Study Title: An Open-Label Study of Lixivaptan in Subjects With Autosomal Dominant Polycystic Kidney Disease Who Previously Experienced Abnormal Liver Chemistry Test Results While Receiving Tolvaptan: The ALERT Study

ClinicalTrials.gov ID: NCT04152837

Clinical Study Protocol Version 4.0, dated 17-Nov-2021

Clinical Study Protocol Version 4.0, dated 17-Nov-2021, Summary of Changes

Clinical Study Protocol Version 3.0, dated 02-Sep-2020, Summary of Changes

Note: the first approved version of the protocol was Version 2.0.

CLINICAL STUDY PROTOCOL

An Open-label Study of Lixivaptan in Subjects with Autosomal Dominant Polycystic Kidney Disease Who Previously Experienced Abnormal Liver Chemistry Test Results While Receiving Tolvaptan: The ALERT Study

Protocol Number: PA-ADPKD-303

IND Number: 136,419

Sponsor:	Palladio Biosciences, Inc. 5 Walnut Grove Drive Suite 120 Horsham, PA 19044
Sponsor Contact:	Neil Shusterman, MD FACP Chief Medical Officer Palladio Biosciences, Inc. 5 Walnut Grove Drive, Suite 120 Horsham, PA 19044 Email: nshusterman@palladiobio.com
Medical Monitor:	David Frid, MD ICON Clinical Research Direct: (215) 616-2504 (including urgent medical issues) Fax: +1 (215) 699-6288 Email: David.Frid@iconplc.com In a study related health emergency, when the assigned Medical Monitor cannot be reached, please refer to Section 7.3.8.3 for further contact information.
Version of Protocol:	4
Date of Protocol:	November 17, 2021

CONFIDENTIAL

Protocol Approval - Sponsor Signature

Protocol Title	An Open-label Study of Lixivaptan in Subjects with Autosomal Dominant Polycystic Kidney Disease Who Previously Experienced Abnormal Liver Chemistry Test Results While Receiving Tolvaptan: The ALERT Study Protocol Number: PA-ADPKD-303
Protocol Number	PA-ADPKD-303
Protocol Version	4
Protocol Date	November 17, 2021

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

November 17, 2021
Date

Declaration of Investigator

I have read and understood all sections of the protocol titled "An Open-label Study of Lixivaptan in Subjects with Autosomal Dominant Polycystic Kidney Disease Who Previously Experienced Abnormal Liver Chemistry Test Results While Receiving Tolvaptan: The ALERT Study"

Protocol Number: PA-ADPKD-303

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 Practice: Integrated Addendum to ICH E6(R1) Guidance for Industry, 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 subjects. I agree to administer study treatment only to subjects 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. Subject 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.

_____ _____

Signature of Investigator Date

_____

Printed Name of Investigator

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PROTOCOL SYNOPSIS

Protocol Number:	**PA-ADPKD-303**
Protocol Title:	An Open-label Study of Lixivaptan in Subjects with Autosomal Dominant Polycystic Kidney Disease Who Previously Experienced Abnormal Liver Chemistry Test Results While Receiving Tolvaptan: The ALERT Study Protocol Number: PA-ADPKD-303
Sponsor:	Palladio Biosciences, Inc. 5 Walnut Grove Drive Suite 120 Horsham, PA 19044
Study Phase:	Phase 3
Study Sites:	Up to approximately 25 global sites
Indication:	Autosomal Dominant Polycystic Kidney Disease (ADPKD)
Rationale:	ADPKD is the most frequent, inherited cause of end-stage renal 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 renal 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. The vasopressin V_2 receptor antagonist, lixivaptan, has also been shown to ameliorate polycystic disease manifestations in animal models of disease. Evidence from Quantitative Systems Toxicology modeling and initial clinical results suggest that lixivaptan does not have the same potential for liver injury. Thus, lixivaptan may represent a safer alternative to tolvaptan with similar efficacy. This Phase 3 study will assess the safety and efficacy of lixivaptan in subjects with ADPKD who experienced abnormal liver chemistry test results on tolvaptan resulting in permanent discontinuation of tolvaptan.
Objectives:	The primary objective of this study is: • To assess the hepatic safety of lixivaptan in subjects with ADPKD who experienced abnormal liver chemistry test results on tolvaptan that resulted in permanent discontinuation of tolvaptan. The secondary objectives of this study are: • To characterize the non-hepatic safety and tolerability of lixivaptan in subjects with ADPKD who previously

	experienced abnormal liver chemistry test results while treated with tolvaptan. • To assess renal function (efficacy) in ADPKD subjects while on lixivaptan using change in estimated glomerular filtration rate (eGFR). The exploratory objectives of this study are: • To explore any possible relationships between liver chemistry abnormalities and trough levels of lixivaptan collected during the study. • To assess potential genetic risk factors for hepatic abnormalities (if and when feasible).

Subject Population:	At Visits 1 and 2, subjects must meet all of the Inclusion criteria and none of the Exclusion criteria. An eligibility review and discussion between the Investigator and the medical monitor will be conducted before each subject is enrolled (prior to Visit 3) at each site. Inclusion criteria: 1. Male or female, between 18 and 65 years of age (inclusive) at the time of Screening. 2. Female subjects 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) must agree to practice appropriate methods of birth control or remain abstinent (only if this is the usual and preferred lifestyle of the participant) during the study and for 30 days after the last dose of study drug. Acceptable forms of contraception include any of the following: • hormonal contraceptives (i.e., oral, intravaginal, transdermal, injectable, implantable) • double barrier methods of non-hormonal contraception are permitted in this study: o male or female condom with spermicide (cream, spray, gel, suppository, contraceptive sponge, or polymer film) o diaphragm, cervical cap, or

	contraceptive sponge with spermicide (with or without a condom) • 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 • bilateral tubal ligation • Essure® procedure (tubal occlusion). 3. Male subjects and their female partners of childbearing potential must also use the contraceptive methods listed above or remain abstinent (only if this is the usual and preferred lifestyle of the participant) during the study and for 30 days after the last dose of study drug. 4. Diagnosis of ADPKD confirmed by previous treatment with tolvaptan specifically for that indication and supported by evidence from any imaging modality or genetic analysis. 5. Screening eGFR ≥ 20 ml/min/1.73 m^2. 6. Body mass index (BMI) between 18 and 35 kg/m^2 (inclusive) at the time of Screening. 7. Documented history of: a. Based on upper limit of normal (ULN): at least 2 elevated alanine aminotransferase (ALT) levels, 1 ALT level >2 times (x) the ULN and 1 ALT level >3 x ULN while the subject was receiving tolvaptan, or within 4 weeks after tolvaptan discontinuation, with no other explanation for the ALT elevations. The 2 elevated ALT measurements could be recorded during the same instance of liver injury or during distinct instances; OR b. Based on the subject's stable baseline as determined by the Investigator: at least 2 elevated ALT levels, 1 ALT level >2 x the subject's stable baseline level and 1 ALT level >3 x the subject's stable baseline level while the subject was receiving tolvaptan, or within 4 weeks after tolvaptan discontinuation, with no other explanation for the ALT elevations; provided that at least one ALT elevation was >2 x ULN. The 2 elevated ALT measurements could be recorded during the same instance of liver injury or during distinct instances;

	OR c. A pattern of ALT elevations deemed by the Investigator to be consistent with tolvaptan liver injury with no other explanation for the ALT elevations and the agreement of the medical monitor and sponsor. 8. Permanent discontinuation of prior tolvaptan treatment because of the abnormal liver chemistry test results. 9. The subject may or may not have been re-challenged with tolvaptan. If re-challenge with tolvaptan was performed, the ALT level should have increased to >2 x ULN upon re-challenge or the ALT level was increasing but tolvaptan was stopped for patient safety reasons before it reached > 2 x ULN after having previously normalized. 10. Appropriate control of hypertension including an angiotensin converting enzyme inhibitor or angiotensin receptor blocker (unless not considered appropriate for the subject) without the use of a diuretic in concert with KDIGO "Clinical Practice Guideline for the Management of Blood Pressure in Chronic Kidney Disease". 11. 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. Exclusion criteria: 1. Known sensitivity, allergy, or idiosyncratic reaction to any compound present in lixivaptan and related compounds. 2. Hypovolemia on physical examination at Screening. 3. Abnormal serum sodium concentration at Screening. 4. Subjects who have taken any investigational drug or used an investigational device within 30 days, or 5 half-lives, whichever is longer, prior to Screening 5. Subjects who are taking, have taken within the 2 weeks prior to Screening, 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. 6. Simvastatin at total daily doses >10 mg or amlodipine at total daily doses >5 mg. 7. Prior use of tolvaptan within the 3 months prior to Screening OR until a previously elevated ALT level has returned to ≤1 x ULN for at least 3 months. 8. Prior use of lixivaptan or participation in a clinical study with lixivaptan within the 3 months of Screening. 9. Prior use of conivaptan, somatostatin analogs (e.g., lanreotide,

	pasireotide, octreotide, etc.), metformin (allowed for diabetes mellitus), nicotinamide, bardoxolone, venglustat, demeclocycline, or mTOR kinase inhibitors (e.g., everolimus, sirolimus, etc.) to treat ADPKD within the 3 months prior to Screening. 10. Requirement for chronic diuretic use. 11. History of advanced diabetes (e.g., glycosylated hemoglobin [HgbA1c] >7.5%, and/or glycosuria by dipstick, significant proteinuria [>300 mcg albumin/mg creatinine], retinopathy), other significant renal disease, transplanted kidney, recent kidney surgery within the 6 months prior to Screening (including cyst drainage or fenestration) or acute kidney injury within 6 months prior to Screening. 12. Clinically significant incontinence, overactive bladder, or urinary retention (e.g., benign prostatic hyperplasia). 13. 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 subject. 14. Clinically significant liver disease or impairment or active chronic hepatitis at Screening. 15. Positive test results for hepatitis B surface antigen (HBsAg) or hepatitis C (HCV) antibody. 16. History of infection with human immunodeficiency virus (HIV) unless the subject is stable and doing well on a non-CYP interacting anti-retroviral therapy (ART) regimen and who has not required more than 2 changes in their ART regimen. 17. Serum ALT or aspartate aminotransferase (AST) values >1.5 x ULN during Screening/Baseline. 18. Total bilirubin values >1.0 x ULN during Screening/Baseline. 19. History of drug or alcohol abuse in the 2 years prior to Screening. 20. Any malignancy within 5 years prior to Screening except for basal cell carcinoma successfully treated with local therapy. 21. Medical history or findings that preclude safe participation in the study or who are likely to be non-compliant with study procedures in the opinion of the Investigator or medical monitor.
Study Design:	This is a Phase 3, open-label, repeat-dose study designed to assess hepatic safety, non-hepatic safety, and efficacy of lixivaptan in subjects who previously experienced abnormal liver chemistry test results while treated with tolvaptan and were permanently discontinued from the drug for that reason. Up to 50 subjects will be

	enrolled and treated. Evaluations will include frequent testing of liver chemistry tests (ALT, AST, total and direct bilirubin, and alkaline phosphatase values every week during the Baseline and Titration Periods and every 4 weeks during the Maintenance Period), physical examinations, vital signs, safety labs (serum chemistry, hematology, urinalysis), eGFR, urine specific gravity and osmolality determinations and trough serum concentration of lixivaptan. After meeting entry criteria during a 1-3-week Screening Period that can extend up to 8 weeks for subjects who need to be discontinued from diuretic therapy and/or have their anti-hypertensive medication adjusted, subjects will enter a 3-week no study drug Baseline Period to obtain baseline measurements followed by a 3-6 week Titration Period during which lixivaptan administered twice daily will be titrated to a dose that is tolerated and results in a reduced trough urine specific gravity (or until the maximum dose level is reached). The minimum dose to enter the Maintenance Period is 100 mg BID. Treatment will continue for up to 52 weeks (12 months) after which study drug will be stopped, and final assessments obtained during the Follow-up Period of 4 weeks. The total study duration will be up to approximately 73 weeks (16.8 months). Note that the study may be interrupted at any time if safety issues compromise the safety of the subjects.
Study Period Description and Estimated Duration:	The study schematic is depicted in Figure 1. Certain visits may be conducted remotely with a home healthcare clinician (HHC), but Visits 1, 5, 17, 24, and 27 are to be conducted in the clinic (with only extraordinary exceptions at the discretion of the medical monitor and sponsor). Some of the remote visits will include a telemedicine component with the Investigator. Details are provided in Table 1. Screening Period (Visits 1, 2): To obtain informed consent and determine subject eligibility, screening assessments will be performed during Visit 1. Subjects whose anti-hypertensive medications in the absence of diuretics are known and documented to have been stable (unchanged) for at least 3 weeks prior to Visit 1 may proceed to Visit 2 in 1 week to confirm eligibility. Subjects for whom the stability of anti-hypertensive medications in the absence of diuretics is unknown or not documented must be observed for 3 weeks during the Screening Period to ensure stable dosing of anti-hypertensive medications and then will be scheduled for Visit 2. For those subjects who need to be discontinued from diuretic therapy and/or have their anti-hypertensive therapy optimized, the Screening Period may be extended up to a total of 8 weeks prior to Visit 2. Subjects who meet all the inclusion criteria and none of the exclusion criteria and achieve stability of background anti-hypertensive medications in the absence of diuretics will either return to the clinic or be scheduled for a remote visit with a HHC for Visit 2 end-of-screening assessments, including body weight,

	vital signs, and serum chemistry testing (if diuretics were discontinued or anti-hypertensive medications were adjusted) and final eligibility determination (utilizing telemedicine if a remote visit). Subjects 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 and sponsor. Immediately following successful completion of Visit 2, subjects will enter the Baseline Period. Baseline Period (Visits 3 – 5): Following completion of all screening assessments and establishment of background medication stability, subjects will enter the 3-week no-study-drug Baseline Period during which time liver chemistry tests and serum creatinine (for eGFR calculation) will be assessed weekly to obtain baseline measurements and assess individual variability. Visits 3 and 4 may be conducted remotely by a HHC. After successful completion of Visit 5 assessments in the clinic, subjects will be dispensed study drug and begin treatment. This completes the Baseline Period and subjects will enter the Titration Period. Titration Period (Visits 6 – 11): During a period of 3-6 weeks, the lixivaptan dose will be increased weekly according to the titration schedule to achieve a level that results in both a tolerable dose and reduced first morning (trough) spot urine specific gravity or until the maximum dose level (Level 4 [200 mg BID]) is reached. Dosing will be started at Level 1 (50 mg BID), but the minimum dose to enter the Maintenance Period is Level 2 (100 mg BID). In general, dose tolerability will be confirmed over an additional week of dosing before the subject advances to the Maintenance Period. At the 2 highest dose levels where aquaretic effects may be problematic in certain subjects, there will be an opportunity to reduce the evening (PM) dose (Level 3a [150 mg AM/100 mg PM] and Level 4a [200 mg AM/150 mg PM]). Liver chemistry tests will be assessed weekly throughout the Titration Period. Urine osmolality and specific gravity will be assessed at all titration visits that occur except Visit 11. All titration visits may be conducted remotely by a HHC with telemedicine. Maintenance Period (Visits 12-24): Following successful completion of the Titration Period, subjects will continue to receive lixivaptan in the Maintenance Period at the dose level achieved at the end of the Titration Period for up to 52 weeks (12 months). In consultation with the medical monitor and sponsor, the Investigator has the option of reducing the dose to address potential hepatic and non-hepatic adverse events. Assessments, including liver chemistry tests, will be performed every 4 weeks during this period. Visits 12, 13, 14, 15, 16,

<table>
<tr><td></td><td>18, 19, 20, 21, 22, and 23 may be conducted remotely by a HHC. If conducted remotely, Visits 14 and 20 will include telemedicine. All other remote maintenance visits may include a telemedicine component if deemed necessary by the Investigator.

Follow-up Period (Visits 25-27): After the last dose of lixivaptan is given, subjects will enter the Follow-up Period which will last 4 weeks. Serum creatinine, to determine final eGFR, will be obtained over three (3) visits starting the 8th day after the final dose of study drug and continuing through the 28th day of the Follow-up Period. Visits 25 and 26 may be conducted remotely by a HHC (using telemedicine if necessary). Other routine final safety assessments will be performed at the last visit on Day 28 following the final dose of study drug.
The total study duration will be up to 73 weeks (16.8 months). Note that the study may be interrupted at any time if safety issues potentially compromise the safety of the subjects.
The Hepatic Events Review Committee (HERC), consisting of 3-4 expert hepatologists, will review safety data in subjects who develop liver abnormalities of concern during the study. This committee will independently determine the probable causality for liver chemistry test abnormalities of concern and the relatedness to lixivaptan.</td></tr>
<tr><td>Study Drug, Dosage, and Route of Administration:</td><td>The sponsor (Palladio Biosciences, Inc.) will provide adequate supplies of lixivaptan 50 mg capsules for use during the study. All doses will be self-administered orally at home. During the Titration Period, dosing will start at Level 1 (50 mg BID) and will be increased periodically through Levels 2, 3, and 4 according to the dosing schedule shown in the table below. 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 aquaresis is limiting the subject’s tolerability at either the 150 mg BID or 200 mg BID dose levels, respectively. Titration will stop when a tolerable dose level at or above the minimum (Level 2) is reached that achieves the goal of lowering trough (first AM) urine specific gravity to ≤1.005 or the highest dose (Level 4 or 4a) is achieved (regardless of urine specific gravity). Subjects tolerating the drug at Levels 2, 3, or 3a, but not achieving the urine specific gravity goal may be allowed to continue in the study after consultation with the medical monitor and sponsor. Throughout the study, subjects will take the allotted capsules BID approximately 10 hours apart.
Dosing Levels during the Titration Period
<table>
<tr><th>Dose Level</th><th>AM Dose</th><th>PM Dose</th></tr>
<tr><td>1*</td><td>50 mg (1 capsule)</td><td>50 mg (1 capsule)</td></tr>
</table>
</td></tr>
</table>

2	100 mg (2 capsules)	100 mg (2 capsules)
3	150 mg (3 capsules)	150 mg (3 capsules)
3a**	150 mg (3 capsules)	100 mg (2 capsules)
4	200 mg (4 capsules	200 mg (4 capsules)
4a***	200 mg (4 capsules)	150 mg (3 capsules)

*Dose Level 1 is for initiation of treatment

**Subjects 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)

***Subjects 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 and reduced urine specific gravity (or tolerability and maximum dose) are achieved, subjects will stay on that dose for 1 additional week to confirm tolerability (except when the dose is reduced to Level 3a or Level 4a after 2 weeks of dosing at Level 3 or Level 4, respectively, when a single final week at the lower dose level is sufficient; see Dosing Diagram below).

