinine and serum sodium will be at the Investigator's discretion.

Clinical laboratory parameters for analysis are presented in Table 8.

**Table 8. Clinical Laboratory Parameters**

| Chemistry | Hematology | Urinalysis |
|---|---|---|
| Albumin | Hematocrit | pH |
| Blood urea nitrogen (urea) | Hemoglobin | Specific gravity |
| Calcium | Red blood cell count | Protein |
| Chloride | Quantitative platelet count | Glucose |
| Carbon dioxide ($CO_2$) | White blood cell (WBC) count with differential (total and %) | Ketones |
| Glucose | | |
| Phosphorous | only if WBC count is abnormal: | Bilirubin |
| Potassium | Neutrophils | Blood |
| Protein | Lymphocytes | Urobilinogen |
| Sodium* | Monocytes | Leukocytes |
| Uric Acid | Eosinophils | Nitrite |
| | Basophils | Microscopic examination (if positive for blood, protein, nitrite, or leukocytes) |
| **Serum Creatinine** | | |
| Creatinine, enzymatic | | |
| Serum cystatin C | | |
| **Serology (Screening)** | **Liver Chemistry** | **Other Tests** |
| Hepatitis B surface antigen | Alkaline phosphatase | Urine osmolality |
| Hepatitis C antibody (reflex HCV RNA for all Hepatitis C antibody positive or reactive results) | ALT | Urine or serum pregnancy test |
| | AST | |
| | Bilirubin (Total and Direct) | |

*Serum sodium will be assessed at all time-points specified in the Schedule of Procedures either as part of chemistry or liver chemistry testing.

### 7.3.5.1 Sample Collections

Instructions regarding the collection, processing, and shipment of laboratory samples are detailed in a separate laboratory manual. All samples will be given a unique identifier. The sample collection date and time will be entered into the eCRF. Participant age and year of birth will be collected at the time of sample collection and reported on the requisition form.

### 7.3.6 Assessment of Tolerability During Placebo Run-in and Lixivaptan Titration Period

In the pre-randomization phase of the study, tolerability will be assessed at the end of the Placebo Run-in Period (Visit 3), to maintain participant blinding and at each visit during the Lixivaptan Titration Period (Visits 4 to 9). A single tolerability question will be asked: "Would you take the study drug for the next 24 months?" If the participant confirms the dose can be tolerated, the participant will be titrated to the next dose level (i.e., the dose will be increased). This assessment of tolerability will continue at weekly intervals until either the maximum dose level is reached (Level 4 [200 mg BID]) or until the participant confirms the dose cannot be tolerated for a 24-month period. In that case, the dose will be reduced by one dose level as described in the Lixivaptan Titration Period (Part 1) — Lixivaptan Titration Flowchart.

### 7.3.7 Assessment of Liver Symptoms, Signs, or Test Abnormalities

Testing for hepatic aminotransferases (ALT/AST), alkaline phosphatase, and total bilirubin will be performed at the timepoints as specified in the Schedule of Procedures – Part 1 and Schedule of Procedures – Part 2. Management of hepatic and liver chemistry test abnormalities are discussed below. *The appearance of any suspicious symptoms or signs of liver dysfunction as described in Appendix 2 (Section 13.2) in a participant at any time during the study should trigger prompt testing of liver chemistry tests (i.e., within 48 hours).*

#### 7.3.7.1 Management of Liver Chemistry Test Abnormalities and Signs or Symptoms of Liver Dysfunction Occurring During Study Treatment

Elevations of liver aminotransferase levels >3 x ULN or total bilirubin levels >2 x ULN should prompt immediate retesting within 48 hours. Although central laboratory testing is preferred, local laboratory testing is acceptable, ideally with a concurrent central laboratory sample for confirmation. After the Investigator consults with the medical monitor and sponsor, temporary reduction in dose or interruption of study drug administration must be instituted if liver aminotransferase levels >3 x ULN or total bilirubin levels >2 x ULN are observed during the Lixivaptan Titration or Double-Blind Randomized Treatment Periods of Part 1 or the Lixivaptan Re-Titration or Maintenance Treatment Periods of Part 2. During the time that values remain elevated, testing should be done at least weekly for the first month, gradually returning to monthly as indicated by the results. In the presence of such liver chemistry test abnormalities, additional clinical testing (examples listed in Appendix 3 (Section 13.3) may be recommended by the medical monitor and sponsor and those results reported according to local guidelines.

Similarly, the appearance of any suspicious symptom or sign of liver dysfunction (see Appendix 2 (Section 13.2)) at any time during the study should trigger prompt testing of liver chemistry (i.e., within 48 hours) and consultation with the medical monitor and sponsor to discuss any changes to dosing with study drug.

All elevations meeting these thresholds or confirmed symptoms or signs of liver dysfunction will be assessed for causality by an independent, blinded Hepatic Events Review Committee (HERC) (see Section 7.3.7.2).

In the case of study drug interruption, re-starting study drug should be encouraged (see except under these circumstances below) after discussion with the medical monitor and sponsor, when liver chemistry tests have normalized or stabilized, and in conjunction with a plan for increased frequency of liver chemistry test monitoring.

Treatment with study drug cannot be resumed in participants when:

- aminotransferase levels >8 × ULN at any time,
- aminotransferase levels >5 × ULN for more than 2 weeks, or
- concurrent elevations of aminotransferase levels >3 × ULN and total bilirubin >2 × ULN

except under these circumstances:

> Participants with these levels of abnormality may be re-challenged with study drug if abnormalities were adjudicated by the HERC as having a ≤50% likelihood of being related to study drug (per DILI Network [DILIN] probability criteria, modified, Appendix 4 (Section 13.4) (Fontana et al, 2009) and the Investigator, medical monitor, and sponsor agree to an intensive monitoring plan to mitigate risk. The participant must also be willing to comply with these monitoring measures, be informed of the potential risks, and agree to study drug re-challenge.

Liver chemistry test increases of a lesser extent (serum aminotransferase levels ≤3 × ULN or total bilirubin ≤2 × ULN) will be discussed with the medical monitor. They may be related to the underlying variability of such test results in ADPKD patients (Watkins et al, 2015). Generally, dosing should continue during such lesser liver chemistry test increases; however, the frequency of laboratory testing should increase, particularly during the Double-Blind Randomized Treatment Period in Part 1 and the Maintenance Treatment Period in Part 2. Slowing of titration may also be considered if the abnormalities occur during the Lixivaptan Titration Period in Part 1 or Lixivaptan Re-Titration Period in Part 2.

For participants exhibiting an increase during the Lixivaptan Titration or Lixivaptan Re-titration Periods, follow the instructions in Section 7.3.7.1.2 (Liver Test Abnormalities during the Placebo Run-in and/or Lixivaptan Titration Period).

#### 7.3.7.1.1 Repeat Liver Testing in Participants with Abnormal Values at Screening

Participants with aminotransferase or total bilirubin values above the values in the exclusion criteria (>1.2 × ULN) during Screening will not proceed further in the trial. However, if evaluation indicates a reversible cause of abnormal values, such participants may be re-screened when their liver chemistry tests return to values allowing entry into the trial.

#### 7.3.7.1.2 Liver Test Abnormalities during the Placebo Run-in and/or Lixivaptan Titration Period

Serum aminotransferase or total bilirubin levels that are ≥1.2 × ULN to 2 × ULN during the Placebo Run-in and/or Lixivaptan Titration Period will require holding the dose constant and a discussion with the medical monitor. Levels exceeding 2 × ULN during the Placebo Run-in and/or Lixivaptan Titration Period will require reducing the dose or discontinuation of study drug (randomization will not occur) depending on the extent of the elevation and in consultation with the medical monitor. Weekly monitoring of liver chemistry tests will be conducted until the values are below the ULN. In consultation with the medical monitor and sponsor, continuation of titration can resume if study drug was continued. If study drug had been stopped, titration can be re-initiated at Level 1 (50 mg BID) with weekly monitoring of liver chemistry tests if the participant agrees to the increased monitoring, is informed of the potential risks, and agrees to re-initiating study drug.

### 7.3.7.2 Hepatic Events Review Committee (HERC) – Special Reporting of Liver Events

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. This additional data will be reported on a liver dysfunction details eCRF. The type of information needed to complete it is provided in Appendix 2 (Section 13.2). The purpose of the liver dysfunction details eCRF and additional testing is to facilitate review and adjudication of each event of clinically significant liver chemistry test abnormalities and/or signs or symptoms of liver dysfunction during the study to determine the probable cause(s) of these abnormalities. The review and adjudication will be performed by an independent, blinded HERC, a committee of 3 to 4 experts experienced in liver disease. The HERC will use the modified DILIN probability criteria (0 to 5% = unrelated; 6 to 25% = unlikely, 25% to 50% = possibly, 51% to 75% = probably, 76% to 95% = very likely, >95% = definite; see Appendix 4: DILI Network Causality Criteria, modified) to assign attribution to each case of potential liver dysfunction. The HERC will independently decide attribution 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. The result of these analyses may be presented separately from the final clinical study report (CSR).

The Investigator must complete and submit a copy of the liver dysfunction details eCRF with the SAE/AESI form within 24 hours of awareness through the SAE reporting pathway (described in Section 7.3.8.3) for any participant who develops an Adverse Event of Special Interest (AESI) related to abnormal liver chemistry test results or clinical signs or symptoms. Specifically, any participant who

- develops ALT or AST levels >3 × ULN,

- develops total bilirubin levels >2 × ULN,
- develops any signs or symptoms of hepatic dysfunction (see Appendix 2 (Section 13.2)),
- discontinues treatment due to a liver-related AE,
- reports a serious liver-related AE.

The SAE/AESI report form should be updated as new information becomes available.

### 7.3.8 Adverse Events

#### 7.3.8.1 Definitions of Adverse Events

The Investigator is responsible for reporting all AEs that are observed or reported during the study, regardless of their relationship to study drug or their clinical significance, as outlined in Section 7.3.8.3 below.

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.

An 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.

#### 7.3.8.2 Eliciting and Documenting Adverse Events and Serious Adverse Events

All AEs will be recorded from the time the participant signs the ICF until study completion.

At every study visit, participants will be asked a standard nonleading question to elicit any medically related changes in their well-being. They will also be asked if they have been hospitalized, had any accidents, used any new medications, used acetaminophen/paracetamol, or changed concomitant medication regimens (both prescription and over-the-counter medications). Tolerability issues including but not limited to aquaretic effects, difficulty swallowing capsules, and abnormal thirst, will be recorded as adverse events.

In addition to participant observations, any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECGs, radiological scans, vital sign measurements, physical examination findings), including those that worsen from baseline, deemed to be CS in the medical and scientific judgment of the Investigator are to be recorded as AEs or SAEs.

#### 7.3.8.3 Reporting Adverse Events, Serious Adverse Events, and Adverse Events of Special Interest

All AEs reported or observed during the study will be recorded on the AE page of the eCRF. Information to be collected includes drug treatment, dose, event term, date of onset, Investigator-specified assessment of severity and relationship to study drug, date of resolution of the event, seriousness, any required treatment or evaluations, and outcome. AEs resulting from concurrent illnesses, reactions to concurrent illnesses, reactions to concurrent medications, or progression of disease states must also be reported. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time the participant is screened, i.e., medical history, but does not deteriorate should not be reported as an AE. However, if it deteriorates at any time during the study, it should be recorded as an AE.

However, any safety assessments that are associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the participant's condition, are not to be reported as AEs or SAEs.

For the purpose of this study, liver events are considered to be AESIs.

Any AE that meets SAE or AESI criteria will be reported to Premier Research Global Pharmacovigilance (Premier PV) immediately (i.e., within 24 hours) after site personnel first learn about the event. Investigators should record all SAE details available, including Investigator causality assessment, on the SAE/AESI form and subsequently record the event on the Adverse Event eCRF within 24 hours of becoming aware of the event. Investigators should record all AESI details available, including Investigator causality assessment, on the SAE/AESI form and subsequently record the event on the Adverse Event eCRF within 24 hours of becoming aware of the event.

Completed SAE/AESI Report Forms should be submitted to Premier PV as an email attachment to the email address below.

| Premier PV | |
|---|---|
| Investigative sites in North America | PVDS-NA@premier-research.com |
| Investigative sites in Rest of World | PVDS-ROW@premier-research.com |

Should the site have questions or concerns regarding SAE/AESI report submission, the site may elect to call the site Clinical Research Associate (CRA) or contact the medical monitor. The site will be asked to provide the following information: protocol number, site number, participant identifiers, event term, study drug information, and relationship of the event to study drug.

In a study-related health emergency, for discussion of urgent protocol medical-related questions when the assigned medical monitor for a study cannot be reached, an on-call physician can be reached 24 hours per day, 7 days per week (24/7) via a Premier Research Call-Center:

**Investigative Sites in North America:** +1 510 722 8910
**Investigative Sites in South America:** +54 911 5479 8678
**Investigative Sites in Europe and Middle East Region:** +44 118 936 4096
**Investigative Sites in Australia, New Zealand:** +61 3921 39875

For the purpose of this study, Adverse Events of Special Interest are liver events as described in Section 7.3.7.2.

The sponsor (or Premier Research) will determine whether the SAE must be reported to regulatory authorities in compliance with local and regional regulations and law (i.e., within 7 or 15 days). If so, the sponsor (or Premier Research) will report the event to the appropriate regulatory authorities and investigators. The investigator will report SAEs to the IRB/IEC per their IRB/IEC policy.

#### 7.3.8.4 Assessment of Severity

AEs should be graded as mild, moderate, or severe, using the following definitions:

- Mild: Awareness of signs or symptoms, but easily tolerated and of a minor irritant type, causing no loss of time from normal activities. Symptoms do not require therapy or a medical evaluation; signs and symptoms are transient.
- Moderate: Events introduce a low level of inconvenience or concern to the participant and may interfere with daily activities but are usually improved by simple therapeutic measures; moderate experiences may cause some interference with functioning.
- Severe: Events interrupt the participant's normal daily activities and generally require systemic drug therapy or other treatment; they are usually incapacitating.

Changes in the severity of an AE should be documented to allow an assessment of the duration of the event at each level of intensity to be performed. AEs characterized as intermittent do not require documentation of onset and duration of each episode.

### 7.3.8.5 Assessment of Causality

The Investigator's assessment of causality must be provided for all AEs. The causality is the determination of whether there exists a reasonable possibility that the study drug itself caused or contributed to an AE.

If the final determination of causality is unknown and the Investigator does not know whether the study drug caused the event, then the event will be handled as "possibly related to study drug" for reporting purposes. If the Investigator's causality is "unknown, but not related to study drug", this should be clearly documented on study records.

The relationship of an AE to the administration of the study drug will be assessed and recorded on the eCRF. Terms used to describe the degree of causality between a study drug/investigational product and an AE are: definitely, probably, possibly, unlikely, or not related. The best estimate at the time of reporting of the causal relationship between the experimental intervention and an AE and the degree of certainty about causality will be graded using the criteria specified in Table 9.

**Table 9. Guideline for Assessment of Adverse Event Causality**

| Relationship to Study Drug | Description |
|---|---|
| Not Related | The AE is clearly due to extraneous causes (e.g., underlying disease, environment) or exposure to the investigational product has not occurred. Such events MUST have an alternative, definitive etiology documented in the participant's medical record. |
| Unlikely Related | A potential relationship between study drug and the AE could exist (i.e., the possibility cannot be excluded), but the AE is most likely explained by causes other than the study drug (e.g., could readily have been produced by the participant's clinical state or could have been due to environmental or other interventions). |
| Possibly Related | The AE and administration of study drug are reasonably related in time and/or follow a known pattern of response, and the AE can be explained equally well by causes other than study drug (e.g., could readily have been produced by the participant's clinical state or could have been due to environmental or other interventions). |
| Probably Related | The AE and administration of study drug are reasonably related in time and/or follow a known pattern of response, and the AE is more likely explained by study drug than other causes. |
| Definitely Related | The AE and administration of study drug are related in time, and a direct association can be demonstrated (e.g., disappears or decreases with reduction in dose or cessation of drug/investigational product and recurs with re-exposure). |

### 7.3.8.6 Procedure for Breaking the Blind

The investigator is encouraged to contact the medical monitor or sponsor to discuss their rationale for unblinding. However, to prevent delays to the investigator or medical personnel responding to a potentially emergent situation, unblinding of study drug will not be dependent upon the investigator receiving approval from the medical monitor or sponsor. If the blind is broken, the medical monitor will be notified immediately or as soon as reasonably possible (but within 24 hours of unblinding) to provide an explanation.

The treatment assignment for the participant can be determined by designated site personnel logging into the IRT system via role-based and password-protected access. Documentation of breaking the blind should be recorded in the participant's medical record with the date and time the blind was broken, and the names of the personnel involved. Once the blind is broken for a given participant, that participant may not reinitiate treatment with study drug.

### 7.3.8.7 Follow-Up of Participants Reporting Adverse Events

All AEs must be reported in detail on the appropriate page of the eCRF and followed to satisfactory resolution, until the Investigator deems the event to be chronic or not CS, or until the participant is considered to be stable.

### 7.3.8.8 Overdose Management

An overdose is any dose of study drug given to a participant or taken by a participant that exceeds the dose described in the protocol. Participants must be instructed to immediately report any overdoses to the Investigator. The Investigator must then promptly report the overdose, irrespective of whether it was associated with an AE. Additionally, ingestion of study drug by anyone other than the participant should immediately be reported to the sponsor. Overdoses without signs or symptoms do not need to be recorded as AEs; in case of any AEs associated with the overdose, these should be reported on relevant AE/SAE sections in the eCRF. Overdoses associated with an SAE must additionally be reported to Premier PV within 24 hours of awareness via the SAE Reporting Form. In the event of suspected overdose, the appropriate supportive clinical care including fluid replacement should be instituted at the discretion of the Investigator or as dictated by the participant's clinical status.

### 7.3.8.9 AE/SAEs Experienced by Nonparticipants Exposed to Study Treatment

Nonparticipants exposed to study treatment are persons who are not enrolled in the study but have been exposed to study treatment, including instances of diversion of study treatment. All such AE/SAEs occurring in nonparticipants from such exposure will be reported to Premier PV personnel on the SAE Reporting Form regardless of whether the event was serious or not. Instructions for completing the form for events experienced by nonparticipants will be provided. SAEs occurring in nonparticipants exposed to study treatment will be processed with the same SAE reporting timelines as described in Section 7.3.8.3. Additionally, the drug accountability source documentation at the site should reflect this occurrence.

### 7.3.8.10 Pregnancy

Pregnancy is not regarded as an AE unless there is a medical/surgical complication. Any pregnancy of a participant or the female partner of a male participant that occurs during study participation must be reported using a Pregnancy Data Collection Form to Premier PV through the SAE reporting pathway. To ensure participant safety, each pregnancy must be reported through this mechanism to Premier PV within 24 hours of learning of its occurrence.

The pregnancy must be followed up to determine outcome (including spontaneous miscarriage, elective termination, normal birth, or congenital abnormality) and status of mother and child, even if the participant was discontinued from the study. Pregnancy complications and terminations of pregnancy will be reported as an SAE if SAE criteria are fulfilled. Spontaneous miscarriages must be reported as an SAE.

Any SAE occurring in association with a pregnancy, brought to the Investigator's attention after the participant has completed the study, and considered by the Investigator as possibly related to the study treatment, must be promptly reported to Premier PV.

### 7.3.9 Clinical Laboratory and Other Safety Assessments

Any abnormal clinical laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECGs, radiological scans, vital sign measurements), including those that worsen from baseline, felt to be CS in the medical and scientific judgment of the Investigator are to be recorded as AEs or SAEs.

However, any safety assessments that are associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the participant's condition, are not to be reported as AEs or SAEs.

### 7.3.10 Independent Data Monitoring Committee

In order to ensure the safety of participants, the sponsor will use an IDMC. An IDMC charter will be 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 report any findings or make recommendations to the sponsor without revealing participant treatment assignment. Adjudication results as determined by the HERC will be reported to the IDMC on an agreed frequency (see Section 7.3.7.2). All IDMC recommendations will be documented in writing, and where required, submitted to the IRB/Ethics Committees (ECs) and respective regulatory/CAs for their review or information.

## 7.4 Population Pharmacokinetic Assessments

Blood samples will be obtained from all participants at designated timepoints as described in the Schedule of Procedures – Part 1 and Schedule of Procedures – Part 2. Sparse sampling will be used to support PopPK analysis of lixivaptan using a validated assay.

### 7.4.1 Pharmacokinetic Blood Sampling

Sparse samples will be taken for determination of plasma lixivaptan concentrations. A single plasma sample will be collected at the timepoints specified in the Schedule of Procedures – Part 1 and Schedule of Procedures – Part 2, further described below. Visit scheduling on PK sampling days should take into account the PK measurement time requirements relative to the last dose of study drug taken. **Note**: At Visit 3, dosing should occur in the clinic to allow for PK sampling following the first dose of single-blind lixivaptan. Visits 12 and 32 should ideally be scheduled in the morning to accommodate pre-dose sampling without a lapse in dosing. At all other visits, the morning dose should not be withheld. **Note**: At Visit 9 and Visit 38 PK samples can be obtained at any time post-dose. Participants who have taken their morning dose at home should not receive a second dose in the clinic for the purposes of PK sampling.

**Part 1:**

- Screening – Visit 2 (any time)
- After first dose of lixivaptan – Visit 3 (0.5 to 1.5 hours post-dose)

- After dose titration – Visit 9 (any time post-dose)
- During the Double-blind, Randomized Treatment Period – Visit 12 (pre-dose); Visit 15 (1 to 6 hours post-dose); Visit 18 (6 to 10 hours post-dose)

**Part 2:**

- After lixivaptan dose re-titration – Visit 29 (1 to 6 hrs post dose)
- During the Maintenance Treatment Period – Visit 32 (pre-dose); Visit 35 (6 to 10 hours post-dose); Visit 38 (any time post-dose)

The date and time of collection of the PK sample and the date and time of administration of the last preceding dose of study drug must be recorded in the eCRF for all samples.

All plasma samples will be shipped frozen to the central clinical laboratory for storage, until analyzed. Detailed handling and shipping instructions are in the laboratory manual.

Blood samples for the PopPK analysis of lixivaptan will be collected and handled according to the specifications in the laboratory manual.

# 8 STATISTICAL PROCEDURES

The statistical and analytical plan for this clinical trial is summarized below. Further details are provided separately in the Statistical Analysis Plan (SAP).

## 8.1 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. In order to compensate for possible dropouts, 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.

A blinded sample size re-estimation (BSSR) to estimate the variance corresponding to the primary efficacy estimand is planned after at least 20% of the total randomized participants have completed Part 1 of the study or before screening for the study has ended (Section 8.2).

**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.

## 8.2 Blinded Sample Size Re-estimation

After at least 20% of the randomized participants (i.e., 270 participants) complete Part 1 of the study (Visit 25) or before screening for the study has ended, a BSSR will be performed by a blinded statistician. Based on this interim data set of blinded eGFR values, the variance corresponding to the primary efficacy estimand will be estimated and the total sample size re-estimated by a blinded statistician. If the BSSR estimates that the standard deviation of the primary efficacy estimand is less than or equal to 6.20 mL per minute per 1.73 $m^2$, then the sample size will remain at 1350 randomized participants. If the BSSR estimates that the standard deviation is greater than 6.20 mL per minute per 1.73 $m^2$, then the total sample size will be re-estimated based on this new estimate of the standard deviation by the sponsor or designee. The BSSR will be based on the original, assumed between-treatment group difference, i.e., there will be no change to the estimate of the between-treatment group difference based on the BSSR. Since a BSSR does not inflate the type 1 error of the primary efficacy analysis, the significance level for the final efficacy analysis will remain at 0.01.

## 8.3 Populations for Analysis

**Part 1:**

The following populations will be used for analyzing the data from Part 1:

Treated Safety Set: The Treated Safety Set consists of all participants who received at least 1 dose of study drug in Part 1.

Randomized Safety Set: The Randomized Safety Set consists of all participants in the Treated Safety Set who are randomized and receive at least 1 dose of randomized study drug.

Primary Efficacy Analysis Set (PEAS): The PEAS consists of all participants in the Randomized Safety Set who have both baseline and at least 1 valid assessment of eGFR in the Follow-up Period.

Secondary Efficacy Analysis Set (SEAS): The SEAS consists of all participants in the Randomized Safety Set who have both baseline and at least 1 on-treatment assessment of eGFR.

**Part 2:**

The following populations will be used for analyzing the data from Part 2:

Long-term Safety Set: The Long-term Safety Set consists of all participants in the Randomized Safety Set of Part 1 who receive at least 1 dose of lixivaptan during Part 2.

Long-term Full Analysis Set: The Long-term Full Analysis Set consists of all participants in the Long-term Safety Set who have at least 1 on-treatment measurement of eGFR in Part 2.

Long-term Efficacy Analysis Set: The Long-term Efficacy Analysis Set consists of all participants in the Long-term Full Analysis Set who were randomized to lixivaptan in Part 1.

## 8.4 Primary Efficacy Analysis

### 8.4.1 Primary Endpoint Analysis

The primary endpoint analysis is based on the eGFR calculated from the CKD-EPIcr_R equation. Baseline eGFR is defined as the mean of the 3 eGFR determinations obtained during the Screening and Placebo Run-in Periods. The primary efficacy analysis will utilize the change from this baseline eGFR to the post-treatment follow-up eGFR, annualized by each participant's treatment duration. The post-treatment follow-up eGFR is defined as the mean of 3 eGFR assessments obtained during the Follow-up Period I or the equivalent off-therapy eGFR determinations if a participant discontinues study drug earlier.

The estimand corresponding to the primary objective is the between-treatment group difference in the change from baseline in eGFR if all the participants in the PEAS had tolerated and adhered to their treatment for 52 weeks. For randomized participants prematurely discontinuing from double-blind treatment, the primary analysis 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 (days) from the median of the baseline eGFR assessments to the median of the 3 eGFR assessments obtained during the post-treatment, follow-up period immediately following study drug discontinuation.

The primary efficacy endpoint, the annualized change from baseline to post-treatment follow-up in mean eGFR, will be analyzed by means of an analysis of covariance model 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. This analysis assumes that data are missing at random. The stratification by CKD stage (Section 8.4.1) will be based on the baseline eGFR creatinine equation in effect at the time of randomization. However, all efficacy analyses of change in eGFR will be based on the CKD-EPIcr_R equation.

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

### 8.4.2 Sensitivity Analysis of the Primary Efficacy Analysis

In particular, in order to address the impact of missing data on the results from the primary efficacy analysis, a number of sensitivity analyses will be performed. The detailed sensitivity analyses for the primary efficacy analysis will be described in the statistical analysis plan (SAP).

Amongst these sensitivity analyses, the main sensitivity analysis of the primary efficacy analysis will use the mean of eGFR values obtained pretreatment and the mean of eGFR values obtained off treatment after the end of the 52-week treatment period, regardless of adherence to randomized treatment and/or withdrawal for all randomized participants, i.e., Intent-To-Treat participants. Missing values at Week 52 will be imputed using multiple imputations (assuming missing at random). Tipping point analyses will be included using a pattern-mixture model to impute missing data at Week 52 (assuming missing not at random) by systematically varying assumptions about the missing outcomes on the two treatment arms – these tipping point analyses will be bi-dimensional, i.e., allow the assumptions about the missing outcomes in the two arms to vary independently, and will include scenarios where the dropouts on the lixivaptan arm have worst outcome compared to placebo dropouts.