The usual dosing scenarios that can be advanced to the Maintenance Period are shown in the Dosing Diagram below. With concurrence of the medical monitor and the sponsor, some subjects may be allowed to enter maintenance at a lower dose if tolerability is an issue after trying a higher dose (regardless of specific gravity result at the lower dose).

Dosing Diagram

Dose Level	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**
Level 2	50mg BID	100mg BID	100mg BID			
Level 3	50mg BID	100mg BID	150mg BID	150mg BID		
Level 3a	50mg BID	100mg BID	150mg BID	150/100 mg (AM/ PM)	150/100 mg (AM/ PM)	
Level 3a (if 2nd week of Level 3 is not tolerated)	50mg BID	100mg BID	150mg BID	150 mg BID	150/ 100mg (AM/ PM)	
Level 4	50mg BID	100mg BID	150mg BID	200mg BID	200mg BID	
Level 4a	50mg BID	100mg BID	150mg BID	200mg BID	200/ 150mg (AM/ PM)	200/ 150mg (AM/ PM)
Level 4a (if 2nd week of	50mg BID	100mg BID	150mg BID	200mg BID	200mg BID	200/ 150mg (AM/

	Level 4 is not tolerated) | | | | | | PM) Subjects who tolerate their optimized dose will then enter the Maintenance Period during which they will continue at the lixivaptan dose level achieved at the end of the Titration Period. During the Maintenance Period, in consultation with the medical monitor and sponsor, the dose may be adjusted downward at the Investigator's discretion if needed to manage hepatic or non-hepatic side effects and may also be increased back to the dose achieved at the end of the Titration Period. The Investigator may temporarily hold the drug for up to 7 days, if necessary, to manage acute intercurrent illness, tolerability issues, planned or unplanned surgical procedures or life situations, e.g., airplane travel, etc. Additionally, subjects who have a longer interruption due to illness, including COVID-19, or other reasons may be able to re-start study drug when medically stable after discussion with the medical monitor and sponsor.
Study Assessments:	**Safety:** Liver chemistry tests (ALT, AST, total bilirubin, direct bilirubin, alkaline phosphatase), clinical laboratory (hematology, non-hepatic clinical chemistry, and urinalysis), 12-lead ECGs, vital signs, physical examination, adverse events (AEs), and serious adverse events (SAEs). **Efficacy:** eGFR **Pharmacokinetic:** Trough measurements of lixivaptan **Additional Investigations:** DNA sample to assess genetic variants associated with hepatic abnormalities (if and when feasible). This is obtained under a biobanking informed consent at Visit 5 (or later).
Criteria for Evaluation:	**Primary Endpoint** *Safety:* Proportion of subjects who develop ALT levels >3 x ULN during the Titration or Maintenance Periods that were assessed by the independent HERC to be at least probably related to lixivaptan and resulted in discontinuation of the study drug. **Secondary Endpoints** *Safety:* Proportion of subjects who develop ALT levels >5 x ULN during the Titration or Maintenance Periods that were assessed by the independent HERC to be at least probably related to lixivaptan and resulted in discontinuation of the study drug. Proportion of subjects who develop ALT levels >3 x ULN during the Titration or Maintenance Periods that were assessed by the independent HERC to be at least probably related to lixivaptan and resulted in dose reduction of the study drug.

	The safety and tolerability of lixivaptan assessed through evaluation of AEs, clinical laboratory findings (clinical chemistry including additional analyses of liver chemistry tests, hematology, and urinalysis), vital signs, and 12-lead ECG. *Efficacy:* Change in eGFR from baseline (mean of 3 eGFR determinations obtained during the Baseline Period) to final assessment (eGFR determination obtained during the Follow-up Period).
Statistical Methods:	**Sample Size:** Up to 50 subjects will be enrolled and treated based on the design of a similar study with a different drug. **Populations for Analysis:** The Enrolled Population is defined as all subjects who meet inclusion/exclusion criteria during Screening Period and complete Visit 3. All general summaries, except safety and efficacy analyses are based on the Enrolled Population, unless specified otherwise. The Safety Population is a subset of the Enrolled Population defined as those subjects who received at least 1 dose of lixivaptan. This population will be utilized for all safety analyses. The Efficacy Population is a subset of the Safety Population defined as those subjects who have at least 1 eGFR determination during the Baseline Period and have at least 1 eGFR determination during the Follow-up Period. This population will be used for all efficacy analyses. The Pharmacokinetic (PK) Population is a subset of the Safety Population defined as those subjects who had at least 1 on-therapy evaluable PK measurement. The Pharmacodynamic (PD) Population is a subset of the Enrolled Population defined as those subjects who have at least 1 set of urine specific gravity and urine osmolality measurements from the same specimen. **Analysis of Primary and Secondary Safety Endpoint:** Descriptive statistics (n and percentage) and 95% confidence intervals will be used to present the proportion of subjects who develop ALT levels >3 x ULN that were assessed by the independent HERC to be at least highly likely related to lixivaptan and resulted in discontinuation of the study drug during the Titration or Maintenance Periods. Similarly, descriptive statistics and 95% confidence intervals will be utilized to present the 2 secondary endpoints: the proportion of subjects who develop ALT levels >5 x ULN during the Titration or Maintenance Periods that were assessed by the independent HERC to be related to lixivaptan and resulted in discontinuation of the study drug and the proportion of subjects who develop ALT levels >3 x

	ULN during the Titration or Maintenance Periods that were assessed by the independent HERC to be related to lixivaptan and resulted in lixivaptan dose reduction. **Analysis of Additional Hepatic Safety Endpoints:** Proportion of subjects who develop during the Titration and Maintenance Periods: • >3 x, 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 • ALT or AST levels >2 x their baseline • Any elevation of total bilirubin to >2 x ULN • Any elevation of alkaline phosphatase (ALP) >2 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. **Analysis of Additional Non-hepatic Safety Endpoints:** The following safety variables will be summarized using appropriate descriptive statistics: Treatment-emergent adverse events, clinical laboratory data, 12-lead ECGs, and vital signs. Potentially clinically significant results in certain clinical laboratory tests, 12-lead ECGs, and vital signs identified using prospectively defined criteria will also be summarized descriptively. **Efficacy Analysis:** Baseline eGFR is defined as the mean of the 3 eGFR assessments obtained during the Baseline Period. The endpoint eGFR is defined as the mean of 3 eGFR assessments obtained during the Follow-up Period. Descriptive statistics (n, mean, standard deviation, median, minimum, and maximum) will be presented for the change from baseline in eGFR at endpoint.

Figure 1. Study Schematic

Table 1 Schedule of Procedures

Assessment	Screening (1-8 weeks)		Baseline (Weekly ± 3 days) (3 weeks)	Titration Period (Weekly ± 3 days) (Up to 6 weeks)						Maintenance Period (every 28 days ± 3 days) for 52 weeks		Follow-up Period (4 weeks after last dose[b])
Visit Number	**V1**	**V2[a]**	**V3, 4, 5**	**V6**	**V7**	**V8**	**V9**	**V10**	**V11**	**V12-V23**	**V24**	**V25, 26, 27**
Informed consent	X											
Demographic information	X											
Medical history/Prior meds	X	X										
Review eligibility (Inclusion/Exclusion)	X	X	V5									
ECG	X		V5							V17	X	V27
Vital signs[c]	X	X	X	X	X	X	X	X	X	X	X	V27
Physical examination[d]	X									V17	X	V27
Body weight/height[e]	X	X	V5	X	X	X	X	X	X		X	
Pregnancy test (WOCBP)[f]	X		V5						X	V14,17,20	X	V27
Chemistry/Serology samples[g]												
Complete Chemistry Panel	X	(X)	V5						X	V14,17,20	X	V27
Liver Chemistry Panel	X	(X)	X	X	X	X	X	X	X	X	X	V27
Serum Creatinine			V3, 4									V25, 26
HBsAg, Anti-HCV	X											
Hematology[h]	X		V5						X	V17	X	V27
Urinalysis	X										X	V27
Urine specific gravity/ osmolality[i]			V5	X	X	X	X	X			X	V27
Trough PK sample[j]			V5						X	(X)	(X)	
DNA sample[k]			V5									
Dosing review/Tolerability				X	X	X	X	X	X	X		
Drug dispensation			V5	X	X	X	X	X	X	X		
Drug reconciliation				X	X	X	X	X	X	X	X	
IRT entry	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events[l]	<----------											---------->
Assess for liver dysfunction				<----------								----------> V25
Concomitant medications	<----------											---------->

NOTES:

- V24 also serves as the Early Termination (ET) visit.
- Remote visits (if available) may occur at V2 (with telemedicine), V3, V4, V6 – V11 (each with telemedicine), V12, V13, and V14 (with telemedicine), V15, V16, V18, V19, and V20 (with telemedicine), V21, V22, V23, V25 and V26. Any remote visit may have a telemedicine component added if needed. With permission from the medical monitor and sponsor, other visits may be done remotely on a case-by-case extraordinary basis.

a V2 should be scheduled as follows: 1 week after V1 for subjects whose anti-hypertensive medications in the absence of diuretics are documented to have been stable for at least 3 weeks prior to V1; 3 weeks after V1 in subjects whose stability of anti-hypertensive medications in the absence of diuretics is unknown or not documented. However, in subjects who have an adjustment to anti-hypertensive medications or discontinuation of diuretics, V2 should be scheduled once the regimen is stable for 3 weeks, but no longer than 8 weeks after V1.

b During the Follow-up Period, 3 visits should be scheduled over 4 weeks. Visit 25 cannot occur earlier than the 8th day (+3) after the last dose of study drug (V24). Visit 26 will occur 7-14 ±3 days after V25. Visit 27 is the last visit in this study and will occur 28±3 days after the last dose of study drug (V24).

c Vital signs after the subject has been sitting for 5 minutes include heart rate, blood pressure, respiratory rate, and temperature at V1. At all subsequent visits include sitting heart rate and sitting blood pressure.

d A full physical examination will be performed at V1 and V24/ET. A brief physical examination will be performed at V17 and V27. Additional physical examinations will be performed only for assessment of signs or symptoms reported by the subject that might require further evaluation.

e Height will only be measured at V1.

f Serum pregnancy test for WOCBP will be obtained at V1. Subsequently, urine pregnancy tests will be performed at V5, V11/Last Titration, V14, V17, V20, V24/ET and V27. All positive urine results will be confirmed by a serum pregnancy test.

g Chemistry blood samples and calculations:

- Complete Chemistry Panel will be obtained at V1, V5, V11/Last Titration, V14, V17, V20, V24/ET, V27. If a subject required adjustment of blood pressure medications during Screening, then a repeat Complete Chemistry Panel will be performed at V2.
- Liver Chemistry Panel will be obtained at V1, V3-V24/ET, and V27. If a subject requires adjustment of blood pressure medications during Screening, then a repeat Liver Chemistry Panel will be performed at V2.
- Serum creatinine will be obtained at V3, V4, V25 and V26.
- eGFR will be calculated and reported whenever serum creatinine is obtained either as part of the Complete Chemistry Panel or when serum creatinine alone is measured.
- Hepatitis B surface antigen (HBsAg) and hepatitis C virus antibody (Anti-HCV) will be obtained at V1 only.

h Hematology blood samples will be obtained at V1, V5, V11/Last Titration, V17, V24/ET, and V27.

i First morning, fasting urine specific gravity and osmolality (AM trough) will be obtained at V5, at every visit during the Lixivaptan Titration Period (except V11/Last Titration) and at V24 and V27. Dispense container and review instructions (including not to take study drug on the morning of the next visit) with subject at V4-V9, V23, and V26.

j Trough (7-10 AM prior to AM dose) plasma specimens for measurement of lixivaptan will be obtained at V5, at the final visit during the Titration Period (V11/Last Titration), and during the Maintenance Period *if* ALT levels >3 x ULN and/or total bilirubin >2 x ULN occur.

k Utilizing a separate informed consent, all subjects will be asked to provide a DNA sample at the end of the Baseline Period (V5) to assess genetic variants associated with hepatic abnormalities (if and when feasible).

l Serious adverse events (SAEs) will be collected throughout the study beginning with signing of informed consent. All non-serious adverse events will be collected beginning at the start of the Titration Period. Adverse Events of Special Interest (AESI) include hepatic events and pregnancy and will be reported on special forms using the SAE pathway of reporting.

LIST OF ABBREVIATIONS

Abbreviation	Definition
ADPKD	autosomal dominant polycystic kidney disease
AE	adverse event
AESI	adverse events of special interest
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ART	anti-retroviral therapy
AST	aspartate aminotransferase
BID	twice per day
BMI	body mass index
cAMP	cyclic adenosine 3',5'-monophosphate
CFR	Code of Federal Regulations
CKD	chronic kidney disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CRO	Contract Research Organization
CS	clinically significant
CT	computerized tomography
DILI	drug induced liver injury
DILIN	Drug Induced Liver Injury Network
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
GCP	Good Clinical Practices
HBsAg	hepatitis B surface antigen
HCV	hepatitis C antibody
HERC	Hepatic Events Review Committee
HgbA1c	glycosylated hemoglobin
HHC	home healthcare clinician

Abbreviation	Definition
HIV	human immunodeficiency virus
IB	Investigator Brochure
ICF	Informed Consent Form
ICH	International Council for Harmonisation
IND	Investigational New Drug
IRB	Institutional Review Board
MedDRA	Medical Dictionary for Regulatory Activities
MRI	magnetic resonance imaging
NCS	not clinically significant
OTC	over-the-counter
PC1	Polycystin 1
PC2	Polycystin 2
PIN	personal identification number
PK	Pharmacokinetic
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
SOC	system organ class
TEAE	treatment-emergent adverse event
ULN	upper limit of normal
U_{osm}	urine osmolality
US	United States
WOCBP	Women of childbearing potential

1 INTRODUCTION

1.1 Background

Lixivaptan (also known as VPA-985, 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 PS 1998).[1] 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 (S_{osm}) as well as restoring normal levels of intracellular cyclic adenosine 3',5'-monophosphate (cAMP) (Chebib FT 2015).[2]

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 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 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 renal cysts (Antignac C 2015).[3] The progressive development and growth of numerous bilateral renal cysts results in fibrosis, renal 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, renal function loss and kidney failure (Antignac C 2015, Chapman AB 2015).[3,4]

The most frequent extrarenal manifestation of ADPKD is polycystic liver disease, which is typically associated with increased renal 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 MC 2015).[5] 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 cause fever, right upper abdominal pain, and possible elevated CA19.9 and alkaline phosphatase (ALP) 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 FA 2004).[6] 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 VE 2005).[7] 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 V2 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 VE 2012).[12] 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 O 2017).[14] 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 VE 2017b).[15]

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 renal cAMP plays a pivotal role in the complex phenotypical manifestation of ADPKD (Belibi FA 2004),[6] 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.[7] 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 X 2008),[8] 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 (Gattone VH 2003)[9] and tolvaptan (Wang X 2005),[10] have proved efficacious in normalizing renal 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 WE 2012).[11]

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 renal 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 VE 2012, Torres VE 2017a).[12,13] 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 renal function during the study (Devuyst O 2017).[14] 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 O 2017).[14]

More recently, the results of REPRISE, a second pivotal Phase 3 trial with tolvaptan for the treatment of ADPKD, were published (Torres VE 2017b).[15] 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 (Wang X 2005).[10] 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 renal volume increase, and delayed renal function decline. These beneficial effects were accompanied by a reduction in renal cAMP levels, as expected.

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.

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 subjects, ADPKD patients, and various other patient populations (patients with hypervolemic and euvolemic hyponatremia) demonstrated that treatment with lixivaptan readily suppresses urinary osmolality to levels below the target threshold of 300 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 O 2017).[14]

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

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. 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. Four identified human metabolites of lixivaptan (WAY-137930, 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.

Studies using rodent genetic models have demonstrated that inhibiting vasopressin signaling is protective against the development of PKD. Rats harboring a renal cyst-inducing PKD mutation genetically crossed to produce offspring with no circulating serum vasopressin do not develop cysts (Wang X 2008).[8] In addition, the V_2 vasopressin receptor antagonists mozavaptan and tolvaptan can normalize renal cAMP and correct disease manifestations in rodent models of ADPKD (Gattone II VH 2003, Wang X 2005).[9,10] Similarly, lixivaptan reduces renal cAMP levels in rodent models of PKD and is also protective against the development of kidney disease manifestations, including reduced cystic burden, reduced renal volume increase, and delayed renal function decline. Additional information regarding the nonclinical evaluation of 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 subjects, 10 Phase 2a studies, and 4 Phase 3 studies in subjects with hyponatremia. More than 1600 subjects received at least one dose of lixivaptan as part of the hyponatremia development program, including 867 who participated in Phase 2 and 3 studies. The extent of exposure ranged from 1 day to 180 days with the average around 28 days. Total daily doses ranged from 1 mg to 800 mg with the average around 169 mg. 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.

Lixivaptan is being tested in ADPKD patients in the ongoing, open-label Phase 2 study, PA-102. This study is being conducted to directly 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 subjects with both ADPKD and CKD stage 1 (CKD1), stage 2 (CKD2), and stage 3 (CKD3). At the dose of 200 mg BID, based on preliminary results, lixivaptan effectively reduced U_{osm} to dilute levels, i.e., levels below 300 mOsm/kg, throughout 24 hours. The effect on U_{osm} was demonstrated in all severities of CKD tested in the study. 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 V2 receptor throughout 24 hours. However, 50 mg BID is an appropriate starting dose to assess tolerability. Lixivaptan was well tolerated by ADPKD subjects; the most common AEs reported in 3 or more subjects were dry mouth, headache, and nausea. No subject was discontinued because of an AE. Additional information regarding the results of this study can be found in the IB.

1.7 Study Rationale

This is a Phase 3, open-label, repeat dose study designed to assess hepatic safety, non-hepatic safety, and efficacy in subjects who previously experienced abnormal liver chemistry test results while treated with tolvaptan. Subjects who had to stop tolvaptan therapy due to abnormal liver chemistry test results do not currently have available therapeutic options. In this study, such subjects will be treated with lixivaptan to determine if it is a safer alternative. Up to 50 subjects will be enrolled and treated. Evaluations will 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.

Lixivaptan as a safer alternative to tolvaptan

Tolvaptan, a vasopressin V_2 receptor antagonist, has been approved in many territories, to slow the progression of renal function deterioration in patients with autosomal dominant polycystic kidney disease (ADPKD). However, drug-induced liver injury (DILI) occurs in a

certain percentage of subjects taking tolvaptan. Acute liver failure requiring liver transplantation has also been reported (Endo M 2019).[24] Subjects 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 has allowed for the assessment of potential abnormal liver chemistry tests prior to conducting large clinical trials (Woodhead JL 2017a).[19] This is a Quantitative Systems Pharmacology model of drug-induced liver injury (DILIsym™). 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 which have been identified as putative mechanisms for DILI.

DILIsym was employed to model tolvaptan-mediated liver injury (Woodhead JL 2017b).[20] DILIsym was able to successfully recapitulate the observed toxicity. The frequency of predicted alanine aminotransferase (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 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 JL 2019).[21] *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 2). Additional information regarding the DILIsym evaluation of lixivaptan can be found in the IB.

Table 2 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	Study not yet conducted	0/285
Tolvaptan	90/30 mg q AM/PM, 24 weeks	4.4%	18/229 7.86%

Therefore, compared to tolvaptan, *in silico* modeling and simulation have demonstrated that lixivaptan does not have the same potential for liver injury.

A safety assessment of the potential for lixivaptan to be associated with clinical hepatotoxicity in the hyponatremia program was included in the Cardiokine NDA. In the healthy volunteer studies, analysis of adverse events and liver-related laboratory tests showed no evidence of liver toxicity with lixivaptan. In the Phase 2 and Phase 3 trials in subjects with hyponatremia, a population prone to liver chemistry abnormalities, there were small mean decreases in ALT, AST, and total bilirubin in the lixivaptan treated subjects. Furthermore, there were no instances of hepatotoxicity meeting the definition of Hy's Law among subjects treated with lixivaptan. The percentage of subjects who had concomitant elevations of ALT >3 x ULN and total bilirubin >2 x ULN was low and numerically lower in the lixivaptan group compared to the placebo group, further suggesting no lixivaptan-related liver toxicity. Analysis of the Phase 2 ADPKD study also did not show any hepatic AEs.

Recently, Palladio has had the opportunity to test the hypothesis of lixivaptan's superior liver safety in a clinical setting. An ADPKD patient who experienced disabling kidney pain was treated with tolvaptan in 2017 with marked clinical improvement. However, tolvaptan-associated liver toxicity manifest as ALT elevation required interruption of tolvaptan treatment. Two subsequent attempts to re-introduce the drug resulted in rapid recurrence of the ALT abnormalities (Figure 2) and the drug was stopped permanently in December 2017. The treating physician reached out to Palladio for access to lixivaptan for treatment of the recurrent disabling pain. Treatment with lixivaptan in this subject was started in 2019 under an expanded access protocol. Pain reduction and improvement in measured quality of life occurred as the dose of lixivaptan was up-titrated. At the time of this writing, there has been no evidence of increased ALT values in this subject after 14 months of therapy with lixivaptan (Figure 3).

Figure 2. Time course of ALT levels in relationship to dosing of tolvaptan in a patient with painful ADPKD

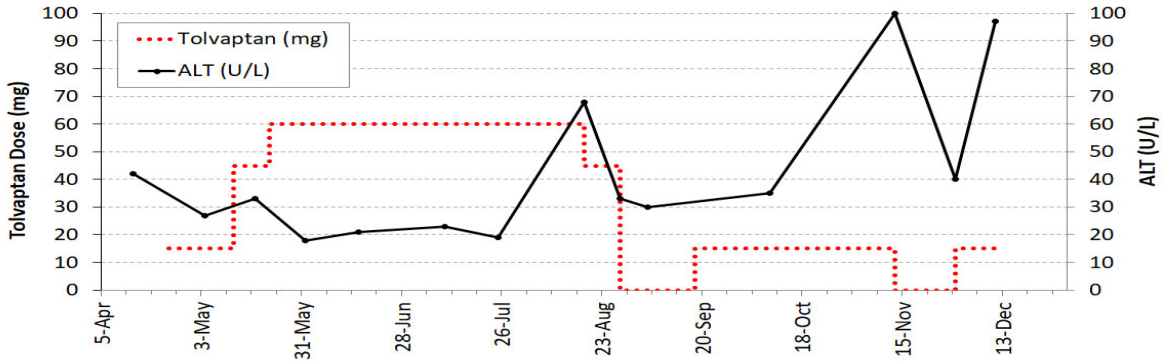
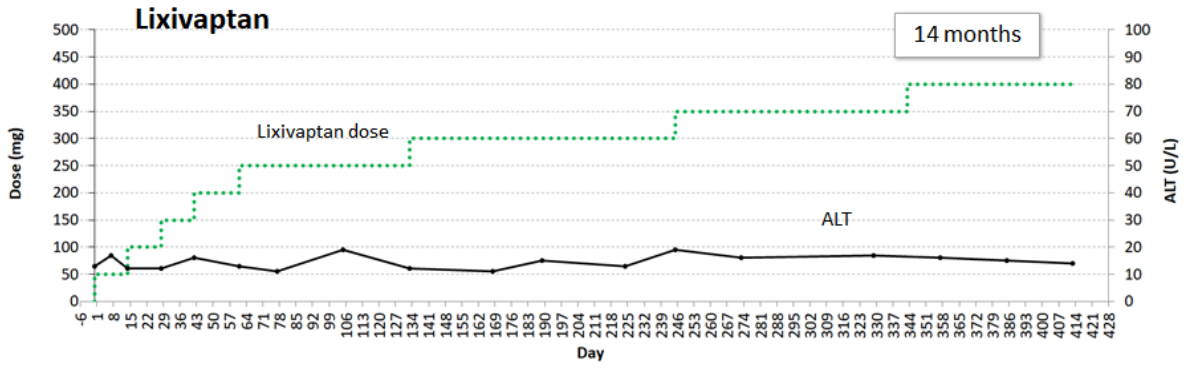


Figure 3. Time course of ALT levels in relationship to dosing of lixivaptan in a patient with painful ADPKD



These results strongly suggest that lixivaptan may represent a safer alternative to tolvaptan not only for the general patient population, but also for those subjects who experienced abnormal liver chemistry test results while receiving tolvaptan. The intent of the PA-ADPKD-303 study is to replicate the experience of hepatic safety with lixivaptan in a larger number of ADPKD patients previously treated with tolvaptan who discontinued the drug permanently because of abnormal liver chemistry test results.

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 previous clinical studies conducted in healthy subjects, in subjects with hyponatremia of various etiologies, and in the PA-102 study of subjects with ADPKD.

The dose range selected for this study is 100 mg BID to 200 mg BID (with 50 mg BID as a starting dose). Doses will be titrated in individual subjects to achieve an appropriate reduction in urine specific gravity (as a point-of-care surrogate for U_{osm}) and tolerability or the highest dose is achieved. The same dose range and dose titration scheme will be used in the Phase 3 pivotal study (PA-ADPKD-301) to assess the efficacy and tolerability of lixivaptan in subjects with ADPKD.

2 STUDY OBJECTIVES AND ENDPOINTS

2.1 Study Objectives

2.1.1 Primary Objectives

The primary objectives of this study are:

- To assess the effect of lixivaptan on hepatic safety in subjects with ADPKD who experienced abnormal liver chemistry test results on tolvaptan that resulted in permanent discontinuation of tolvaptan.

2.1.2 Secondary Objectives

The key secondary objectives of this study are

- To characterize the non-hepatic safety and tolerability of lixivaptan in subjects with ADPKD who previously experienced abnormal liver chemistry test results while treated with tolvaptan.
- To assess renal function (efficacy) in ADPKD subjects while on lixivaptan using change in estimated glomerular filtration rate (eGFR).

2.1.3 Exploratory Objectives

- To evaluate the relationship between abnormal liver chemistry test results and dose/exposure of lixivaptan as data permit.
- To assess potential genetic risk factors for hepatic abnormalities (if and when feasible).

2.2 Study Endpoints

2.2.1 Primary Endpoint

Safety:

- Proportion of subjects who develop ALT levels >3 x ULN during the Titration or Maintenance Periods that were assessed by the independent Hepatic Events Review Committee (HERC) to be at least probably related to lixivaptan and resulted in discontinuation of the study drug.

2.2.2 Secondary Endpoints

Safety:

- Proportion of subjects who develop ALT levels >5 x ULN during the Titration or Maintenance Periods that were assessed by the independent HERC to be at least probably related to lixivaptan and resulted in discontinuation of the study drug.
- Proportion of subjects who develop ALT levels >3 x ULN during the Titration or Maintenance Periods that were assessed by the independent HERC to be at least probably related to lixivaptan and resulted in dose reduction of the study drug.

- The safety and tolerability of lixivaptan assessed through evaluation of AEs, clinical laboratory findings (clinical chemistry including additional analyses of liver chemistry tests, hematology and urinalysis), vital signs, and 12-lead electrocardiograms (ECG).

Efficacy:

- Change in eGFR from baseline (mean of 3 eGFR determinations obtained during Screening and Baseline Periods) to final assessment (mean of 3 eGFR determinations obtained during the Follow-up Period).

2.2.3 Exploratory Endpoints

- Correlation of abnormal liver chemistry test results to plasma levels of lixivaptan.
- Assessment of genetic variants (if and when feasible) to understand potential genetic risk factors for hepatic abnormalities.

3 INVESTIGATIONAL PLAN

3.1 Study Design

3.1.1 Overview of Study Design

This is a Phase 3, open-label, repeat dose study designed to assess hepatic safety, non-hepatic safety, and efficacy of lixivaptan in subjects who previously experienced abnormal liver chemistry test results while treated with tolvaptan that resulted in permanent discontinuation of tolvaptan for that reason. Up to 50 subjects will be enrolled and treated. Evaluations will include frequent testing of liver chemistry tests (every week during the Baseline and Titration Periods, every 4 weeks during the Maintenance Period, and at the final Follow-up Visit), physical examinations, vital signs, safety labs (non-hepatic serum chemistry, hematology, urinalysis), eGFR, urine osmolality determinations, and trough serum concentrations of lixivaptan. A DNA sample to assess genetic variants associated with hepatic abnormalities (if and when feasible) will be obtained under a separate biobanking informed consent at Visit 5 or later in the study. After meeting study entry criteria during a 1-3-week Screening Period that can extend up to 8 weeks (in subjects who need adjustment of anti-hypertensive medications), subjects will enter a 3-week no study drug Baseline Period to obtain baseline serum creatinine measurements (for eGFR determinations) and repeat liver chemistry tests to assess baseline variability, followed by a 3-6 week Titration Period during which lixivaptan administered twice daily will be titrated to a dose that is tolerated and results in a reduced trough urine specific gravity (or until the maximum dose level is reached) as described below. The minimum dose to enter the Maintenance Period is Level 2 (100 mg BID). Treatment will continue for up to 52 weeks (12 months) after which study drug will be stopped, and final safety and efficacy assessments obtained during the Follow-up Period of 4 weeks. The maximum study duration will be 73 weeks (16.8 months).

All assessments and the relative timings are listed in the Schedule of Study Procedures (Table 1). Certain visits may be conducted remotely with a home healthcare clinician (HHC), but Visits 1, 5, 17, 24, and 27 are to be conducted in the clinic (with only extraordinary exceptions at the discretion of the medical monitor and sponsor). Some of the remote visits will include a telemedicine component with the Investigator while other remote visits may have that option if deemed necessary for a medical reason. Details are provided in Table 1. Study procedures by visit are outlined in Section 6. A comprehensive description of study procedures can be found in Section 7. Dosing procedures are outlined in Section 5.

Note that the study may be interrupted at any time if safety issues compromise the safety of the subjects.

The study schematic is depicted in Figure 1.

3.1.2 Detailed Study Design

Screening Period (Visits 1, 2): After obtaining Informed consent, screening assessments will be performed at Visit 1 to determine subject eligibility. Subjects whose anti-hypertensive medications in the absence of diuretics are known and documented to have been stable

(unchanged) for at least 3 weeks prior to Visit 1 may proceed to Visit 2 in 1 week to confirm eligibility. Subjects for whom the stability of anti-hypertensive medications in the absence of diuretics is unknown or not documented must be observed for 3 weeks during the Screening Period to ensure stable dosing of these medications and then will be scheduled for Visit 2. For other subjects, diuretic therapy may be discontinued and/ or the antihypertensive therapy adjusted during the Screening Period; however, following any adjustments, the dose for these medications must remain stable for a minimum of 3 weeks prior to the completion of the Screening Period (Visit 2). As such, the Screening Period (from Visit 1 to Visit 2) may be extended up to 8 weeks for those subjects who need to discontinue diuretic therapy and/or have their anti-hypertensive therapy optimized.

Subjects who meet all the inclusion criteria and none of the exclusion criteria and achieve stability of background medications will be scheduled for end-of-screening assessments including body weight, vital signs, and a Complete Chemistry Panel (only if blood pressure medications were adjusted) at Visit 2 (which may be conducted remotely by a HHC with telemedicine to determine final eligibility). Eligible subjects will then enter the Baseline Period immediately at the completion of Visit 2. Subjects who fail inclusion/exclusion criteria because of temporary or correctable reasons may be re-screened up to 2 times after obtaining new informed consents with the approval of the medical monitor and sponsor.

Baseline Period (Visits 3 - 5): Following completion of all Screening assessments and establishment of background medication stability, subjects will enter the 3-week no study drug treatment Baseline Period during which time liver chemistry tests and serum creatinine (for eGFR determinations) will be assessed weekly to establish a baseline and assess individual variability. Baseline trough spot urine specific gravity (as a point-of-care surrogate for urine osmolality) and U_{osm} will be determined only at Visit 5. Visits 3 and 4 may be conducted remotely by a HHC. At the successful completion of the Baseline Period (Visit 5), subjects will be dispensed study drug and begin treatment. This completes the Baseline Period and subjects will enter the Titration Period.

Titration Period (Visits 6 - 11): During a period of 3-6 weeks, the lixivaptan dose will be increased weekly according to the titration schedule (Table 4) to achieve a level that results in both a tolerable dose and reduced first morning (trough) spot urine specific gravity (as a point-of-care surrogate for urine osmolality) or until the maximum dose level (Level 4 [200 mg BID]) is reached. Dosing will be started at Level 1 (50 mg BID), but the minimum dose to enter the Lixivaptan Maintenance Period is Level 2 (100 mg BID). In most circumstances, dose tolerability will be confirmed during an additional week of dosing before the subject advances to the Maintenance Period. At the 2 highest dose levels (Level 3 and Level 4) where aquaretic effects may be problematic in certain subjects, there will be an opportunity to reduce the evening (PM) dose (Level 3a [150 mg AM/100 mg PM] and Level 4a [200 mg AM/150 mg PM]; see Section 5.2). Liver chemistry tests will be assessed weekly during the Titration Period. Urine specific gravity and U_{osm} will be measured at all titration visits that occur except Visit 11. All titration visits may be conducted remotely by a HHC with telemedicine.

Maintenance Period (Visits 12 - 24): Subjects will continue to receive lixivaptan at the dose achieved at the end of the Titration Period for up to 52 weeks (12 months). The investigator will have the ability to lower the dose if necessary, to improve tolerability. Assessments, including liver chemistry tests, will be performed every 4 weeks during this period. Visits 12, 13, 14, 15, 16, 18, 19, 20, 21, 22, and 23 may be conducted remotely by a HHC. If conducted remotely, Visits 14 and 20 will include telemedicine and the other visits may include telemedicine if deemed necessary by the Investigator.

Follow-up Period (Visits 25 - 27): After the last dose of lixivaptan is given, the subjects will enter the Follow-up Period which will last 4 weeks. Three assessments of serum creatinine to determine eGFR will be obtained starting as early as the 8th day after the final dose of study medication and continuing through the 28th day of the Follow-up Period. Visits 25 and 26 may be conducted remotely by a HHC (using telemedicine if deemed necessary by the Investigator). Other routine safety assessments will be performed at the last visit (Visit 27).

Study assessments including clinical laboratory tests, spot urine collections, physical examination findings, vital signs, ECGs, trough PK sampling, and monitoring of AEs will be performed at the timepoints presented in the Schedule of Procedures (Table 1). Serious AEs (SAEs) will be recorded from the time the subject signs the Informed Consent Form (ICF) until exit from the study. All other AEs will be recorded from the start of the Titration Period until exit from the study.

The maximum study duration is 73 weeks (16.8 months). Note that the study may be interrupted at any time if safety issues compromise the safety of the subjects.

3.2 Rationale for Study Design

This study design is similar to the study design used to assess the safety of ambrisentan in subjects with pulmonary arterial hypertension (PAH) who had discontinued endothelin receptor antagonist therapy due to abnormal liver chemistry test results (McGoon MD 2009).[22] Subjects in this study will have previously discontinued tolvaptan permanently due to abnormal liver chemistry test results. The rationale for selecting a similar design is twofold.

1) Selection of an enriched population of subjects

It is only in the ADPKD population that abnormal liver chemistry tests resulting in drug discontinuation have been observed with tolvaptan. Liver injury has not been detected following exposure to tolvaptan in non-ADPKD patients including subjects with cirrhosis, congestive heart failure, or hyponatremia (Watkins PB 2015).[23] Although other factors might have contributed to tolvaptan's toxicity, it is possible that subjects with ADPKD may be more susceptible to tolvaptan-associated liver injury (Watkins PB 2015).[23] Because the liver injury may be severe, tolvaptan is no longer a treatment option for ADPKD subjects experiencing this side effect of tolvaptan. Therefore, subjects who discontinue from tolvaptan due to liver injury are a higher risk population where the hepatic safety profile of lixivaptan may be appropriately tested and where the potential benefit of vaptan therapy may be provided in the absence of other therapeutic choices.

2) Sample size consideration

The sample size of up to 50 subjects selected for this study is similar to that used in the ambrisentan hepatic safety study (McGoon MD 2009).[22] Although, a placebo-controlled, double-blind assessment of lixivaptan is the preferred study design, a placebo-controlled trial would not be feasible in this patient population due to the low frequency of patients with abnormal liver chemistry test results following treatment with tolvaptan. Conversely, a 52-week placebo-controlled, double-blind trial with an additional 52 weeks of open-label lixivaptan treatment to assess the efficacy and safety of lixivaptan for the treatment of ADPKD (PA-ADPKD-301) in a broader patient population will be conducted approximately in parallel to this study. The number of subjects planned to be enrolled in the PA-ADPKD-301 trial is 1200. A comparison of the frequency of serum ALT elevations between the lixivaptan and placebo arms of that trial will provide complementary data to support the hepatic safety profile of lixivaptan. Additionally, subjects in the placebo arm of the double-blind phase of that study will receive lixivaptan during the open-label phase and be studied for an additional 52 weeks, thus, providing further hepatic safety information. Safety data from the pivotal efficacy and safety trial (PA-ADPKD-301) and this hepatic safety study (PA-ADPKD-303) will be used to support the predicted hepatic safety profile of lixivaptan. Therefore, both studies will contribute to understanding the safety of lixivaptan in ADPKD patients, particularly any effects on liver chemistry tests.