## 8.5 Secondary Efficacy Analyses.

If the primary efficacy analysis is statistically significant at the 1% level of significance, then the secondary efficacy endpoints of (1) annualized rate of change (slope) in eGFR based on all on-treatment eGFR determinations during the Double-blind, Randomized Treatment Period calculated from the CKD-EPIcr_R equation for serum creatinine; and (2) annualized change in TKV as measured by MRI will be analyzed. Each will be tested at the 1% level of significance in sequential order using a closed testing procedure on the SEAS. The details of the testing procedure will be provided in the SAP for the study.

### 8.5.1 Annualized Rate of Change (Slope) in eGFR on Treatment

In order to assess the linear effect (slope) of lixivaptan based on the on-treatment eGFR data in all randomized participants, an analysis utilizing a mixed-effects model for repeated measures (MMRM) will be performed on the observed changes in eGFR from baseline (mean of the 3 eGFR determinations obtained during the Screening and Placebo Run-in Periods) to all Double-Blind Treatment Period visits up to Week 52. The MMRM will include treatment group, visit, treatment group-by-visit, and randomization stratification

factors as fixed effects baseline as a covariate, and the baseline-by-visit interaction. An unstructured variance-covariance matrix is assumed for the repeated measures. A linear contrast of the treatment slope differences for these 12 monthly visits will be used for this analysis.

### 8.5.2 Annualized Change in TKV

The 2nd secondary efficacy endpoint is the change in height-adjusted TKV (normalized as a percentage) from baseline for lixivaptan relative to placebo. In order to handle the variability in the time of post-baseline MRI acquisition, time to post-baseline MRI from randomization will be treated as a continuous variable, expressed as years from date of randomization to date of MRI visit (date of MRI visit – date of randomization + 1, Baseline MRI will have a time of 0). In addition, in order to reduce heterogeneity in variance and achieve linearity over time, $\log_{10}$ transformation will be applied to the total kidney volume data.

ANCOVA will be used to analyze the log transformed TKV data. Further details will be provided in the statistical analysis plan.

A significance level of 0.01 (two-sided) will be used to declare statistical significance at the final analysis. In addition, estimate of the contrast and its 99% CI will be obtained. Anti-log of these statistics will provide an estimate of the ratio of the geometric means of annual percent changes (expressed as a ratio of the predicted one-year TKV divided by the predicted baseline TKV) from baseline for the 2 treatment groups and its 99% CI.

## 8.6 Key Comparison Analysis

### 8.6.1 Key Comparison Endpoint Analysis

Baseline eGFR in Part 2 is defined as the mean of the 3 eGFR assessments obtained during Follow-up Period I.

Descriptive statistics will be presented for the annualized changes from this baseline (Follow-up Period I in Part 1) to the mean of the 3 eGFR measurements during Follow-up Period II in Part 2 for participants in the Long-term Efficacy Analysis Set. The mean annualized change from baseline for Part 2 will be compared to corresponding mean annualized change in eGFR for Part 1 for the same analysis set.

In addition, 2 supportive analyses will be performed for Part 2:

To assess the transition from treatment with placebo in Part 1 to treatment with lixivaptan in Part 2, the same quantity will be calculated for participants in the Long-term Full Analysis Set who were randomized to placebo in Part 1 to be compared to the corresponding quantity in Part 1 for participants randomized to placebo.

In addition to assess the effect of delaying treatment, the same quantity will be calculated for participants in the Long-term Full Analysis Set who were randomized to placebo in Part 1 to be compared to the corresponding quantity in Part 1 for participants randomized to lixivaptan.

### 8.6.2 Sensitivity Analysis of the Key Comparison Analysis

In order to assess the effect of missing data on the key comparison analysis in Part 2, sensitivity analyses for the descriptive statistics will be performed for all available annualized changes from baseline (the mean of the 3 eGFR assessments obtained during Follow-up Period I in Part 1) to the mean of the 3 off-treatment eGFRs after Week 104.

## 8.7 Other Comparison Analyses

### 8.7.1 Annualized Rate of Change (Slope) in eGFR on Treatment

In order to assess the linear effect (slope) of lixivaptan based on the on-treatment eGFR data in Part 2, an analysis utilizing a MMRM will be performed on the observed changes from baseline (mean of the 3 eGFR assessments obtained during Follow-up Period I) to Weeks 52 to 104 (Visit 29 to Visit 42) of the Maintenance Treatment Period in eGFR. The MMRM will include visit, and randomization stratification factors as fixed effects, and baseline (Follow-up Period I in Part 1) as a covariate, and the baseline-by-visit interaction. An unstructured variance-covariance matrix is assumed for the repeated measures. The estimated annualized rate of change (slope) from this analysis will be compared to the corresponding results in Part 1.

### 8.7.2 Annualized Change in TKV

The height-adjusted TKV measurements at Follow-up Periods I and II will be $\log_{10}$ transformed. The mean and 99% CI of the difference of the $\log_{10}$-transformed value at Follow-up Period II from the $\log_{10}$-transformed value at Follow-up Period I will be calculated for all participants in the Long-term Efficacy Analysis Set. Anti-log of these statistics will provide an estimate of the geometric mean of annual percent changes (expressed as a ratio of the predicted one-year TKV divided by the predicted baseline TKV) from baseline of the 2 treatment groups and its 99% CI. This will be compared to the corresponding quantity from Part 1 for the same participants.

## 8.8 Population Pharmacokinetics Analyses

Plasma concentrations of lixivaptan will be used for PopPK analysis that will be described in a separate report.

## 8.9 Health Outcomes Analyses

### 8.9.1 Medical Resource Utilization

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.

### 8.9.2 ADPKD-IS, ADPKD-PDS, and ADPKD-UIS

Detailed descriptions of analyses of these questionnaires will be specified in the SAP.

## 8.10 Exploratory Analyses

### 8.10.1 Annualized Change in LV

The between-treatment group difference in LV for Part 1 and 2 will be examined using appropriate statistical methods. Details for the planned analyses will be provided in the SAP.

### 8.10.2 Urinary Osmolality

Descriptive statistics for the change from baseline in morning spot urine osmolality will be presented by treatment group for the Treated Safety Set in Part 1 and overall, for the Long-term Safety Analysis Set in Part 2 at each scheduled time point. 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.

### 8.10.3 Annualized Change in eGFR Assessed by Serum Creatinine and Cystatin-C (CKD-EPIcr-cys_R)

This analysis is based on the change from the baseline eGFR to the post-treatment follow-up eGFR calculated from the CKD-EPIcr-cys_R equation, annualized by each participant's treatment duration. Baseline eGFR is defined as the mean of the 3 eGFR determinations obtained during the Screening and Placebo Run-in Periods. The post-treatment follow-up eGFR is defined as the mean of 3 eGFR assessments obtained during the Follow-up Period I or the equivalent off-therapy eGFR determinations if a participant discontinues study drug earlier.

This annualized change from baseline to post-treatment follow-up in mean eGFR will be analyzed by means of an analysis of covariance model with fixed effects for treatment group and the randomization stratification factors and the baseline eGFR as a covariate.

## 8.11 Subgroup Analyses

Descriptive statistics will be presented for the primary and secondary efficacy analyses for age (≤50 years, >50 years), sex, 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) and baseline TKV (≤median, >median).

## 8.12 Analysis of Demographic and Baseline Characteristics

**Part 1:**

Descriptive statistics for demographic (e.g., sex, age, race, and ethnicity) and baseline (e.g., eGFR, TKV, and LV) characteristics will be presented by treatment group and for all participants for the Randomized Safety Set and PEAS.

**Part 2:**

Descriptive statistics for demographic and baseline characteristics will be presented for all

participants for the Long-term Safety and Long-term Full Analysis Sets.

## 8.13 Safety Analyses

All Safety Analyses will be performed separately for Parts 1 and 2 for the following:

- Single-Blind Placebo Run-in Period for the Treated Safety Set (Part 1)
- Lixivaptan Titration Period for the Treated Safety Set (Part 1) and Lixivaptan Re-titration Period for the Long-term Safety Set (Part 2)
- The Double-blind, Randomized Treatment and Follow-up I Periods for the Randomized Safety Set (Part 1) and the Maintenance Treatment and Follow-up II Periods for the Long-term Safety Set (Part 2)

### 8.13.1 Liver Safety Analyses

#### 8.13.1.1 Liver Safety Analysis for Key Safety Endpoint

The incidence of participants who develop serum ALT levels >3 times the ULN will be summarized by treatment group for Part 1 and for all participants for Part 2. Additional summaries of causality of such cases as determined by the HERC will be included.

#### 8.13.1.2 Additional Liver Safety Analyses

The incidence of participants in Parts 1 and 2 who develop:

- >5 x-, 10 x-, and 20 x ULN elevations for ALT
- >3 x-, 5 x-, 10 x-, and 20 x ULN elevations for AST
- >3 x-, 5 x-, 10 x-, and 20 x ULN elevations for either ALT or AST
- Any elevation of bilirubin; elevated serum total bilirubin (TBL) to >2 x ULN
- Any elevation of alkaline phosphatase >1.5 x ULN
- Elevation of aminotransferase (>3 x ULN) accompanied by elevated bilirubin (>2 x ULN) and displayed as evaluation of Drug-Induced Serious Hepatotoxicity (eDISH) plots
- Elevation of aminotransferase in temporal association with nausea, vomiting, anorexia, abdominal pain, or fatigue
- Possible liver-related deaths and liver-related treatment discontinuations.

### 8.13.2 Adverse Events

For Part 1, the number and percentage of participants and the number of events will be presented by treatment group for treatment-emergent adverse events (TEAEs), serious adverse events (SAEs), treatment-related TEAEs, and TEAEs leading to premature withdrawal according to the system organ class (SOC) and preferred term (PT) assigned to the event using MedDRA. In addition, TEAEs will also be summarized by maximum severity and relationship to study drug. Listings of deaths and serious TEAEs will be

provided.

For Part 2, the number and percentage of participants and the number of events will be presented for all participants for TEAEs, SAEs, treatment-related TEAEs, and TEAEs leading to premature withdrawal according to the SOC and PT assigned to the event using MedDRA. In addition, TEAEs will also be summarized by maximum severity and relationship to study drug. Listings of deaths and serious TEAEs will be provided.

For Part 1 and Part 2, all AE data will be listed for all participants. Both the Investigator's verbatim terms and the MedDRA preferred terms will be listed for each participant. Listings will also include whether the adverse events are SAEs, AESIs, date of AEs, relationship to study drug, severity, and action taken for the AEs. Adverse events with a relationship to study drug considered possibly, probably, or definitely related will be considered related.

### 8.13.3 Clinical Laboratory Tests

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.

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.

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.

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

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).

For Part 1 and Part 2, individual data listings of clinical laboratory results will be presented for each participant. Values outside of the clinical laboratory's reference range (i.e., those with low or high values) will be flagged in the clinical laboratory listings.

### 8.13.4 ECGs

For Part 1, 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 using descriptive statistics (n, mean, SD, median, minimum, and maximum).

A summary shift table comparing the baseline (last value observed prior to the first dose of study drug) interpretation (normal, abnormal - NCS, abnormal - CS) to the Investigator interpretation at each post-baseline time point will also be presented by treatment group.

A listing will be provided for Investigator-identified ECG abnormalities from safety ECGs. The overall evaluation of safety ECGs will be summarized by treatment group using frequency counts and percentage of participants as normal or abnormal, and the relevance of the abnormality will be summarized as CS or NCS.

For Part 2, observed values and changes from baseline (last value observed during Follow-up Period I in Part 1) at each post-baseline assessment timepoint for continuous ECG parameters including heart rate, ventricular rate, PR interval, QRS duration, QT interval (uncorrected), and QTcF interval will be summarized for all participants using descriptive statistics (n, mean, SD, median, minimum, and maximum).

A summary shift table comparing the baseline (last value observed during Follow-up Period I in Part 1) interpretation (normal, abnormal - NCS, abnormal - CS) to the Investigator interpretation at each post-baseline time point will be presented for all participants.

A listing will be provided for Investigator-identified ECG abnormalities from safety ECGs. The overall evaluation of safety ECGs will be summarized for all participants using frequency counts and percentage of participants as normal or abnormal, and the relevance of the abnormality will be summarized as CS or NCS.

### 8.13.5 Body Height, Weight and BMI

For Part 1, changes in 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 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 by each participant based on the respective analysis population.

### 8.13.6 Vital Signs

For Part 1, changes from baseline (last value observed prior to the first dose of study drug) in vital signs at each post-baseline timepoint will be summarized by treatment group using descriptive statistics (n, mean, SD, median, minimum, and maximum).

For Part 2, changes from baseline (last value observed during Follow-up Period I in Part 1) in vital signs at each post-baseline timepoint 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. All vital sign data will be listed individually by each participant based on the respective analysis population.

## 8.14 Statistical Analysis Methodology

Statistical analysis will be performed using SAS® software Version 9.4 or higher. All continuous variables will be summarized using the following descriptive statistics: number of non-missing observations (n), mean, standard deviation (SD), median, minimum, and maximum. Categorical variables will be summarized using the following descriptive statistics: frequency counts and percentages. All data will be listed in data listings.

Further details of the statistical analyses, methods, and data conventions are described in the SAP. The SAP may modify the plans outlined in the protocol; however, any major modifications of the primary analysis will also be reflected in a protocol amendment. Additional statistical analyses other than those described in this section may be performed if deemed appropriate in the plan.

## 8.15 Data Quality Assurance

Designated study site personnel will be responsible for maintaining quality assurance and quality control to ensure that the study conduct as well as data collection and documentation are performed in compliance with the study protocol, GCP requirements, and applicable regulatory requirements.

Clinical data will undergo source document verification by the CRA in accordance with the Study-Specific Monitoring Plan and the principles of Risk-based Monitoring. Clinical data will be reviewed by Data Management in accordance with the Data Management Plan prior to database lock. Programmed edit checks are also implemented to check for missing data, data inconsistencies, data ranges, etc. Corrections are made prior to database lock. Electronic CRFs can be printed directly from the database. Each eCRF will be reviewed and signed electronically by the Investigator.

### 8.15.1 Data Management

An eCRF is required and should be completed for each included participant. The completed original eCRFs are the sole property of the sponsor and should not be made available in any form to third parties, except for authorized representatives of the sponsor or appropriate regulatory authorities, without written permission from the sponsor.

As part of the responsibilities assumed in conducting the study, the Investigator agrees to

maintain adequate case histories for the participants treated as part of the research under this protocol. The investigator has ultimate responsibility for the accuracy, authenticity, and timely collection and reporting of all clinical, safety, laboratory data entered on the eCRFs and any other data collection forms. The eCRFs must be signed by the investigator to attest that the data contained on the eCRFs are true. Any corrections to entries made on the eCRFs must be dated, initialed, and explained (if necessary) and should not obscure the original entry.

In most cases, the source documents are the hospital's or the physician's participant chart. In these cases, data collected on the eCRFs must match the data in those charts.

In some cases, the eCRF, or part of the eCRF, may also serve as source documents. In these cases, a document should be available at the investigator's site as well as at the sponsor and clearly identify those data that will be recorded on the eCRF, and for which the eCRF will stand as the source document.

The Investigator agrees to maintain accurate eCRFs and source documentation as part of the case histories. These source documents may include laboratory reports, ECGs, etc.

Investigative site personnel will enter participant data into eCRFs using the EDC system. The analysis data sets will be a combination of these data and data from other sources (e.g., laboratory data) and follow Clinical Data Interchange Standards Consortium (CDISC) standard.

Clinical data management will be performed in accordance with applicable standards and data cleaning procedures of the CRO to ensure the integrity of the data, e.g., removing errors and inconsistencies in the data. Adverse events and concomitant medication terms will be coded using the MedDRA and World Health Organization Drug Dictionaries, respectively.

After database lock, each study site will receive all site-specific eCRF data for the study, including full discrepancy and audit history. Additionally, a copy of all of the study site's data from the study will be created and sent to the sponsor for storage. The CRO will maintain a duplicate copy for their records. In all cases, participant initials will not be collected or transmitted to the sponsor.

# 9 ETHICS

## 9.1 Institutional Review Board (IRB)/Ethics Committee (EC)

It is the responsibility of the investigator to have prospective approval of the study protocol, protocol amendments, ICFs, and other relevant documents, e.g., written participant instructions, recruitment advertisements, if applicable, from the IRB/EC before participation of human participants in research studies. All correspondence with the IRB/EC should be retained in the Investigator Site File. Copies of IRB/EC approvals should be forwarded to the sponsor.

All IRB/EC approvals should be signed by the IRB/EC chairperson or designee and must identify the IRB/EC name and address, the clinical protocol by title or protocol number or both, and the date approval or a favorable opinion was granted.

The Investigator is responsible for providing written summaries of the progress and status of the study at intervals not exceeding 1 year or otherwise specified by the IRB/EC. The Investigator must promptly supply the sponsor or its designee, the IRB/EC, and, where applicable, the institution, with written reports on any changes significantly affecting the conduct of the study or increasing the risk to participants.

## 9.2 Ethical Conduct of the Study

The study will be performed in accordance with the ethical principles that have their origin in the Declaration of Helsinki and all applicable regulations. In addition, the study will be conducted in accordance with the protocol, the International Conference on Harmonisation (ICH) tripartite guideline E6 (R2), Good Clinical Practice (GCP), and applicable local regulatory requirements and laws.

## 9.3 Participant Information and Consent

Written informed consents that meet the requirements of 21 Code of Federal Regulations (CFR) 50, local regulations, ICH guidelines, privacy and data protection laws applicable in the respective participating countries and the IRB/IEC or study center shall be obtained from each participant before entering the study, upon re-screening, and when performing any unusual or non-routine procedure that involves risk to the participant. Informed consent templates may be provided by the sponsor to investigative sites. If any institution-specific modifications to study-related procedures are proposed or made by the site, the consents should be reviewed by the sponsor or its designee or both before IRB/EC submission. Once reviewed, the consents will be submitted by the Investigator to his or her IRB/EC for review and approval before the start of the study. If the ICF is revised during the course of the study, all active participating participants must sign the revised form.

Before recruitment and enrollment, each prospective participant or his or her legal guardian will be given a full explanation of the study and be allowed to read the approved ICF. Once the Investigator is assured that the participant/legal guardian understands the implications of participating in the study, the participant/legal guardian will be asked to give consent to participate in the study by signing the ICF. Participants will be made aware that they may

withdraw from the study at any time for any reason.

The Investigator shall retain the signed original ICF(s) and give a copy of the signed original form to the participant or legal guardian.

# 10 INVESTIGATOR'S OBLIGATIONS AND STUDY PERSONNEL

The study will be conducted by qualified investigators under the sponsorship of Palladio Biosciences, Inc. (the sponsor).

The contact information for the sponsor's Chief Medical Officer, the CRO's medical monitor, other sponsor and CRO personnel (Premier Research is the designated CRO), and other study vendors are listed in the Investigator Site File provided to each site.

The following administrative items are meant to guide the Investigator in the conduct of the study but may be subject to change based on industry and government standard operating procedures, working practice documents, or guidelines. Changes will be reported to the IRB but will not result in protocol amendments.

## 10.1 Confidentiality

All laboratory specimens, evaluation forms, reports, and other records will be identified in a manner designed to maintain participants' confidentiality. All records will be kept in a secure storage area with limited access. Clinical information will not be released without participants' (or legal guardians') written permission, except as necessary for monitoring and auditing by the sponsor, its designees, the US Food and Drug Administration (FDA), European Medicines Agency (EMA) or other health or regulatory authority or the IRB/EC.

The Investigator and all employees and coworkers involved with this study may not disclose or use for any purpose other than performance of the study any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the study. Prior written agreement from the sponsor or its designee must be obtained for the disclosure of any said confidential information to other parties.

## 10.2 Financial Disclosure and Obligations

Investigators and sub-investigators will provide the sponsor with sufficient, accurate financial information as requested to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. In addition, Investigators and sub-investigators must provide to the sponsor a commitment to promptly update this information if any relevant changes occur during the course of the investigation and for 1 year following the completion of the study.

Neither the sponsor nor sponsor's representatives are financially responsible for further testing or treatment of any medical condition that may be detected during the screening process. In addition, in the absence of specific arrangements, neither the sponsor nor sponsor's representatives are financially responsible for further treatment of the participant's disease.

## 10.3 Investigator Documentation

Before beginning the study, the Investigator will be asked to comply with ICH E6 (R2) Section 10.4 and Title 21 of the CFR by providing the following essential documents, including but not limited to:

- IRB/EC written approval and any other local approval, as appropriate
- Original signed Investigator agreement page of the protocol
- Form FDA 1572, fully executed (where applicable), and all updates on a new fully executed Form FDA 1572
- Curriculum vitae for the Investigator and each Sub-Investigator
- Financial disclosure information to allow the sponsor to submit complete and accurate certification or disclosure statements to the appropriate regulatory authorities. In addition, the Investigators must provide to the sponsor a commitment to promptly update this information if any relevant changes occur during the course of the investigation and for 1 year after the completion of the study.
- IRB/EC-approved informed consent, samples of site advertisements for recruitment for this study, and any other written information regarding this study that is to be provided to the participant or legal guardian, and
- Laboratory certifications and normal ranges for any local laboratories used by the site.

## 10.4 Study Conduct

The Investigator agrees that the study will be conducted according to the principles of ICH E6 (R2). The Investigator will conduct all aspects of this study in accordance with all national, state, and local laws or regulations. Study information from this protocol will be posted on publicly available clinical trial registers before enrollment of participants begins.

## 10.5 Adherence to Protocol

The Investigator agrees to conduct the study as outlined in this protocol in accordance with ICH E6 (R2) and all applicable guidelines and regulations.

## 10.6 Adverse Events and Study Report Requirements

By participating in this study, the Investigator agrees to submit reports of SAEs according to the timeline and method outlined in the protocol. In addition, the Investigator agrees to submit annual reports to the study site IRB/EC as appropriate.

## 10.7 Investigator's Final Report

Upon completion of the study, the Investigator, where applicable, should inform the institution; the Investigator/institution should provide the IRB/EC with a summary of the study's outcome and the sponsor and regulatory authority with any reports required.

## 10.8 Records Retention

To enable evaluations and/or audits from regulatory authorities or the sponsor, the investigator agrees to keep records, including the identity of all participating participants

(sufficient information to link records, e.g., eCRFs and hospital records), all original signed ICFs, copies of all eCRFs, SAE forms, source documents, and detailed records of treatment disposition, and adequate documentation of relevant correspondence (e.g., letters, meeting minutes, telephone calls reports). The records should be retained by the investigator according to ICH, local regulations, or as specified in the Clinical Study Agreement, whichever is longer.

If the investigator becomes unable for any reason to continue to retain study records for the required period (e.g., retirement, relocation), the sponsor should be prospectively notified. The study records must be transferred to a designee acceptable to the sponsor, such as another investigator, another institution, or to the sponsor. The investigator must obtain the sponsor's written permission before disposing of any records, even if retention requirements have been met.

Essential documents should be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region, or at least 2 years have elapsed since the formal discontinuation of clinical development of the study drug. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by an agreement with the sponsor. It is the responsibility of the sponsor to inform the Investigator/institution as to when these documents no longer need to be retained.

## 10.9 Publications

After completion of the study, the data may be considered for reporting at a scientific meeting or for publication in a scientific journal. In these cases, the study steering committee will be responsible for these activities and will work with the Investigators to determine how the manuscript is written and edited, the number and order of authors, the publication to which it will be submitted, and other related issues. The steering committee has final approval authority over all such issues.

Data are the property of the sponsor, but data and publication thereof will not be unduly withheld.

# 11 STUDY MANAGEMENT

## 11.1 Monitoring

### 11.1.1 Monitoring of the Study

The Clinical Research Associate (CRA) and/or designee, as a representative of the sponsor, has the obligation to follow the study closely. In doing so, the CRA will visit the Investigator and study site at periodic intervals, or conduct central or remote monitoring, in addition to maintaining necessary telephone, email and letter contact. The CRA will maintain current personal knowledge of the study through observation, review of study records and source documentation, and discussion of the conduct of the study with the Investigator and personnel.

All aspects of the study will be carefully monitored, by the sponsor or its designee, for compliance with applicable government regulation with respect to current GCP and current standard operating procedures.

### 11.1.2 Inspection of Records and Quality Assurance

Investigators and institutions involved in the study will permit study-related monitoring, audits, IRB/EC review, and regulatory inspections by providing direct access to all study records. In the event of an audit or inspection, the Investigator agrees to allow the sponsor, representatives of the sponsor, or a regulatory agency (e.g., FDA, EMA or other regulatory agency) access to all study records.

It is important that the investigator(s) and their relevant personnel are available during the monitoring visits and possible audits or inspections and that sufficient time is devoted to the process.

The Investigator should promptly notify the sponsor and/or its designee of any inspections scheduled by any regulatory authorities and promptly forward copies of any related reports received to the sponsor.

## 11.2 Management of Protocol Amendments and Deviations

### 11.2.1 Modification of the Protocol

Any changes in this research activity, except those necessary to remove an apparent, immediate hazard to the participant, must be reviewed and approved by the sponsor or its designee. Amendments to the protocol must be submitted in writing to the Investigator's IRB/EC for approval before participants can be enrolled into an amended protocol, and before the changes can be implemented.

## 11.3 Protocol Deviations

The Investigator or designee must document and explain in the participant's source documentation any deviation from the approved protocol. The Investigator may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard to study participants without prior IRB/EC approval. As soon as possible after such an occurrence,

the implemented deviation or change, the reasons for it, and any proposed protocol amendments should be submitted to the IRB/EC for review and approval, to the sponsor for agreement, and to the regulatory authorities, if required.

A protocol deviation is defined as an unintended or unanticipated departure from the procedures or processes approved by the sponsor and the IRB and agreed to by the Investigator. A major deviation occurs when there is not adherence to the protocol by the participant or Investigator that results in a significant, additional risk to the participant. Major deviations can include not adhering to inclusion or exclusion criteria or not adhering to FDA regulations or ICH GCP guidelines, and will lead to the participant being withdrawn from the study (Section 4.2.5).

Protocol deviations will be documented by the Clinical Monitor throughout the course of monitoring visits. Investigators will be notified in writing by the monitor of deviations. The IRB/EC should be notified of all protocol deviations in a timely manner.

## 11.4 Management of Study Termination

Although the sponsor has every intention of completing the study, the sponsor reserves the right to discontinue the study at any time for clinical or administrative reasons.

The end of the study is defined as the date on which the last participant completes his/her last visit.

Whether the study is completed or prematurely terminated, the sponsor will ensure that the clinical study report is prepared and provided to the regulatory agency as required by the applicable regulatory requirement(s). The sponsor will also ensure that the clinical study report in marketing applications meet the standards of the ICH harmonized tripartite guideline E3: Structure and content of clinical study reports.

Where required by applicable regulatory requirements, an Investigator signatory will be identified for the approval of the clinical study report. The Investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results.