4 SUBJECT SELECTION AND WITHDRAWAL CRITERIA

4.1 Selection of Study Population

Approximately 100-200 subjects will be screened in order to enroll and treat up to approximately 50 subjects at up to approximately 25 sites globally. An eligibility review and discussion between the Investigator and the medical monitor will be conducted before each subject is enrolled (completes Visit 3) at each site. Subjects will be enrolled only if they meet all the inclusion criteria and none of the exclusion criteria. With the approval of the medical monitor and sponsor, subjects who fail inclusion/exclusion criteria due to temporary or correctable reasons may be re-screened up to 2 times after re-consenting each time.

Deviations from the inclusion and exclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability, or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

4.1.1 Inclusion Criteria

In order to qualify for enrollment in this study, each subject must meet the following inclusion criteria:

1. Male or female, between 18 and 65 years of age (inclusive) at the time of Screening.
2. Female subjects 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) must agree to practice appropriate methods of birth control or remain abstinent (only if this is the usual and preferred lifestyle of the participant) during the study and for 30 days after the last dose of study drug. Acceptable forms of contraception include any of the following:
        - hormonal contraceptives (i.e., oral, intravaginal, transdermal, injectable, implantable)
        - double barrier methods of non-hormonal contraception are permitted in this study.
            - male or female condom with spermicide (cream, spray, gel, suppository, contraceptive sponge, or polymer film)
            - diaphragm, cervical cap, or contraceptive sponge with spermicide (with or without a condom)
        - 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

- bilateral tubal ligation
- Essure® procedure (tubal occlusion).

3. Male subjects and their female partners of childbearing potential must also use the contraceptive methods listed above or remain abstinent (only if this is the usual and preferred lifestyle of the participant) during the study and for 30 days after the last dose of study drug.
4. Diagnosis of ADPKD confirmed by previous treatment with tolvaptan specifically for that indication and supported by evidence from any imaging modality or genetic analysis.
5. Screening eGFR ≥20 ml/min/1.73m^2.
6. Body mass index (BMI) between 18 and 35 kg/m^2 (inclusive) at the time of Screening.
7. Documented history of:
    a. Based on upper limit of normal (ULN): at least 2 elevated alanine aminotransferase (ALT) levels, 1 ALT level >2 times (x) the ULN and 1 ALT level >3 x ULN while the subject was receiving tolvaptan, or within 4 weeks after tolvaptan discontinuation, with no other explanation for the ALT elevations. The 2 elevated ALT measurements could be recorded during the same instance of liver injury or during distinct instances;

    OR

    b. Based on the subject's stable baseline as determined by the Investigator: at least 2 elevated ALT levels, 1 ALT level >2 x the subject's stable baseline level and 1 ALT level >3 x the subject's stable baseline level while the subject was receiving tolvaptan, or within 4 weeks after tolvaptan discontinuation, with no other explanation for the ALT elevations; provided that at least one ALT elevation was >2 x ULN. The 2 elevated ALT measurements could be recorded during the same instance of liver injury or during distinct instances;

    OR

    c. A pattern of ALT elevations deemed by the Investigator to be consistent with tolvaptan liver injury with no other explanation for the ALT elevations and the agreement of the medical monitor and sponsor.
8. Permanent discontinuation of prior tolvaptan treatment because of the abnormal liver chemistry test results.
9. The subject may or may not have been re-challenged with tolvaptan. If re-challenge was performed, the ALT level should have increased to >2 x ULN upon rechallenge or the ALT level was increasing but tolvaptan was stopped for patient safety reasons before it reached > 2 x ULN after having previously normalized.
10. Appropriate control of hypertension including an angiotensin converting enzyme inhibitor or angiotensin receptor blocker (unless not considered appropriate for the subject) without the use of a diuretic in concert with KDIGO "Clinical Practice

Guideline for the Management of Blood Pressure in Chronic Kidney Disease".[17]

11. 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

Subjects meeting any of the following exclusion criteria are not eligible for study enrollment.

1. Known sensitivity or idiosyncratic reaction to any compound present in lixivaptan and related compounds.
2. Hypovolemia on physical examination at Screening.
3. Abnormal serum sodium concentration at Screening.
4. Subjects who have taken any investigational drug or used an investigational device within 30 days, or 5 half-lives, whichever is longer, prior to Screening.
5. Subjects who are taking, have taken within the 2 weeks prior to Screening, 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.
6. Simvastatin at total daily doses >10 mg or amlodipine at total daily doses >5 mg.
7. Prior use of tolvaptan within the 3 months prior to Screening OR until a previously elevated ALT level has returned to ≤1 x ULN for at least 3 months.
8. Prior use of lixivaptan or participation in a clinical study with lixivaptan within the 3 months of Screening.
9. Prior use of conivaptan, somatostatin analogs (e.g., lanreotide, pasireotide, octreotide, etc.), metformin (allowed for diabetes mellitus), nicotinamide, bardoxolone, venglustat, demeclocycline, or mTOR kinase inhibitors (e.g., everolimus, sirolimus, etc.) to treat ADPKD within the 3 months prior to Screening.
10. Requirement for chronic diuretic use.
11. History of advanced diabetes (e.g., glycosylated hemoglobin [HgbA1c] >7.5%, and/or glycosuria by dipstick, significant proteinuria [>300 mcg albumin/mg creatinine], retinopathy), other significant renal disease, transplanted kidney, recent kidney surgery within the 6 months prior to Screening (including cyst drainage or fenestration) or acute kidney injury within 6 months prior to Screening.
12. Clinically significant incontinence, overactive bladder, or urinary retention (e.g., benign prostatic hyperplasia).
13. New York Heart Association Functional Class 3 or 4 heart failure or other significant cardiac or ECG findings that could pose a safety risk to the subject.
14. Clinically significant liver disease or impairment or active chronic hepatitis at Screening.
15. Positive test results for hepatitis B surface antigen (HBsAg) or hepatitis C (HCV) antibody.
16. History of infection with human immunodeficiency virus (HIV) unless the subject is stable and doing well on a non-CYP interacting anti-retroviral therapy (ART) regimen and who has not required more than 2 changes in their ART regimen.

17. ALT or aspartate aminotransferase (AST) values >1.5 x ULN during Screening/Baseline.
18. Total bilirubin values >1.0 x ULN during Screening/Baseline.
19. History of drug or alcohol abuse in the 2 years prior to Screening.
20. Any malignancy within the 5 years prior to Screening except for basal cell carcinoma successfully treated with local therapy.
21. Medical history or findings that preclude safe participation in the study or who are likely to be non-compliant with study procedures in the opinion of the Investigator or medical monitor.

4.2 Completion and Withdrawal of Subjects from the Study

4.2.1 Definition of Completed Subjects

A completed treatment subject is one who has completed the procedures beginning with Screening through the last Maintenance Period visit (Visit 24). A completed study subject is one who has completed the procedures beginning with Screening through the last Follow Up Period visit (Visit 27). The end of the study is defined as the last subject's last visit.

4.2.2 Screening and Baseline Failures

Subjects 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 and Baseline Periods, subjects who withdraw their consent or fail to meet all the entry criteria to participate in the study will be designated as "Screen failures" or "Baseline failures", respectively. Screen or Baseline failure subjects will be recorded as such on the electronic Case Report Form (eCRF) and do not need to enter the Follow-up Period unless a SAE is ongoing, in which case they will be scheduled for Visit 27 in 4 weeks. Screen failure subjects who do not meet study entry requirements due to temporary or correctable reasons may be re-screened up to 2 times with the written approval of the medical monitor and sponsor.

4.2.3 Subject Withdrawal Criteria

A subject may withdraw from the study at any time at his/her own request or may be withdrawn at any time at the discretion of the Investigator for safety, behavioral, or administrative reasons.

Subjects who discontinue or are discontinued after the Baseline Period will be considered as a withdrawal, regardless of whether they have received study drug. A subject that withdraws from study drug will be treated as a study withdrawal unless they are being followed for safety reasons.

All premature discontinuations and their causes must be carefully documented in the eCRF, and in the AE form (if applicable).

Subjects not completing the entire study on study drug should be encouraged to continue with the visit schedule, a modified visit schedule, or telephone follow-up as agreed between the subject and the Investigator. At a minimum, subjects should be encouraged to complete the Early Termination Visit (Visit 24 – in the clinic) at the time of study drug discontinuation and the Follow-up Visits (Visits 25 – 27) over the 4 weeks after the last dose of study drug. Subjects with ongoing SAEs or AEs believed to be at least possibly related to study medication will continue to be followed until resolution or for 28 days as warranted by the nature of the AE. If the AE is related to abnormal liver chemistry test results, see Section 7.3.7 for additional follow-up requirements.

4.2.4 Reasons for Withdrawal

Reasons for withdrawal (subjects who refuse to return for any remaining study visits) or discontinuation (subjects who prematurely stop the treatment) at any time during the study may include, but are not limited, to the following:

1. No longer meet the protocol inclusion or exclusion criteria, e.g., require a prohibited concomitant medication.
2. Noncompliance with the protocol.
3. Lack of efficacy.
4. A serious or intolerable AE that, in the Investigator's opinion, requires 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 of an intercurrent illness that justifies withdrawal. If the AE is related to abnormal liver chemistry test results, see Section 7.3.7 for additional follow-up requirements. Mild elevations in serum creatinine or serum sodium are known pharmacodynamic effects of vasopressin antagonists like lixivaptan and are transient and reversible. Therefore, subjects should not be withdrawn from study participation solely due to mildly elevated serum creatinine or serum sodium. Cases where eGFR has declined by 25% or more from baseline should be discussed with the medical monitor and sponsor.
5. Death
6. Lost to follow-up.
7. Other (e.g., pregnancy, study burden, development of contraindication to use of study drug).
8. Withdrawal of consent (partial or complete).
9. Investigator's or Sponsor's decision to discontinue the subject's participation in the study.
10. Sponsor termination of the study.

Upon occurrence of an SAE or intolerable AE, the Investigator will confer with the medical monitor and Sponsor. If a subject is discontinued from study drug because of an AE, the

event will be followed until it is resolved or stable or up to 28 days as determined by the Investigator. Any subject may withdraw his or her consent at any time. Subjects' safety will be closely monitored throughout the study, and the study will be conducted following Good Clinical Practices (GCP). The entire study may be stopped at any time at the discretion of the Sponsor.

4.2.5 Methods to Prevent Lost to Follow-up

The Investigator must make every attempt to follow-up subjects who have withdrawn from the study at any time and for any reason. When a subject 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 subject 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 subject cannot be reached, they should be identified as "lost to follow-up" in the eCRF.

4.2.6 Replacements

Subjects who withdraw from the study will not be replaced.

4.2.7 Withdrawals Due to Abnormal Liver Chemistry Test Results

Guidance for management of abnormal liver chemistry test results is provided in Section 7.3.7. In some cases, it will be advisable to discontinue the study drug and under other circumstances dose reduction might be appropriate. These actions in response to abnormal liver chemistry test results must be discussed with the medical monitor and sponsor prior to taking any action (except in an emergency). If study drug is withdrawn, re-starting study drug should generally be encouraged 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. In certain circumstances described in Section 7.3.7.2, restarting study drug should only occur when the HERC believes the relationship between the abnormal liver chemistry test results and the study drug is less than 50% according to the DILIN criteria, modified, Section 13.2 (Fontana RJ 2009).[18]

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 3.

Table 3. Investigational Product

	Lixivaptan
Strength	50 mg
Formulation	Capsules, packaged 30 per bottle
Inactive ingredients	Polyethylene glycol 400, NF/EP; Polyethylene glycol 1000, NF/EP; Povidone (K-17), USP/EP; Polysorbate 80, NF/EP; Butylated hydroxytoluene; Butylated hydroxyanisole
Manufacturer	PMRS, Inc. 202 Precision Road Horsham, PA 19044
Packager	PCI Pharma Services 4545 Assembly Drive Rockford, IL 61109

5.1.2 Route of Administration

The study drug (lixivaptan capsule) is intended to be taken orally, i.e., to be swallowed whole with water. The capsule should NOT be opened.

5.1.3 Study Drug Packaging, Labeling, and Storage

Study drug will be packaged and labeled by PCI Pharma Services, Rockford, IL, USA according to all local legal requirements. The study drug will be labeled in accordance with applicable regulatory requirements.

All study drug supplies should be stored at the study site in its original packaging in a secure, locked area under the responsibility of the Investigator or other authorized individual. At the study site, lixivaptan should be stored in accordance with the specifications detailed in the study pharmacy manual. Once lixivaptan is dispensed, the subject should be instructed to store it in its original packaging and in accordance with lixivaptan treatment labelling at all times until ready to take. For subjects who utilize remote visits, study drug will be dispensed and shipped from the study site to the subject's home, or to an alternate location pre-specified by the subject, via an experienced courier approved by the Sponsor. Subjects 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 HHC arrives.

5.1.4 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 and used by each subject 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 drug 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. During the Lixivaptan Titration Period, 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 4. 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 subjects' tolerability at either the 150 mg BID or 200 mg BID dose levels, respectively. Titration will stop when a tolerable dose level is reached that achieves the goal of lowering trough (first AM) fasting spot urine specific gravity to ≤1.005 (as a point-of-care surrogate for U_{osm}) *or* the highest dose (Level 4 or 4a) is achieved (regardless of trough urine osmolality). Subjects tolerating the drug at Level 2, 3, and 3a, but not achieving the urine specific gravity goal may be allowed to continue in the study after consultation with the medical monitor and sponsor.

Table 4 Dosing Levels during the Lixivaptan Titration Period

Dose Level	AM Dose	PM Dose*
1[†]	50 mg (1 capsule)	50 mg (1 capsule)
2	100 mg (2 capsules)	100 mg (2 capsules)
3	150 mg (3 capsules)	150 mg (3 capsules)
3a[‡]	150 mg (3 capsules)	100 mg (2 capsules)
4	200 mg (4 capsules)	200 mg (4 capsules)
4a[#]	200 mg (4 capsules)	150 mg (3 capsules)

*PM dose to be administered approximately 10 hours after the AM dose.
[†]Dose Level 1 is only for initiation of treatment.
[‡]Subjects having difficulty with aquaretic effects at Dose Level 3 can drop back to 150 mg in the AM and 100 mg in the PM (Dose Level 3a: 150/100 mg).
[#]Subjects having difficulty with aquaretic effects at 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 and reduced trough urine specific gravity are achieved (or tolerability and maximum dose), subjects will stay on that dose for 1 additional week to confirm tolerability (except when the dose is reduced to Level 3a or Level 4a after 2 weeks of dosing at Level 3

or Level 4, respectively, when a single final week at the lower dose level is sufficient; see Table 5). The most common dosing scenarios that can be advanced to the Maintenance Period are shown in Table 5. With concurrence of the medical monitor and the sponsor, some subjects may be allowed to enter the Maintenance Period at a lower dose (but not less than 100 mg BID) if tolerability is an issue after trying a higher dose (regardless of specific gravity result at the lower dose):

Table 5. Dosing Diagram in the Lixivaptan Titration Period

Dose Level	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
2	50 mg BID	100 mg BID	100 mg BID			
3	50 mg BID	100 mg BID	150 mg BID	150 mg BID		
3a	50 mg BID	100 mg BID	150 mg BID	150/100 mg (AM/PM)	150/100 mg (AM/PM)	
3a (when 2nd week of Level 3 is not tolerated)	50 mg BID	100 mg BID	150 mg BID	150 mg BID	150/100 mg (AM/PM)	
4	50 mg BID	100 mg BID	150 mg BID	200 mg BID	200 mg BID	
4a	50 mg BID	100 mg BID	150 mg BID	200 mg BID	200/150 mg (AM/PM)	200/150 mg (AM/PM)
4a (when 2nd week of Level 4 is not tolerated)	50 mg BID	100 mg BID	150 mg BID	200 mg BID	200 mg BID	200/150 mg (AM/PM)

Subjects will continue to adhere to the recommended fluid intake regimen outlined in Section 5.8.

Subjects who tolerate their optimized dose will then enter the Maintenance Period during which time they will continue at the lixivaptan dose level achieved at the end of the Titration Period. During the Maintenance Period, the dose may be adjusted downward at the Investigator's discretion if needed to manage non-hepatic side effects and may also be increased back to the dose achieved at the end of the Titration Period. The Investigator may instruct the subject to hold the study drug for up to 7 days, if necessary, to manage acute intercurrent illness, tolerability issues, surgical procedures or life situations, e.g., airplane travel, etc. Additionally, subjects who have a longer interruption due to illness, including COVID-19, or other reasons may be able to re-start study drug when medically stable after discussion with the medical monitor and sponsor. Temporary or permanent study drug discontinuation for management of abnormal liver chemistry test results is described further in Section 7.3.7.

5.3 Method of Assigning Subjects to Identification Numbers

Each investigator will be assigned a unique 5-digit site number (XXXXX). This site number will be concatenated with a leading protocol number (303) and a following 3-digit subject

number to assure that each subject will be uniquely identified in the clinical database. Thus, a prototypical subject number in this study will look like 303-XXXXX-YYY. As subjects are screened, the next subject qualified chronologically at a site will be assigned the next number in ascending numerical order. Thus, the subject identification number for the first subject screened at the first site activated will be 303-10001-001. The first subject screened at the second site activated will be 303-10002-001 and so on. The subject identification number assigned at Screening will be used for that subject throughout the study, including if the subject re-screens.

5.4 Blinding

This will be an open-label study.

5.5 Randomization and Treatment Assignment

This study will not be randomized.

5.6 Treatment Compliance

Dispensing of study drug and reconciliation will be done at each visit during the Titration Period and every 4 weeks during the Maintenance Period. A final reconciliation will be done by the site once each subject completes study drug dosing. Subject compliance will be monitored by capsule counts as study drug is returned.

The dates of all study medication dosing, including interruptions, missed doses or overdose, must be recorded in the eCRF. If the subject 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 subject should be re-educated regarding the correct study drug doses to be administered. However, if the Investigator, medical monitor, and sponsor have agreed to a treatment interruption (Section 5.2), then this period of non-compliance will not be considered a protocol deviation.

5.7 Prior and Concomitant Therapy

Subjects will be allowed to take their medications to treat chronic conditions unless excluded by the protocol (Section 5.7.1.2) and provided that their chronic medication therapy meets the conditions outlined in Section 5.7.1.1, including remaining constant throughout the duration of the study (unless there is a documented medical reason to change the dose).

Use of all prior medications within 1 month prior to study drug administration will be recorded in the subject's eCRF. However, for those medications specified in Exclusion Criteria 7, 8, and 9 it will be necessary to check medical records during the prior 3 months. The minimum requirement is that drug name and 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 subject's eCRF.

Any concomitant medication deemed necessary for the well-being of the subject during the study may be given at the discretion of the Investigator after consideration of the clinical situation. 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 subject's eCRF: name of medication, dose administered, dates and time of administration, and reason for medication use.

Upon entering the study, each subject will be instructed about the importance of not taking any medication (including herbal supplements and OTC medications) without consulting the Investigator.

5.7.1.1 Permitted Therapy

Allowed medications include those typically prescribed to treat ADPKD, CKD, and their complications such as hypertension. These include angiotensin II receptor blockers (e.g., valsartan, candesartan, telmisartan, irbesartan) and angiotensin-converting enzyme inhibitors (e.g., enalapril, lisinopril). However, certain medications are prohibited. Those are detailed in Section 5.7.1.2 below.

Acetaminophen, at doses ≤3 g per day, is permitted for use any time during the study in subjects who usually take acetaminophen to control episodic pain. Because of their effect on renal function, use of non-steroidal anti-inflammatory drugs should be minimized.

Non-sedating antihistamines (other than loratadine) and decongestants are permitted on an as-needed basis. Low-dose aspirin (up to 325 mg per day) is permitted.

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 5.7.1.2). All medications should be stable for at least 3 weeks before and/or during the Screening Period. Subsequently, doses of such medications should remain as stable as possible allowing for minor adjustments per the standard of care to treat medical conditions. The stability of certain drugs, such as insulin and warfarin, should be based on the standard of care rather than on a stable dose.

As lixivaptan has the potential to inhibit the metabolism of CYP3A4 and CYP2C8 substrates, care should be exercised when administering lixivaptan in combination with these substrates (Section 5.7.1.2) due to the potential for drug-drug interactions. Caution should be exercised when concomitantly administering simvastatin or amlodipine with lixivaptan. In order to minimize the potential for AEs, the Investigator should consider potential dose adjustments or alternative therapeutic options that are not CYP3A4 or CYP2C8 substrates (e.g. rosuvastatin to replace simvastatin) or that have a high therapeutic index. If subjects are receiving simvastatin as background medication and it is not possible to replace simvastatin

with rosuvastatin, the dose of simvastatin should be decreased to ≤10 mg daily. If amlodipine is needed for clinical care, the dose should not exceed 5 mg daily.

5.7.1.2 Prohibited Therapy

Subjects should not be entered into the study until all abnormal liver chemistry test results related to tolvaptan have resolved for at least 3 months prior to the first Screening Visit.

The following medications are prohibited during the 3 months prior to Screening Visit 1 and throughout the study:

- Conivaptan
- Somatostatin analogs (e.g. lanreotide, pasireotide, octreotide, etc.)
- Metformin (for ADPKD; allowed for diabetes mellitus)
- Nicotinamide (for ADPKD)
- Bardoxolone
- Venglustat
- Demeclocycline
- mTOR kinase inhibitors (e.g., everolimus, sirolimus, etc.)
- SGLT2 inhibitors (e.g., canagliflozin, dapagliflozin, empagliflozin, etc.)
- HIF-PH inhibitors
- Any investigational drug or device.

The following medications are excluded within 2 weeks of Screening Visit 1 (except diuretics) and prohibited throughout the study:

- Diuretics (which should be discontinued during the Screening Period when anti-hypertensive medications are being adjusted);
- 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, posaconazole, quinupristin/dalfopristin, remdesivir, ritonavir and ritonavir-containing products, telaprevir, teriflunomide, tofisopam, troleandomycin, verapamil, and voriconazole;
- 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.

5.8 Diet, Fluid, Activity, and Lifestyle Considerations

Restriction of excess dietary sodium and cooked meat protein may prove beneficial to subjects 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.

Subjects 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 subjects with ADPKD. Given the potential for dehydration with lixivaptan treatment, all subjects 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 Titration Period, all subjects 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 Titration Period and continue through the end of the study. Additionally, subjects 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 subject self-assessment of symptoms such as lightheadedness or dizziness. Starting at the end of the Baseline Period (Visit 5) and continuing during the Titration Period, subjects will be weighed weekly at their visits. Acute decreases of >3% of body weight over any 7-day period should be noted by the staff and the subject should be counseled to increase fluid intake.

Daily activity including exercise should remain stable during the study and subjects 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 Table 1. Certain visits must occur in the clinic. Other visits may occur in the clinic or remotely by a HHC (if available).

6.1 Screening Period

To determine subject eligibility, informed consent will be obtained, and screening assessments will be performed during Visit 1. It will be particularly important to review previous treatment history with tolvaptan and documentation of all liver chemistry tests during and after treatment with that drug. Subjects not known to the Investigator should be prepared to bring medical records. Such documentation must include evidence of the decision to discontinue tolvaptan permanently on the basis of prior abnormal liver chemistry test results.

Following Visit 1, subjects whose anti-hypertensive medications in the absence of diuretics are known and documented to have been stable (unchanged) for at least 3 weeks prior to Visit 1 may proceed to Visit 2 in 1 week to confirm eligibility. Subjects for whom the stability of anti-hypertensive medications in the absence of diuretics is unknown or not documented must be observed for 3 weeks during the Screening Period to ensure stable dosing of these medications and then will be scheduled for Visit 2. At Visit 2 (which may be conducted remotely by a HHC and telemedicine to determine eligibility) screening test results will be reviewed, and additional assessments obtained as per the Schedule of Procedures.

Subjects who need to have diuretics discontinued or anti-hypertensive regimens intensified will undergo those changes and be monitored as medically appropriate. Once the doses of those medications have been stabilized for 3 weeks, the subject will be scheduled for Visit 2. Under these circumstances, the Screening Period should not exceed 8 weeks.

Subjects who successfully complete the Screening Period will immediately enter the Baseline Period. Subjects who fail inclusion/exclusion criteria will be discontinued but may be re-screened and new informed consents obtained with the approval of the medical monitor and sponsor at a later time up to 2 times if the screen failure reason was temporary or correctable.

6.1.1 Visit 1

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

- Collect and review demographic information
- Collect and review medical history information (including documented history of abnormal liver chemistry test results while taking tolvaptan resulting in discontinuation for that reason)
- Collect and review prior and concomitant medication information with particular emphasis on collection of prior tolvaptan use and dosing and protocol-prohibited medications
- Perform a complete physical examination
- Collect body weight and height measurements

- Check vital signs (includes sitting blood pressure, heart rate, respiratory rate, and temperature)
- Perform a 12-lead ECG
- Collect blood samples for
    - Complete Chemistry Panel
    - Liver Chemistry Panel
    - Hepatitis B surface antigen (HBsAg) and hepatitis C virus antibody (HCV Ab)
    - Hematology
    - Serum pregnancy testing for WOCBP
- Collect urine for urinalysis
- Determine whether the subject is eligible to participate in the study based on meeting all the Inclusion Criteria (Section 4.1.1) and none of the Exclusion Criteria (Section 4.1.2) except for those related to diuretic use and BP control.
- Contact the IRT to assign the subject an identification number as described in Section 5.3. The Investigator or designee must use his or her own user identification and personal identification number (PIN) when contacting the IRT. PIN must not be shared between users and should be maintained in a secure location.
- If no medication changes are necessary and anti-hypertensive medications in the absence of diuretics have been stable for at least 3 weeks prior to Visit 1, schedule Visit 2 one (1) week later (with an extension of up to 2 weeks for scheduling purposes).
- If no medication changes are necessary but it is unknown or undocumented whether anti-hypertensive medications in the absence of diuretics have been stable for at least 3 weeks prior to Visit 1, instruct the subject to maintain stable medications and schedule Visit 2 three (3) weeks later (with an extension of up to 2 weeks for scheduling purposes).
- For subjects who need medication changes (discontinuation of diuretics or revision of anti-hypertensive medications), proceed with those changes per medical standard of care. The subject should then be observed on stable medications for 3 weeks and Visit 2 should be scheduled. The full duration of screening should not exceed 8 weeks.

6.1.2 Visit 2

Subjects will either report to the clinic for Visit 2 or be scheduled for a remote visit with a HHC to review tests from Visit 1 and finalize screening assessments. Telemedicine will be used to determine final eligibility by the investigator.

The following procedures will be performed at Visit 2:

- Collect and review any additional medical history since prior visit
- Collect and review concomitant medication information
- Determine if any SAE occurred since the prior visit
- Collect body weight

- Check vital signs (sitting blood pressure and heart rate)
- *Only for those subjects who had extended screening to allow for adjustment of blood pressure medications,* collect a blood sample for the following laboratory testing:
    - Complete chemistry panel
    - Liver Chemistry Panel
- Determine whether the subject is still eligible to participate in the study.
    - If the subject is not eligible to participate, the site will record the subject as a Screen Failure and contact the IRT to discontinue the subject from the study and complete the appropriate eCRFs. Subjects 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 and sponsor.
    - If the subject is eligible to participate, contact the IRT to update the subject's status, instruct the subject not to change any concomitant medications, and Schedule Visit 3 for 1 week later.

6.2 Baseline Period

The 3-week Baseline Period begins at the conclusion of Visit 2 and consists of 3 visits – Visits 3, 4, and 5. It is designed to collect baseline data to record variability of each subject's liver chemistry test results and to obtain 3 serum creatinine values to determine baseline renal function (eGFR) according to the CKD-EPI equation. Visits will be weekly (Visits 3 and 4 may be done remotely if available), and no study drug will be administered during this time.

6.2.1 Visit 3

Prior to Visit 3 the eligibility review by the medical monitor will be completed for each subject. Subjects will either report to the clinic for Visit 3 or be scheduled for a remote visit with a HHC. The following procedures will be performed at Visit 3:

- Collect and review concomitant medication information
- Check SAEs
- Check vital signs (sitting blood pressure and heart rate)
- Collect a blood sample for the following laboratory testing:
    - Liver Chemistry Panel
    - Serum creatinine
- Contact the IRT to record the visit
- Schedule the subject for Visit 4 to occur in 1 week.

6.2.2 Visit 4

Subjects will either report to the clinic for Visit 4 or be scheduled for a remote visit with a HHC. The following procedures will be performed at Visit 4:

- Collect and review concomitant medication information

- Check SAEs
- Check vital signs (sitting blood pressure and heart rate)
- Collect a blood sample for the following laboratory testing:
    - Liver Chemistry Panel
    - Serum creatinine
- Contact the IRT to record the visit
- Provide the subject with a urine collection container and written instructions (See Section 7.2.1 for more detailed information) to produce a first morning, fasting (8 hours except for water) spot urine specimen to be brought in at Visit 5 for specific gravity and U_{osm} determination.
- Schedule the subject to return to the clinic for Visit 5 to occur in 1 week.

6.2.3 Visit 5

Subjects will report to the clinic in the morning of Visit 5 (after a minimum 8-hour overnight fast) to complete the Baseline Period and begin the Lixivaptan Titration Period.

The following procedures will be performed at Visit 5:

- Collect and review concomitant medication information
- Check SAEs
- Check vital signs (Sitting blood pressure and heart rate)
- Measure body weight
- Process an aliquot of first AM spot urine for specific gravity
- Send the remaining AM spot urine for U_{osm} determination
- Collect urine to conduct a pregnancy test in WOCBP
- Collect a blood sample for:
    - Complete chemistry panel
    - Liver Chemistry Panel
    - Hematology
    - DNA sample (to be obtained under a separate informed consent for the purposes of biobanking to assess genetic variants associated with hepatic abnormalities [if and when feasible]. This may be obtained at any later visit if the subject initially declines permission but agrees at a later time.)
- Collect a plasma sample for measurement of lixivaptan. Note date and time of sample on the requisition form. The sample must be collected before the first dose of lixivaptan is administered.
- Perform a 12-lead ECG
- If all study entry criteria are met, contact the IRT to complete subject's participation in the Baseline Period, obtain kit assignment, and enter the subject into the Titration Period and proceed as follows:
    - Dispense lixivaptan (Level 1) and have subject take the first dose when they return home from the clinic. Instruct the subject to take 1 capsule twice a day approximately 10 hours apart, e.g., 8 AM and 6 PM.

    - Educate the subject to expect aquaretic effects and to maintain fluid intake to prevent dehydration.
- Provide subject with a urine collection container and written instructions to produce a first morning, fasting (8 hours except for water) spot urine specimen to be brought in at Titration Period Visit 6 or provided to the HHC if the visit will be conducted remotely for specific gravity and U_{osm} determination.
- Schedule the subject for Visit 6 to occur in 1 week. Provide the subject with an appointment reminder card and any written instructions.
- If the subject does not meet the study entry criteria, contact the IRT to discontinue the subject. No follow-up visit is needed unless an SAE is ongoing.

6.3 Titration Period

The Titration Period will last from 3 to 6 weeks. Subjects will self-administer lixivaptan at home. All subjects will start at Level 1 (50 mg BID) and will be increased weekly over 2-5 weeks 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 4. If aquaretic effects limit a subject's tolerability at Level 3 (150 mg BID) or Level 4 (200 mg BID), dose levels can be adjusted to Level 3a and Level 4a, respectively, which reduce the evening (PM) dose by 50 mg (from 150 mg to 100 mg for Level 3 to 3a, and from 200 mg to 150 mg for Level 4 to 4a). Titration will stop when a tolerable dose level is reached that achieves the goal of lowering trough (first morning, fasting except for water) urine specific gravity to ≤1.005 or the highest dose (Level 4 or 4a) is achieved (regardless of trough urine osmolality). The minimum dose to enter the Lixivaptan Maintenance Treatment Period is Level 2. Once tolerability and reduced trough urine specific gravity are achieved (or tolerability and maximum dose), subjects will stay on that dose for 1 additional week under most circumstances to confirm tolerability (Table 5). Subjects will then proceed to Visit 11/Last Titration Visit.

6.3.1 Visits 6 to 10

Visits 6, 7, 8, 9, and 10 will occur weekly ± 3 days *as needed* for titration and may be conducted remotely by a HHC. Subjects will either report to the clinic or interact with a HHC in the morning for their visits (after a minimum 8-hour overnight fast except for water) to undergo Titration Period assessments. Note that telemedicine will be utilized at these visits for the Investigator to assess the subject's status and tolerability of the study drug and to make a titration decision. Any other issues may also be discussed with the subject.

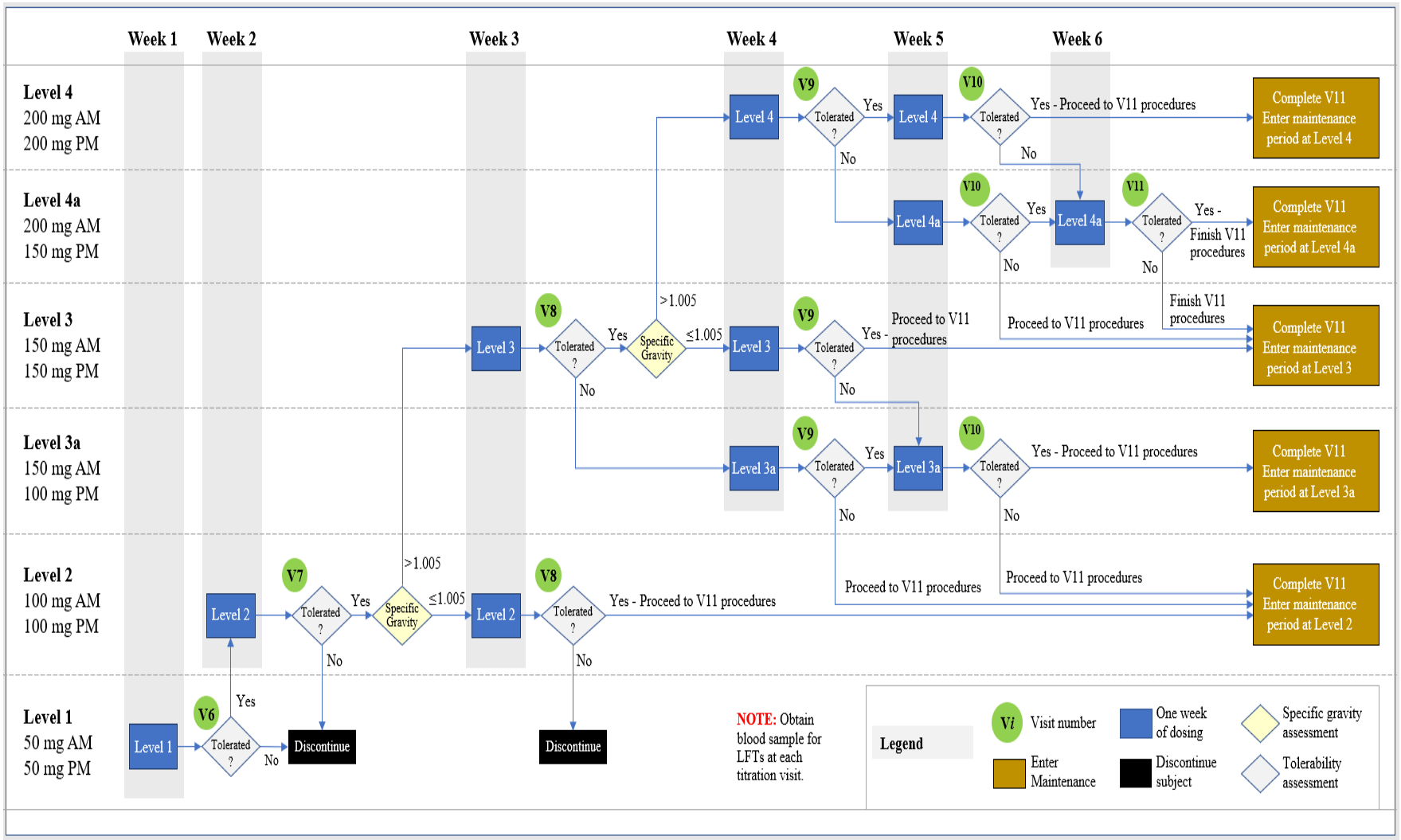
Subjects should bring into the clinic, or provide to the HHC, any remaining study drug. Remind the subjects not to take their morning dose of lixivaptan on the morning of the visit in order to obtain a trough PK sample should they advance to Visit 11 procedures.