Upon completion of the clinical study report, the sponsor will provide the Investigator with the full summary of the study results. The Investigator is encouraged to share the summary results with the study participants, as appropriate. The study results will be posted on publicly available clinical trial registers.

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# 13APPENDICES

## 13.1 Appendix 1: Chronic Kidney Disease Classification Criteria

As of protocol Version 2, this study will replace measurement of eGFR as calculated by the 2009 CKD-EPI creatinine equation[1] with the 2021 CKD-EPI equation refit without race variable (CKD-EPIcr_R) (Delgado et al, 2021), which is recommended by the National Kidney Foundation-American Society of Nephrology Task Force.

The CKD-EPIcr_R equation is:

$$eGFR = 142 \times \min\left(\frac{Scr}{\kappa}, 1\right)^{\alpha} \times \max\left(\frac{Scr}{\kappa}, 1\right)^{-1.200} \times 0.9938^{Age} \times 1.012 \text{ (if female)}$$

Abbreviations/Units

eGFR (estimated glomerular filtration rate) = mL/min/1.73 $m^2$

Scr (standardized serum creatinine) = mg/dL

$\kappa$ = 0.7 (females) or 0.9 (males)

$\alpha$ = -0.241 (females) or -0.302 (males)

min = indicates the minimum of Scr / or 1

max = indicates the maximum of Scr / or 1

age = years

---

[1]

$$eGFR = 141 \times \min\left(\frac{Scr}{\kappa}, 1\right)^{\alpha} \times \max\left(\frac{Scr}{\kappa}, 1\right)^{-1.209} \times 0.993^{Age} \times 1.018 \text{ (if female)} \times 1.159 \text{ (if black)},$$

Abbreviations/Units

eGFR (estimated glomerular filtration rate) = mL/min/1.73 $m^2$

Scr (standardized serum creatinine) = mg/dL

$\kappa$ = 0.7 (females) or 0.9 (males)

$\alpha$ = -0.329 (females) or -0.411 (males)

min = indicates the minimum of Scr/$\kappa$ or 1

max = indicates the maximum of Scr/$\kappa$ or 1

age = years

**Serum Creatinine and Cystatin C (CKD-EPIcr-cys_R):**

In this study eGFR will also be assessed from a novel serum creatinine and serum cystatin C equation refit without the race variable (CKD-EPIcr-cys_R) (Delgado et al, 2021). To reduce potential decision errors, eGFR calculated from this equation will not be shared with sites as all eligibility and treatment decisions will be based on eGFR calculated from the CKD-EPIcr_R equation.

The CKD-EPIcr-cys_R equation is:

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

Abbreviations/Units

eGFR (estimated glomerular filtration rate) = mL/min/1.73 $m^2$

Scr (serum creatinine) = mg/dL

Scys (serum cystatin C) = mg/L

= 0.7 (females) or 0.9 (males)

α = -0.219 (females) or -0.144 (males)

min = indicates the minimum of Scr/k or 1 OR Scys/0.8 or 1

max = indicates the maximum of Scr/k or 1 OR Scys/0.8 or 1

age = years

## 13.2 Appendix 2: Liver Dysfunction Checklist

The following items will be included in a checklist to be completed by the site (Part 1- Symptoms and Part 2- Signs) when directed by the protocol.

Part 1 - Symptoms
Did the participant develop the following symptoms since the last visit?

| Symptom |
|---|
| Fatigue |
| Weakness |
| Malaise |
| Loss of appetite |
| Nausea |
| Vomiting |
| Pain in the right upper part of the abdomen |
| Urine darker than usual |
| Itching |
| Yellowing of skin |
| Yellowing of eyes |
| Fever |
| Rash |

Part 2 - Signs
Did the participant develop the following signs since the last visit?

| Sign |
|---|
| Jaundice |
| Ascites |
| Rash |
| Fever |
| Right upper quadrant tenderness or liver enlargement |
| Encephalopathy |

The Investigator is responsible for adverse event reporting of any symptoms, signs, or associated diagnosis deemed clinically significant based on information entered in this checklist. The Investigator will determine whether a constellation of symptoms and signs in the context of all available information including laboratory data indicate the potential presence of liver dysfunction. Otherwise, individual symptoms and signs need to be

recorded as other AEs or as an alternative diagnosis. *For example,* fatigue alone may be a non-specific AE with no clear cause. *As another example,* fatigue, weakness, and vomiting occurring together may indicate a gastroenteritis. Liver dysfunction should only be considered when the totality of the evidence makes that a plausible diagnosis.

If liver dysfunction is suspected, the Investigator will communicate with the medical monitor and additional testing detailed in Section 7.3.7.1 may be recommended. Those tests are listed in Appendix 3 (Section 13.3). Additionally, the following supplemental information to complete the liver dysfunction eCRF will be collected by the site:

- Was there evidence of a concomitant viral illness?
- Did the participant's alcohol intake increase since the previous visit? If so,
    - on what date did it start?;
    - on what date did it end?;
    - what type of alcohol was ingested?; and
    - what was the total daily alcohol intake during this time?
- Did the participant start any new medications or increase any concomitant medications including herbal medications, prescribed medications, or over-the-counter medications?
- Was any AE related to liver dysfunction serious?
- Did any AE related to liver dysfunction result in discontinuation of study drug, either temporarily or permanently?
- What is the participant's nationality?

## 13.3 Appendix 3: Additional Potential Hepatic Testing if Liver Chemistry Abnormalities Occur

The medical monitor may request the following additional testing if liver chemistry test abnormalities occur:

- Hematology/coagulation:
  - CBC with Diff
  - International normalized ratio (INR)

- Viral hepatitis serology:
  - Hepatitis A immunoglobulin M (IgM) antibody
  - Hepatitis B surface antigen
  - Hepatitis B core antibody
  - Hepatitis C RNA
  - Hepatitis E IgM antibody (hepatitis E RNA, if available)
  - Epstein-Barr viral capsid antigen IgM antibody
  - Cytomegalovirus IgM antibody

- Autoimmune serology:
  - Total serum immunoglobulin G
  - Anti-nuclear antibody
  - Anti-smooth muscle antibody

- Ultrasound of the liver and gallbladder

## 13.4 Appendix 4: DILI Network Causality Criteria, modified

Causality assessment scoring in the Drug-Induced Liver Injury Network (DILIN) prospective study, modified (Fontana et al, 2009).

| Causality score | Likelihood (%) | Description |
|---|---|---|
| 1 = definite | >95 | Liver injury is typical for drug or herbal product ('signature' or pattern of injury, timing of onset, recovery).<br>The evidence for causality is "beyond a reasonable doubt". |
| 2 = highly likely | 75-95 | The evidence for causality is 'clear and convincing' but not definite. |
| 3 = probable | 50-74 | The causality is supported by 'the preponderance of evidence' as implicating the drug but the evidence cannot be considered definite or highly likely. |
| 4 = possible | 25-49 | The causality is not supported by 'the preponderance of evidence'; however, one cannot definitely exclude the possibility. |
| 5 = unlikely | 6-24 | The evidence for causality is 'highly unlikely' based upon the available information. |
| 6 = unrelated | 0-5 | An alternate cause was clearly established. |
| 7 = insufficient data | Not applicable | Key elements of the drug exposure history, initial presentation, alternative diagnoses and/or diagnostic evaluation prevent one from determining a causality score. |

## 13.5 Appendix 5: Minimization of Missing Data: Study and Study Treatment Modification

In all cases of impending study drug discontinuation or withdrawal of consent, Investigators should meet and discuss with the participant the options for continuing in the study, preferably on therapy. The Investigator should ensure understanding and documentation of the reasons for the participants' desire to withdraw consent.

If a participant wishes to withdraw from the study (Part 1 or Part 2) at any time following randomization:

1) The Investigator should seek to understand the participant's motivation and wherever possible make accommodations to prevent treatment discontinuation or complete withdrawal of consent and maintain compliance with the protocol assessments (e.g., provide necessary travel reimbursement, in-home visits, alternate visit scheduling including during weekends). If the participant's wish is to discontinue study drug only, proceed to Step 2.

2) Ask the participant, "Are you willing to continue if your dose of study drug is lowered?" If the answer is "Yes", titrate the dose down one level. Repeat this step if the participant continues to request withdrawal of consent. If the answer is "No" or if, once the Level 1 dose is reached, the participant continues to request withdrawal of consent, go to Step 3.

3) Ask the participant, "If we temporarily interrupt your study drug would you be willing to later resume medication and continue with regular visits?" If the answer is "Yes", interrupt study drug but continue to follow all other trial procedures for the participant until the participant restarts treatment. If the participant does not resume treatment with study drug after 7 days, perform the treatment interruption assessments and follow-up specified in Section 4.2.3, and then resume study drug and continue with all other monthly assessments to the end of the trial. If the answer is "No" and the participant still wishes to withdraw from the trial, go to Step 4.

4) Ask the participant, "If we discontinue your study drug permanently, would you continue with all visits and sample collections?" The Investigator should discuss the scientific value of continued data collection even in the absence of study treatment. If the answer is "Yes", the participant is willing to continue with all visits and sample collections, discontinue study drug, perform an ET visit (Visit 22/ET if in Part 1 or Visit 42/ET if in Part 2), complete the Follow-up Period assessments, and continue with all other monthly assessments to the end of the trial to reduce missing data. If the answer is "No" go to Step 5.

5) If the above steps including interruption or permanent discontinuation of study drug do not resolve the participant's issues, further accommodations such as less frequent visits or blood draws may be used (as long as adequate safety monitoring can be ensured for participants continuing study drug). Less frequent visits or procedures must be offered only if they are required to maintain access to the participant's medical information and/or to encourage the participant to continue in the trial.

## 13.6 Appendix 6: Procedures to Prevent Loss to Follow-up

The Investigator must make every effort to contact participants who fail to return for scheduled visits to prevent participants from being "lost to follow-up". The following procedures should be followed at a minimum, with additional measures taken if unsuccessful.

1) Contact all numbers for the participant and their listed contacts (to be collected in source document at the participant's entry into the trial). This includes making calls after normal business hours or on holidays and weekends. Notify the CRA and/or medical monitor promptly after participant fails to return for a scheduled visit.

2) Contact the participant's primary care physician, referring specialist, pharmacist or other health-care professional (using the contacts provided by the participant at entry to the trial).

3) Send a text, e-mail and postal mail with certified (return-receipt requested) letters to all the participant's addresses and all contacts (as provided by the participant at entry to the trial).

4) In-home visit at last address given.

5) Review available medical records/notes for details of hospitalizations, clinic visits or other procedures which may indicate the status of participants (as allowed through release of medical record forms to be completed by participant at trial entry).

6) Conduct an internet search for additional contact information (e.g., reverse directory for phone numbers or new address information; social media outlets (e.g., Facebook, LinkedIn or other social media for status updates).

7) Check local, regional and national public records to locate the participant or search for mortality status as allowed by law.

Once all these actions have been exhausted (and documented), then the CRA or medical monitor should be contacted for additional guidance.

**Overview of Changes in the Conduct of the Study**

Due to the staggered nature of regulatory submissions and approvals around the world, two versions of the protocol were active and being used at the time of early termination. The design in Version 3.0 was being used in two countries (Bulgaria and Poland) and Version 1.0 (original protocol) was being used in all other countries. After Version 2.0 was completed and released, but before implementation anywhere in the world, it was realized that additional clarifications and improvements to the text would result in an enhanced protocol. Thus, Version 3.0 was created from Version 2.0, and, in particular, details about the primary endpoint analyses, more typically provided only in the Statistical Analysis Plan, were incorporated into the main body of the study protocol. The major changes from V1.0 to V3.0 are provided below. Details of all the changes are provided in the subsequent 2 documents.

1) to increase study power from 85% to 90% resulting in a sample size increase from 1200 randomized to 1350 randomized participants, to meet the mainstream industry standard for powering phase 3 studies, and a direct result of the merging of Palladio into the newly formed Centessa Pharmaceuticals. This was decided before any participant had enrolled into the ACTION study.
2) to update the equation used in the calculation of the primary endpoint from the 2009 CKD-EPI equation to the 2021 CKD-EPIcr_R equation. This updated equation removes the race variable from the calculation of eGFR, per the recent update to this standard.
3) to add exploratory endpoints for eGFR calculated from a combination of serum creatinine and serum cystatin C based on the 2021 CKD-EPIcr-cys_R equation in recognition of the potential for this to be the standard equation in the future.
4) to increase frequency of serum sodium assessments to enhance safety monitoring as recommended by members of the Steering Committee based on the experience in the REPRISE study of tolvaptan.
5) to remove suppression of post-randomization eGFR results to facilitate the Blinded Sample Size Re-estimation process and enhance safety monitoring.
6) to add a benefit/risk paragraph.
7) to incorporate additional guidance about the importance of study participants staying on study drug or continuing in the study off study drug.

# Study PA-ADPKD-301: The ACTION Study

# Clinical Study Protocol Version 3.0, Dated 14 February 2022

# Summary of Changes

This Summary of Changes document reflects revisions incorporated into Protocol Amendment 02, Version 3.0 of the protocol. It should be used in conjunction with the tracked-changes version of the clinical study protocol amendment. Page numbers reported herein refer to the **tracked-changes** version. Revisions in Version 3 are shown in Track Changes. Minor editorial and document formatting revisions have not been summarized.

The amendment is a substantial amendment because these changes affect 1) the safety or rights of the participant and 2) the reliability and robustness of the data generated in the clinical trial.

The main reasons for this amendment are clarification and/or codification of text to clarify activities and/or to reduce variance; and providing further insight into the statistical analyses that will be conducted.

## Administrative Changes

There are no Administrative Changes to this version of the protocol.

## Text Clarifications

This section lists corrections of typos, text changes for consistency or other clarifications. These changes do not affect clinical decision-making or consent.

| Page | Section | Version 2.0, Dated 01 Feb 2022 | Version 3.0; Dated 14 Feb 2022 | Explanation |
|---|---|---|---|---|
| Multiple | Global | Maintenance Period | ~~Maintenance Period~~Maintenance Treatment Period | For consistency of 'Maintenance Treatment Period' throughout the |

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| | | | | protocol |
| Multiple | Global | Lixivaptan Dose Titration Period | Lixivaptan ~~Dose~~ Titration Period | For consistency of 'Lixivaptan Titration Period' throughout the protocol |
| 20 | Synopsis, Study Design: | At the beginning of Part 2, participants will start lixivaptan during the Lixivaptan Re-titration Period to re-establish the dose level that was tolerated during Part 1. | At the beginning of Part 2, all participants will start lixivaptan during the Lixivaptan Re-titration Period to re-establish the dose level that was tolerated during Part 1 | To clarify re-establishment of dose level applies to all participants (both lixivaptan-treated participants in Part 1 and placebo treated participants in Part 1) |
| 20 | Synopsis, Study Period Description and Estimated Duration: | Screening Period (Visits 1a, 1b, and 2): To obtain informed consent and determine initial participant eligibility, screening assessments will be performed at Visit 1a | Screening Period (Visits 1a, 1b, and 2): ~~To~~ After obtaining informed consent screening assessments will be performed at Visit 1a to ~~and~~ determine initial participant eligibility~~, screening assessments will be performed at Visit 1a~~. | To clarify that informed consent will be obtained prior to any assessments at Screening Visit 1a. |
| 23 | Synopsis, Study Period Description and Estimated Duration: | Part 2, Lixivaptan Re-titration Period (Visits 26 to 29):<br>….For participants randomized to placebo in Part 1, lixivaptan dosing will be titrated to the final blinded dose level achieved during the Double-Blind Treatment Period in accordance with Table 2. | Part 2, Lixivaptan Re-titration Period (Visits 26 to 29):<br>…..For participants randomized to placebo in Part 1, lixivaptan dosing will be titrated to the final blinded inferred dose level (the dose level equal to the active dose level had the participant been randomized to the active arm) achieved during the Double-Blind Treatment Period in accordance with Table 2. | To clarify the target dose level that participants randomized to placebo in Part 1 during re-titration in Part 2. |
| 25 | Synopsis, Study Drug, Dosage, and Route of Administration: | In an effort to minimize missing data, wherever practicable, participants who experience a study drug interruption of 7 or more days should 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. | In an effort to minimize missing data, wherever practicable, participants who experience a study drug interruption of 7 or more days ~~should~~ 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. | To strengthen the protocol emphasis on obtaining blood draws for eGFR determinations at the same clock time during baseline eGFR and off-treatment timepoints; important primary efficacy endpoints. |
| 26 | Synopsis, Table 2, footnote * | Periodic attempts should be made to re-establish dosing at Level 2 (100 mg BID) during the open-label phase | ~~Periodic a~~Attempts ~~should~~ will be made every 4 to 8 weeks to re-establish dosing at | To clarify and codify the frequency of attempts to re-establish dose level 2 |

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| | | | Level 2 (100 mg BID) during the open-label phase. | during open-label phase in Part 2 for participants who had a reduction in dose to level 1. |
| 34 | Table 3. Schedule of Procedures – Part 1, footnote e | e. As participants transition into the Double-Blind Randomized Treatment Period, with visits scheduled every 4 weeks, participant follow-up via a telephone contact is strongly encouraged. Telephone contacts should be repeated throughout the study as needed. Any contact with the participant via telephone will be recorded in the source document | e. ~~As participants transition into the Double-Blind Randomized Treatment Period, with visits scheduled every 4 weeks, participant follow-up via a telephone contact is strongly encouraged.~~ To encourage study compliance, ~~D~~during the Double-Blind Randomized Treatment Period, t~~T~~elephone contacts will~~should~~ be made 2 weeks (± 5 days) after every scheduled visit.~~repeated throughout the study as needed~~. ~~Any~~ All telephone contacts with the participant ~~via telephone~~ will be recorded in the source document. | To clarify and codify the frequency and schedule of follow-up telephone contacts in order to encourage study compliance. |
| 38 | Table 4. Schedule of Procedures – Part 2, footnote l | l. Participant follow-up via a telephone contact between study visits is strongly encouraged. Telephone contacts should be repeated throughout the study as needed.<br>Any contact with the participant via telephone will be recorded in the source document | l. During the Maintenance Treatment Period, p~~P~~articipant follow-up via a telephone contact will be made 2 weeks (± 5 days) after every scheduled visit ~~between study visits is strongly encouraged~~. ~~Telephone contacts should be repeated throughout the study as needed. Any~~ All telephone contacts with the participant ~~via telephone~~ will be recorded in the source document | To clarify and codify the frequency and schedule of telephone contacts. |
| 58 | 3.1.2 Detailed Study Design; Part 2: | | Lixivaptan Re-Titration Period (Visits 26 to 29):<br>**Note**: during the Re-titration Period of Part 2, all participants are re-titrated with lixivaptan to the last actual dose level if they had received lixivaptan during the Double-blind, Randomized Treatment Period, 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 | Relocated text from Maintenance Treatment Period section of protocol to the more appropriate location in the Lixivaptan Re-titration Period section. |

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| | | | 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. If no adjustment is made to the inferred dose during the Double-Blind, Randomized Treatment Period, the last inferred dose for a participant randomized to placebo will be equal to the maximal tolerated lixivaptan dose established during the Titration Period in Part 1. | |
| 59 | 3.1.2 Detailed Study Design; Part 2: | Maintenance Treatment Period (Visits 30 to 42):<br><br>~~**Note**: during the Re-titration Period of Part 2, all participants are re-titrated with lixivaptan to the last actual dose level if they had received lixivaptan during the Double-blind, Randomized Treatment Period, 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). This will be performed by the IRT and the site, participant, and sponsor maintain a blinded status to the prior treatment assignment in Part 1. If no adjustment is made to the inferred dose during the Double-Blind, Randomized Treatment Period, the last inferred dose for a participant randomized to placebo will be equal to the maximal tolerated lixivaptan dose established during the Titration Period in Part 1.~~ | | Relocated text from Maintenance Treatment Period section of protocol to the more appropriate location in the Lixivaptan Re-titration Period section. |

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| 65 | 4.2.3 Treatment Interruption | If a study drug interruption is expected to last 7 or more consecutive days, the participant should be scheduled to have 3 separate serum creatinine and serum cystatin C samples obtained (minimally 24 hrs. apart) beginning on Day 8 after the last dose of study drug for determination of eGFR in the event the study drug interruption leads to permanent discontinuation of treatment. In such cases, the 3rd sample should be collected up to Day 28 (± 3 days) after the last dose of study drug. If, in the Investigator's opinion, the treatment interruption is expected to be prolonged (i.e., >4 weeks in duration) or permanent, then a postbaseline MRI should also be obtained. | If a study drug interruption is expected to last 7 or more consecutive days, the participant will~~should~~ be scheduled to have 3 separate serum creatinine and serum cystatin C samples obtained (minimally 24 hrs. apart) beginning on Day 8 after the last dose of study drug for determination of eGFR in the event the study drug interruption leads to permanent discontinuation of treatment. In such cases, the 3rd sample will~~should~~ be collected up to Day 28 (± 3 days) after the last dose of study drug. If, in the Investigator's opinion, the treatment interruption is expected to be prolonged (i.e., >4 weeks in duration) or permanent, then a postbaseline MRI will~~should~~ also be obtained. | To clarify if the interruption is expected to last 7 or more days, the per protocol mandate to obtain 3 blood draws for determination of serum creatinine and serum cystatin C (used for eGFR calculations) at least 24 hours apart beginning on Day 8 after the last dose of study drug. If interruption is expected to last > 4 weeks, an MRI will be obtained. |
| 67 | 4.2.4 Treatment Discontinuation | Participants may elect to stop treatment permanently before the end of the study for various reasons. | Participants may elect to stop treatment, (i.e., stop taking their study drug)~~,~~ permanently before the end of the study for various reasons. This is separate and distinct from study discontinuation and/or withdrawal (~~,~~Section 4.2.5)~~,~~ although the reason(s) for study drug discontinuation may be identical and/or similar to reason(s) for study discontinuation. | To clarify and distinguish between Treatment Discontinuation, i.e., cessation of study drug treatment, and study discontinuation and/or study withdrawal. |
| 78<br><br>79<br>80<br>81<br>83<br><br>90<br><br>91 | 6. Timing of Study Procedures;<br>6.1.1 Visit 1a;<br>6.1.2 Visit 1b;<br>6.1.3 Visit 2;<br>6.2 Placebo Run-in Period and Visit 3;<br>6.4.2 Visit 22/Early Termination;<br>6.5.1 Visit 23 and Visit 24; | For a given participant, every attempt should be made to draw blood samples for eGFR determinations at approximately the same clock time during Visits 1a/1b, 2, and 3 and Visits 23, 24, and 25, and again at Visits 43, 44, and 45. | For a given participant, every attempt will~~should~~ be made to draw blood samples for eGFR determinations at approximately the same clock time during Visits 1a/1b, 2, and 3 and Visits 23, 24, and 25, and again at Visits 43, 44, and 45. | To strengthen the protocol emphasis on obtaining blood draws for eGFR determinations at the same clock time during baseline eGFR and off-treatment timepoints; important primary efficacy endpoints. |

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| 92<br>97<br><br>98<br>99<br>101 | 6.5.2 Visit 25;<br>6.7.2 Visit 42/Early Termination (Part 2);<br>6.8.1 Visit 43 and 44;<br>6.8.2 Visit 45;<br>7.1.1 eGFR Determination | | | |
| 122 | 8.5.1 Annualized Rate of Change (Slope) in eGFR on Treatment | A linear contrast of the treatment differences for these 12 monthly visits will be used for this analysis. | A linear contrast of the treatment slope differences for these 12 monthly visits will be used for this analysis. | To clarify that the treatment slope difference will be used for the analysis. |
| 136 | 11.4 Study Termination | 11.4 Study Termination | 11.4 Management of Study Termination | Section heading revised to reflect the content of the section. |

## Clinically-relevant Changes

This section describes changes that affect safety or study conduct.

| Page | Section | Version 2.0, Dated 01 Feb 2022 | Version 3.0; Dated 14 Feb 2022 | Rationale |
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| Multiple | Global | **Note:** In the event of newly emerging tolerability issues at any time during the study, dosing may be decreased or temporarily stopped. During the Double-Blind Treatment Period, periodic attempts should be made to re-establish the previously achieved dose level from Visit 9, if medically appropriate. | **Note:** In the event of ~~newly emerging~~ tolerability issues at any time during the study, dosing may be decreased or temporarily stopped. If dosing is resumed during the Double-Blind Treatment Period at a dose level less than that achieved at Visit 9 ~~During the Double-Blind Treatment Period~~, ~~periodic~~ attempts ~~should~~ will be made every 4 to 8 weeks to re-establish the previously achieved dose level from Visit 9, if medically appropriate. If study drug is interrupted for longer than 8 weeks, then study drug treatment will be permanently discontinued. | To clarify and codify 1) the frequency of attempts to re-establish end-of-titration dose level and 2) the maximum duration of a temporary drug interruption is 8 weeks after which study drug treatment will be permanently discontinued. |
| Multiple | Global | As participants transition into the Double-Blind Randomized Treatment Period, with visits scheduled every 4 weeks, participant follow-up via a telephone contact is strongly encouraged. Telephone contacts should be repeated throughout the study as needed. Any contact with the participant via telephone will be recorded in the source document | ~~As participants transition into the Double-Blind Randomized Treatment Period, with visits scheduled every 4 weeks, participant follow-up via a telephone contact is strongly encouraged.~~ To encourage study compliance, ~~D~~during the Double-Blind Randomized Treatment Period, t~~T~~elephone contacts will~~should~~ be made 2 weeks (± 5 days) after every scheduled visit.~~repeated throughout the study as needed~~. ~~Any~~ All telephone contacts with the participant ~~via telephone~~ will be recorded in the source document. | To codify the frequency and schedule of telephone contacts and clarify that all telephone contacts will be documented. |

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| Multiple | Global | Note: In the event of newly emerging tolerability issues at any time during the study including the Part 2 Lixivaptan Re-titration Period or during the Maintenance Period, dosing may be decreased or temporarily stopped. Periodic attempts should be made to re-establish the previously achieved dose level from Part 1, if medically appropriate. | **Note**: In the event of ~~newly emerging~~ tolerability issues at any time during the study including the Part 2 Lixivaptan Re-titration Period or during the ~~Maintenance Period~~Maintenance Treatment Period, dosing may be decreased or temporarily stopped. ~~Periodic a~~Attempts ~~should~~ 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~~from~~ Part 1, if medically appropriate. If study drug is interrupted for longer than 8 weeks, then study drug treatment will be permanently discontinued. | To clarify and codify 1) during the Maintenance Treatment Period, the frequency of attempts to re-establish the dose level at the end of the Double-blind, Randomized Treatment Period and 2) the maximum duration of a temporary drug interruption, after which study drug treatment will be permanently discontinued. |
| Multiple | Global | | If study drug is interrupted for longer than 8 weeks, then study drug treatment will be permanently discontinued. | Text added to establish a maximum duration of study drug interruption, after which study drug treatment will be permanently discontinued. |
| 17<br><br>62 | Synopsis, Study Population, Inclusion Criteria<br><br>4.1.1, Inclusion Criteria | 7.b**. …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 and should be discussed between the Investigator and the potential participant. | 7.b. .. **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 ~~should~~ be discussed ~~between~~ with the ~~Investigator and the~~ potential participant. | To elaborate the discussion with women of child-bearing potential (WOCBP) participants with severe polycystic liver disease (PLD) regarding estrogen and potential effects on development and growth of liver cysts and progression of PLD disease. The investigator is inherent on all activities of the protocol so unnecessary to state it. |