The following procedures will be performed at each visit unless otherwise noted:

- Collect and review concomitant medication information
- Review study drug compliance/reconciliation
- Assess study drug tolerability by asking the subject, "Are you able to continue this dose for the next 12 months?"
- Review lab results from prior visit

    - For subjects exhibiting an increase in ALT or AST during the Titration Period (>3 x ULN) or total bilirubin (>2 x ULN), follow the instructions in Section 7.3.7.
- Check AEs and SAEs
- Assess clinically for liver dysfunction (symptoms, signs [if a clinic visit]) in accordance with the checklist and instructions in Appendix 4 (Section 13.4).
- Check vital signs (sitting blood pressure and heart rate)
- Measure body weight and counsel subject to increase fluid intake if weight decreases >3%
- Process an aliquot of first morning, fasting urine for specific gravity and assess if ≤1.005 has been reached (except if Level 4 or 4a)
- Send the remaining first morning, fasting urine for U_{osm} determination
- Collect a blood sample:
    - Liver Chemistry Panel
- Follow the flowchart in Figure 4 to determine whether to increase the dose, decrease the dose, advance the subject to Visit 11 procedures to initiate the Maintenance Period, or discontinue the subject. For remote visits, the HHC will call the Investigator to receive direction on this decision. The Investigator may want to utilize telemedicine to interact with the subject.
    - Based on the decision made from the flowchart, contact the IRT to receive the kit assignment for the next period of time
    - Dispense study drug and instruct subject how many capsules to take based on the dose level guide in Table 4
    - For remote visits, the site will contact the IRT sufficiently in advance of the scheduled visit day to obtain kit assignment and to allow sufficient time for study drug to be shipped to arrive at subject's home prior to the visit date.
- At each visit, remind the subject to take adequate fluids, provide a urine collection container, and instruct the subject to collect a first morning, fasting (8 hours except for water) urine specimen on the morning of their next visit, which they will bring with them (if a clinic visit) or provide to the HHC if a remote visit is conducted.
- Provide the subject with an appointment reminder card and any written instructions.

Figure 4. Titration Flowchart



6.3.2 Visit 11/Last Titration Visit

Visit 11/Last Titration Visit is intended to confirm that the subject tolerates lixivaptan at the dose determined during the Titration Period and to collect additional data before entering the Maintenance Period. The procedures specified for Visit 11 may be performed at an earlier titration visit if the subject qualifies to enter the Maintenance Period at that time. The last titration visit may be in the office or conducted remotely by a HHC. If conducted remotely, telemedicine will be used for the Investigator to interact with the subject and confirm that the subject can continue into the Maintenance Period.

Subjects will either report to the clinic in the morning or interact with the HHC, if a remote visit is conducted, to undergo the assessments for the last visit of the Titration Period. Subjects should bring any remaining study drug into the clinic or provide to the HHC. Remind the subjects not to take their morning dose of lixivaptan on the morning of the visit. Dosing instructions will occur during the visit.

The following procedures will be performed:

- Collect and review concomitant medication information
- Review study drug compliance/reconciliation
- Assess study drug tolerability
- Check AEs and SAEs
- Assess clinically for liver dysfunction (symptoms, signs [if a clinic visit]) in accordance with the checklist and instructions in Appendix 4 (Section 13.4).
- Review lab results from prior visit
    - For subjects exhibiting an increase in ALT or AST during the Titration Period (>3 x baseline) or total bilirubin (>2 x ULN), follow the instructions in Section 7.3.7
- Measure body weight and counsel subject to increase fluid intake if weight decreases >3%
- Check vital signs (sitting blood pressure and heart rate)
- Collect urine to conduct a pregnancy test in WOCBP
- Collect blood for the following laboratory testing:
    - Complete chemistry panel
    - Liver chemistry panel
    - Hematology
- Collect a trough blood sample for measurement of lixivaptan. Note date and time of last dose from the previous day and the date and time of the sample on the requisition. The sample must be collected before the dose of lixivaptan is administered.
- The site will contact the IRT to complete subject's participation in the Titration Period, obtain new kit assignment, and enter the subject into the Maintenance Period and proceed as follows:
    - Dispense new kits. Have the subject take the first dose at home. Instruct the subject to take the appropriate dose twice a day approximately 10 hours apart, e.g., 8 AM and 6 PM.

  - For remote visits, the site will contact the IRT sufficiently in advance of the scheduled visit day to obtain kit assignment and to allow sufficient time for study drug to be shipped to arrive at subject's home prior to the visit date.
- Schedule the subject for their next visit to occur in 4 weeks and remind the subject to take adequate fluids. Provide the subject with an appointment reminder card and any written instructions. Note that the next visit may be conducted remotely (if available).

6.4 Maintenance Period

The Maintenance Period will last 52 weeks (Visit 12 to Visit 24). If available, the following visits may be done remotely: Visits 12, 13, 14 (with telemedicine), 15, 16, 18, 19, 20 (with telemedicine), 21, 22, and 23. Note that telemedicine may be added to any other visit if the Investigator judges that additional interaction or information is needed to assess the subject's status.

6.4.1 Visits 12 to 23

Visits 12 to 23 will occur every 4 weeks ± 3 days. For visits not done remotely, subjects will report to the clinic in the morning or afternoon on visit days. Subjects should bring any remaining study drug into the clinic or provide to the HHC.

The following procedures will be performed at each visit unless otherwise noted:

- Check vital signs (sitting blood pressure and heart rate)
- Review lab results from prior visit
  - For subjects exhibiting an increase in ALT or AST during the Maintenance Period (>3 x ULN) or total bilirubin (>2 x ULN), follow the instructions in Section 7.3.7.
- Check AEs and SAEs and inquire about tolerability of the study drug
- Assess clinically for liver dysfunction (symptoms, signs [if a clinic visit]) in accordance with the checklist and instructions in Appendix 4 (Section 13.4)
- Review study drug compliance/ reconciliation
- Collect and review concomitant medication information
- Perform a brief physical exam (Visit 17)
- Perform a 12-lead ECG (Visit 17)
- Collect urine to conduct a pregnancy test in WOCBP (Visits 14, 17, and 20 only).
- Collect a blood sample for the following laboratory testing:
  - Complete Chemistry Panel (Visits 14, 17, and 20 only)
  - Liver Chemistry Panel (all visits)
  - Hematology (Visit 17)
- Collect a plasma sample for trough measurement of lixivaptan *if* ALT > 3 x ULN and/or total bilirubin > 2 x ULN. Note date and time of last dose from the previous day and the date and time of the sample on the requisition form.

- At Visit 23, provide subject 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 24 for specific gravity and U_{osm} determination.
- The site will contact the IRT at each visit to obtain kit number assignment and complete the visit. Dispense assigned kits to subject for the next 4 weeks. Instruct the subject to take the appropriate dose twice a day approximately 10 hours apart, e.g., 8 AM and 6 PM.
- For remote visits, the site must contact the IRT sufficiently in advance of the scheduled visit day to obtain kit assignment and to allow sufficient time for drug to be shipped to arrive at subject's home prior to the visit date.
- Schedule the subject to return for their next visit or confirm if visit will be done remotely. The visit is to occur in 4 weeks. Provide the subject with an appointment reminder card and any written instructions.

6.4.2 Visit 24/Early Termination

Visit 24 will occur at Week 52 ± 3 days.

Subjects will report to the clinic in the morning to undergo last Maintenance Visit assessments. These procedures will also be conducted on subjects that terminate the study early (Section 6.5.4).

Subjects should bring in any remaining study drug with them.

The following procedures will be performed:

- Collect and review concomitant medication information
- Review study drug compliance/reconciliation and collect all remaining study drug
- Review lab results from prior visit
    - For subjects exhibiting an increase in ALT or AST during the Maintenance Period (>3 x ULN) or total bilirubin (>2 x ULN), follow the instructions in Section 7.3.7.
- Check AEs and SAEs
- Assess clinically for liver dysfunction (symptoms, signs) in accordance with the checklist and instructions in Appendix 4 (Section 13.4).
- Perform a full physical examination
- Measure body weight
- Check vital signs (sitting blood pressure and heart rate)
- Perform a 12-lead ECG
- Process an aliquot of first morning, fasting urine for specific gravity
- Send the remaining first morning, fasting for U_{osm} determination
- Collect urine for urinalysis
- Collect urine sample for a urine pregnancy test in WOCBP
- Collect blood for the following laboratory testing:
    - Complete chemistry panel

  - Liver chemistry panel
  - Hematology
- Collect a plasma sample for trough measurement of lixivaptan *if* ALT > 3 x ULN and/or total bilirubin > 2 x ULN. Note date and time of last dose from the previous day and the date and time of the sample on the requisition form.
- Contact the IRT to complete the visit.
- Schedule the subject to return to the clinic for the first follow-up visit (Visit 25) or schedule as a remote visit if available.

6.5 Follow-up Period

The Follow-up Period will last 28 days and include 3 visits (Visit 25, Visit 26, and Visit 27). If available, Visits 25 and 26 may be done remotely by a HHC (with telemedicine if necessary).

6.5.1 Visit 25

Visit 25 will occur no earlier than 8 (+ 3) days after Visit 24. The following procedures will be performed at Visit 25:

- Check AEs and SAEs
- Review lab results from prior visit
  - For subjects exhibiting an increase in ALT or AST (>3 x ULN) or total bilirubin (>2 x ULN) at the prior visit, follow the instructions in Section 7.3.7.
- Assess clinically for liver dysfunction (symptoms, signs [if a clinic visit]) in accordance with the checklist and instructions in Appendix 4 (Section 13.4).
- Collect and review concomitant medication information
- Collect a blood sample:
  - Serum creatinine
- Schedule the subject to return to the clinic for V26, in 7-14±3 days or confirm if it will be done remotely
- The site will contact the IRT to complete the visit

6.5.2 Visit 26

Visit 26 will occur 7-14±3 days after Visit 25. The following procedures will be performed at Visit 26:

- Check AEs and SAEs
- Collect and review concomitant medication information
- Collect a blood sample:
  - Serum creatinine
- Schedule the subject to return to the clinic for V27, 28±3 days after V24 (last dose of study drug) and provide a urine collection container and instructions for first morning, fasting (8 hours except for water) urine specific gravity and U_{osm} determination

- The site will contact the IRT to complete the visit

6.5.3 Visit 27 (End of Study Visit)

Visit 27 is the last visit in this study and will occur 28±3 days after the last dose of study drug (Visit 24). The visit will be conducted in the clinic. The following procedures will be performed at Visit 27:

- Check vital signs (sitting blood pressure and heart rate)
- Perform a brief physical exam
- Perform a 12-lead ECG
- Check AEs and SAEs
- Collect and review concomitant medication information
- Process an aliquot of first morning, fasting urine for specific gravity
- Send the remaining first morning, fasting urine for U_{osm} determination
- Collect urine for urinalysis
- Collect urine sample for a urine pregnancy test in WOCBP
- Collect a blood sample for the following laboratory testing:
    - Complete chemistry panel
    - Liver chemistry panel
    - Hematology
- Contact the IRT to complete subject's participation in PA-ADPKD-303

6.5.4 Early Termination Procedures

Subjects who terminate the study during the Screening or Baseline Periods will not have follow up unless there is an ongoing SAE. See Section 4.2.2.

Subjects who terminate the study during the Titration or Maintenance Periods will have final procedures and follow up performed as described in Section 4.2.3.

7 DESCRIPTION OF STUDY ASSESSMENTS AND PROCEDURES

Before performing any study procedures, all potential subjects will sign the primary ICF. Separate informed consent will be obtained at the end of Baseline or later in the study to allow biobanking of a DNA sample to assess genetic variants associated with hepatic abnormalities (if and when feasible). Subjects will have the opportunity to have any questions answered before signing the ICFs. The Investigator must address all questions raised by the subject.

The study assessments to be performed at each visit are specified in the study Schedule of Procedures (Table 1).

Visits that occur following an overnight fast (8 hours except for water) will occur in the morning. Other visits may occur in the morning or afternoon. To avoid aquaretic effects while traveling, study drug will be administered at home.

7.1 Efficacy Assessments

7.1.1 eGFR

The eGFR values reported to Investigators from the central laboratory will be calculated according to the CKD-EPI formula from the serum creatinine concentrations taken at Screening and at the visits specified in the Schedule of Procedures (Table 1) when either the Complete Chemistry Panel or serum creatinine is listed. During the Baseline Period, serum creatinine assessments will be used to determine the average eGFR. The averaged value will be used as the eGFR baseline.

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 5.8.

7.2 Pharmacodynamic Assessments

7.2.1 Specific Gravity and Urine Osmolality

First AM, fasting urine collections will be obtained at the timepoints specified in the Schedule of Procedures (Table 1). An 8-hour overnight fast (except for water, which is encouraged to satisfy thirst and replace any overnight urine production) is required prior to collection. Morning medications should not be taken until the urine collection has been obtained. Subjects will be provided a urine collection container and instructions in advance of each visit where specific gravity and U_{osm} are determined. Subjects will be asked to completely empty their bladders upon arising in the morning and then provide a spot urine specimen within the next 20-40 minutes for the determination of specific gravity and U_{osm}. They will either bring that specimen to their clinic visit or provide to a HHC if a remote visit is conducted, where specific gravity will be measured by dipstick using an automated device. The remainder of the urine specimen will be sent to the central laboratory for U_{osm} determination.

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 study Schedule of Procedures (Table 1). Assessment windows are presented in Table 1.

7.3.1 Body Height, Weight, and BMI

Body height (inches or centimeters) and weight (pounds or kilograms) will be measured at Screening 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 (Table 1). 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 subject wearing shoes.

7.3.2 Demographics and Medical History

Medical history and demographic data, including initials, sex, age, race, and ethnicity will be recorded at the Screening Visit 1 (Table 1).

Medical and medication history will be assessed as related to the eligibility criteria in Section 4.1. Medical history will include documented history of abnormal liver chemistry test results while on tolvaptan, history of permanent discontinuation of tolvaptan due to abnormal liver chemistry test results, history of any re-challenge with tolvaptan or development of DILI on tolvaptan. As data permit, information on the duration of tolvaptan use prior to discontinuation should be included.

Other than tolvaptan, medication history will include all medications taken within 30 days prior to Screening (except for the longer periods specified for Exclusion Criteria 7, 8, and 9; see Section 5.7) and history of drug, alcohol, and tobacco use.

An update to medical history, including medication review, will be performed at Screening Visit 2 to verify subject eligibility.

7.3.3 Physical Examinations

A complete physical examination will be performed during Screening Visit 1 and the final Maintenance/Early Termination Visit (Visit 24). Brief physical examination will subsequently be performed at the timepoints specified in the study Schedule of Procedures (Table 1).

Complete physical examinations will include, at a minimum, height and weight (Visit 1 only, Visit 24 will include weight only), 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 and Baseline 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 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 subjects experiencing AEs.

7.3.4 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 study Schedule of Procedures (Table 1). Respiratory rate and body temperature will be measured once at Screening.

Subjects will remain at rest in a seated position for a minimum of 5 minutes before vital sign measurements are obtained. For all subjects, 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 must be recorded as medical history during the Screening and Baseline Periods and as an AE after the start of study drug. If other procedures are scheduled at the same timepoint, vital signs should be obtained first, before an ECG and/or blood draw.

7.3.5 12-Lead ECGs

Standard 12-lead ECGs will be performed at the timepoints specified in the study Schedule of Procedures (Table 1).

Safety 12-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 subject throughout the study.

ECGs will be obtained with the subject remaining in a supine or semi-recumbent position following 5 minutes of rest. All ECGs throughout the study for a given subject 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.

For all subjects, ECGs will be reviewed, signed, and dated by the Investigator or a qualified designee. 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 will be reported as medical history during the Screening and Baseline Periods and as AEs after the start of study drug.

Additional ECGs may be performed if deemed medically appropriate.

7.3.6 Clinical Laboratory Tests

Blood and urine specimens will be collected for the clinical laboratory (hematology, clinical chemistry, and urinalysis) and pregnancy tests (all female subjects of child-bearing potential) at the timepoints specified in the study Schedule of Procedures (Table 1). Except where noted, all laboratory testing will be performed at the central laboratory, which will return results with normal ranges to the clinical sites. A confirmatory assessment through the central lab should be obtained as soon as possible for any CS abnormal laboratory parameter if deemed necessary by the Investigator. 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 be discussed first by the Investigator with the medical monitor before such tests are ordered. All routine testing will be conducted in the morning after an overnight fasted state (minimum of 8 hours except for water). 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 6.

Macroscopic urinalysis will be performed via dipstick. Microscopic examination is needed only if macroscopic results are positive for blood or protein.

Additional liver chemistry tests should be collected if the subject experiences liver chemistry test abnormalities of concern or has suspicious symptom or sign of liver dysfunction (Section 7.3.7).

Table 6 Clinical Laboratory Parameters

Complete Chemistry Panel	Hematology	Urinalysis
Albumin	Hematocrit	pH
Blood urea nitrogen (urea)	Hemoglobin	Specific gravity
Calcium	Red blood cell count	Protein
Chloride	Quantitative platelet count	Glucose
CO_2	WBC count	Ketones
Creatinine	with differential (total and %) only if	Bilirubin
Glucose	WBC count is abnormal:	Blood
Phosphorous	Neutrophils	Urobilinogen
Potassium	Lymphocytes	Leukocytes
Protein	Monocytes	Nitrite
Sodium	Eosinophils	Microscopic examination (if positive for blood, protein, nitrite, or leukocytes)
Uric acid	Basophils	
Liver Chemistry Panel	**Other Tests**	
Alanine aminotransferase	Urine specific gravity (local to guide titration)	
Alkaline phosphatase		
Aspartate aminotransferase	Urine osmolality	
Bilirubin Total and Direct	Urine or serum pregnancy test (female subjects)	
	Specimen for potential DNA analysis	

Abbreviations: CO_2 = carbon dioxide; WBC = white blood cell.

7.3.6.1 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.

7.3.7 Assessment of Liver Symptoms, Signs, or Test Abnormalities

Testing for hepatic transaminase (ALT/AST), alkaline phosphatase, and total and direct bilirubin will be performed at the timepoints as specified in the Schedule of Procedures (Table 1). 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 4 (Section 13.4) in a subject at any time during the study should trigger prompt testing of liver chemistry tests (i.e., within 48 hours).*

7.3.7.1 Requirements for Additional Liver Chemistry Testing

To determine when repeat liver chemistry testing is necessary, follow the instructions in Table 7:

Table 7 Additional Liver Chemistry Testing and Other Testing

Subjects with Liver Chemistry Values Exceeding Eligibility Requirements at Screening or Baseline	Subjects with Eligible Liver Chemistry Values at Screening or Baseline
Subjects with transaminase (ALT/AST) or total bilirubin values above the values in the exclusion criteria (>1.5 x ULN and >1.0 x ULN, respectively) during the Screening or Baseline Periods will not proceed further in the study. Any required follow up should be part of routine clinical care. However, if evaluation indicates a reversible cause of liver disease, such subjects may be re-screened in consultation with the medical monitor and sponsor when such liver chemistry test results return to values allowing entry into the study.	Any transaminase value >3 x ULN or total bilirubin >2 x ULN during the Titration or Maintenance Periods requires immediate retesting within 48 hours. Local laboratory testing is acceptable with collection of a concurrent central laboratory sample for confirmation whenever possible. 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. Individual cases that either resolve quicker or slower should be discussed with the medical monitor and sponsor. The medical monitor will discuss with the Investigator any additional laboratory testing or imaging depending on the case circumstances. Examples of such testing are shown in Appendix 3 (Section 13.3).