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| 28 | Synopsis, Statistical Methods: | | Primary Efficacy Analysis: Part 1: Sensitivity analyses of the primary efficacy analysis will include the use of the mean of eGFR values obtained pretreatment and the mean of eGFR values obtained off treatment after the end of the 52-week treatment period, regardless of adherence to randomized treatment and/or withdrawal for all randomized participants, i.e., Intent-To-Treat participants. Week 52 missing values will be imputed using multiple imputations (assuming missing at random). Bi-directional tipping point analyses will be included using a pattern-mixture model to impute missing data at Week 52 (assuming missing not at random); allowing varying assumptions about the missing outcomes on the two treatment arms. | Text added to describe planned sensitivity analyses of the primary efficacy analysis. |
| 49 | 1.9, Benefit/Risk Assessment | Safety data gathered to date in participants with ADPKD are consistent with the robust safety experience from studies in earlier indications investigated for lixivaptan—congestive heart failure, liver cirrhosis with ascites, or syndrome of inappropriate antidiuretic hormone secretion. The primary adverse effects of lixivaptan are related to the exaggerated pharmacology of blockade of the renal concentrating mechanism. This results in urinary dilution with the production of increased amounts and frequency of urine output and, secondarily, the stimulation of thirst. No adverse effects on other body | Safety data gathered to date in participants with ADPKD are consistent with the robust safety experience from lixivaptan treatment in studies in earlier indications investigated for lixivaptan—congestive heart failure, liver cirrhosis with ascites, or syndrome of inappropriate antidiuretic hormone secretion. The most common (≥~~>~~ 2%)~~primary~~ adverse effects of lixivaptan are related to the exaggerated pharmacology of blockade of the renal concentrating mechanism. This results in urinary dilution with the production of increased amounts and frequency of urine output and, secondarily, the stimulation of thirst. No adverse | To clarify and elaborate the risks, specifically of the potential risk for drug-induced liver injury (DILI) and the associated redundancy, by design, of safety monitoring. |

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| | | systems have been noted. Additionally, pharmacodynamic data generated for vasopressin V2 receptor antagonists that assess the mechanism of action, primarily through the development program for tolvaptan, have been confirmed in Phase 2 studies with lixivaptan and support continued development of lixivaptan for this indication. As there additionally remains a large unmet medical need to address chronic treatment in patients with ADPKD, particularly those who have experienced liver chemistry abnormalities leading to discontinuation of treatment with tolvaptan, continued investigation of this drug for ADPKD is warranted. Based on the totality of evidence generated to date on lixivaptan, the benefit/risk assessment of lixivaptan remains favorable. | effects on other body systems have been noted. Lixivaptan as a vasopressin V2 receptor antagonist is in the same pharmacological class as tolvaptan. ~~Drug-induced liver injury (~~DILI~~)~~ has been observed during the use of tolvaptan for ADPKD and JINARC / Jynarque® (brand names for tolvaptan) have boxed warnings for DILI in their Summary of Product Characteristics/US Prescribing Information label. Although there have been no reports of DILI related to lixivaptan to-date, the potential risk of DILI during lixivaptan treatment exists; this protocol has been designed to frequently monitor the participants for liver test abnormalities, has established an ~~Independent Data Monitoring Committee~~IDMC for safety oversight and has established an independent Hepatic Event Review Committee to monitor and review any participant's signs, symptoms, and/ laboratory tests that meet hepatic criteria, and described procedures to prevent liver toxicity. One of the two primary objectives of this study is to determine the risk of DILI as a result of lixivaptan treatment for ADPKD. | |
| 56 | 3.1.2 Detailed Study Design; Part 1: | Participants who were originally enrolled based on the 2009 CKD-EPI equation (Appendix 1 (Section 13.1)) will not be discontinued from the study should their recalculated baseline eGFR, based on the CKD-EPIcr_R equation, yield a mean eGFR value outside of the range of 25 to 90 mL/min/1.73 $m^2$ | Participants who were ~~originally~~ enrolled based on the 2009 CKD-EPI equation ((~~referenced in~~ Appendix 1 (Section 13.1~~)~~) will not be discontinued from the study should their recalculated baseline eGFR, based on the CKD-EPIcr_R equation, yield a mean eGFR value outside of the range of 25 to 90 mL/min/1.73 $m^2$. The stratification by CKD stage | Clarification that the CKD stratification at time of randomization will be based on the baseline eGFR creatinine equation (CKD-EPIcr or CKD-EPIcr_R) at the time of randomization. All efficacy assessments will be based on eGFR based on CKD-EPIcr_R equation. |

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| | | | (Section 8.4.1) will be based on the baseline eGFR creatinine equation in effect at the time of randomization. However, all efficacy analyses of change in eGFR will be based on the CKD-EPIcr_R equation. | |
| 59 | 3.1.2 Detailed Study Design; Part 2: | Maintenance Treatment Period (Visits 30 to 42):<br><br>If the participant's dose was decreased during the Lixivaptan Re-Titration Period, periodic attempts should be made to re-establish the previously achieved dose level from Part 1 | Maintenance Treatment Period (Visits 30 to 42):<br><br>If the participant's dose was decreased during the Lixivaptan Re-Titration Period, ~~periodic~~ attempts ~~should~~ will be made every 4 to 8 weeks to re-establish the previously achieved dose level (actual or inferred) at the end of the Double-blind, Randomized Treatment Period of~~from~~ Part 1. | To codify and clarify 1) that attempts to re-establish end-of-titration dose level every 4-8 weeks and 2) the target dose is actual or inferred dose at the end of the Double-blind, Randomized Treatment Period of Part 1. |
| 65 | 4.2.3 Treatment Interruption | If a participant's study drug must be interrupted for medical or surgical reasons, liver chemistry test abnormalities, tolerability issues, temporary use of a prohibited concomitant medication, life situations, e.g., airplane travel, etc. or other reasons; the participant's study drug should be resumed as early as the situation allows and the interruption recorded as a "Treatment Interruption" on the eCRF. | If a participant's study drug must be interrupted for medical or surgical reasons, liver chemistry test abnormalities, tolerability issues, temporary use of a prohibited concomitant medication, life situations, e.g., airplane travel, etc.. or other reasons, the interruption should be recorded as a "Treatment Interruption" on the eCRF.; ~~the~~ The participant's study drug should be resumed as early as the situation allows, e.g., airplane travel ends or recovery from surgery allows re-initiation/re-establishment of study drug treatment, ~~and the interruption recorded as a "Treatment Interruption" on the eCRF~~. The maximum duration of an interruption is 8 weeks; if interruption | To clarify resumption of study drug after a treatment interruption and to establish a maximum duration of study drug interruption of 8 weeks, after which study drug treatment will be permanently discontinued. |

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| | | | is greater than 8 weeks, study drug will be permanently discontinued. | |
| 66 | 4.2.3 Treatment Interruption | Participants with a prolonged study drug interruption (i.e., greater than 4 weeks in duration), who are re-initiating treatment are required to re-establish their last tolerated dose over a 2-week interval. | Participants with a prolonged study drug interruption (i.e., greater than 4 weeks in duration) equal to or less than 8 weeks), who are re-initiating treatment are required to re-establish their last tolerated dose over a 2-week interval. | To establish a maximum duration of study drug interruption of 8 weeks. |
| 67 | 4.2.3 Treatment Interruption | | Participants with a prolonged study drug interruption of greater than 8 weeks will not be allowed to re-initiate and/or re-establish study drug treatment i.e., study drug treatment will be permanently discontinued. These participants should be encouraged to continue their participation in the study following the procedures described in Section 4.2.7 and Appendix 5 (Section 13.5). | Text added to establish a maximum duration of study drug interruption of 8 weeks after which study drug treatment will be discontinued. Study participants should be encouraged to continue in the study. |
| 73 | 5.2 Dose Administered | | The participant's study drug should be resumed as early as the situation allows, e.g., airplane travel ends or recovery from surgery allows re-initiation/re-establishment of study drug treatment. The maximum duration of an interruption is 8 weeks; if interruption is greater than 8 weeks, study drug treatment will be permanently discontinued (Section 4.2.3). | Text added to clarify resumption of study drug administration after a temporary interruption and to establish a maximum duration of study drug interruption. |

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| 74 | 5.4 Treatment Compliance | If the participant is not ≥ 80% compliant with the prescribed study drug doses during the study overall, it will be noted as a significant protocol deviation. | If the participant is not ≥ 80% compliant with the prescribed study drug doses during the study overall, it will be noted as a ~~significant~~ protocol deviation. | To clarify the protocol deviation of study drug treatment compliance is <80% during the study overall. |
| 75 | 5.5 Prior and Concomitant Therapy | **Note**: in female participants with severe polycystic liver disease, contraceptives (and hormone replacement therapy) containing estrogen may be involved in the development and growth of liver cysts and polycystic liver disease progression and should be discussed between the Investigator and the potential participant | **Note**: in female participants with severe polycystic liver disease, contraceptives (and hormone replacement therapy) containing estrogen 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 ~~and should~~ will be discussed ~~between the~~with ~~Investigator and~~ the potential participant. | To elaborate, with regards to, the discussion with women of child-bearing potential (WOCBP) participants with severe polycystic liver disease (PLD) regarding estrogen and potential effects on development and growth of liver cysts and progression of PLD disease. The investigator is inherent on all activities of the protocol so unnecessary to state it. |
| 76 | 5.5.1.2 Prohibited Therapy | strong or moderate CYP3A4 or CYP2C8 inhibitors, including…. | strong or moderate CYP3A4 or CYP2C8 inhibitors, including…. voriconazole, | Voriconazole added to the list of strong or moderate CYP3A4 or CYP2C8 inhibitors |
| 109 | 7.3.7.1 Management of Liver Chemistry Test Abnormalities and Signs or Symptoms of Liver Dysfunction Occurring During Study Treatment | Liver chemistry test increases of a lesser extent (serum aminotransferase levels ≤3 × ULN or total bilirubin ≤2 × ULN) should be discussed with the medical monitor. They may be related to the underlying variability of such test results in ADPKD patients (Watkins et al, 2015). | Liver chemistry test increases of a lesser extent (serum aminotransferase levels ≤3 × ULN or total bilirubin ≤2 × ULN) will~~should~~ be discussed with the medical monitor. They may be related to the underlying variability of such test results in ADPKD patients (Watkins et al, 2015). | To mandate, by protocol, discussion of liver chemistry test abnormalities with the medical monitor. |

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| 112 | 7.3.8.2 Eliciting and Documenting Adverse Events and Serious Adverse Events | | Tolerability issues including but not limited to aquaretic effects, difficulty swallowing capsules, and abnormal thirst, will be recorded as adverse events. | Text added to clarify and provide examples of tolerability issues. |
| 119 | 8.2 Blinded Sample Size Re-estimation | After at least 20% of the randomized participants (i.e., 270 participants) complete Part 1 of the study (Visit 25) or before screening for the study has ended, a BSSR will be performed by a blinded, independent statistician. Based on this interim data set, the variance corresponding to the primary efficacy estimand will be estimated and the total sample size re-estimated. If the BSSR estimates that the standard deviation of the primary efficacy estimand is less than or equal to 6.20 mL per minute per 1.73 $m^2$, then the sample size will remain at 1350 randomized participants. If the BSSR estimates that the standard deviation is greater than 6.20 mL per minute per 1.73 $m^2$, then the total sample size will be re-estimated based on this new estimate of the standard deviation and the resulting revised sample size estimate will be provided to the sponsor for consideration. The BSSR will be based on the original, assumed between-treatment group difference, i.e., there will be no change to the estimate of the between-treatment group difference based on the BSSR. Since it is felt that the impact of the BSSR on the significance level will be negligible, | After at least 20% of the randomized participants (i.e., 270 participants) complete Part 1 of the study (Visit 25) or before screening for the study has ended, a BSSR will be performed by a blinded~~, independent~~ statistician. Based on this interim data set of blinded eGFR values, the variance corresponding to the primary efficacy estimand will be estimated and the total sample size re-estimated by a blinded statistician. If the BSSR estimates that the standard deviation of the primary efficacy estimand is less than or equal to 6.20 mL per minute per 1.73 $m^2$, then the sample size will remain at 1350 randomized participants. If the BSSR estimates that the standard deviation is greater than 6.20 mL per minute per 1.73 $m^2$, then the total sample size will be re-estimated based on this new estimate of the standard deviation~~ and the resulting revised sample size estimate will be conducted by the sponsor provided to~~ by the sponsor or designee~~for consideration~~. The BSSR will be based on the original, assumed between-treatment group difference, i.e., there will be no change to the estimate of the between-treatment group difference based on the BSSR. Since ~~it is felt that the impact of the~~ a | To clarify that the BSSR will be performed by a blinded statistician using blinded eGFR values and that the BSSR does not inflate the type 1 error of the primary efficacy analysis. |

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| | | the significance level for the final test will remain at 0.01. | BSSR does not inflate the type 1 error of the primary efficacy analysis~~on the significance level will be negligible~~, the significance level for the final ~~test~~ efficacy analysis will remain at 0.01. | |
| 120 | 8.4.1 Primary Endpoint Analysis | The estimand corresponding to the primary objective is the between-treatment group difference in the change from baseline in 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 baseline eGFR assessments to the median of the 3 eGFR assessments made during the post-treatment, follow-up assessments in days. | The estimand corresponding to the primary objective is the between-treatment group difference in the change from baseline in eGFR if all the participants in the PEAS had tolerated and adhered to their treatment for 52 weeks. ~~Potential bias due to~~For randomized participants prematurely discontinuing from double-blind treatment, the primary analysis 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 (days) from the median of the baseline eGFR assessments to the median of the 3 eGFR assessments ~~made~~ obtained during the post-treatment, follow-up period immediately following study drug discontinuation~~assessments in days~~.<br>~~For participants who prematurely discontinue study drug prior to Week 52, the follow-up data from the period immediately following study drug discontinuation will be used for the primary efficacy endpoint.~~ | To clarify the method of primary analysis for randomized participants who discontinue from study drug treatment during the double-blind treatment period. |

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| 121 | 8.4.1 Primary Endpoint Analysis | | The stratification by CKD stage (Section 8.4.1) will be based on the baseline eGFR creatinine equation in effect at the time of randomization. However, all efficacy analyses of change in eGFR will be based on the CKD-EPIcr_R equation. | Text added to clarify that CKD stage stratification will be based on whichever baseline eGFR equation was used at the time of randomization. To clarify that all efficacy analyses will be based on eGFR calculated using the 2021 CKD-EPIcr-R equation. |
| 121 | 8.4.2 Sensitivity Analysis of the Primary Efficacy Analysis | In order to address the impact of missing data on the results from the primary efficacy analysis, a number of sensitivity analyses will be performed. The sensitivity analyses for the primary efficacy analysis will be described in the statistical analysis plan (SAP). | In particular, in order to address the impact of missing data on the results from the primary efficacy analysis, a number of sensitivity analyses will be performed. The detailed sensitivity analyses for the primary efficacy analysis will be described in the statistical analysis plan (SAP).<br><br>Amongst these sensitivity analyses, the main sensitivity analysis of the primary efficacy analysis will use the mean of eGFR values obtained pretreatment and the mean of eGFR values obtained off treatment after the end of the 52-week treatment period, regardless of adherence to randomized treatment and/or withdrawal for all randomized participants, i.e., Intent-To-Treat participants. Missing values at Week 52 will be imputed using multiple imputations (assuming missing at random). Tipping point analyses will be included using a pattern-mixture model to impute missing data at Week 52 (assuming missing not at random) by systematically varying assumptions about the missing outcomes on the two treatment arms – these tipping point analyses will be bi-dimensional, | Text added to elaborate planned sensitivity analyses of the primary efficacy analysis of the Intent-To-Treat population. Multiple imputations will be used to impute missing values at Week 52 (assuming missing at random) and tipping point analysis will be used to impute missing value at Week 52 (assuming missing not at random). |

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| | | | i.e., allow the assumptions about the missing outcomes in the two arms to vary independently, and will include scenarios where the dropouts on the lixivaptan arm have worst outcome compared to placebo dropouts. | |
| 123 | 8.6.2 Sensitivity Analysis of the Key Comparison Analysis | In order to assess the effect of missing data on the key comparison analysis in Part 2, sensitivity analyses for the descriptive statistics described in the corresponding section of Section 8.6.1, will be performed. The sensitivity analyses for the key comparison analysis in Part 2 will be described in the SAP. | In order to assess the effect of missing data on the key comparison analysis in Part 2, sensitivity analyses for the descriptive statistics ~~described in the corresponding section of Section 8.6.1,~~ will be performed for all available annualized changes from baseline (the mean of the 3 eGFR assessments obtained during Follow-up Period I in Part 1) to the mean of the 3 off-treatment eGFRs after Week 104. ~~The sensitivity analyses for the key comparison analysis in Part 2 will be described in the SAP.~~ | To clarify the endpoints that will be used for the key comparison analyses. |

# Study PA-ADPKD-301: The ACTION Study

# Clinical Study Protocol Version 2.0, Dated 01 February 2022

# Summary of Changes

This Summary of Changes document reflects revisions incorporated into Protocol Amendment 01, Version 2.0 of the protocol. It should be used in conjunction with the tracked-changes version of the clinical study protocol amendment. Page numbers reported herein refer to the **tracked-changes** version. Revisions in Version 2.0 are shown in Track Changes. Minor editorial and document formatting revisions have not been summarized.

The amendment is a substantial amendment because these changes affect 1) the safety or rights of the participant and 2) the reliability and robustness of the data generated in the clinical trial.
The main reasons for this substantial amendment are as follows:

1) to increase study power to 90% resulting in a sample size increase from 1200 randomized to 1350 randomized participants;
2) to update the equation used in the calculation of the primary endpoint from the 2009 CKD-EPI equation to the 2021 CKD-EPIcr_R . This updated equation removes the race variable from the calculation of eGFR;
3) to add exploratory endpoints for eGFR calculated from a combination of serum creatinine and serum cystatin C based on the 2021 CKD-EPIcr-cys_R equation;
4) to increase frequency of serum sodium level assessment to enhance safety monitoring;
5) to remove suppression of post-randomization eGFR results to facilitate safety monitoring and allow the sponsor to conduct the Blinded Sample Size Re-estimation (BSSR);
6) to add a benefit/risk section;
7) to incorporate additional messaging about the importance of study participants staying on study drug or continuing in the study off of study drug; and
8) to incorporate protocol administrative letters #1 (06-Jul-2021) and #2 (04-Aug-2021).

## Administrative Changes

This section lists administrative changes, including changes of names or roles of companies or personnel and/or contact information. There are no changes included that affect clinical decision making or consent.

| Page | Section | Version 1.0, Dated 30JUN2021 | Version 2.0, Dated 01FEB2022 | Rationale |
|---|---|---|---|---|
| 1, 4, 14 | Title Page: Sponsor, Palladio Logo, Sponsor Contact,<br><br>Protocol Approval Signature Page,<br><br>Protocol Synopsis | Palladio Biosciences, Inc., a Centessa Pharmaceuticals Company | Palladio Biosciences, Inc., ~~a Centessa Pharmaceuticals Company~~ | Clarified that Palladio Biosciences, Inc is the legal entity. This update incorporates the change noted in Protocol Administrative Letter 01 (06-Jul-2021).<br>The logo has been revised to only show Palladio Biosciences. |
| 1, 115 | Title Page: Emergency Telephone Number<br><br>Protocol, Section, 7.3.8.3, Reporting Adverse Events, Serious Adverse Events, and Adverse Events of Special Interest | **North America:** +1 510 722 8910<br>**South America:** +54 911 5479 8678<br>**Europe, South Africa:** +44 118 936 4096<br>**Australia:** +61 3921 39875<br><br>**Investigative Sites in North America:** +1 510 722 8910<br>**Investigative Sites in South America:** +54 911 5479 8678<br>**Investigative Sites in Europe, South Africa:** +44 118 936 4096<br>**Investigative Sites in Australia:** +61 3921 39875 | **North America:** +1 510 722 8910<br>**South America:** +54 911 5479 8678<br>**Europe~~,~~ ~~South Africa~~ and Middle East Region:** +44 118 936 4096<br>**Australia, New Zealand:** +61 3921 39875<br><br>**Investigative Sites in North America:** +1 510 722 8910<br>**Investigative Sites in South America:** +54 911 5479 8678<br>**Investigative Sites in Europe~~,~~ ~~South Africa~~ and Middle East Region:** +44 118 936 4096<br>**Investigative Sites in Australia, New Zealand:** +61 3921 39875 | List of country/regional emergency telephone numbers have been updated to reflect current study regional locations. |
| 41 | List of Abbreviations | | CKD-EPIcr_RChronic Kidney Disease Epidemiology Collaboration eGFR creatinine equation refit without the race variable | Added abbreviations for the updated equations for eGFR which will be used in the primary and exploratory |

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| | | | CKD-EPIcr-cys R Chronic Kidney Disease Epidemiology Collaboration eGFR creatinine and cystatin C equation refit without the race variable. | endpoints. |
| 112 | Protocol Section, 7.3.7.2. Hepatic Events Review Committee (HERC)-Special Reporting of Liver Events | The Investigator must complete the liver dysfunction details eCRF within 24 hours of awareness through the SAE reporting pathway (described in Section 7.3.8.3) for any participant who develops an Adverse Event of Special Interest (AESI) related to abnormal liver chemistry test results or clinical signs or symptoms….<br>….The AESI report form should be updated as new information becomes available. | The Investigator must complete and submit a copy of the liver dysfunction details eCRF with the SAE/AESI form within 24 hours of awareness through the SAE reporting pathway (described in Section 7.3.8.3) for any participant who develops an Adverse Event of Special Interest (AESI) related to abnormal liver chemistry test results or clinical signs or symptoms….<br>….The SAE/AESI report form should be updated as new information becomes available. | Clarified the procedures for AESI reporting.<br>Updated the name of the report form to SAE/AESI report form. |
| 114 | Protocol, Section, 7.3.8.3, Reporting Adverse Events, Serious Adverse Events, and Adverse Events of Special Interest | Any AE that meets SAE or AESI criteria will be reported to Premier Research Global Pharmacovigilance (Premier PV) immediately (i.e., within 24 hours) after site personnel first learn about the event. Investigators should record all SAE details available, including Investigator causality assessment, on the SAE form and subsequently record the event on the Adverse Event eCRF within 24 hours of becoming aware of the event. Investigators should record all AESI details available, including Investigator causality assessment, on the AESI form and subsequently record the event on the Adverse Event eCRF | Any AE that meets SAE or AESI criteria will be reported to Premier Research Global Pharmacovigilance (Premier PV) immediately (i.e., within 24 hours) after site personnel first learn about the event. Investigators should record all SAE details available, including Investigator causality assessment, on the SAE/AESI form and subsequently record the event on the Adverse Event eCRF within 24 hours of becoming aware of the event. Investigators should record all AESI details available, including Investigator causality assessment, on the SAE/AESI form and subsequently record the event on the Adverse Event eCRF within 24 hours of becoming aware of the event. | Text revised to reflect that there is a combined SAE/AESI form for event reporting. |

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| | | within 24 hours of becoming aware of the event.<br><br>Completed SAE Report Forms and AESI Report Forms should be submitted to Premier PV as an email attachment to the email address below.<br><br>….<br><br>Should the site have questions or concerns regarding SAE report submission, the site may elect to call the site Clinical Research Associate (CRA) or contact the medical monitor. The site will be asked to provide the following information: protocol number, site number, participant identifiers, event term, study drug information, and relationship of the event to study drug. | Completed SAE/AESI Report Forms ~~and AESI Report Forms~~ should be submitted to Premier PV as an email attachment to the email address below.<br><br>….<br>Should the site have questions or concerns regarding SAE/AESI report submission, the site may elect to call the site Clinical Research Associate (CRA) or contact the medical monitor. The site will be asked to provide the following information: protocol number, site number, participant identifiers, event term, study drug information, and relationship of the event to study drug. | |
| 139 | Reference List | | 7. Delgado, C., Baweja, M., Crews, D. C., Eneanya, N. D., Gadegbeku, C. A., Inker, L. A., Mendu, M. L., Miller, W. G., Moxey-Mims, M. M., Roberts, G. V., St Peter, W. L., Warfield, C., & Powe, N. R. 2021. A Unifying Approach for GFR Estimation: Recommendations of the NKF-ASN Task Force on Reassessing the Inclusion of Race in Diagnosing Kidney Disease. J Am Soc Nephrol. 2021 Sep 23:ASN.2021070988. doi: 10.1681/ASN.2021070988. Epub ahead of print. | Updated the reference list with the reference for the 2021 CKD-EPI equation refit without race variable (CKD-EPIcr_R). Reference List renumbered as a result of this change. |
| 139 | Reference List | 8.Di Mise A, Wang X, Ye H, Pellegrini L, Torres VE, Valenti G: Dual Targeting of the G Protein-Coupled Receptors CaSR and V2R for Treating Autosomal | ~~8.~~9. Di Mise A, Wang X, Ye H, Pellegrini L, Torres VE, Valenti G. 2021. Pre- clinical evaluation of dual targeting of the GPCRs CaSR and V2R as therapeutic strategy for | Updated the Di Mise reference from the abstract to the full manuscript. |

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| | | Dominant Polycystic Kidney Disease (ADPKD) [Abstract]. J Am Soc Nephrol. 30, 2019: Page 628. | autosomal dominant polycystic kidney disease. FASEB J 2021: 2021;35:e21874.. kwwsv=22gr1ruj243143<92in153543 3::7U 35(10): e21256 ~~8.Di Mise A, Wang X, Ye H, Pellegrini L, Torres VE, Valenti G: Dual Targeting of the G Protein-Coupled Receptors CaSR and V2R for Treating Autosomal Dominant Polycystic Kidney Disease (ADPKD) [Abstract]. J Am Soc Nephrol. 30, 2019: Page 628~~. | |
| 140 | Reference List | 14. International Society of Nephrology. Kidney International Supplements. KDIGO. 2012. KDIGO Clinical Practice Guideline for the Management of Blood Pressure in Chronic Kidney Disease. 2(5). Available at: http://www.kidney-international.org. | ~~14. International Society of Nephrology. Kidney International Supplements. KDIGO. 2012. KDIGO Clinical Practice Guideline for the Management of Blood Pressure in Chronic Kidney Disease. 2(5). Available at: http://www.kidney-international.org.~~ ~~14.~~ 15.International Society of Nephrology. Kidney International Supplements. KDIGO 2021 Clinical Practice Guideline for the Management of Blood Pressure in Chronic Kidney Disease. Kidney Int. 99(3S):S1-S87. DOI: https://doi.org/10.1016/j.kint.2020.11.003. | Updated the reference list with the KDIGO 2021 guideline for blood pressure control to align with Inclusion Criterion #5. |

## Text Clarifications

This section lists corrections of typos, text changes for consistency or other clarifications. These changes do not affect clinical decision-making or consent.

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| Multiple | Global | KDIGO | the 2021 KDIGO guideline | Clarified version of the KDIGO blood pressure guideline used. |
| Multiple | Global | Mayo Clinic ADPKD Classification | Mayo Clinic ADPKD Image Classification. | Clarified that ADPKD classification is based on MRI imaging. |
| Multiple | Global | | Note: In the event of newly emerging tolerability issues at any time during the study, dosing may be decreased or temporarily stopped. During the Double-Blind Treatment Period, periodic attempts should be made to re-establish the previously achieved dose level from Visit 9, if medically appropriate. | Clarified that dosing may be reduced or temporarily stopped at any time during the study with the caveat that the dose should be re-established only if medically appropriate. |
| Multiple | Global | | Note: In the event of newly emerging tolerability issues at any time during the study including the Part 2 Lixivaptan Re-titration Period or during the Maintenance Period, dosing may be decreased or temporarily stopped. Periodic attempts should be made to re-establish the previously achieved dose level from Part 1, if medically appropriate | Clarified that dosing may be reduced or temporarily stopped at any time during the study, including the Lixivaptan Re-titration or Maintenance Periods of Part 2 with the caveat that the dose should be re-established only if medically appropriate. |
| Multiple | Global | Visit 24 must be scheduled to occur 12 to 24 days after the last dose of double-blind study drug and a minimum of 24 hours apart from either Visit 23 or Visit 25. | Visit 24 must be scheduled to occur at least 24 hours after Visit 23 and at least 24 hours prior to Visit 25 ~~12 to 24 days after the last dose of double-blind study drug and a minimum of 24 hours apart from either Visit 23 or Visit 25.~~ | Changed visit timing to increase operational efficiency. |
| Multiple | Global | Visit 44 must be scheduled to occur 12 to 24 days after the last dose of study drug and a minimum of 24 hours apart | Visit 44 must be scheduled to occur at least 24 hours after Visit 43 and at least 24 hours prior to Visit 45. ~~12 to 24 days after the last~~ | Changed visit timing to increase operational efficiency. |

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| | | from either Visit 43 or Visit 45. | ~~dose of study drug and a minimum of 24 hours apart from either Visit 43 or Visit 45.~~ | |
| Multiple | Global | Schedule participant for the post-baseline MRI for assessment of TKV and LV to occur 28 ± 3 days after the last dose; the MRI should occur up to 3 days prior to or on the same day as Visit 25. | Schedule participant for the post-baseline MRI for assessment of TKV and LV to occur between 25 days and 31 ~~28 ± 3~~ days after the last dose of study drug; the MRI ~~should occur up to 3 days~~ must be completed prior to or on the same day as all other Visit 25 study procedures | Text regarding timing of MRI at Visit 25 was revised for clarity. |
| Multiple | Global | Schedule participant for the post-baseline MRI for assessment of TKV and LV to occur 28 ± 3 days after the last dose; the MRI should occur up to 3 days prior to or on the same day as Visit 45. | Schedule participant for the post-baseline MRI for assessment of TKV and LV to occur between 25 days and 31 ~~28 ± 3~~ days after the last dose of study drug; the MRI ~~should occur up to 3 days~~ must be completed prior to or on the same day as all other Visit 45 study procedures | Text regarding timing of MRI at Visit 45 was revised for clarity. |
| Multiple | Global | …The baseline MRI, which may be completed before, at, or after Visit 1b, is to be completed minimally 2 weeks prior to Visit 2 to allow for receipt of the Mayo Clinic ADPKD classification from the central imaging vendor… | …The baseline MRI, which may be completed before, at, or after Visit 1b, is to be completed ~~minimally 2 weeks~~ approximately 1 week prior to Visit 2 to allow for receipt of the Mayo Clinic ADPKD Image ~~classification~~ from the central imaging vendor (**Note**: Visit 2 should be scheduled approximately 1 week after the baseline MRI and approximately 2 weeks after completion of Visit 1b, whichever occurs later)… | Timing of Visit 2 relative to the completion of Visit 1b for participants requiring blood pressure/blood pressure medication optimization has been revised for operational efficiency, including time for determination of Mayo Image Classification. |
| | | Participants who do not require modification to medical treatment should be scheduled for the baseline MRI and should proceed to Visit 2 in 3 to 5 weeks following Visit 1a. **Note:** Visit 2 should be scheduled minimally 2 weeks following completion of the MRI to allow for receipt of the Mayo Clinic ADPKD classification from the central imaging vendor… | Participants who do not require modification to medical treatment should be scheduled for the baseline MRI and should proceed to Visit 2 in 3 to 5 weeks following Visit 1a. **Note:** Visit 2 should be scheduled ~~minimally 2 weeks~~ approximately 1 week following completion of the MRI to allow for receipt of the Mayo Clinic ADPKD Image ~~classification~~ Classification from the central imaging vendor. Participants must have adequate BP control for a minimum of 3 weeks prior to enrollment (Visit 2)… | Timing of Visit 2 relative to the completion of Visit 1b for participants requiring blood pressure/blood pressure medication optimization has been revised for operational efficiency, including time |

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| | | | | for determination of Mayo Image Classification. |
| Multiple | Global | Designated visits may be done remotely by a home healthcare clinician (HHC) (where available and locally approved by the Competent Authority (CA) and/or IRB/EC) and telehealth (e.g., telemedicine virtual visit, telephone or video call (without recording)) with the study site, as applicable. | If agreeable to the participant and at the discretion of the investigator, designated visits may be done remotely by a home healthcare clinician (HHC) (where available and locally approved by the Competent Authority (CA) and/or IRB/EC). Some remote visits ~~and~~ may also include telehealth (e.g., telemedicine virtual visit, telephone or video call (without recording)) with the study site, as applicable. Remote visits may also be conducted by qualified site personnel who have been delegated the authority to carry out the procedures required at remote visits by the Investigator. | Added framework around permitting remote visits and indicated that some visits will include telehealth with the Investigator.<br><br>Clarified that remote visits may also be conducted by qualified site staff, where available and approved. |
| 16, 53 | Protocol Synopsis, Objectives<br><br>Protocol Section, 2.1.2 Objectives of the Open-label Phase (Part 2/Year 2) | | The population pharmacokinetics (PopPK) objective of Part 2 is:<br>• To further characterize the PK profile of lixivaptan utilizing PopPK based on sparse plasma sampling. | For completeness, an objective related to population PK has been added to Part 2 of the study. |
| 17 | Protocol Synopsis, Inclusion Criteria | 2. Diagnosis of ADPKD by modified Pei criteria:<br>3. For participants with family history of ADPKD, by ultrasound:<br>• 18-39 years: ≥3 cysts, unilateral or bilateral;<br>• 40-59 years: ≥2 cysts in each kidney;<br>• 60 years: ≥4 cysts in each kidney…. | 2. Diagnosis of ADPKD by modified Pei criteria:<br>~~3.~~ For participants with family history of ADPKD, by ultrasound:<br>• 18-39 years: ≥3 cysts, unilateral or bilateral;<br>• 40-59 years: ≥2 cysts in each kidney;<br>• 60 years: ≥4 cysts in each kidney… | Corrected from a separately numbered inclusion criterion to a sub-bullet. This update incorporates the change noted in Protocol Administrative Letter 2 (04-Aug-2021) and harmonizes with numbering of criteria.<br>As a result, all inclusion criteria after Inclusion Criterion 2 appearing in |

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| | | | | the protocol synopsis were renumbered. |
| 17, 64 | Protocol Synopsis, Study Population<br><br>Protocol Section 4.1.1, Inclusion Criteria | • 5. eGFR ≥25 mL/min/1.73 $m^2$ and ≤90 mL/min/1.73 $m^2$ based on the mean of 2 eGFR determinations…**Note:** This criterion will preliminarily be reviewed at Visit 2 based on Visit 1a or Visit 1b results (if Visit 1b is required). The criterion must be re-evaluated no later than Visit 3 when results for Visits 1a and 2 or Visits 1b and 2 are available. | • ~~5~~ 4.eGFR ≥25 mL/min/1.73 $m^2$ and ≤90 mL/min/1.73 $m^2$ based on the mean of 2 eGFR determinations… **Note**: This criterion will preliminarily be reviewed at Visit 2 based on Visit 1a or Visit 1b results (if Visit 1b is required). The criterion must be re-evaluated no later than Visit 3 when results for Visits 1a and 2 or Visits 1b and 2 are available to confirm that the participant remains eligible for participation. | Clarification added that eligibility based on eGFR should be re-evaluated once Visit 2 lab results become available by Visit 3. |
| 19, 65 | Protocol Synopsis, Study Population<br><br>Protocol, Section 4.1.2, Exclusion Criteria.<br>4 | 4. History of infection with human immunodeficiency virus (HIV) unless the participant is stable and doing well on a non-CYP interacting ART regimen and the participant has not required more than 2 changes in their ART regimen since treatment inception. | 4. History of infection with human immunodeficiency virus (HIV) unless the participant is clinically stable and doing well on a non-CYP interacting ART regimen and the participant has not required more than 2 changes in their ART regimen since treatment inception | "Clinically" added to criterion for clarity |
| 21 | Protocol Synopsis, Study Period Description and Estimated Duration: | • Participants with appropriate blood pressure control…and not receiving a diuretic will have a baseline MRI exam scheduled and will proceed to Visit 2, 3 to 5 weeks after Visit 1a and after completion of the baseline MRI. (Screening may be extended to a maximum of 8 weeks, in the event of extenuating circumstances. Prior approval from the medical monitor is required.)<br><br>• Participants who need to be discontinued from diuretic therapy and/or have their anti-hypertensive | • Participants with appropriate blood pressure control…and not receiving a diuretic will have a baseline MRI exam scheduled and will proceed to Visit 2, 3 to 5 weeks after Visit 1a, and after completion of the baseline MRI (allow approximately 1 week after completion of the MRI, however, a 3-week minimum between Visit 1a and Visit 2 is required). (Screening may be extended to a maximum of 8 weeks, in the event of extenuating circumstances. Prior approval from the medical monitor is required.) | Clarified visit timing during the Screening Period for participants who do not require optimization of blood pressure/blood pressure medications as well as for those requiring optimization of blood pressure/blood pressure medication.<br><br>Clarified that optimization of blood pressure/blood pressure medications for those who require it should |

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| | | therapy optimized due to inadequate BP control (as defined by KDIGO) will return for Visit 1b to have additional serum chemistry testing once their treatment and blood pressure have been stable for a minimum of 3 weeks. The baseline MRI exam should be scheduled after completion of Visit 1a but may be performed before, at, or after Visit 1b (but prior to Visit 2) depending on scheduling. Upon attaining stability of antihypertensive therapy and completion of the baseline MRI, the participant will be scheduled for Visit 2 (a minimum of 2 weeks following the MRI and a minimum of 1 week following Visit 1b.) The total duration of the Screening Period may be extended up to 8 weeks, including the time to completion of Visit 2.<br><br>Participants who meet all of the inclusion criteria…will have Visit 2 end-of-screening assessments and eligibility determination (final eGFR eligibility will be delayed until Visit 2 serum creatinine results are obtained). Participants who fail inclusion/exclusion criteria… | • Participants who need to be discontinued from diuretic therapy and/or have their anti hypertensive therapy optimized due to inadequate BP control (as defined by the 2021 KDIGO guideline) will return for unscheduled visits for blood pressure monitoring following Visit 1a. Once their treatment and blood pressure have been stable for a minimum of 3 weeks, they will return for Visit 1b to have additional serum chemistry testing ~~once their treatment and blood pressure have been stable for a minimum of 3 weeks~~.The baseline MRI exam should be scheduled after completion of Visit 1a but may be performed before, at, or after Visit 1b (but prior to Visit 2) depending on scheduling. Upon attaining stability of antihypertensive therapy and completion of the baseline MRI, the participant will be scheduled for Visit 2 (approximately 1 week ~~minimum of 2 weeks~~ following the MRI and ~~a minimum of~~ approximately ~~1~~ 2 weeks following Visit 1b, whichever occurs later.) The total duration of the Screening Period may be extended up to 8 weeks, including the time to completion of Visit 2.<br><br>Participants who meet all of the inclusion criteria…will have Visit 2 end-of-screening assessments and eligibility determination (final eGFR eligibility will be delayed until Visit 2 serum creatinine results are obtained); fasting morning spot (i.e., discard first void and sample will be obtained from next void within 30 to 45 minutes) urine osmolality will also be measured. Participants who fail inclusion/exclusion criteria… | occur at Unscheduled Visits prior to Visit 1b.<br><br>Text added to clarify procedure for urine osmolality at Visit 2. |

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| 22, 59 | Protocol Synopsis Study Period Description and Estimated Duration (Part 1)<br><br>Protocol Section 3.1.2, Detailed Study Design (Part 1) | Lixivaptan Dose Titration Period (Visits 4 to 9): During a period of 5 to 6 weeks, the lixivaptan dose will be increased in a single-blind fashion according to the titration schedule in Table 1 to achieve the maximally tolerated dose up to the maximum dose allowed (Level 4 [200 mg BID]); as 4 capsules BID. The minimum dose to enter the Double-blind, Randomized Treatment Period is Level 2 (100 mg BID). Once the maximally…. | Lixivaptan Dose Titration Period (Visits 4 to 9): During a period of 5 to 6 weeks for participants who complete the Placebo Run-in Period, the lixivaptan dose will be increased in a single-blind fashion according to the titration schedule in Table 1 to achieve the maximally tolerated dose up to the maximum dose allowed (Level 4 [200 mg BID]); as 4 capsules BID. The minimum dose to enter the Double-blind, Randomized Treatment Period is Level 2 (100 mg BID). Once the maximally…. | Text revised for clarity. |
| 23 | Protocol Synopsis Study Period Description and Estimated Duration (Part 2) | Lixivaptan Re-titration Period (Visits 26 to 29): Titration of lixivaptan will occur over a 2 to 4-week re-titration period and will start at a dose of 50 mg BID beginning at the conclusion of Visit 25. The dose will be increased at weekly intervals until the dose level from the end of the Double-blind, Randomized Treatment Period in Part 1 is achieved. In order to maintain blinding of the treatment assignment from Part 1, the dose level assignment for Part 2 will continue to be managed by the IRT. The dose-titration schedule for this part of the study is described in Table 2. | Lixivaptan Re-titration Period (Visits 26 to 29): Titration of lixivaptan will occur over a 2 to 4-week re-titration period and will start at a dose of 50 mg BID beginning at the conclusion of Visit 25. The dose will be increased at weekly intervals until the dose level from the end of the Double-blind, Randomized Treatment Period in Part 1 is achieved. For participants randomized to placebo in Part 1, lixivaptan dosing will be titrated to the final blinded dose level achieved during the Double-Blind Treatment Period in accordance with Table 2. In order to maintain blinding of the treatment assignment from Part 1, the dose level assignment for Part 2 will continue to be managed by the IRT. The dose-titration schedule for this part of the study is described in Table 2. | Text has been revised to provide clarity around re-titration in Part 2, particularly for participants previously randomized to placebo in Part 1. |
| 24, 61 | Study Synopsis, Study Period Description and Estimated Duration: | The total duration of participation in the study will be approximately 123 to 131 weeks (up to 71 weeks in Part 1 and up to 60 weeks in Part 2) depending on the length of Screening (3 to 8 weeks), Titration (5 to 6 weeks), and Re-titration | The total duration of participation in the study will be approximately 123 to 131 weeks (up to 71 weeks in Part 1 and up to 60 weeks in Part 2) depending on the length of Screening (3 to 8 weeks), Titration (5 to 6 weeks), and Re-titration (2 to 4 weeks). Note that the | Text clarified to reflect that the study may be interrupted at any time based on findings from the IDMC and the sponsor. |

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| | | (2 to 4 weeks). Note that the study may be interrupted at any time if safety issues identified by an IDMC potentially compromise the safety of the participants. | study may be interrupted at any time if safety issues identified by an Independent Data Monitoring Committee (IDMC) and sponsor potentially compromise the safety of the participants. | |
| | Protocol Section, 3.1.2 Detailed Study Design | The maximum duration of participation in this study is 131 weeks (up to 71 weeks in Part 1 and up to 60 weeks in Part 2) depending on the length of Screening (3 to 8 weeks), Titration (5 to 6 weeks) and Re-titration (2 to 4 weeks). Note that the study may be interrupted at any time if safety issues identified by an Independent Data Monitoring Committee potentially compromise the safety of the participants. | The maximum duration of participation in this study is 131 weeks (up to 71 weeks in Part 1 and up to 60 weeks in Part 2) depending on the length of Screening (3 to 8 weeks), Titration (5 to 6 weeks) and Re-titration (2 to 4 weeks). Note that the study may be interrupted at any time if safety issues identified by an ~~Independent Data Monitoring Committee~~ IDMC and sponsor potentially compromise the safety of the participants. | |
| 25 | Protocol Synopsis, Study Drug, Dosage, and Route of Administration | Participants who are unable to tolerate the minimum dose for study entry (100 mg BID/ Level 2) will be discontinued from the study. | Participants who are unable to tolerate the minimum dose for ~~study~~ entry into the Double-blind, Randomized Treatment Period (100 mg BID/ Level 2) will be discontinued from the study. | Revised text for clarity. |
| 75 | Protocol Section 5.2, Dose Administered | 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, 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 | 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, 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 | |

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| | | period. Participants who are unable to tolerate the minimum dose for study entry (100 mg BID/ Level 2) will be discontinued from the study. | minimum dose for ~~study~~ entry into the Double-Blind, Randomized Treatment Period, (100 mg BID/ Level 2) will be discontinued from the study. | |
| 26 | Protocol Synopsis, Study Drug Dosage, and Route of Administration (Part 2) | **Part 2:** During the Lixivaptan Re-titration Period, dosing will start at Level 1 (50 mg BID) and will be increased weekly until the dose level taken at the end of the Double-blind, Randomized Treatment Period is achieved. This is shown in Table 2. That dose will be continued for the remainder of Part 2…. | **Part 2:** 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. This is shown in Table 2. That dose will be continued for the remainder of Part 2. | Clarified that all participants (lixivaptan- and placebo-treated participants from Part 1 who participate in Part 2) will initiate re-titration at lixivaptan (50 mg BID). |
| 35 | Table 3, Schedule of Procedures, Part 1 (table note) | Not all participants will need these visits<br>Visits 4 to 7, 10, 11, 13, 14, 16, 17, 19 to 21, 23 and 24 may be conducted in the clinic or remotely by a home healthcare clinician | Not all participants will need these visits<br>Visits 4 to 7, 10, 11, 13, 14, 16, 17, 19 to 21, 23 and 24 may be conducted in the clinic or remotely by a home healthcare clinician or qualified site personnel with the agreement of the participant and the discretion of the investigator, and where it is available and locally approved by the Competent Authority and/or Ethics Committee/Institutional Review Board. | Clarified that remote visits may also be conducted by qualified site staff where available and approved.<br>. |
| 39 | Table 4, Schedule of Procedures, Part 2 (table note) | Visits 26 to 28, 30, 31, 33, 34, 36, 37, 39 to 41, 43, and 44 may be conducted in the clinic or remotely by a home healthcare clinician. Remote visits may include a telehealth visit with the investigator as needed. | Visits 26 to 28, 30, 31, 33, 34, 36, 37, 39 to 41, 43, and 44 may be conducted in the clinic or remotely by a home healthcare clinician or qualified site personnel with the agreement of the participant and the discretion of the investigator, and where it is available and locally approved by the Competent Authority and/or Ethics Committee/Institutional Review Board. Remote visits may include a telehealth visit with the investigator as needed. | |
| 39 | Table 4, Schedule of Procedures, Part 2, (footnote b) | During the study drug-free Follow-up Period I, 3 visits will occur to obtain the 3 serum creatinine values for | During the study drug-free Follow-up Period I, 3 visits will occur to obtain the 3 serum creatinine values for calculation of eGFR to | Corrected endpoint for Part 2 of the study. |

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| | | calculation of eGFR to determine the primary endpoint. At the final… | determine the ~~primary endpoint~~ key comparison endpoint. At the final… | |
| 54 | Protocol Section 2.2.1.3, Secondary Efficacy Endpoint | The annualized rate of change (slope) in on-treatment eGFR, based on all on-treatment eGFR determinations during the Double-Blind, Randomized Treatment Period in Part 1, calculated from the CKD-EPI equation for serum creatinine;<br><br>The annualized rate of change from baseline in TKV, determined by MRI, during Follow-up Period I. | The annualized rate of change (slope) from baseline in on-treatment eGFR, based on all on-treatment eGFR determinations during the Double-Blind, Randomized Treatment Period in Part 1, calculated from the CKD-EPI equation (CKD-EPIcr_R) for serum creatinine.<br><br>The annualized ~~rate of~~ change from baseline in TKV, determined by MRI, during Follow-up Period I. | Clarified the method of calculating the eGFR for the primary endpoint.<br><br>Removed reference to rate of change from baseline for TKV analysis. |
| 56 | Protocol Section 2.2.2.6, Population Pharmacokinetics | | **2.2.2.6 Population Pharmacokinetics**<br>• Area under the plasma concentration-time curve during the dosing interval.<br>• Maximum plasma concentration. | For completeness, a population PK-related endpoint has been added for Part 2 of the study. |
| 57 | Protocol Section 3.1.1, Overview of Study Design | Approximately 2000 participants with ADPKD will be screened in order to randomize 1200 participants to lixivaptan or placebo in a 2:1 ratio in Part 1 of the study….. | Approximately ~~2000~~ 2250 participants with ADPKD will be screened in order to randomize ~~1200~~ 1350 participants to lixivaptan or placebo in a 2:1 ratio in Part 1 of the study….<br><br>Participants who have not discontinued due to an adverse event or withdrawn consent will continue into the 2- to 4-week Lixivaptan Re-titration Period to initiate Part 2 of the study. Dosing with lixivaptan 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. Lixivaptan treatment will continue for 52 weeks during the Maintenance Treatment Period after which study drug will be held, and final assessments obtained off-treatment over | Text to briefly summarize Part 2 of the study has been added for completeness. |

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| | | | 3 visits starting on the 8th day after the last dose of double-blind study drug through the 28th day after the last dose of study drug. | |
| 59 | Protocol Section 3.1.2, Detailed Study Design | Placebo Run-in Period (Visit 3): During the 1-week Placebo Run-in Period, participants will receive single-blind placebo and take 4 capsules twice daily. At the end of the week, participants will attend Visit 3… | Placebo Run-in Period (Visit 3): During the 1-week Placebo Run-in Period, participants will receive single-blind placebo and take 4 capsules twice daily. At the end of the 1-week Placebo Run-in Period, participants will attend Visit 3… | Clarification of study period |
| 62 | Protocol Section 3.2, Rationale for Study Design | A placebo-controlled, double-blind assessment of lixivaptan is the preferred study design for demonstrating the efficacy and safety of the drug in the treatment of patients with ADPKD. This type of design was used both in the TEMPO 3:4 and REPRISE studies to assess the efficacy and safety of tolvaptan. Furthermore, following the double-blind phase of the study with a 52-week open label treatment phase allows for the demonstration of the durability of effect of lixivaptan…. | A placebo-controlled, double-blind assessment of lixivaptan is the preferred study design for demonstrating the efficacy and safety of the drug in the treatment of patients with ADPKD. This type of design was used both in the TEMPO 3:4 and REPRISE studies to assess the efficacy and safety of tolvaptan. Furthermore, following the double-blind phase of the study with a 52-week open label treatment phase allows for the demonstration of the durability of effect of lixivaptan as well as assessment of long-term safety…. | Clarified that long term safety will also be assessed as part of Part 2 of the study. |
| 67 | Protocol Section 4.2.2, Screening and Run-in Titration Failures | **4.2.2 Screening and Run-in/Titration Failures**<br>…. During the Screening Period, participants who withdraw their consent or fail to meet all of the entry criteria to participate in the study will be designated as "Screen failures." Screen failures will be recorded as such on the electronic Case Report Form (eCRF)... | **4.2.2 Screening and Placebo Run-in/Lixivaptan Titration Failures**<br>…. During the Screening Period, participants who withdraw their consent or fail to meet all of the entry criteria to participate in the study will be designated as "Screen failures." These participants will not complete an ET visit (Visit 22). Screen failures will be recorded as such on the electronic Case Report Form (eCRF)... | Text revised to clarify that screen failures do not undergo early termination procedures.<br><br>Text revised for consistency and to align with the case report form. |

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| | | Participants who discontinue or are discontinued after screening but before randomization (i.e., during the Placebo Run-in Period or Lixivaptan Titration Period) will be considered "Run-in/Titration failures." A participant may be considered a "Run-in/Titration failure" for the following reasons:<br>• Participant does not meet entry requirements (i.e., participant cannot tolerate study drug as specified for a particular pre-randomization period); or<br>• Participant decides to formally withdraw consent and/or fails to return for subsequent appointments at the trial site; or<br>• Participant experiences an AE or SAE that precludes further participation; or<br>• The Investigator considers the participant unsuitable for further participation.<br>Run-in/Titration failures will be recorded as such on the eCRF. Run-in/Titration Failures will complete an ET visit (Visit 22) upon withdrawal from the trial and within 7 days of the last dose of study drug. The ET visit assessments for Part 1 are delineated in the Schedule of Procedures – Part 1. Run-in/Titration Failures are not required to complete Follow-up Period I, however, Run-in/Titration failures with ongoing AEs will continue to be followed according to the instructions in Section 7.3.8.7. | Participants who discontinue or are discontinued after screening but before randomization (i.e., during the Placebo Run-in Period or Lixivaptan Titration Period) will be considered "Placebo Run-in/Lixivaptan Titration failures." ~~A participant may be considered a "Run-in/Titration failure" for the following reasons:~~<br>~~• Participant does not meet entry requirements (i.e., participant cannot tolerate study drug as specified for a particular pre-randomization period); or~~<br>~~• Participant decides to formally withdraw consent and/or fails to return for subsequent appointments at the trial site; or~~<br>~~• Participant experiences an AE or SAE that precludes further participation; or~~<br>~~The Investigator considers the participant unsuitable for further participation~~<br>~~Run-in/Titration failures will be recorded as such on the eCRF.~~ Placebo Run-in/Lixivaptan Titration Failures will complete an ET visit (Visit 22) upon withdrawal from the trial and within 7 days of the last dose of study drug. The ET visit assessments for Part 1 are delineated in the Schedule of Procedures – Part 1. Placebo Run-in/Lixivaptan Titration failures will not ~~are not required to~~ participate in ~~complete~~ Follow-up Period I, however, Placebo Run-in/Lixivaptan Titration failures with ongoing AEs will continue to be followed according to the instructions in Section 7.3.8.7. | |