7.3.7.2 Changes in Study Drug Dosing if Liver Chemistry Test Abnormalities Occur During the Titration or Maintenance Periods

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 transaminase levels >3 x ULN or total bilirubin levels >2 x ULN are observed during the Titration or Maintenance Periods. All elevations meeting these thresholds will be assessed the HERC. In the case of study drug interruption, re-starting study drug should be encouraged (see exception 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.

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.

Treatment with study drug cannot be resumed in subjects when:

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

except under these circumstances:

> Subjects with these levels of abnormality may be re-challenged with study drug if abnormalities were adjudicated as having a ≤50% likelihood of being related to study drug (per DILI Network [DILIN] probability criteria, modified, Section 13.2 (Fontana RJ 2009)[18] by the HERC, and the Investigator, medical monitor, and sponsor agree to an intensive monitoring plan to mitigate risk. The subject must also be willing to comply with these monitoring measures, be informed of the potential risks, and consent to study drug re-challenge.

Liver chemistry test increases of a lesser extent should be discussed with the medical monitor. They may be related to the underlying variability of such tests in ADPKD patients. Generally, dosing should continue, but the frequency of laboratory testing should increase, particularly during the Maintenance Period. Slowing of titration may also be considered if the abnormalities occur during the Titration Period.

7.3.7.3 Special Reporting of Liver Events

A liver dysfunction details eCRF will be utilized during the study. The type of information needed to complete it is provided in Appendix 4 (Section13.4). The purpose of the liver dysfunction details eCRF and additional testing is to facilitate review of each subject who develops 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 will be performed by an independent HERC using DILIN probability criteria, modified (0 to 5% = unrelated; 6 to 25% = unlikely, 25% to 50% = possibly, 51% to 75% = probably, 76% to 95% = very likely, >95% = definite) (Fontana RJ 2009).[18] The HERC will independently decide attribution and will communicate in writing with the sponsor. The results of the HERC review will be used in assessing the primary and secondary endpoints. The result of these analyses may be presented separately from the final clinical study report (CSR).

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

- develops any signs or symptoms of hepatic dysfunction (see Appendix 4 [Section 13.4])
- discontinues treatment due to a liver-related AE,
- reports a serious liver-related AE,
- develops ALT or AST levels >3 × ULN,
- develops total bilirubin levels >2 × ULN.

The 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 7.3.8.2 below.

An AE is defined as any untoward medical occurrence in a subject 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-subject 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 subject 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 SAEs will be recorded from the time the ICF is signed until study completion. Non-serious AEs will be recorded from the start of study treatment until study completion. Other changes in health or new symptoms occurring during the Screening or Baseline Periods will be recorded as medical history.

At every study visit, subjects will be asked a standard non-leading 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, or changed concomitant medication regimens (both prescription and over-the-counter medications).

In addition to subject 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 subject is screened or during baseline, 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 subject's condition, are not to be reported as AEs or SAEs.

For the purpose of this study, liver events are considered to be AESIs. Liver events will be reported directly from the electronic data capture [EDC] system.

Pregnancy events will need to be reported on special pregnancy forms to ICON PVSS.

Any AE that meets SAE or AESI criteria will be reported to ICON Pharmacovigilance & Safety Services (PVSS) immediately (i.e., within 24 hours) after site personnel first learn about the event. Investigators should record all SAE/AESI details available, including Investigator causality assessment, on an Adverse Event eCRF or appropriate AESI forms and submit the report via the electronic data capture (EDC) system within 24 hours of becoming aware of the event. Notification of SAE/AESI entry will be generated and sent to ICON PVSS via the EDC system. Pregnancy events will also be reported to ICON PVSS within 24 hours of recognition of the event, but on the special forms provided.

In the event the EDC system is unavailable, a completed SAE Report Form should be submitted to ICON PVSS as an email attachment or by fax to the email address / fax number below. The EDC system must be updated with the information on the paper report as soon as the EDC is available. The preferred method of reporting on paper is via email:

ICON PVSS Email Address: ICON-Safety-CentralReceipt@iconplc.com

or via fax to:

ICON PVSS Fax: 1-215-616-3096 (within US)

ICON PVSS Fax: +44 (0)208 100 5005 (outside US)

Should the site have questions or concerns regarding SAE report submission, the site may elect to call the ICON PVSS SAE Hotline number. The site will be asked to provide the following information: protocol number, site number, subject identifiers, event term, study drug information, relationship of the event to study drug.

- ICON PVSS SAE Hotline Number: +1-888-426-8801 (within US) – Toll Free Number or +1-281-295-4889 (outside of US)

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 via an ICON Call-Center:

- Telephone: +1 857-957-5013
  (chargeable telephone number allowing a global reach from both landlines and mobile phones)
- https://icophone.iconplc.com/24-7-Medical.pdf

On this internet page, a list of country-specific toll-free telephone numbers is provided. It should be noted that not all countries globally have access to toll-free numbers as indicated on the "24/7 Medical Help desk" index. Countries without toll-free numbers need to dial the chargeable number as indicated above. Furthermore, there may be restrictions when dialling toll-free numbers from a mobile phone.

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 8.

Table 8 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 subject'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 subject'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 subject'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 Follow-Up of Subjects 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 subject is considered to be stable.

7.3.8.7 Overdose Management

An overdose is any dose of study drug given to a subject or taken by a subject that exceeds the dose described in the protocol. Any overdose, with or without associated AEs, must be promptly reported to the Investigator and also 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. 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 subject's clinical status.

7.3.8.8 Pregnancy

Pregnancy is not regarded as an AE unless there is a medical/surgical complication. Any pregnancy of a subject or the partner of a subject that occurs during study participation must be reported using a clinical study pregnancy reporting form. To ensure subject safety, each pregnancy must be reported through this mechanism to ICON PVSS 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 subject 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 subject has completed the study, and considered by the Investigator as possibly related to the study treatment, must be promptly reported to ICON PVSS.

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 subject's condition, are not to be reported as AEs or SAEs.

7.3.10 Hepatic Events Review Committee (HERC)

The HERC consists of 3-4 expert hepatologists who will review demographic, medical and medication history, safety data, PK data and any other relevant data as available on subjects who develop liver abnormalities of concern during the study.

This committee will independently determine the probable causality for instances of suspected liver dysfunction and liver chemistry test abnormalities of concern (Section 7.3.7) and of relatedness to lixivaptan.

The results of these reviews will be captured on an eCRF and used for analysis of the primary and secondary safety endpoints. A full report may be provided separately from the clinical study report.

The HERC will also assess the relationship to tolvaptan of prior instances of liver dysfunction and liver chemistry test abnormalities that occurred while enrolled subjects were previously receiving tolvaptan.

7.4 Pharmacokinetic Assessments

Blood samples will be obtained to measure plasma levels of lixivaptan.

7.4.1 Pharmacokinetic Blood Sampling

Blood samples for PK will be taken for determination of plasma lixivaptan concentrations. A 4 mL plasma sample will be collected at the timepoints specified in the study Schedule of Procedures (Table 1) and here:

- Baseline
- At trough (between 7-10 AM prior to AM dosing) at the last titration visit (Visit 11).
- At trough (between 7-10 AM prior to AM dosing) at the time of a liver chemistry test abnormality (any transaminase value >3 x ULN or total bilirubin >2 x ULN).

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 to the central clinical laboratory for storage. Detailed handling and shipping instructions are in the laboratory manual.

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

8 STATISTICAL AND ANALYTICAL PLAN

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

8.1 Sample Size

Up to 50 subjects will be enrolled and treated. This is based on a similar study conducted with ambrisentan in subjects who had previous liver chemistry test abnormalities on other endothelin receptor antagonists for the treatment of pulmonary arterial hypertension (McGoon MD 2009).[22]

8.2 Populations for Analysis

The following populations will be used for analyzing the data:

The Enrolled Population is defined as all subjects who meet inclusion/exclusion criteria during the Screening Period and complete Visit 3. All general summaries, except safety and efficacy analyses are based on the Enrolled Population, unless specified otherwise.

The Safety Population is a subset of the Enrolled Population defined as those subjects who received at least 1 dose of lixivaptan. This population will be utilized for all safety analyses.

The Efficacy Population is a subset of the Safety Population defined as those subjects who have at least 1 eGFR determination during the Baseline Period and have at least 1 eGFR determination during the Follow-up Period. This population will be used for all efficacy analyses.

The Pharmacokinetic (PK) Population is a subset of the Safety Population defined as those subjects who had at least 1 on-therapy evaluable PK measurement.

The Pharmacodynamic (PD) Population is a subset of the Enrolled Population defined as those subjects who have at least 1 set of urine specific gravity and urine osmolality measurements from the same specimen.

8.3 Analysis of Demographic and Baseline Characteristics

Descriptive statistics for demographic (e.g., age, race, and sex) and baseline (e.g., eGFR) characteristics will be presented for the Safety and Efficacy Populations.

8.4 Safety Analyses

8.4.1 Primary Hepatic Safety Endpoint

Descriptive statistics (n, percentage, 95% confidence interval) will be used to summarize the proportion of subjects who develop ALT levels >3 x ULN that were assessed by the independent HERC to be at least probably related to lixivaptan and resulted in discontinuation of the study drug during the Titration or Maintenance Periods.

8.4.2 Secondary Hepatic Safety Endpoints

Similarly, descriptive statistics (n, percentage, 95% confidence interval) will be utilized to present the 2 secondary endpoints:

The proportion of subjects who develop ALT levels >5 x ULN that were assessed by the independent HERC to be at least probably related to lixivaptan and resulted in

discontinuation of the study drug during the Titration or Maintenance Periods; and

The proportion of subjects who develop ALT levels >3 x ULN that were assessed by the independent HERC to be at least probably related to lixivaptan and resulted in lixivaptan dose reduction during the Titration or Maintenance Periods.

8.4.3 Additional Hepatic Safety Endpoints

The proportion of subjects who develop the following abnormalities during the Titration and Maintenance Periods will be tabulated and presented descriptively:

- >3 x, 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
- ALT or AST levels >2 x their baseline
- Any elevation of total bilirubin (TBL) >2 x ULN
- Any elevation of alkaline phosphatase (ALP) >2 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.

8.4.4 Adverse Events

The number and percentage of subjects and the number of events during the Titration and Maintenance Periods will be presented for TEAEs, 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 SOC, and PT separately by maximum severity and relationship to study drug. Listings of deaths and serious TEAEs will be provided. Similarly, number and percentage of subjects will be presented for TEAEs, SAEs, and treatment-related TEAEs during the Follow Up Period.

All AE data will be listed for all subjects. Both the Investigator's verbatim terms and the MedDRA preferred terms will be listed for each subject. Listings will also include the SAEs, start and end time and 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.

8.4.5 Clinical Laboratory Tests

Descriptive statistics (n, mean, standard deviation [SD], median, minimum, and maximum) for clinical laboratory data and changes from baseline will be presented for each scheduled time point.

The baseline value is the last value observed prior to first administration of study drug and any information taken 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 scheduled time point. For categorical data, change from baseline will be summarized using frequency and proportion at each scheduled timepoint.

Individual data listings of clinical laboratory results will be presented for each subject. 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.

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

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

8.4.6 ECGs

Observed values and changes from baseline for continuous ECG parameters including heart rate, ventricular rate, PR interval, QRS duration, QT interval (uncorrected), and QTcF interval will be summarized over each scheduled timepoint in terms of absolute values using descriptive statistics (n, mean, SD, median, minimum, and maximum). In addition, a summary shift table comparing baseline interpretation (normal, abnormal - NCS, abnormal - CS) to the Investigator interpretation at each time point will also be presented.

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

8.4.7 Vital Signs

Changes from baseline in vital signs at each scheduled timepoint will be summarized using descriptive statistics (n, mean, SD, median, minimum, and maximum). The baseline value is defined as the last value observed prior to first administration of study drug. The change from baseline is defined as the post-baseline value minus the baseline value.

8.5 Efficacy Analysis

Descriptive statistics (n, mean, SD, median, minimum, and maximum) will be presented for observed values at baseline and endpoint, as well as change from baseline in eGFR at the endpoint. Baseline eGFR is defined as the mean of the 3 eGFR assessments obtained during the Baseline Period. However, if any values are missing, the remaining values will be used to determine the baseline eGFR. The endpoint eGFR is defined as the mean of 3 eGFR assessments obtained during the Follow-up Period. However, if any values are missing, the remaining values will be used to determine the endpoint eGFR

8.6 Exploratory Analyses

8.6.1 Relationship of Liver Chemistry Test Abnormalities to Drug Levels

Trough levels of lixivaptan will be listed. Any possible relationships between liver chemistry test abnormalities and trough levels of lixivaptan collected during the study will be explored

if sufficient data exist. Details will be provided in the statistical analysis plan.

8.6.2 Assessment of Genetic Variants

In the event that specific genetic variants confirming increased or decreased risk to hepatic abnormalities in subjects with DILI become known during the time of the study, testing for such variants may be performed on stored specimens of those individuals who provided consent.

8.6.3 Pharmacodynamic Assessments

Relationships between urine specific gravity and U_{osm} will be explored. Details will be provided in the statistical analysis plan.

8.7 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, SD, median, minimum, and maximum. Categorical variables will be summarized using the following descriptive statistics: frequency counts and percentages and 95% confidence intervals for the primary and secondary safety endpoints. 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.

8.8 Data Quality Assurance

Standard operating procedures are available for all activities performed at the study sites relevant to the quality of this study. 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.

All clinical data will undergo source document verification by the Clinical Research Associate and data review by Data Management 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.

8.8.1 Data Management

An eCRF is required and should be completed for each included subject. 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 subjects 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 subject 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 subject data into eCRFs using the EDC system designated by the sponsor. 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 contract research organization (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 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, subject 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 subject instructions, recruitment advertisements, if applicable, from the IRB/EC before participation of human subjects 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 subjects.

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 Subject Information and Consent

Written informed consents in compliance with US Title 21 Code of Federal Regulations (CFR) Part 50 shall be obtained from each subject before entering the study, upon re-screening, and when performing any unusual or non-routine procedure that involves risk to the subject. 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 subjects must sign the revised form.

Before recruitment and enrollment, each prospective subject 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 subject/legal guardian understands the implications of participating in the study, the subject/legal guardian will be asked to give consent to participate in the study by signing the ICF.

The Investigator shall retain the signed original ICF(s) and give a copy of the signed original form to the subject 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 and the CRO's medical monitor are listed on the cover page of this protocol. Contact information for other sponsor personnel, ICON Clinical Research Limited, the designated CRO, and other 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 subjects' confidentiality. All records will be kept in a secure storage area with limited access. Clinical information will not be released without subjects' (or legal guardians') written permission, except as necessary for monitoring and auditing by the Sponsor, its designee, the FDA, 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 are required to provide financial disclosure information to allow the Sponsor to submit the complete and accurate certification or disclosure statements required under 21 CFR 54. In addition, the Investigator 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 is 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 is financially responsible for further treatment of the subject'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, and all updates on a new fully executed Form FDA 1572
- Curriculum vitae for the Investigator and each Sub-Investigator listed on Form FDA 1572
- Financial disclosure information to allow the Sponsor to submit complete and accurate certification or disclosure statements required under 21 CFR 54. 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 subject or legal guardian, and
- Laboratory certifications and normal ranges for any local laboratories used by the site, in accordance with 42 CFR 493

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 subjects 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 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 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 subjects (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 Sponsor 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 Sponsor has final approval authority over all such issues.

Data are the property of the Sponsor and cannot be published without prior authorization from 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 Monitor and/or designee, as a representative of the Sponsor, has the obligation to follow the study closely. In doing so, the Monitor 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 monitor 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 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 subject, 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 subjects can be enrolled into an amended protocol, and before the changes can be implemented.

11.2.2 Protocol Deviations

The Investigator or designee must document and explain in the subject'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 subjects 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 subject or Investigator that results in a significant, additional risk to the subject. Major deviations can include not adhering to inclusion or exclusion criteria, enrollment of the subject without prior Sponsor approval, or not adhering to FDA regulations or ICH GCP guidelines, and will lead to the subject being withdrawn from the study (Section 4.2).

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.

11.3 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 subject completes the final end of study assessment.

11.4 Final Report

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 subjects, as appropriate. The study results will be posted on publicly available clinical trial registers.

12 REFERENCE LIST

1. Chan PS, Coupet J, Park HC, Lai F, Hartupee, D, Cervoni P, Dusza JP, Albright JD, Ru X, Mazandarani H, Tanikella T, Shepherd C, Ochalski L, Bailey T, Lock YYW, Ning X, Taylor JR, Spinelli W. 1998. VPA-985, a nonpeptide orally active and selective vasopressin V_2 receptor antagonist. Adv Exp Med Biol. 449:439-443.
2. Chebib FT, Sussman CR, Wang X, Harris PC, Torres VE. 2015. Vasopressin and disruption of calcium signaling in polycystic kidney disease. Nat Rev Nephrol. 11(8):451-464.
3. Antignac C, Calvet JP, Germino GG, Grantham JJ, Guay-Woodford LM, Harris PC, Hildebrandt F, Peters DJM, Somlo S, Torres VE, Walz G, Zhou J, Yu ASL. 2015. The Future of Polycystic Kidney Disease Research — As Seen By the 12 Kaplan Awardees. J Am Soc Nephrol (26):2081-2095.
4. Chapman AB, Devuyst O, Eckardt K-U, Gansevoort RT, Harris T, Horie S, Kasiske BL, Odland D, Pei YP, Perrone RD, Pirson Y, Schrier RW, Torra R, Torres VE, Watnick T, Wheeler, DC. 2015. Autosomal-dominant polycystic kidney disease (ADPKD): executive summary from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference. Kidney Int. 88(1):17-27
5. Hogan MC, Abebe K, Torres VE, Chapman AB, Bae KT, Tao C, Sun H, Perrone RD, Steinman TI, Braun W, Winklhofer FT, Miskulin DC, Rahbari-Oskoui F, Brosnahan G, Masoumi A, Karpov IO, Spillane S, Flessner M, Moore CG, Schrier RW. 2015. Liver involvement in early autosomal dominant polycystic kidney disease. Clin Gastroenterol Hepatol. 13(1):155-164.
6. Belibi FA, Reif G, Gail R, Wallace DP, Yamaguchi T, Olsen L, Li H, Helmkamp GM, Grantham, JJ. 2004. Cyclic AMP promotes growth and secretion in human polycystic kidney epithelial cells. Kidney Int. 66(3):964-973.
7. Torres VE. 2005. Vasopressin antagonists in polycystic kidney disease. Kidney Int. 68(5):2405-2418.
8. Wang X, Wu Y, Ward CJ, Harris PC, Torres VE. 2008. Vasopressin directly regulates cyst growth in polycystic kidney disease. J Am Soc Nephrol. 2008 Jan; 19(1):102-108.
9. Gattone II VH, Wang X, Harris PC, Torres VE. 2003. Inhibition of renal cystic disease development and progression by a vasopressin V_2 receptor antagonist. Nat Med. 9(10):1323-1326.
10. Wang X, Gattone V, 2nd, Harris PC, Torres VE. 2005. Effectiveness of vasopressin V_2 receptor antagonists OPC-31260 and OPC-41061 on polycystic kidney disease development in the PCK rat. J Am Soc Nephrol. 16(4):846-851.
11. Boertien WE, Meijer E, Zittema D, van Dijk MA, Rabelink TJ, Breuning MH, Struck J, Bakken SJL, Peters DJM, de Jong PE, Gansevoort RT. 2012. Copeptin, a surrogate marker for vasopressin, is associated with kidney function decline in subjects with autosomal dominant polycystic kidney disease. Nephrol Dial Transplant 27:4131-4137.