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| 81 | Protocol Section 6.1.1 Visit 1a | • Collect body weight and height measurements; enter in the eCRF to calculate BMI. | • Collect body weight and height measurements; ~~enter in the eCRF to calculate~~ BMI will be auto-calculated by the EDC system. | Text revised to reflect that height is not entered in the eCRF (entered in IRT); upon entering height in IRT and weight in the eCRF, BMI will be auto-calculated by the EDC system. |
| 84 | Protocol Section 6.2, Placebo Run-in Period and Visit 3 | • Review the mean eGFR from Visits 1a and 2 or Visits 1b and 2, if Visit 1b was required. If less than 25 ml/min/1.73 $m^2$ or greater than 90 ml/min/1.73 $m^2$, complete procedures for ET visit (Visit 22) and contact the IRT to discontinue the participant from the study. Participant will not be required to complete the Follow-up Period. | • Review the mean eGFR (based on serum creatinine) from Visits 1a and 2 or Visits 1b and 2, if Visit 1b was required. If less than 25 ml/min/1.73 $m^2$ or greater than 90 ml/min/1.73 $m^2$, complete procedures for ET visit (Visit 22) and contact the IRT to discontinue the participant from the study and complete early termination procedures (Section 6.4.2). Participant will not ~~be required to complete~~ participate in the Follow-up Period.<br>• Review ALT, AST, and total bilirubin from Visit 2. If ALT, AST, or total bilirubin >1.2 x ULN complete procedures for ET visit (Visit 22) and contact the IRT to discontinue the participant from the study and complete early termination procedures (Section 6.4.2). Participant will not participate in the Follow-up Period. | Text clarifications added regarding eGFR and liver enzyme-related eligibility criteria.<br><br>Text added to clarify that participants discontinuing after enrollment, but prior to randomization undergo early termination procedures (V22) only, without subsequently entering the follow-up period. |
| 87 | Protocol Section 6.3.1, Visits 4 to 8 (Lixivaptan Titration Period) | At Visit 4, document CKD Stage based on mean eGFR calculated from Visits 1a, 2, and 3 or Visits 1b, 2, and 3 (if Visit 1b was required).<br>○ CKD Stage 2: eGFR 60 to 89 ml/min/1.73 $m^2$ | At Visit 4, document CKD Stage based on mean eGFR calculated from Visits 1a, 2, and 3 or Visits 1b, 2, and 3 (if Visit 1b was required).<br>○ CKD Stage 2: eGFR 60 to ~~89~~ 90 ml/min/1.73 $m^2$ | Expand threshold for CKD Stage 2 to be inclusive of 90 ml/min/1.73 $m^2$ for stratification purposes. |

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| 98 | Protocol Section 6.7.2 Visit 42/Early Termination (Part 2) | Visit 42 is the end of the Maintenance Period and will also serve as the Early Termination visit for Part 2 of the study. For participants completing the Maintenance Period, the visit will occur at Week 52 ± 3 days. | Visit 42 is the end of the Maintenance Period and will also serve as the Early Termination visit for Part 2 of the study. For participants completing the Maintenance Period, the visit will occur at Week ~~52~~ 104 ± 3 days. | Correction of typo |
| 104 | Protocol Section 7.1.2, MRI Assessment | The MRI acquisition protocol will be detailed in the study imaging manual. MRI images will be sent to a central imaging vendor for a) quality control; b) determination of Mayo Clinic ADPKD classification (Screening only); and c) measurement of TKV and LV. | The MRI acquisition protocol will be detailed in the study imaging manual. MRI images will be sent to a blinded central imaging vendor for a) quality control; b) determination of Mayo Clinic ADPKD Image Classification (Class 1 [typical] or Class 2 [atypical] at Screening only); and c) measurement of TKV and LV. | Text revised to clarify that central imaging vendor will be blinded to study treatment and to specify the Mayo Clinic ADPKD Image Classification that the central imaging vendor will provide. |
| 105 | Protocol Section 7.2.3 ADPKD- Pain and Discomfort Scale<br>Protocol Section 7.2.4, ADPKD- Urinary Impact Scale<br>Protocol Section 7.2.4, ADPKD- Urinary Impact Scale | | In this study, participants will directly record their responses into an electronic data capture device (i.e., tablet). During remote visits, participants will record their responses on a paper copy, which will later be transcribed into the database by site personnel. | Added clarity regarding collection of patient-reported outcome (PRO) measures. |
| 107 | Protocol Section 7.3.3, Vital Sign Measurements | Any confirmed, clinically significant vital sign measurements must be recorded as medical history during the Screening and Baseline Periods and as an AE after the start of study drug. | Any confirmed, clinically significant vital sign measurements ~~must~~ should be recorded as medical history ~~during the~~ at Screening Visit 1a ~~and Baseline Periods~~ and as an AE thereafter ~~the start of study drug.~~ | Clarified the guidance for recording clinically significant vital sign measurements as medical history vs. adverse events. |
| 108 | Protocol Section 7.3.4, 12-Lead ECGs | ECGs will be transmitted electronically to the central ECG laboratory for analysis according to the instructions provided by the laboratory to be read by a cardiologist….All CS findings will be reported as medical history during the Screening Period and as AEs after the start of study drug. | ECGs will be transmitted electronically to the central ECG laboratory for analysis according to the instructions provided by the laboratory to be read by a cardiologist….All CS findings ~~will~~ should be reported as medical history ~~during the~~ at Screening ~~Period~~ Visit 1a and as AEs thereafter ~~the start of study drug~~. | Clarified the guidance for recording clinically significant events as medical history versus adverse events. |

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| 108 | Protocol Section 7.3.5, Clinical Laboratory Tests | Blood and urine specimens will be collected for clinical laboratory determinations…. A confirmatory assessment through the central laboratory should be obtained as soon as possible for any CS abnormal laboratory parameter if deemed necessary by the Investigator…. | Blood and urine specimens will be collected for clinical laboratory determinations… A confirmatory assessment through the central laboratory should be obtained as soon as possible for any CS abnormal laboratory parameter if deemed necessary by the Investigator. All CS findings should be reported as medical history at Screening Visit 1a and as AEs thereafter….. | Added guidance regarding reporting of clinically significant events as medical history versus adverse events |
| 109 | Protocol Section 7.3.5.1, Sample Collections. | | Participant age and year of birth will be collected at the time of sample collection and reported on the requisition form. | Clarified that participant age and year of birth will be collected and reported on each requisition form at the time of laboratory sample collection. |
| 112 | Protocol Section 7.3.7.1.2, Liver Test Abnormalities during the Lixivaptan Titration Period | **7.3.7.1.2 Liver Test Abnormalities during the Lixivaptan Titration Period**<br>Serum aminotransferase or total bilirubin levels that are 1× ULN to 2 × ULN during the Lixivaptan Titration Period will require holding the dose constant and a discussion with the medical monitor. Levels exceeding 2 × ULN during the Lixivaptan Titration Period will require reducing the dose or discontinuation of study drug (randomization will not occur) depending on the extent of the elevation and in consultation with the medical monitor.... | **7.3.7.1.2 Liver Test Abnormalities during the Placebo Run-in and/or Lixivaptan Titration Period**<br>Serum aminotransferase or total bilirubin levels that are ~~1~~ ≥1.2× ULN to 2 × ULN during the Placebo Run-in and/or Lixivaptan Titration Period will require holding the dose constant and a discussion with the medical monitor. Levels exceeding 2 × ULN during the Placebo Run-in and/or Lixivaptan Titration Period will require reducing the dose or discontinuation of study drug (randomization will not occur) depending on the extent of the elevation and in consultation with the medical monitor... | Text has been updated to clarify liver enzyme thresholds apply during the Placebo Run-in Period in addition to the Lixivaptan Titration Period. |
| 123 | Protocol Section 8.5.1, Annualized Rate of Change (Slope) in eGFR on Treatment | In order to assess the linear effect (slope) of lixivaptan based on the on-treatment eGFR data, an analysis utilizing a mixed-effects model for repeated measures (MMRM) will be performed on the observed changes from baseline (mean of the 3 eGFR determinations obtained during the | In order to assess the linear effect (slope) of lixivaptan based on the on-treatment eGFR data in all randomized participants, an analysis utilizing a mixed-effects model for repeated measures (MMRM) will be performed on the observed changes in eGFR from baseline (mean of the 3 eGFR determinations obtained during the Screening | The planned secondary analysis of the annualized rate of change (slope) in eGFR during treatment has been clarified. |

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| | | Screening and Placebo Run-in Periods) to Weeks 0 to 52 of the Double-Blind, Randomized Treatment Period in eGFR…. | and Placebo Run-in Periods) ~~to Weeks 0~~ to all Double-Blind Treatment Period visits up to Week 52 ~~of the Double-Blind, Randomized Treatment Period in eGFR~~…. | |
| 123 | Protocol Section 8.5.2, Annualized Rate of Change in TKV | **8.5.2 Annualized Rate of Change in TKV**<br>The 2nd secondary efficacy endpoint is the rate of change in TKV (normalized as a percentage) from baseline for lixivaptan relative to placebo….<br><br>A linear mixed effect model will be fitted to the log transformed TKV data. Further detail will be provided in the statistical analysis plan. | **8.5.2 Annualized ~~Rate of~~ Change in TKV**<br>The 2nd secondary efficacy endpoint is the ~~rate of~~ change in height-adjusted TKV (normalized as a percentage) from baseline for lixivaptan relative to placebo….<br><br>~~A linear mixed effect model will be fitted to the log transformed TKV data~~ ANCOVA will be used to analyze the log transformed TKV data. Further details will be provided in the statistical analysis plan. | Clarified that height-adjusted TKV will be analyzed and clarified the analysis to be used for these data. |
| 125 | Protocol Section 8.7.1, Annualized Rate of Change (Slope) in eGFR on Treatment | In order to assess the linear effect (slope) of lixivaptan based on the on-treatment eGFR data in Part 2…. A linear contrast of the treatment differences for these 12 monthly visits will be used for this analysis. | In order to assess the linear effect (slope) of lixivaptan based on the on-treatment eGFR data in Part 2…. ~~A linear contrast of the treatment differences for these 12 monthly visits will be used for this analysis.~~The estimated annualized rate of change (slope) from this analysis will be compared to the corresponding results in Part 1. | Text regarding analysis of annualized rate of change (slope) in eGFR treatment has been clarified. |
| 125 | Protocol Section 8.7.2, Annualized Rate of Change in TKV | **8.7.2 Annualized Rate of Change in TKV**<br>The TKV measurements at Follow-up Periods I and II will be $\log_{10}$ transformed. | **8.7.2 Annualized ~~Rate of~~ Change in TKV**<br>The height-adjusted TKV measurements at Follow-up Periods I and II will be $\log_{10}$ transformed. | Corrected title/clarified analysis |
| 125 | Protocol Section 8.9.2, ADPKD-IS, ADPKD-PDS, and ADPKD-UIS | Detailed descriptions of analyses of these questionnaires will be specified in a prospective health outcomes analysis plan. | Detailed descriptions of analyses of these questionnaires will be specified in ~~a prospective health outcomes analysis plan~~ the SAP. | Clarified that the details surrounding the health-related quality of life questionnaires will be included in the SAP. |
| 125 | Protocol Section 8.10.1, Annualized Rate of Change in LV | **Protocol, Section 8.10.1, Annualized Rate of Change in LV** | **Protocol, Section 8.10.1, Annualized ~~Rate of~~ Change in LV** | Corrected title/clarified analysis. |

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| 125 | Protocol Section 8.10.2, Urine Osmolality | Descriptive statistics for the change from baseline in morning spot urine osmolality will be presented by treatment group for the Safety Analysis Set in Part 1 and overall, for the Long-term Safety Analysis Set in Part 2 at each scheduled time point. | Descriptive statistics for the change from baseline in morning spot urine osmolality will be presented by treatment group for the Treated Safety ~~Analysis~~ Set in Part 1 and overall, for the Long-term Safety Analysis Set in Part 2 at each scheduled time point. | Corrected name of the analysis set for consistency. |
| 126 | Protocol Section 8.11, Subgroup Analyses | Descriptive statistics will be presented for the primary efficacy estimand for age (≤50 years, >50 years)…. | Descriptive statistics will be presented for the primary and secondary efficacy ~~estimand~~ analyses for age (≤50 years, >50 years)…. | Clarified that subgroup analyses will also be performed for secondary efficacy endpoints. |

## Clinically-relevant Changes

This section describes changes that affect safety or study conduct.

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| Multiple | Global | Visit 3 will occur at the end of the Placebo Run-in Period (± 3 days) | Visit 3 will occur at the end of the Placebo Run-in Period (± ~~3~~ 2 days) | Visit windows for the Placebo Run-in Period were shortened to maintain consistency with visit windows for the Lixivaptan Titration period. |
| Multiple | Global | Visits 4, 5, 6, 7, and 8 (Visit 8 only if needed) will occur weekly ± 3 days for titration… | Visits 4, 5, 6, 7, and 8 (Visit 8 only if needed) will occur weekly ± ~~3~~ 2 days for titration… | Visit windows shortened for visits occurring at weekly intervals to ensure at least 5 days of exposure per dose level, allowing more confidence in the assessment of tolerability at each dose. |
| Multiple | Global | …Visits 26 to 29 will occur weekly ± 3 days... | …Visits 26 to 29 will occur weekly ± ~~3~~ 2 days... | Visit windows shortened for visits occurring at weekly intervals to ensure a minimum of 5 days exposure at each dose level. |
| Multiple | Global | … calculated by the Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI) from serum creatinine values… | … calculated by the 2021 Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) eGFR creatinine equation refit without the race variable (CKD-EPIcr_R) ~~equation~~ from serum creatinine values | Updated the CKD-EPI equation for calculation of serum creatinine- based eGFR from the 2009 CKD-EPI equation to the 2021 CKD-EPIcr_R equation, without the race variable. eGFR calculated from this revised equation will be used for eligibility determination as well as all efficacy analyses. |
| Multiple | Global | Participants with appropriate blood pressure control (as defined by KDIGO) including those on ACEi or ARB treatment (unless not considered appropriate) and not receiving a diuretic will proceed to V2 | Participants with appropriate blood pressure control for a minimum of 3 weeks prior to enrollment (Visit 2) (as defined by the 2021 KDIGO guideline) including those on stable ACEi or ARB treatment (unless not considered | Added timeframe for appropriate blood pressure control for study eligibility for participants who do not require blood pressure medication optimization and clarified it for |

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| | | 3 to 5 weeks after Visit 1a…<br>Participants who need to be discontinued from diuretic therapy and/or have their anti-hypertensive therapy optimized due to inadequate BP control (as defined by KDIGO) will return for V1b… | appropriate) and not receiving a diuretic will proceed to V2 3 to 5 weeks after Visit 1a…<br><br>Participants who need to be discontinued from diuretic therapy and/or have their anti-hypertensive therapy optimized due to inadequate BP control (as defined by the 2021 KDIGO guideline) will return for unscheduled visits for blood pressure monitoring following Visit 1a. Once their treatment and blood pressure have been stable for a minimum of 3 weeks, they will return for V1b… | those who require blood pressure medication optimization.<br><br>Those participants requiring blood pressure medication optimization will attend Unscheduled Visits for blood pressure monitoring until blood pressure has been stable for a minimum of 3 weeks, at which time Visit 1b will be completed. |
| Multiple | Global | | serum cystatin C<br><br>Part 1: Added serum cystatin C to blood samples collected during the Screening Period (V1a, V1b, V2), Placebo Run-in Period (V3), and during the Follow-up Period (V23, V24, and V25) to support exploratory efficacy analyses.<br>Serum cystatin C will be assessed at the time-points specified in the Schedule of Procedures - Part 1 (Table 3) of the study protocol.<br>Protocol text has been updated globally to reflect collection of blood samples for serum cystatin C assessments as applicable.<br>Part 2: Added serum cystatin C to blood samples collected during Follow-up Period II (V43, V44, and V45) to support exploratory efficacy analyses. | Collection of serum cystatin C, in addition to serum creatinine for the determination of eGFR for exploratory analyses based on a novel equation, CKD-EPIcr-cys has been added to the study at Visits 1a, 1b, 2, 3, 23, 24, 25, 43, 44, 45. Serum cystatin C is also to be collected at the same time-points as serum creatinine in the event of a treatment interruption expected to last 7 or more days (Protocol Section 4.2.3). |

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| | | | Serum cystatin C will be assessed at the time-points specified in the Schedule of Procedures - Part 2 (Table 4) of the study protocol.<br>Protocol text has been updated to reflect collection of blood samples for serum cystatin C assessments as applicable. | |
| Multiple | Global | | serum sodium<br><br>Part 1: Added serum sodium to chemistry blood samples collected during the Screening Period (V1a, V1b, V2), Placebo Run-in Period (V3), Lixivaptan Titration Period (V9), Double-blind Randomized Treatment Period (V10-21 and V22), and during the Follow-up Period (V25).<br>Serum sodium will be assessed at the time-points specified in the Schedule of Procedures - Part 1 (Table 3) of the study protocol either as part of chemistry or liver chemistry testing.<br>Protocol text has been updated to reflect additional serum sodium assessments as applicable.<br>Part 2: Added serum sodium to blood chemistry samples collected during the Lixivaptan Re-titration Period (V29), during the Maintenance Treatment Period (V30-V41 and V42/ET), and during the Follow-up Period II (V45).<br>Serum sodium will be assessed at the time-points specified in the Schedule of Procedures - Part 2 (Table 4) of the study protocol either as part of | Serum sodium assessments have been added during additional study visits in Parts 1 and 2 of the study for increased safety monitoring. |

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| | | | chemistry or liver chemistry testing. Protocol text has been updated to reflect additional serum sodium assessments as applicable | |
| Multiple | Global | 1200 randomized, Part 1 (estimated 1080 will continue into Part 2) | ~~1200~~1350 randomized, Part 1 (estimated ~~1080~~ 1215 will continue into Part 2) | Sample size increased as a result of increasing the power in the statistical analysis to 90%. |
| 14 | Protocol Synopsis, Study Sites | Approximately 200 sites worldwide | Approximately ~~200~~ 250 sites worldwide | Increase the number of sites to 250 to meet increased sample size requirements |
| 16, 53, 54 | Protocol Synopsis, Objectives [Exploratory] [Part 1 (Year 1)]<br><br>Protocol Section 2.1.1, Objectives of the Double-Blind Phase (Part 1/Year 1)<br><br>Protocol Synopsis, Objectives [Exploratory [Part 2 (Year 2)]<br><br>Protocol Section 2.1.2, Objectives of the Open-Label Phase (Part 2/Year 2) | | To evaluate the effect of lixivaptan on kidney function as assessed by eGFR using a novel serum creatinine and serum cystatin C-based Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation refit without the race variable (CKD-EPIcr-cys_R) compared with placebo.<br><br>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. | Exploratory objectives related to eGFR based on a novel equation utilizing serum creatinine and serum cystatin C, (CKD-EPIcr-cys) have been added to Parts 1 and 2 of the study. |
| 17, 63 | Protocol Synopsis, Inclusion Criteria<br><br>Protocol Section 4.1.1, Inclusion Criteria | 4. Mayo Clinic ADPKD classification of 1C, 1D, or 1E based on age and height-adjusted total kidney volume as determined by kidney MRI obtained during Screening by the central imaging vendor. | ~~4~~ 3. At risk for rapid progression of ADPKD as based on the Mayo Clinic Image ADPKD classification of 1C, 1D, or 1E based on age and height-adjusted total kidney volume (TKV) as determined by kidney MRI obtained during Screening, where class (class 1 [typical] ~~as assessed~~ versus class 2 [atypical]) and TKV are determined by | This criterion has been clarified to include participants at risk of rapid progression of ADPKD, which in this study is being assessed by Mayo Image Classification (MIC). In MIC, class 1 is typical ADPKD and class 2 is atypical ADPKD. The MIC and TKV are determined by |

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| | | | ~~the~~ a central imaging vendor. | a central imaging vendor. |
| 17, 64 | Protocol Synopsis,<br><br>Protocol Section 4.1.1, Inclusion Criteria | 5. eGFR ≥25 mL/min/1.73 $m^2$ 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 Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI) from serum creatinine values obtained during Screening… | ~~5~~ 4. eGFR ≥25 mL/min/1.73 $m^2$ 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) equation from serum creatinine values obtained during Screening... | Calculation of eGFR in the inclusion criteria updated from the CKD-EPI to the CKD-EPIcr_R, which removes the race variable from the calculation of eGFR. |
| 17, 64 | Protocol Synopsis,<br><br>Protocol Section 4.1.1, Inclusion Criteria | 6. Appropriate control of hypertension including an angiotensin converting enzyme inhibitor or angiotensin receptor blocker (unless not considered appropriate for the participant) as suggested by the 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.~~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. | Added a 3-week minimum timeframe of BP control for all participants. |
| 18, 64, 77 | Protocol Synopsis,<br><br>Protocol Section 4.1.1, Inclusion Criteria<br><br>Protocol Section 5.5, Prior and Concomitant Therapy | 7. Female participants must:<br>• not be pregnant, lactating, or breastfeeding.<br>• 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 (WOCBP)…<br>• hormonal contraceptives: | 7. Female participants must:<br>a. not be pregnant, lactating, or breastfeeding.<br>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- | Text added within the Inclusion Criteria and protocol body to highlight the potential risk of estrogen-containing contraceptives and hormone replacement therapy on the risk of cyst growth and polycystic liver disease progression.<br><br>Criterion text in Protocol |

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| | | 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).<br><br>Any concomitant medication deemed necessary for the well-being of the participant during the study may be given at the discretion of the Investigator after consideration of the clinical situation (Section 5.5). The Investigator is responsible… | bearing potential (WOCBP)…<br>• 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 and should be discussed between the Investigator and the potential participant.<br><br>Any concomitant medication deemed necessary for the well-being of the participant during the study may be given at the discretion of the Investigator after consideration of the clinical situation (Section 5.5). **Note**: in female participants with severe polycystic liver disease, contraceptives (and hormone replacement therapy) containing estrogen may be involved in the development and growth of liver cysts and polycystic liver disease progression and should be discussed between the Investigator and the potential participant. The Investigator is responsible… | Section 4.1.1 has been harmonized with text for same criterion in the Protocol Synopsis. |

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| 20, 66 | Protocol Synopsis, Study Population<br><br>Protocol, Section 4.1.2, Exclusion Criteria. | 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.) 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, ~~or~~ 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. | Updated inclusion criterion to reflect additional supplements that may confound safety or efficacy assessments in this study. |
| 20, 57 | Protocol Synopsis, Study Design<br><br>Protocol, Section 3.1.1, Overview of Study Design | Approximately 2000 participants with ADPKD meeting the entry criteria will be screened in order to randomize 1200 participants to lixivaptan or placebo in a 2:1 ratio in Part 1 of this study. | Approximately ~~2000~~ 2250 participants with ADPKD ~~meeting the entry criteria~~ will be screened in order to randomize ~~1200~~1350 participants to lixivaptan or placebo in a 2:1 ratio in Part 1 of this study. | Sample size increased as a result of increasing the power in the statistical analysis to 90%. |
| 26 | Protocol Synopsis, Study Drug, Dosage, and Route of Administration (Table 2) | **Table 2. Dose Titration Based on Dose Achieved at End of Double-blind Randomized Treatment Period in Part 1**<br><br>Dose in Part I* / Week 1 / Week 2 / Week 3 / Week 4<br>100mg BID / 50mg BID / 100mg BID / / <br>150mg BID / 50mg BID / 100mg BID / 150mg BID / <br>150/100mg (AM/PM) / 50mg BID / 100mg BID / 150/100mg (AM/PM) / <br>200mg BID / 50mg BID / 100mg BID / 150mg BID / 200mg BID<br>200/150mg (AM/PM) / 50mg BID / 100mg BID / 150mg BID / 200/150mg (AM/PM)<br><br>*Participants whose dose was reduced to Level 1 (50 mg BID) during the double-blind phase as a result of tolerability issues or treatment-emergent AEs can continue on that dose during the open-label phase. These participants will initiate dosing at 50mg BID and remain on that dose for 2 weeks during the Re-titration Period and initiate the open-label phase at that dose. Periodic attempts should be made to re-establish dosing at Level 2 (100 mg BID) during the open-label phase. | **Table 2. Dose Titration (Part 2) Based on Dose Level Achieved at End of Double-blind Randomized Treatment Period in Part 1 for Participants Assigned to Lixivaptan or Placebo in Part 1**<br><br>Dose Level in Part I* / Week 1 / Week 2 / Week 3 / Week 4<br>Level 1 100mg BID or Placebo / 50mg BID / 100mg BID / / <br>Level 2 150mg BID or Placebo / 50mg BID / 100mg BID / 150mg BID / <br>Level 3a 150/100mg (AM/PM) or Placebo / 50mg BID / 100mg BID / 150/100mg (AM/PM) / <br>Level 4 200mg BID or Placebo / 50mg BID / 100mg BID / 150mg BID / 200mg BID<br>Level 4a 200/150mg (AM/PM) or Placebo / 50mg BID / 100mg BID / 150mg BID / 200/150mg (AM/PM)<br><br>*Participants whose dose was reduced to Level 1 (50 mg BID lixivaptan or placebo) during the double-blind phase as a result of tolerability issues or treatment-emergent AEs can continue on that dose during the open-label phase. These participants will initiate dosing with lixivaptan at 50mg BID and remain on that dose for 2 weeks during the Re-titration Period and initiate the open-label phase at that dose. Periodic attempts should be made to re-establish dosing at Level 2 (100 mg BID) during the open-label phase. | Table 2 has been revised to clarify dose re-titration during the Lixivaptan re-titration Period in Part 2, for participants in either the lixivaptan treatment arm in Part 1 or the placebo treatment arm in Part 1. |
| 27 | Protocol Synopsis, Study Assessments | **Safety:** Liver chemistry tests (ALT, AST, total bilirubin, direct bilirubin, alkaline phosphatase), clinical laboratory parameters (hematology, non-hepatic clinical chemistry, and | **Safety:** Liver chemistry tests (ALT, AST, total bilirubin, direct bilirubin, alkaline phosphatase), clinical laboratory parameters (hematology, non-hepatic clinical chemistry~~,~~ | Added serum sodium as an additional safety assessment at designated visits. |

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| | | urinalysis), 12 lead ECGs, vital signs, physical examinations, adverse events (AEs), and serious adverse events (SAEs) | including serum sodium and urinalysis), 12-lead ECGs, vital signs, physical examinations, adverse events (AEs), and serious adverse events (SAEs) | |
| 27 | Protocol Synopsis, Study Assessments | **Exploratory:** LV by MRI, urine osmolality | **Exploratory:** LV by MRI, urine osmolality, serum creatinine with cystatin C- based eGFR | Added serum creatinine with cystatin C- based eGFR as additional exploratory assessments. |
| 27, 54 | Protocol Synopsis, Criteria for Evaluation [Primary Efficacy Endpoint]<br><br>Protocol Section 2.2.1.1, Primary Efficacy Endpoint- | **Primary Efficacy Endpoint:** Annualized change in eGFR calculated from the CKD-EPI equation for serum creatinine from baseline…<br><br>The primary endpoint is the annualized change in eGFR calculated from the CKD-EPI equation for serum creatinine from baseline… | **Primary Efficacy Endpoint:** Annualized change in eGFR calculated from the 2021 CKD-EPI equation (CKD-EPIcr_R) for serum creatinine from baseline…<br><br>The primary endpoint is the annualized change in eGFR calculated from the ~~CKD-EPI~~ CKD-EPIcr_R equation for serum creatinine from baseline… | Updated the CKD-EPI equation for calculation of serum creatinine- based eGFR for the primary efficacy endpoint from the 2009 CKD-EPI equation to the 2021 CKD-EPIcr_R equation, without the race variable. |
| 27, 121 | Protocol Synopsis, Statistical Methods, Sample Size (Part 1) | 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 1155 participants (385 placebo participants and 770 lixivaptan participants) is required to achieve 85% power at a significance level of 0.01. In order to compensate for possible missing data, this estimate has been adjusted up to 1200 participants (400 placebo participants and 800 lixivaptan participants). It is estimated that approximately 2000 participants will need to be screened in order to randomize 1200 participants. | 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 ~~1155~~1314 participants (~~385~~ 438 placebo participants and ~~770~~ 876 lixivaptan participants) is required to achieve ~~85~~ 90% power at a significance level of 0.01. In order to compensate for possible ~~missing data~~ drop outs, this estimate has been adjusted up to ~~1200~~1350 participants (~~400~~ 450 placebo participants and ~~800~~ 900 lixivaptan participants). It is estimated that approximately ~~2000~~ 2250 participants will need to be screened | Sample size increased as a result of increasing the power in the statistical analysis to 90%. |

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| | Protocol Section 8.1, Sample Size | **Part I:**<br><br>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 1155 participants (385 placebo-treated participants and 770 lixivaptan-treated participants) is required to achieve 85% power at a significance level of 0.01. In order to compensate for possible missing data, this estimate has been adjusted up to 1200 participants (400 placebo participants and 800 lixivaptan participants). It is estimated that approximately 2000 participants will need to be screened in order to randomize 1200 participants.<br><br>A blinded sample size re-estimation (BSSR) to estimate the variance corresponding to the primary efficacy estimand is planned after 20% of the total randomized participants have completed Part 1 of the study or before screening for the study has ended. (Section 8.1).<br><br>**Part 2:** | in order to randomize ~~1200~~1350 participants.<br><br>**Part ~~I~~1:**<br><br>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 ~~1155~~ 1314 participants (~~385~~ 438 placebo-treated participants and ~~770~~ 876 lixivaptan-treated participants) is required to achieve ~~85~~ 90% power at a significance level of 0.01. In order to compensate for possible ~~missing data~~ drop outs, this estimate has been adjusted up to ~~1200~~ 1350 participants (~~400~~ 450 placebo participants and ~~800~~ 900 lixivaptan participants). It is estimated that approximately ~~2000~~ 2250 participants will need to be screened in order to randomize ~~1200~~ 1350 participants.<br><br>A blinded sample size re-estimation (BSSR) to estimate the variance corresponding to the primary efficacy estimand is planned after at least 20% of the total randomized participants | |

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| | | This is a convenience sample based on the number of participants who elect to continue into Part 2. It is estimated that approximately 1200 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% (1080) of Part 1 participants are anticipated to continue into Part 2. | have completed Part 1 of the study or before screening for the study has ended. (Section 8.1).<br><br>**Part 2:**<br><br>This is a convenience sample based on the number of participants who elect to continue into Part 2. It is estimated that approximately ~~1200~~ 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% (~~1080~~ 1215) of Part 1 participants are anticipated to continue into Part 2. | |
| 28, 54, 122 | Protocol Synopsis, Statistical Methods [Primary Efficacy Analysis Part 1] | Baseline eGFR is defined as the mean of the 3 eGFR assessments obtained during the Screening and single-blind, Placebo Run-in Periods... The post-treatment follow-up eGFR is defined as the mean of 3 eGFR assessments obtained during the Follow-up Period I or the equivalent off-therapy eGFR determinations if a participant discontinues study drug earlier. | Baseline eGFR is defined as the mean of the 3 eGFR assessments obtained during the Screening and single-blind, Placebo Run-in Periods… The post-treatment follow-up eGFR is defined as the mean of 3 eGFR assessments obtained during the Follow-up Period I or the equivalent off-therapy eGFR determinations if a participant discontinues study drug earlier. The eGFR for the primary analysis will be based on serum creatinine using the CKD-EPIcr_R equation. | Clarified that the primary endpoint analysis will be based on the CKD-EPIcr_R equation. |
| | Protocol Section 2.2.1.1, Primary Efficacy Endpoint | The primary endpoint is the annualized change in eGFR calculated from the CKD-EPI equation for serum creatinine from baseline (mean of 3 eGFR determinations obtained during | The primary endpoint is the annualized change in eGFR calculated from the ~~CKD-EPI~~ CKD-EPIcr_R equation for serum creatinine from baseline (mean of 3 eGFR determinations obtained during | |

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| | Protocol Section 8.4.1, Primary Endpoint Analysis | Screening and Placebo Run-in Periods (Visits 1a/1b (if required), Visit 2, and Visit 3) to final assessment…<br>Baseline eGFR is defined as the mean of the 3 eGFR determinations obtained during the Screening and Placebo Run-in Periods. The primary efficacy analysis will utilize… | Screening and Placebo Run-in Periods (Visits 1a/1b (if required), Visit 2, and Visit 3) to final assessment…<br><br>The primary endpoint analysis is based on the eGFR calculated from the CKD-EPIcr_R equation. Baseline eGFR is defined as the mean of the 3 eGFR determinations obtained during the Screening and Placebo Run-in Periods. The primary efficacy analysis will utilize… | |
| 33, 82, 83 | Table 3. Schedule of Procedures – Part 1<br><br>Protocol Section 6.1.2, Visit 1b<br><br>Protocol Section 6.1.3, Visit 2 | • Collect and review any additional medical history since prior visit. | Schedule of Procedures updated to remove Medical History review from visits 1b and 2.<br>• ~~Collect and review any additional medical history since prior visit~~. | Removed collection of medical history from Visits 1b and 2 as all medical history should be collected at Visit 1a.. |
| 34, 36, 38, 40, 89 | Table 3. Schedule of Procedures - Part 1 (footnote e) | V10-V21 | V10-V21[e]<br><br>Telephone contact[e]<br><br>e. As participants transition into the Double-Blind Randomized Treatment Period, with visits scheduled every 4 weeks, participant follow-up via a telephone contact is strongly encouraged. Telephone contacts should be repeated throughout the study as needed. Any contact with the participant via telephone will be recorded in the source document | Added optional telephone contacts as distinct study assessments at Visits 10 to 25. Telephone contacts are strongly encouraged throughout the study, as needed.<br><br>Added footnote “e” to encourage sites to contact participants to assess tolerability as they transition into DBTP where visits are 4 weeks apart (and throughout the study as needed). |

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| | Table 4. Schedule of Procedures - Part 2 (footnote l)<br><br>Protocol Section 6.3.2 Visit 9/Last Titration Visit | | Telephone contact[l]<br><br>l. Participant follow-up via a telephone contact between study visits is strongly encouraged. Telephone contacts should be repeated throughout the study as needed. Any contact with the participant via telephone will be recorded in the source document.<br><br>**Note**: As participants transition into the Double-Blind Randomized Treatment Period with visits scheduled every 4 weeks, it is strongly encouraged to follow up with participants via a telephone contact to ascertain continued tolerability. Telephone contacts should be repeated throughout the study as needed and recorded in the source document. | As result of this insertion, all subsequent footnotes have re-ordered.<br><br>Added optional telephone contacts as a distinct study assessments at Visits 26 to 44. Telephone contacts are strongly encouraged throughout the study, as needed.<br><br>Added footnote "l" to Table 4 to encourage telephone contacts<br><br>Corresponding text has been added for consistency to Section 6.3.2. |
| 36, 39, 68, 92, 100 | Table 3. Schedule of Procedures – Part 1 (footnote f) | f. Participants who discontinue study drug or withdraw from the study at any time after the start of the Placebo Run-In Period should undergo V22/ET procedures within 7 days of the last dose of study drug. Randomized participants should additionally continue into Follow-up Period I for final assessments of | f. Participants who discontinue study drug or withdraw from the study at any time after the start of the Placebo Run-In Period should undergo V22/ET procedures within 7 days of the last dose of study drug. Randomized participants should additionally continue into Follow-up Period I | Following Regulatory Authority feedback, MRI data to be collected for all participants with a study drug interruption expected to last 7 or more days. Relevant text for Parts 1 and 2 of the study have been updated accordingly. |

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| | | safety and efficacy. For participants who permanently discontinue treatment prior to Visit 12 (Week 12), the post-baseline MRI scheduled for Visit 25 is not required. | for final assessments of safety and efficacy. ~~For participants who permanently discontinue treatment prior to Visit 12 (Week 12), the post-baseline MRI scheduled for Visit 25 is not required.~~ | |
| | Table 4. Schedule of Procedures – Part 2 (footnote c) | c. Participants who discontinue from the study should undergo V42/ET procedures within 7 days of the last dose of lixivaptan and subsequently continue into Follow-up Period II for final assessments of safety and efficacy. For participants who permanently discontinue study drug prior to Visit 32 (Week 64), the post-baseline MRI scheduled for Visit 45 is not required. | c. Participants who discontinue from the study should undergo V42/ET procedures within 7 days of the last dose of lixivaptan and subsequently continue into Follow-up Period II for final assessments of safety and efficacy. ~~For participants who permanently discontinue study drug prior to Visit 32 (Week 64), the post-baseline MRI scheduled for Visit 45 is not required.~~ | |
| | Protocol Section 4.2.3 Treatment Interruption | If a study drug interruption is expected to last 7 or more consecutive days… If, in the Investigator's opinion, the treatment interruption is expected to be prolonged (i.e., >4 weeks in duration) or permanent, then a postbaseline MRI should also be obtained if the study drug interruption occurs at or after Visit 12 (Week 12) in Part 1 or at or after Visit 32 (Week 64) in Part 2. | If a study drug interruption is expected to last 7 or more consecutive days…If, in the Investigator's opinion, the treatment interruption is expected to be prolonged (i.e., >4 weeks in duration) or permanent, then a postbaseline MRI should also be obtained ~~if the study drug interruption occurs at or after Visit 12 (Week 12) in Part 1 or at or after Visit 32 (Week 64) in Part 2~~. | |
| | Protocol Section 4.2.4 Treatment Discontinuation | Participants may elect to stop treatment permanently before the end of the study for various reasons...Participants who permanently discontinue study drug prior to Visit 12 (Week 12 in Part 1) or Visit 32 (Week 64 in Part 2) will forego | Participants may elect to stop treatment permanently before the end of the study for various reasons...~~Participants who permanently discontinue study drug~~ | |

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| | | the postbaseline MRI scheduled for Visit 25 or Visit 45, in Part 1 and Part 2, respectively. Those discontinuing at or after Visit 12 in Part 1 or Visit 32 in Part 2 should complete the postbaseline MRI either up to 3 days prior to or on the same day as the other Visit 25 or Visit 45 study assessments.<br><br>….<br>• Schedule participant for the post-baseline MRI for assessment of TKV and LV to occur 28 ± 3 days after the last dose; the MRI should occur up to 3 days prior to or on the same day as Visit 25 (**Note**: Participants who permanently discontinue treatment prior to Visit 12 (Week 12) are not required to undergo a post-baseline MRI). | ~~prior to Visit 12 (Week 12 in Part 1) or Visit 32 (Week 64 in Part 2) will forego the postbaseline MRI scheduled for Visit 25 or Visit 45, in Part 1 and Part 2, respectively. Those discontinuing at or after Visit 12 in Part 1 or Visit 32 in Part 2 should complete the postbaseline MRI either up to 3 days prior to or on the same day as the other Visit 25 or Visit 45 study assessments.~~<br><br>….<br>• **Randomized Participants:** Schedule participant for the post-baseline MRI for assessment of TKV and LV to occur between 25 days and 31 days post last dose of study drug and must be completed prior to or on the same day as all other Visit 25 study procedures ~~(**Note**: Participants who permanently discontinue treatment prior to Visit 12 (Week 12) are not required to undergo a post-baseline MRI)~~. | |
| | Protocol Section 6.4.2 Visit 22/Early Termination | | | |
| | Protocol Section 6.7.2 Visit 42/Early Termination (Part 2) | • Schedule participant for the post-baseline MRI for assessment of TKV and LV to occur 28 ± 3 days after the last dose… (**Note**: Participants who permanently discontinue treatment prior to Visit 32 (Week 64) are not required to undergo a post-baseline MRI). | • Schedule participant for the post-baseline MRI for assessment of TKV and LV to occur between 25 days and 31 days after the last dose… ~~(**Note**: Participants who permanently discontinue treatment prior to Visit 32 (Week 64) are not required to undergo a post-baseline MRI)~~. | |

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| 37 | Table 3, Schedule of Procedures, Part 1, (footnote k) | k. Liver chemistry includes alkaline phosphatase; ALT; AST; bilirubin (total and direct). Blood samples for serum creatinine will be collected… Although serum creatinine results will be provided to Investigators as part of the chemistries to allow safety evaluation, eGFRs derived from the serum creatinine results post-randomization (Visit 9) will NOT be shared with the participant, the investigator, the clinical sites, the sponsor, or the contract research organization (CRO) because of the potential for unblinding. Laboratory test results from samples collected as specified in Table 3 will be reviewed and assessed. | ~~k~~ l. Liver chemistry includes alkaline phosphatase; ALT; AST; bilirubin (total and direct). Blood samples for serum creatinine and serum cystatin C will be collected…~~Although serum creatinine results will be provided to Investigators as part of the chemistries to allow safety evaluation, eGFRs derived from the serum creatinine results post-randomization (Visit 9) will NOT be shared with the participant, the investigator, the clinical sites, the sponsor, or the contract research organization (CRO) because of the potential for unblinding……~~ Laboratory test results from samples collected as specified in Table 3 ~~will~~ are to be reviewed and assessed. | To facilitate safety oversight of participants, and to enable the Sponsor to conduct the Blinded Sample Size Re-estimation, eGFR values will be provided to sites post-randomization. |
| 37 | Table 3, Schedule of Procedures – Part 1, (footnote m) | Serology (HBsAg, Anti-HCV) | Serology (HBsAg, Anti-HCV)[m]<br>Participants with positive test results for hepatitis C (HCV) antibody (Anti-HCV), will require a negative HCV RNA titer reflex test prior to enrollment.<br>HCV seropositive participants will undergo reflex testing at an Unscheduled Visit prior to Visit 2. | Added clarification that retest after a positive HCV antibody should be performed at an unscheduled visit before Visit 2. |
| 51 | Protocol Section 1.9 Benefit/Risk Assessment | | Safety data gathered to date in participants with ADPKD are consistent with the robust safety experience from studies in earlier indications investigated for lixivaptan—congestive heart failure, liver cirrhosis with ascites, or syndrome of inappropriate antidiuretic hormone secretion. The primary | A benefit/risk assessment has been included for Investigators' reference. |

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| | | | adverse effects of lixivaptan are related to the exaggerated pharmacology of blockade of the renal concentrating mechanism. This results in urinary dilution with the production of increased amounts and frequency of urine output and, secondarily, the stimulation of thirst. No adverse effects on other body systems have been noted. Additionally, pharmacodynamic data generated for vasopressin V2 receptor antagonists that assess the mechanism of action, primarily through the development program for tolvaptan, have been confirmed in Phase 2 studies with lixivaptan and support continued development of lixivaptan for this indication. As there additionally remains a large unmet medical need to address chronic treatment in patients with ADPKD, particularly those who have experienced liver chemistry abnormalities leading to discontinuation of treatment with tolvaptan, continued investigation of this drug for ADPKD is warranted. Based on the totality of evidence generated to date on lixivaptan, the benefit/risk assessment of lixivaptan remains favorable. | |
| 54, 56 | Protocol Section 2.2.1.4 Secondary Safety Endpoints<br><br>Protocol Section 2.2.2.4, Secondary Safety | Clinical laboratory findings (clinical chemistry including additional analyses of liver chemistry, hematology and urinalysis); | Clinical laboratory findings (clinical chemistry including additional analyses of liver chemistry~~,~~ and serum sodium, hematology and urinalysis); | Added serum sodium clinical laboratory findings as part of secondary safety endpoints to Parts 1 and 2 of the study. |

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| | Endpoints | | | |
| 55, 56 | Protocol Section 2.2.1.7, Exploratory Endpoints<br><br>Protocol Section 2.2.2.7 Exploratory Endpoints [Part 2/Year 2] | The annualized rate of change from baseline in LV determined by MRI during Follow-up Period I;<br><br>… | The annualized ~~rate of~~ change from baseline in LV determined by MRI during Follow-up Period I;<br><br>…<br>The annualized change in eGFR calculated from the CKD-EPIcr-cys_R equation from baseline to final assessment. | Clarified endpoint related to liver volume (LV)<br><br>Added additional exploratory endpoint. |
| 55 | Protocol Section 2.2.2.1, Key Comparison Endpoint | The primary endpoint of Part 2 is the annualized change in eGFR calculated from the CKD-EPI equation for serum creatinine from baseline… | The primary endpoint of Part 2 is the annualized change in eGFR calculated from the CKD-EPI equation (CKD-EPIcr_R) for serum creatinine from baseline… | Clarified the method of calculating the eGFR for the key comparison endpoint |
| 55 | Protocol Section 2.2.2.3, Other Comparison Endpoints | The annualized rate of change in eGFR (calculated from the CKD-EPI equation for serum creatinine) from baseline (mean of 3 eGFR determinations obtained during Follow-up Period I) to Follow-up Period II;<br>The annualized rate of change in TKV determined by MRI. | The annualized rate of change (slope) from baseline (mean of 3 eGFR determinations obtained during Follow-up Period I) in on-treatment eGFR, based on all on-treatment eGFR determinations during the Maintenance Treatment Period in Part 2, calculated from the CKD-EPI equation (CKD-EPIcr_R) for serum creatinine~~The annualized rate of change in eGFR (calculated from the CKD-EPI equation for serum creatinine) from baseline (mean of 3 eGFR determinations obtained during Follow-up Period I) to Follow-up Period II~~;<br>The annualized ~~rate~~ of change in TKV determined by MRI. | Clarified the method of calculating the rate of change in eGFR and change in TKV for other comparison endpoint |

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| 58 | Protocol Section 3.1.2, Detailed Study Design, Part 1 | Participants are eligible for enrollment if they are between the ages of 18 to 60 years (inclusive)… and meet all other study entry criteria (Section 4.1). Participants who fail inclusion/exclusion criteria due to temporary or correctable reasons… | Participants are eligible for enrollment if they are between the ages of 18 to 60 years (inclusive)…and meet all other study entry criteria (Section 4.1). Participants who were originally enrolled based on the 2009 CKD-EPI equation (Appendix 1 (Section 13.1)) will not be discontinued from the study should their recalculated baseline eGFR, based on the CKD--EPIcr_R equation, yield a mean eGFR value outside of the range of 25 to 90 mL/min/1.73 $m^2$. Participants who fail inclusion/exclusion criteria due to temporary or correctable reasons... | Clarified that any participants enrolled under the eGFR criterion of the original protocol (Version 1.0), would remain in the study, if their recalculated baseline eGFR based on the CKD-EPIcr_R equation yielded a value outside the range for study enrollment. |
| 59 | Protocol Section 3.1.2 Detailed Study Design Part 1: | Tolerability will be assessed at each titration visit (Visit 4 to Visit 9, as applicable). If the participant confirms the dose can be tolerated, the participant will be titrated to the next dose level (i.e., the dose will be increased). This assessment of tolerability will continue at weekly intervals until either the maximum dose level is reached (Level 4 [200 mg BID]) or until the participant confirms the dose cannot be tolerated for a 24-month period. If the dose cannot be tolerated, it will be reduced by one dose level as described in the… | Tolerability will be assessed at each titration visit (Visit 4 to Visit 9, as applicable). If the participant confirms the dose can be tolerated, the participant will be titrated to the next dose level (i.e., the dose will be increased). This assessment of tolerability will continue at weekly intervals until either the maximum dose level is reached (Level 4 [200 mg BID]) or until the participant confirms the dose cannot be tolerated for a 24-month period. If the dose cannot be tolerated, it will be reduced by one dose level per titration visit as described in the… | To clarify that reduction of one dose level is per titration visit. |
| 61 | Protocol Section 3.1.2, Detailed Study Design | Maintenance Period (Visits 30 to 42): Following completion of the Lixivaptan Re-titration Period, participants will | Maintenance Period (Visits 30 to 42): Following completion of the Lixivaptan Re-titration Period, | Text has been revised for clarity on dosing during Part 2 of the study. including an explanation |

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| | | continue to receive lixivaptan in the Maintenance Period for 52 weeks at the same dose achieved at the end of the Re-titration Period in Part 2. Visits will be performed every 4 weeks during this period. Assessments at each visit are detailed in the Schedule of Procedures – Part 2. | participants will continue to receive lixivaptan in the Maintenance Period for 52 weeks at the same dose achieved at the end of the Re-titration Period in Part 2. If the participant's dose was decreased during the Lixivaptan Re-Titration Period, periodic attempts should be made to re-establish the previously achieved dose level from Part 1. Visits will be performed every 4 weeks during this period. Assessments at each visit are detailed in the Schedule of Procedures – Part 2. **Note**: during the Re-titration Period of Part 2, all participants are re-titrated with lixivaptan to the last actual dose level if they had received lixivaptan during the Double-blind, Randomized Treatment Period, 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). This will be performed by the IRT and the site, participant, and sponsor maintain a blinded status to the prior treatment assignment in Part 1. If no adjustment is made to the inferred dose during the Double-Blind, Randomized Treatment Period, the last inferred dose for a participant randomized to placebo will be equal to the maximal tolerated lixivaptan | of re-titration with emphasis on participants who had been assigned to the placebo treatment arm during Part 1 of the study. |

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| | | | dose established during the Titration Period in Part 1…. | |
| 63 | Protocol Section 4.1, Selection of Study Population | Approximately 2000 participants will be screened in order to randomize 1200 participants at approximately 200 sites globally. | Approximately ~~2000~~ 2250 participants will be screened in order to randomize ~~1200~~ 1350 participants at approximately ~~200~~ 250 sites globally. | Sample size increased as a result of increasing the power in the statistical analysis to 90%. |
| 67 | Protocol Section 4.2.3, Treatment Interruption | The participant should immediately inform the Investigator of any missed doses reaching or expected to reach 2 days or more so that the Investigator can continue to monitor the participant's treatment and prepare for a possible extended study drug interruption...<br><br>…In the event of a prolonged study drug interruption (i.e., greater than 4 weeks in duration), participants re-initiating treatment are required to re-titrate to their last tolerated dose over 2 weeks. Re-initiation of treatment will begin with study drug at Level 2 for one week and will then be increased to the highest level the participant was receiving prior to the | The participant should be instructed to immediately inform the Investigator of any missed doses reaching or expected to reach 2 days or more so that the Investigator can continue to monitor the participant's treatment and prepare for a possible extended study drug interruption. The Investigator and/or site staff will notify the CRA and/or medical monitor of a potential interruption within 24 hours…<br><br>Participants with a study drug interruption 4 weeks or less in duration should resume study treatment at their last dispensed dose (or at a lower dose, if medically appropriate) without the need for stepwise dose re-establishment.<br><br>Participants with ~~In the event of~~ a prolonged study drug interruption (i.e., greater than 4 weeks in duration), ~~participants~~ who are re-initiating treatment are required to ~~re-titrate~~ ~~re-~~establish their last tolerated dose over a 2-week interval~~s~~. Re-initiation of treatment will begin with study drug at Level 2 for one week and will then be increased to the highest level the | Included notification of the CRA and/or medical monitor in the treatment interruption procedures in an effort to mitigate potential missing data...<br><br>Clarified that drug interruptions 4 weeks or less in duration do not require stepwise dose re-establishment, unless medically necessary.<br><br>Changed terminology from "re-titrate" to "re-establish" in text and title of Table 6.<br><br>Included a reference to the pharmacy manual for additional details related to dispensing study drug following a prolonged study drug interruption.<br>Added a footnote to Table 6 that specifies that a lower dose than that shown in the table may be assigned during Week 2 of dose re-establishment if clinically indicated. |

<table>
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<tr><td></td><td></td><td>study drug interruption during the second week as described in Table 6. Re-titrations will be managed by the IRT system.<br><br>Table 6. Dose Re-titration Following a Prolonged (> 4-week) Study Drug Interruption<br><table><tr><th>Last tolerated study drug dose level/ dose*</th><th>Week 1</th><th>Week 2**</th></tr><tr><td>Level 2/<br>100mg BID</td><td>100mg BID</td><td>100mg BID</td></tr><tr><td>Level 3/<br>150mg BID</td><td>100mg BID</td><td>150mg BID</td></tr><tr><td>Level 3a/<br>150/100mg (AM/PM)</td><td>100mg BID</td><td>150/100mg<br>(AM/PM)</td></tr><tr><td>Level 4/<br>200mg BID</td><td>100mg BID</td><td>200mg BID</td></tr><tr><td>Level 4a/<br>200/150mg (AM/PM)</td><td>100mg BID</td><td>200/150mg (AM/PM)</td></tr></table>*Participants whose last tolerated dose level was 50mg BID prior to the prolonged treatment interruption, will re-initiate study drug dosing at Level 1/ 50mg BID.</td><td>participant was receiving prior to the study drug interruption during the second week as described in Table 6 (see pharmacy manual for further details related to study drug dispensing following a prolonged drug interruption.) The IRT system will be used to manage re-establishment of dose. The dose level assigned during Week 2 may be lowered based on Investigator discretion and clinical need. ~~Re-titrations will be managed by~~ The IRT system will be used to manage re-establishment of dose.<br><br>Table 6. Dose Re~~-titration~~ establishment Following a Prolonged (> 4-week) Study Drug Interruption<br><table><tr><th>Last tolerated study drug dose level/ dose*</th><th>Week 1</th><th>Week 2**</th></tr><tr><td>Level 2/<br>100mg BID</td><td>100mg BID</td><td>100mg BID</td></tr><tr><td>Level 3/<br>150mg BID</td><td>100mg BID</td><td>150mg BID</td></tr><tr><td>Level 3a/<br>150/100mg (AM/PM)</td><td>100mg BID</td><td>150/100mg<br>(AM/PM)</td></tr><tr><td>Level 4/<br>200mg BID</td><td>100mg BID</td><td>200mg BID</td></tr><tr><td>Level 4a/<br>200/150mg (AM/PM)</td><td>100mg BID</td><td>200/150mg (AM/PM)</td></tr></table>*Participants whose last tolerated dose level was 50mg BID prior to the prolonged treatment interruption, will re-initiate study drug dosing at Level 1/ 50mg BID.<br>**A lower dose level may be assigned at Week 2 if clinically indicated.</td><td></td></tr>
<tr><td>69</td><td>Protocol Section 4.2.4, Treatment Discontinuation</td><td>Participants may elect to stop treatment permanently before the end of the study for various reasons. Every effort should be made to keep randomized participants in the study and offer them options for continued treatment and participation to the</td><td>Participants may elect to stop treatment permanently before the end of the study for various reasons. Every effort should 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</td><td>Added text to emphasize retention of participants in the study even after treatment discontinuation in an effort to minimize missing data. A reference to the protocol appendix related to minimizing</td></tr>
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| | | degree possible as described in Section 4.2.7 in order to minimize missing data. Participants who permanently discontinue study drug will be recorded as study drug discontinuations on the eCRF. They will have an ET visit (Visit 22 if in Part 1 or Visit 42 if in Part 2), which should be scheduled within 7 days of the participant's last dose of study drug, to collect data listed in the Schedule of Procedures – Part 1 and Schedule of Procedures – Part 2. Participants will immediately proceed into the 4-week Follow-up Period and will complete the 3 visits in the Follow-up Period… | degree possible as described in Section 4.2.7 and Appendix 5 (Section 13.5) in order to minimize missing data. Participants who permanently discontinue study drug will ~~be recorded as~~ have the reason for study drug discontinuation~~s~~ (e.g., adverse event) recorded on the eCRF. They will have an ET visit (Visit 22 if in Part 1 or Visit 42 if in Part 2), which should be scheduled within 7 days of the participant's last dose of study drug, to collect data listed in the Schedule of Procedures – Part 1 and Schedule of Procedures – Part 2. Randomized ~~P~~participants who permanently discontinue treatment will immediately proceed into the 4-week Follow-up Period… | missing data was added in this section as an additional resource. |
| 70 | Protocol Section 4.2.5, Reasons for Treatment/Study Discontinuation | Participants may discontinue or may be discontinued from study drug or from the study for any of the following reasons…<br><br>…<br><br>Upon occurrence of an SAE or intolerable AE, the Investigator will confer with the medical monitor and sponsor… The entire study may be stopped at any time at the discretion of the sponsor. | Participants who permanently discontinue study treatment before the end of the study, should be encouraged to continue in the study through the last study visit. ~~Participants~~ They may discontinue or may be discontinued from study drug or from the study for any of the following reasons…<br><br>…<br><br>Upon occurrence of an SAE or intolerable AE, the Investigator will confer with the medical monitor and sponsor… ~~The entire study may be stopped at any time at the discretion of the sponsor.~~ | Additional text added to distinguish treatment discontinuation from study discontinuation (i.e., participants who discontinue treatment should be encouraged to continue in the study through study completion).<br><br>Text on study termination deleted as a new protocol section (4.2.6) on study termination has been added. |

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| 71 | Protocol Section 4.2.6, Methods to Prevent Loss to Follow-up | **4.2.6 Methods to Prevent Loss to Follow-up**<br><br>The Investigator must make every attempt to follow-up participants who have withdrawn from the study at any time and for any reason. When a participant is "lost to follow-up" (i.e., fails to return for study visits or complete one or more scheduled remote site visits), a reasonable effort (3 documented phone calls or 2 phone calls and 2 text messages (if the participant has a mobile phone), on separate occasions, and a follow-up letter sent by registered mail) should be made to contact him/her to determine a reason for the failure to return or complete remote visit(s). If the participant cannot be reached, they should be identified as "lost to follow-up" in the eCRF. | **~~4.2.6~~ 4.2.7 Methods to Prevent Loss to Follow-up**<br><br>The Investigator must make every attempt to contact participants who fail to return for scheduled visits to prevent participants from being "lost to follow-up" and follow-up with participants who have withdrawn from the study at any time and for any reason. Appendix 6 (Section 13.6) specifies the steps expected to be taken to prevent loss to follow-up. All attempts to follow-up with participants in accordance with Section 13.6, must be documented in the source document. ~~When a participant is "lost to follow-up" (i.e., fails to return for study visits or complete one or more scheduled remote site visits), a reasonable effort (3 documented phone calls or 2 phone calls and 2 text messages (if the participant has a mobile phone), on separate occasions, and a follow-up letter sent by registered mail) should be made to contact him/her to determine a reason for the failure to return or complete remote visit(s).~~<br>Only after all measures to mitigate loss to follow-up have been exhausted should participants ~~If the participant cannot be reached, they should~~ be identified as "lost to follow-up" in the eCRF. | Protocol Section numbering updated to reflect inclusion of a new section 4.2.6.<br><br>Updated the procedures related to contacting participants that are lost to follow-up. |
| 71 | (New) Protocol Section 4.2.6, Study Termination | | **4.2.6 Study Termination** | Text added to clarify reasons why the study may be terminated. |

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| | | | The study may be stopped at a study site at any time by the site investigator or the sponsor. The sponsor may stop the study for any reason with appropriate notification, including inability to enroll a sufficient number of participants to meet study goals, an untoward safety signal as recommended by the IDMC and sponsor, or discontinuation of development of lixivaptan for ADPKD. The study may also be stopped by a regulatory authority. | |
| 72 | Protocol Section 4.2.8, Withdrawal of Consent | …The Investigator should then follow the procedures outlined in Appendix 5 (Section 13.5) to determine if the participant can continue participation in the trial if modifications to his/her treatment and/or schedule of procedures can be accommodated, including the potential to continue in the study at a lower dose level. Participants who withdraw their permission for all of the follow-up procedures listed above are considered to have completely withdrawn their consent to participate in the study. | …The Investigator should then follow the procedures outlined in Appendix 5 (Section 13.5) to determine if the participant can continue participation in the trial if modifications to his/her treatment and/or schedule of procedures can be accommodated, including the potential to continue in the study at a lower dose level. The scientific value of collecting data and information even after study drug is discontinued should be discussed with a participant who is at risk of withdrawing consent. Participants who withdraw their permission for all of the follow-up procedures listed above are considered to have completely withdrawn their consent to participate in the study. | Text added to emphasize the scientific importance of continuing to collect complete data on all participants, even for those who discontinue study treatment. |
| 74 | Protocol Section 5.1.4, Study Drug Packaging, Labeling, and Storage | In such cases, participants will be provided with written instructions to securely store the study drug in its original packaging under the conditions described above and not to open the package until the home | In such cases, participants will be provided with written instructions to securely store the study drug in its original packaging under the conditions described above and not to open the package until the home | Added the option to allow qualified site staff conducting remote visits to directly dispense study drug at the remote visit. |

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| | | healthcare clinician (HHC)/ arrives. | healthcare clinician (HHC)/qualified site personnel arrives. Alternatively, where permissible and approved by the sponsor or sponsor designee, qualified study staff conducting remote visits may dispense study drug directly to participants at the time of the remote visit. | |
| 76 | Protocol Section 5.4, Treatment Compliance | The dates of all study drug dosing, including interruptions, missed doses or overdose, must be recorded in the eCRF. If the participant is not ≥ 80% compliant with the prescribed study drug doses during the study, then the period of non-compliance should be noted as significant protocol deviation and the sponsor should be notified. The participant should be re-educated regarding the correct study drug doses to be administered. However, if the Investigator, medical monitor… | The dates of all study drug dosing, including interruptions, missed doses or overdose, must be recorded in the ~~eCRF~~ source document and study drug accountability forms. Compliance is defined as a minimum of 80% dosing compliance during any study interval. If the participant is not ≥ 80% compliant with the prescribed study drug doses during the study overall, ~~then the period of non-compliance should~~ will be noted as a significant protocol deviation ~~and the sponsor should be notified~~. The participant should be re-educated regarding the correct study drug doses to be administered whenever compliance is < 80%. However, if the Investigator, medical monitor… | Clarified minimum compliance expected to guide protocol deviation reporting. |

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| 78 | Protocol Section 5.5.1.2, Prohibited Therapy | | • diuretics (must be discontinued during Screening when anti-hypertensive medications are being adjusted) except for a short course (up to 4 weeks at a time) of loop diuretic therapy to manage a temporary fluid retention condition;<br><br>• strong or moderate CYP3A4 or CYP2C8 inhibitors, including aprepitant, boceprevir…isoniazid, itraconazole, josamycin, ketoconazole, nefazodone, Paxlovid™ (nirmatrelvir co-packaged with ritonavir), posaconazole, quinupristin/dalfopristin, remdesivir, ritonavir and ritonavir-containing products, telaprevir, teriflunomide, tofisopam, troleandomycin, verapamil, and voriconazole;<br><br>• KetoCitra™ or any beta-hydroxybutyrate (BHB) containing supplements;<br><br>• medications, supplements, and herbal preparations that may interfere with the accurate measurement of serum creatinine including cimetidine, trimethoprim, pyrimethamine, phenacemide, and aspirin (aspirin dose above 150 mg per | The list of prohibited therapy has been updated to reflect additional medications that may confound safety or efficacy assessments in this study. |

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| | | | day);<br>• vasopressin or drugs with vasopressin activity ~~any other drug/treatment including medications, supplements, and herbals with known activity on serum creatinine or interference with serum creatinine assays and/or vasopressin activity or interference with serum creatinine assays and/or vasopressin activity~~. | |
| 81 | Protocol, Section 6.1.1 Visit 1a | serology: HbsAg and Anti-HCV | serology: HbsAg and Anti-HCV (Note: A positive anti-HCV test requires a negative HCV RNA titer reflex test prior to enrollment. Participants should undergo reflex testing at an Unscheduled Visit prior to Visit 2). | |
| 81, 107 | Protocol Section 6.1.1, Visit 1a<br><br>Protocol Section 7.3.2 Physical Examinations and Medical History | • Collect and review medical history information..<br><br>Medical history and demographic data will be recorded at the timepoints specified in the Schedule of Procedures – Part 1 and Schedule of Procedures – Part 2. | • Collect and review medical history information, including smoking and alcohol history.<br><br>Medical history, smoking and alcohol history, and demographic data will be recorded at ~~the timepoints specified in the~~ Visit 1a as specified in the Schedule of Procedures – Part 1 ~~and Schedule of Procedures – Part 2~~. | Added collection of smoking and alcohol history at Visit 1a as part of the medical history. |
| 84, 85, 88, 89, 91, 95, 96, 97, 98, | Protocol Section 6.1.3, Visit 2<br>Protocol Section 6.2, Placebo Run-in Period and Visit 3<br>6.3.1, Visit 4 to 8 | | • Remind the participant to increase fluid intake to prevent dehydration | Added instructions to remind the participant at each dispensing visit, to increase fluid intake to prevent dehydration. |

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| | (Lixivaptan Titration Period)<br>Protocol Section 6.3.2, Visit 9/Last Titration Period<br>Protocol Section 6.4.1, Visits 10 to 21<br>Protocol Section Protocol 6.5.2, Visit 25<br>Protocol Section 6.6.1, Visits 26 to 28<br>Protocol Section 6.6.2, Visit 29, Last Re-titration Visit<br>Protocol Section 6.7.1, Visits 30 to 41 | | | |
| 95 | Protocol Section 6.6, Lixivaptan Re-titration Period – Part 2 | During the 2-to-4-week Lixivaptan Re-titration Period, participants will be re-titrated to the dose of lixivaptan last taken during the Double-blind, Randomized Treatment Period in Part 1. All participants will initiate re-titration at a lixivaptan dose of 50 mg BID (at Visit 25). Visits 26 to 29 will occur weekly ± 2 days as needed to achieve the last dose level. | During the 2-to-4-week Lixivaptan Re-titration Period, all participants in Part 2 will be re-titrated to the dose level ~~of lixivaptan~~ last taken during the Double-blind, Randomized Treatment Period in Part 1 (i.e., the last actual dose level if they had received lixivaptan during the Double-blind, Randomized Treatment Period, or if they received placebo during the Double-blind, Randomized Treatment Period, the last inferred dose level (the dose level equal to the active dose level had the participant been randomized to the active arm)) in accordance with Table 2. This will be performed by the IRT and the site, participant, and sponsor maintains a blinded status. For example, if no adjustment is made to the inferred dose during the Double-Blind, Randomized | Text has been revised to clarify re-titration during Part 2 of the study, with emphasis on re-titration for participants randomized to placebo in Part 1 of the study. |

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| | | | Treatment Period, the last inferred dose for a participant randomized to placebo will be equal to the maximal tolerated lixivaptan dose established during the Titration Period in Part 1. | |
| 103 | Protocol Section 7.1.1, eGFR Assessments | The eGFR values will be calculated according to the CKD-EPI formula from the serum creatinine concentrations taken during the Screening Period and at the visits specified in the Schedule of Procedures Part-1 and Schedule of Procedures- Part 2 of the study. The eGFRs will be reported to Investigators up to and including Visit 9 however, to maintain blinding, eGFR values will not be reported to the Investigators after randomization (Visit 9). Although serum creatinine results will be provided to the Investigators as part of the chemistries to allow safety evaluation at the visits specified in the Schedule of Procedures Part 1, eGFR values derived from the serum creatinine results post-randomization (Visit 9) will NOT be shared with the participant, the Investigator, the clinical sites, the sponsor, or the contract research organization (CRO) because of the potential for unblinding. During the Screening Period, serum creatinine assessments… Every attempt should be made to draw blood samples for eGFR determination around the same clock time during Visits 1a/1b, 2, and 3 and Visits 23, | The eGFR values will be calculated according to the 2021 CKD-EPI formula (CKD-EPIcr_R) (Appendix 1 (Section 13.1)) from the serum creatinine concentrations taken during the Screening Period and at the visits specified in the of the Schedule of Procedures Part-1 and Schedule of Procedures- Part 2 of the study. ~~The eGFRs will be reported to Investigators up to and including Visit 9 however, to maintain blinding, eGFR values will not be reported to the Investigators after randomization (Visit 9). Although serum creatinine results will be provided to the Investigators as part of the chemistries to allow safety evaluation at the visits specified in the Schedule of Procedures – Part 1, eGFR values derived from the serum creatinine results post-randomization (Visit 9) will NOT be shared with the participant, the Investigator, the clinical sites, the sponsor, or the contract research organization (CRO) because of the potential for unblinding.~~<br><br>During the Screening Period, serum creatinine assessments… Every attempt should be made to draw blood samples for eGFR determination around the same clock time during Visits 1a/1b, 2, and 3 and Visits 23, | The eGFR calculations from the central lab will no longer be suppressed post Visit 9 to enhance safety monitoring.<br><br>Additionally, eGFR results calculated from the newly added exploratory endpoint utilizing serum creatinine and serum cystatin C (CKD-EPIcr-cys_R) will not be shared with study sites to reduce the potential for decision errors, as eligibility and all treatment decisions will be made based on the eGFR calculated from CKD-EPIcr_R equation. |

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| | | 24, and 25, and again at Visits 43, 44, and 45 for a given participant. | 24, and 25, and again at Visits 43, 44, and 45 for a given participant.<br><br>As an exploratory endpoint, eGFR will also be assessed by the measurement of serum creatinine and serum cystatin C using a novel CKD-EPI equation (CKD-EPIcr-cys_R) (Appendix 1 (Section 13.1)). Along with serum creatinine concentrations, serum cystatin C will be obtained at Visits 1a, 2 and 3 or Visits 1b, 2 and 3 and during Follow-up Period 1 (Visits 23, 24, and 25) and during Follow-up Period II (Visits 43, 44 and 45). The mean of the 3 eGFR measurements calculated from the CKD-EPcr-cys equation during each period will be used for the baseline and follow-up period for analyses of the exploratory eGFR endpoint. To reduce potential decision errors, eGFR calculated from the CKD-EPIcr-cys_R equation will not be shared with sites as all eligibility and treatment decisions will be based on eGFR calculated from the CKD-EPIcr_R equation. | |
| 109 | Table 8, Clinical Laboratory Parameters | Sodium | Sodium*<br><br>* Serum sodium will be assessed at all time-points specified in the Schedule of Procedures either as part of chemistry or liver chemistry testing.<br><br>… Serum cystatin C | Footnote added to table of clinical laboratory parameters to clarify sampling for serum sodium.<br><br>Serum cystatin C added to clinical laboratory parameters collected.to support exploratory |

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| | | | | endpoints. |
| 112 | Protocol Section 7.3.7.1.2, Liver Test Abnormalities during the Lixivaptan Titration Period | Serum aminotransferase or total bilirubin levels that are 1 × ULN to 2 × ULN during the Lixivaptan Titration Period will require holding the dose constant and a discussion with the medical monitor. | Serum aminotransferase or total bilirubin levels that are >1.2 × ULN to 2 × ULN during the Lixivaptan Titration Period will require holding the dose constant and a discussion with the medical monitor. | To align with inclusion criteria, liver enzyme thresholds for holding lixivaptan dose constant during Placebo Run-in or Lixivaptan Titration periods have been increased. |
| 121 | Protocol Section 8.2, Blinded Sample Size Re-estimation | After 20% of the randomized participants (i.e., 240 participants) complete Part 1 of the study (Visit 25) or before screening for the study has ended, a BSSR will be performed by a blinded, independent statistician… If the BSSR estimates that the standard deviation of the primary efficacy estimand is less than or equal to 6.20 mL per minute per 1.73 $m^2$, then the sample size will remain at 1200 randomized participants… | After at least 20% of the randomized participants (i.e., 270 ~~240~~ participants) complete Part 1 of the study (Visit 25) or before screening for the study has ended, a BSSR will be performed by a blinded, independent statistician. Based on this interim data set, the variance corresponding to the primary efficacy estimand will be estimated and the total sample size re-estimated. If the BSSR estimates that the standard deviation of the primary efficacy estimand is less than or equal to 6.20 mL per minute per 1.73 $m^2$, then the sample size will remain at ~~1200~~ 1350 randomized participants… | Increased the number of participants that need to complete Part 1 of the study before the BSSR can be conducted to reflect the increase in sample size. |
| 123 | Protocol Section 8.5, Secondary Efficacy Analyses | If the primary efficacy analysis is statistically significant at the 1% level of significance, then the secondary efficacy endpoints of (1) annualized rate of change (slope) in eGFR based on all on-treatment eGFR determinations during the Double-blind, Randomized Treatment Period calculated from the CKD-EPI equation for serum creatinine; and (2) annualized rate of change in TKV as | If the primary efficacy analysis is statistically significant at the 1% level of significance, then the secondary efficacy endpoints of (1) annualized rate of change (slope) in eGFR based on all on-treatment eGFR determinations during the Double-blind, Randomized Treatment Period calculated from the CKD-EPIcr_R equation for serum creatinine; and (2) annualized ~~rate of~~ change in TKV as | Updated the equation used in the assessment of the secondary efficacy analysis from the CKD-EPI to the CKD-EPIcr_R and also clarified the analysis of TKV. |

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| | | measured by MRI. | measured by MRI will be analyzed. | |
| 126 | (New) Protocol Section 8.10.3, Annualized Change in eGFR Assessed by Serum Creatinine and Cystatin-C (CKD-EPIcr-cys_R) | | **8.10.3 Annualized Change in eGFR Assessed by Serum Creatinine and Cystatin-C (CKD-EPIcr-cys_R)**<br><br>This analysis is based on the change from the baseline eGFR to the post-treatment follow-up eGFR calculated from the CKD-EPIcr-cys_R equation, annualized by each participant's treatment duration. Baseline eGFR is defined as the mean of the 3 eGFR determinations obtained during the Screening and Placebo Run-in Periods. The post-treatment follow-up eGFR is defined as the mean of 3 eGFR assessments obtained during the Follow-up Period I or the equivalent off-therapy eGFR determinations if a participant discontinues study drug earlier.<br>This annualized change from baseline to post-treatment follow-up in mean eGFR will be analyzed by means of an analysis of covariance model with fixed effects for treatment group and the randomization stratification factors and the baseline eGFR as a covariate.. | Analysis added to correspond with new exploratory objective and endpoint. |
| 143 | Protocol Section 13.1, Appendix 1: Chronic Kidney Disease Classification Criteria | This study uses the 2009 CKD-EPI creatinine equation (Levey et al, 2009), which is recommended by the KDIGO Clinical Practice Guidelines for Management of Chronic Kidney Disease.<br>The CKD-EPI equation is: | As of protocol Version 2, ~~This~~ this study will replace measurement of eGFR as calculated by ~~uses~~ the 2009 CKD-EPI creatinine equation[1] with the 2021 CKD-EPI equation refit without race variable (CKD-EPIcr_R) (~~Levey et al, 2009~~ Delgado et al, 2021), which is recommended by the ~~KDIGO~~ | The calculation of eGFR has changed from the 2009 CKD-EPI creatinine equation to the 2021 CKD-EPI equation refit without the race variable (CKD-EPIcr_R). The 2009 CKD-EPI creatinine equation was moved to footnote 1. |

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| | | $eGFR = 141 \times min\left(\frac{Scr}{\kappa}, 1\right)^{\alpha} \times \max\left(\frac{Scr}{\kappa}, 1\right)^{-1.209} \times 0.993^{Age} \times 1.018$ (if fe × 1.159 (if black),<br>Abbreviations/Units<br>eGFR (estimated glomerular filtration rate) = mL/min/1.73 m2<br>SCr (standardized serum creatinine) = mg/dL<br>κ = 0.7 (females) or 0.9 (males)<br>α = -0.329 (females) or -0.411 (males)<br>min = indicates the minimum of SCr /κ or 1<br>max = indicates the maximum of SCr /κ or 1<br>age = years | ~~Clinical Practice Guidelines for Management of Chronic Kidney Disease~~ National Kidney Foundation American Society of Nephrology Task Force.<br><br>The CKD-EPIcr_R equation is:<br>$eGFR = 142 \times \min\left(\frac{Scr}{\kappa}, 1\right)^{\alpha} \times \max\left(\frac{Scr}{\kappa}, 1\right)^{-1.200} \times 0.9938^{Age} \times 1.012$ (if female)<br>Abbreviations/Units<br>eGFR (estimated glomerular filtration rate) = mL/min/1.73 m2<br>Scr (standardized serum creatinine) = mg/dL<br>= 0.7 (females) or 0.9 (males)<br>α = -0.241 (females) or -0.302 (males)<br>min = indicates the minimum of Scr / or 1<br>max = indicates the maximum of Scr / or 1<br>age = years<br><br>**Serum Creatinine and Cystatin C (CKD-EPIcr-cys_R):**<br>In this study eGFR will also be assessed from a novel serum creatinine and serum cystatin C equation refit without the race variable (CKD-EPIcr-cys_R) (Delgado et al, 2021). To reduce potential decision errors, eGFR calculated from this equation will not be shared with sites as all eligibility and treatment decisions will be based on eGFR | Formula was added for the calculation of eGFR based on serum creatinine and serum cystatin C (CKD-EPIcr-cys_R) to correspond with the new exploratory objectives and endpoints.<br>Note added to clarify that eGFR based on this equation will not be shared with sites as eligibility and all treatment decisions will be made based on the CKD-EPIcr_R equation. |

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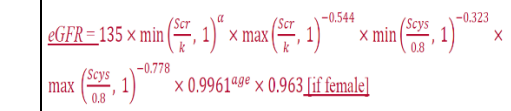
| | | | calculated from the CKDEPIcr_R equation.<br><br>The CKD-EPIcr-cys_R equation is:<br><br>  <br><br>Abbreviations/Units<br>eGFR (estimated glomerular filtration rate) = mL/min/1.73 m2<br>Scr (serum creatinine) = mg/dL<br>Scys (serum cystatin C) = mg/L<br>= 0.7 (females) or 0.9 (males)<br>α = -0.219 (females) or -0.144 (males)<br>min = indicates the minimum of Scr/k or 1 OR Scys/0.8 or 1<br>max = indicates the maximum of Scr/k or 1 OR Scys/0.8 or 1<br>age = years | |
| 146 | Protocol Section 13.2 Appendix 2: Liver Dysfunction Checklist | The following items will be included in a checklist to be completed by the HHC for remote visits (Part 1- Symptoms section only) and by the site for clinic visits (Part 1- Symptoms and Part 2- Signs) when directed by the protocol. For remote visits where there are findings on the Part 1- Symptoms section of the checklist, telehealth will be utilized (e.g., telemedicine virtual visit, telephone or video call (without recording)) to determine if a clinic visit is needed to complete the Part 2-Signs section.<br><br>… | The following items will be included in a checklist to be completed by the ~~HHC for remote visits (Part 1- Symptoms section only) and by the~~ site ~~for clinic visits~~ (Part 1- Symptoms and Part 2- Signs) when directed by the protocol. ~~For remote visits where there are findings on the Part 1- Symptoms section of the checklist, telehealth will be utilized (e.g., telemedicine virtual visit, telephone or video call (without recording)) to determine if a clinic visit is needed to complete the Part 2-Signs section.~~<br><br>… | Revised instructions for completion of Liver Dysfunction checklist for clarity and consistency with the protocol. |

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| | | Any symptoms, signs, or an associated diagnosis deemed clinically significant based on information entered in Part 1-Symptoms or Part 2-Signs during either a remote visit or a clinic visit will be entered in the eCRF by the site as adverse events. | The Investigator is responsible for adverse event reporting of ~~Any~~ symptoms, signs, or an associated diagnosis deemed clinically significant based on information entered in ~~Part 1-Symptoms or Part 2-Signs~~ this checklist~~during either a remote visit or a clinic visit will be entered in the eCRF by the site as adverse events~~. | |
| 150 | Protocol Section 13.5 Appendix 5: Minimization of Missing Data: Study and Study Treatment Modification | 4) Ask the participant, "If we discontinue your study drug permanently, would you continue with all visits and sample collections?" If the answer is "Yes", discontinue study drug, perform an ET visit (Visit 22/ET if in Part 1 or Visit 42/ET if in Part 2), complete the Follow-up Period assessments, and continue with all other monthly assessments to the end of the trial to reduce missing data. If the answer is "No" go to Step 5. | 4) Ask the participant, "If we discontinue your study drug permanently, would you continue with all visits and sample collections?" The Investigator should discuss the scientific value of continued data collection even in the absence of study treatment. If the answer is "Yes", the participant is willing to continue with all visits and sample collections, discontinue study drug, perform an ET visit (Visit 22/ET if in Part 1 or Visit 42/ET if in Part 2), complete the Follow-up Period assessments, and continue with all other monthly assessments to the end of the trial to reduce missing data. If the answer is "No" go to Step 5. | Text added to clarify that there should be a discussion with the participant of the merits of continued study participation, despite not continuing on study treatment. |
| 151 | (New) Protocol Section 13.6; Appendix 6: Procedures to Prevent Loss to Follow-up | | **13.6 Appendix 6: Procedures to Prevent Loss to Follow-up**<br><br>The Investigator must make every effort to contact participants who fail to return for scheduled visits to prevent participants from being "lost to follow-up". The following | Additional instructions to limit missing data have been added. |

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| | | | procedures should be followed at a minimum, with additional measures taken if unsuccessful.<br>1) Contact all numbers for the participant and their listed contacts (to be collected in source document at the participant's entry into the trial). This includes making calls after normal business hours or on holidays and weekends. Notify the CRA and/or medical monitor promptly after participant fails to return for a scheduled visit.<br><br>2) Contact the participant's primary care physician, referring specialist, pharmacist or other health-care professional (using the contacts provided by the participant at entry to the trial).<br><br>3) Send a text, e-mail and postal mail with certified (return-receipt requested) letters to all the participant's addresses and all contacts (as provided by the participant at entry to the trial).<br><br>4) In-home visit at last address given.<br><br>5) Review available medical records/notes for details of hospitalizations, clinic visits or other procedures which may indicate the status of participants (as allowed through release of medical record forms to be completed by participant at trial entry). | |

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| | | | 6) Conduct an internet search for additional contact information (e.g., reverse directory for phone numbers or new address information; social media outlets (e.g., Facebook, LinkedIn or other social media for status updates).<br><br>7) Check local, regional and national public records to locate the participant or search for mortality status as allowed by law. Once all these actions have been exhausted (and documented), then the CRA or medical monitor should be contacted for additional guidance. | |