12. Torres VE, Chapman AB, Devuyst O, Gansevoort RT, Grantham JJ, Higashihara E, Perrone RD, Krasa HB, Ouyang J, Czerwiec FS; TEMPO 3:4 Trial Investigators. 2012. Tolvaptan in patients with autosomal dominant polycystic kidney disease. N Engl J Med. 367(25):2407-2418.

13. Torres VE, Chapman AB, Devuyst O, Gansevoort RT, Perrone RD, Dandurand A, Ouyang J, Czerwiec FS, Blais JD; TEMPO 4:4 Trial Investigators. 2017a. Multicenter, open-label, extension trial to evaluate the long-term efficacy and safety of early versus delayed treatment with tolvaptan in autosomal dominant polycystic kidney disease: the TEMPO 4:4 Trial. Nephrol Dial Transplant. 32(7):1262.

14. Devuyst O, Chapman AB, Gansevoort RT, Higashihara E, Perrone RD, Torres VE, Blais JD, Zhou W, Ouyang J, Czerwiec FS. 2017. Urine osmolality, response to tolvaptan, and outcome in autosomal dominant polycystic kidney disease: results from the TEMPO 3:4 trial. J Am Soc Nephrol. 28(5):1592-1602.

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

16. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J. 2009. A new equation to estimate glomerular filtration rate. Ann Intern Med. 150:604-612.

17. 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.

18. Fontana RJ, Watkins PB, Bonkovsky HL, Chalasani N, Davern T, Serrano J, Rochon J; DILIN Study Group. 2009. Drug-Induced Liver Injury Networ k (DILIN) prospective study: rationale, design and conduct. Drug Saf. 32(1):55-68.

19. Woodhead JL, Watkins PB, Howell BA, Siler SQ, Shoda LKM. 2017a. The role of quantitative systems pharmacology modeling in the prediction and explanation of idiosyncratic drug-induced liver injury. Drug Metabolism Pharmacokinetics. 32(1):40-45.

20. Woodhead JL, Brock WJ, Roth SE, et al. 2017b. Application of a Mechanistic Model to Evaluate Putative Mechanisms of Tolvaptan Drug-Induced Liver Injury and Identify Patient Susceptibility Factors. Toxicological Sciences 155:61-74.

21. Woodhead JL, Pellegrini L, Shoda LKM, Howell BA. 2019. Comparison of the Hepatotoxic Potential of Two Treatments for Autosomal-Dominant Polycystic Kidney Disease Using Quantitative Systems Toxicology Modeling. Pharm Res., in press.

22. McGoon MD, Frost AE, Oudiz RJ, Badesch DB, Galie N, Olschewski H, McLaughlin VV, Gerber MJ, DuftonC, Despain DJ, Rubin LJ. 2009. Ambrisentan therapy in patients with pulmonary arterial hypertension who discontinued bosentan or sitaxsentan due to liver function test abnormalities. Chest 135:122-129.

23. Watkins PB, Lewis JH, Kaplowitz N, Alpers DH, Blais JD, Smotzer DM, Krasa H, Ouyang J, Torres VE, Czerwiec, Zimmer CA. 2015. Clinical Pattern of Tolvaptan-Associated Liver Injury in Subjects with Autosomal Dominant Polycystic Kidney Disease: Analysis of Clinical Trials Database. Drug Saf 38:1103-1113.

24. Endo M, Katayama K, Matsuo H, et al. 2019. Role of liver transplantation in tolvaptan-associated acute liver failure. Kidney Int Rep. 4:1653-57.

13 APPENDICES

13.1 Appendix 1: Chronic Kidney Disease Classification Criteria

This study uses the 2009 CKD-EPI creatinine equation (Levey A,[16] which is recommended by the KDIGO Clinical Practice Guidelines for Management of Chronic Kidney Disease.

The CKD-EPI equation is:

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

where Scr is serum creatinine, κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr or 1.

13.2 Appendix 2: DILI Network Causality Criteria, modified

Causality assessment scoring in the Drug-Induced Liver Injury Network (DILIN) prospective study, modified (Fontana RJ et al)[18]

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). 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.

13.3 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)

- Clinical chemistry
  - Glutamate dehydrogenase (GLDH)

- 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: Liver Dysfunction Checklist

The following items will be included in a checklist to be completed by the HHC for remote visits (Part 1 only) and by the site for clinic visits (Part 1 and Part 2) when directed by the protocol. For remote visits where there are findings on Part 1 of the checklist, telemedicine will be utilized to determine if a clinic visit is needed to complete Part 2.

Part 1 - Symptoms
Did the subject 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 subject develop the following signs since the last visit?

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

Any symptoms, signs, or an associated diagnosis deemed clinically significant based on information entered in Part 1 or Part 2 during either a remote visit or a clinic visit will be entered in the eCRF by the site as adverse events. 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 subject'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 subject 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 subject's country of birth?

Study PA-ADPKD-303: The ALERT Study
Clinical Study Protocol Version 4 Dated November 17, 2021
Summary of Changes

This Summary of Changes document reflects revisions incorporated into Protocol Amendment 3, Version 4 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 to Version 3 are shown in Track Changes. Minor editorial and document formatting revisions have not been summarized.

Administrative Changes

This section lists administrative changes, including changes of names or roles of companies or personnel and/or contact information. No changes listed here affect clinical decision making or consent.

Page	Section	Version 3 dated September 2, 2020	Version 4	Explanation
26	List of Abbreviations	ACEi angiotensin converting enzyme inhibitors ..... ARB angiotensin receptor blocker	~~ACEi angiotensin converting enzyme inhibitors~~ ..... ~~ARB angiotensin receptor blocker~~	These abbreviations are no longer used in text and have been removed from the List of Abbreviations.
48	5.1.1 Identity of the Investigational Product	The investigational product, lixivaptan capsule, is formulated as a white hard gelatin capsule with a clear band (without markings) containing 50 mg of lixivaptan and the inactive ingredients listed in Table 3.	The investigational product, lixivaptan capsule, is formulated as a white, banded, hard gelatin capsule ~~with a clear band (without markings)~~ containing 50 mg of lixivaptan and the inactive ingredients listed in Table 3.	Investigational product description revised to allow for multiple drug lots to be used, which may differ in capsule banding.

Page	Section	Version 3 dated September 2, 2020	Version 4	Explanation
93	References	17. 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.	17. 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.	Reference updated to reflect current guidelines (2021).

Text Clarifications

This section lists text changes for consistency or other clarifications. These changes do not affect clinical decision making or consent.

Page(s)	Section	Version 3 dated September 2, 2020	Version 4	Explanation
13	SYNOPSIS Subject Population	9. If re-challenge with tolvaptan was performed, the ALT level should have increased to >2 x ULN upon re-challenge or the ALT level was increasing but tolvaptan was stopped for patient safety reasons before it reached > 2 x ULN after having previously normalized.	9. The subject may or may not have been re-challenged with tolvaptan. If re-challenge with tolvaptan was performed, the ALT level should have increased to >2 x ULN upon re-challenge or the ALT level was increasing but tolvaptan was stopped for patient safety reasons before it reached > 2 x ULN after having previously normalized.	Text has been updated to make consistent with wording in Inclusion #9 in Section 4.1.1
14	SYNOPSIS Subject Population	11. History of advanced diabetes (e.g., glycosylated hemoglobin [HgbA1c] >7.5%, and/or glycosuria by dipstick, significant proteinuria [>300 mcg	11. History of advanced diabetes (e.g., glycosylated hemoglobin [HgbA1c] >7.5%, and/or glycosuria by dipstick, significant proteinuria [>300 mcg albumin/mg creatinine], retinopathy), other	Text has been updated to make consistent with wording in

Page(s)	Section	Version 3 dated September 2, 2020	Version 4	Explanation
		albumin/mg creatinine]), other significant renal disease, transplanted kidney, recent kidney surgery within the 6 months prior to Screening (including cyst drainage or fenestration) or acute kidney injury within 6 months prior to Screening.	significant renal disease, transplanted kidney, recent kidney surgery within the 6 months prior to Screening (including cyst drainage or fenestration) or acute kidney injury within 6 months prior to Screening.	Exclusion #11 in Section 4.1.2
44	Section 4.1.1 Inclusion Criteria	10. Appropriate control of hypertension including an ACEi or ARB (unless not considered appropriate for the subject) without the use of a diuretic in concert with KDIGO "Clinical Practice Guideline for the Management of Blood Pressure in Chronic Kidney Disease".[17]	10. Appropriate control of hypertension including an ~~ACEi~~ angiotensin converting enzyme inhibitor or ~~ARB~~ angiotensin receptor blocker (unless not considered appropriate for the subject) without the use of a diuretic in concert with KDIGO "Clinical Practice Guideline for the Management of Blood Pressure in Chronic Kidney Disease".[17]	Abbreviation removed and replaced with complete text for consistency
61	Figure 4. Titration Flowchart	Visit 9: Level 3a; Tolerated?; No	Visit 9: Level 3a; Tolerated?; No/Yes	Flowchart updated to reflect all dosing options at Visit 9.

Clinically-relevant Changes

This section describes changes that affect the type or frequency of protocol procedures, or the addition of information to the protocol that influences clinical decision making or consent.

Page	Section	Version 3 dated September 2, 2020	Version 4	Explanation
11	SYNOPSIS Subject Population	2. Female subjects 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) must agree to practice appropriate methods of birth control or remain abstinent during the study and for 30 days after the last dose of study drug. Acceptable forms of contraception include any of the following: i. oral contraceptives ii. double barrier methods of non-	2. Female subjects 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) must agree to practice appropriate methods of birth control or remain abstinent (only if this is the usual and preferred lifestyle of the participant) during the study and for 30 days after the last dose of study drug. Acceptable forms of contraception include any of the following: • hormonal	List of contraceptive methods has been aligned with global standard for "acceptable methods" of contraception. Abstinence, as a method of birth control, has been clarified.

Page	Section	Version 3 dated September 2, 2020	Version 4	Explanation
		hormonal contraception are permitted in this study: 1. female condom with spermicide (cream, spray, gel, suppository, contraceptive sponge, or polymer film) 2. diaphragm with spermicide (with or without a condom) 3. cervical cap with spermicide (with or without a condom) 4. male sexual partner who agrees to use a male	contraceptives (i.e., oral, intravaginal, transdermal, injectable, implantable) ~~i. oral contraceptives~~ ● double barrier methods of non-hormonal contraception are permitted in this study: ~~1.~~o male or female condom with spermicide (cream, spray, gel, suppository, contraceptive sponge, or polymer film) ~~2.~~ o diaphragm, cervical cap, or contraceptive sponge with spermicide (with or without a condom) ~~3.cervical cap with spermicide (with or without a condom)~~ ~~4.male sexual partner~~	

Page	Section	Version 3 dated September 2, 2020	Version 4	Explanation
		condom in addition to female subject's use of spermicide (cream, spray, gel, suppository, contraceptive sponge, or polymer film) iii. intrauterine device (IUD), including progestin-containing intrauterine devices iv. 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 v. bilateral tubal ligation vi. Essure® procedure. 3. Male subjects and their female partners of childbearing potential	~~who agrees to use a male condom in addition to female subject's use of spermicide (cream, spray, gel, suppository, contraceptive sponge, or polymer film)~~ ~~iii.~~ ● intrauterine device (IUD), including progestin-containing intrauterine devices • intrauterine hormone-releasing system (IUS) ~~iv.~~ ● 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 ~~v.~~ ● bilateral tubal ligation ~~vi.~~ ● Essure® procedure (tubal occlusion).	

Page	Section	Version 3 dated September 2, 2020	Version 4	Explanation
		must also use the contraceptive methods listed above during the study and for 30 days after the last dose of study drug.	3. Male subjects and their female partners of childbearing potential must also use the contraceptive methods listed above or remain abstinent (only if this is the usual and preferred lifestyle of the participant) during the study and for 30 days after the last dose of study drug.	
41	4.1.1 Inclusion Criteria	2. Female subjects 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) must agree to practice appropriate methods of birth control or remain abstinent during the study and for 30 days after the last dose of study drug. Acceptable forms of contraception include any of the following: • oral contraceptives • double barrier	2. Female subjects 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) must agree to practice appropriate methods of birth control or remain abstinent (only if this is the usual and preferred lifestyle of the participant) during the study and for 30 days after the last dose of study drug. Acceptable forms of contraception include any of the following: • hormonal	List of contraceptive methods has been aligned with global standard for "acceptable methods" of contraception. Abstinence, as a method of birth control, has been clarified.

Page	Section	Version 3 dated September 2, 2020	Version 4	Explanation
		methods of non-hormonal contraception are permitted in this study. ○ female condom with spermicide (cream, spray, gel, suppository, contraceptive sponge, or polymer film) ○ diaphragm with spermicide (with or without a condom) ○ cervical cap with spermicide (with or without a condom) ○ male sexual partner who agrees to use	contraceptives (i.e., oral, intravaginal, transdermal, injectable, implantable) ~~• oral contraceptives~~ • double barrier methods of non-hormonal contraception are permitted in this study. ○ male or female condom with spermicide (cream, spray, gel, suppository, contraceptive sponge, or polymer film) ○ diaphragm, cervical cap, or contraceptive sponge with spermicide	

Page	Section	Version 3 dated September 2, 2020	Version 4	Explanation
		a male condom in addition to female subject's use of spermicide (cream, spray, gel, suppository, contraceptive sponge, or polymer film) • intrauterine device (IUD), including progestin-containing intrauterine devices • 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 • bilateral tubal ligation • Essure® procedure	(with or without a condom) o ~~cervical cap with spermicide (with or without a condom)~~ o ~~male sexual partner who agrees to use a male condom in addition to female subject's use of spermicide (cream, spray, gel, suppository, contraceptive sponge, or polymer film)~~ • intrauterine device (IUD), including progestin-containing intrauterine devices	

Page	Section	Version 3 dated September 2, 2020	Version 4	Explanation
		3. Male subjects and their female partners of childbearing potential must also use the contraceptive methods listed above during the study and for 30 days after the last dose of study drug.	• 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 • bilateral tubal ligation • Essure® procedure (tubal occlusion). 3. Male subjects and their female partners of childbearing potential must also use the contraceptive methods listed above or remain abstinent (only if this is the usual and preferred lifestyle of the participant) during the study and for 30 days after the last dose of study drug.	
48	5.1.3 Study Drug Packaging, Labeling, and Storage	All study drug supplies should be stored at the study site in its original packaging in a secure, locked area under the responsibility of the Investigator or other authorized individual. At the study site, the study drug should be stored refrigerated	All study drug supplies should be stored at the study site in its original packaging in a secure, locked area under the responsibility of the Investigator or other authorized individual. At the study site, lixivaptan should be stored in accordance	Drug storage details revised based on updated CMC data; details will be reflected in the pharmacy manual.

Page	Section	Version 3 dated September 2, 2020	Version 4	Explanation
		(between 2-8°C [35-46°F]) and protected from extreme conditions of temperature, light, and humidity. Once study drug is dispensed, the subject should be instructed to store it in its original packaging at refrigerated temperatures between 2-8°C [35-46°F]), protected from extreme conditions of temperature, light, and humidity.	with the specifications detailed in the study pharmacy manual. ~~At the study site, the study drug should be stored refrigerated (between 2-8°C [35-46°F]) and protected from extreme conditions of temperature, light, and humidity.~~ Once ~~study drug~~ lixivaptan is dispensed, the subject should be instructed to store it in its original packaging ~~at refrigerated temperatures between 2-8°C [35-46°F]), protected from extreme conditions of temperature, light, and humidity~~ and in accordance with lixivaptan treatment labelling at all times until ready to take.	

Page	Section	Version 3 dated September 2, 2020	Version 4	Explanation
53	5.7.1.2 Prohibited Therapy	The following medications are prohibited during the 3 months prior to Screening Visit 1 and throughout the study: • Conivaptan • Somatostatin analogs (e.g. lanreotide, pasireotide, octreotide, etc.) • Metformin (for ADPKD; allowed for diabetes mellitus) • Nicotinamide (for ADPKD) • Bardoxolone • Venglustat • Demeclocycline • mTOR kinase inhibitors (e.g., everolimus, sirolimus, etc.) • Any investigational drug or device.	The following medications are prohibited during the 3 months prior to Screening Visit 1 and throughout the study: • Conivaptan • Somatostatin analogs (e.g. lanreotide, pasireotide, octreotide, etc.) • Metformin (for ADPKD; allowed for diabetes mellitus) • Nicotinamide (for ADPKD) • Bardoxolone • Venglustat • Demeclocycline • mTOR kinase inhibitors (e.g., everolimus, sirolimus, etc.) • SGLT2 inhibitors (e.g., canagliflozin, dapagliflozin, empagliflozin, etc.) • HIF-PH inhibitors • Any investigational drug or device.	The list of prohibited medications has been revised to reflect additional medications that may confound safety or efficacy assessments in this study.

Study PA-ADPKD-303: The ALERT Study
Clinical Study Protocol Version 3 Dated September 2, 2020
Summary of Changes

This Summary of Changes document 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 are shown in Track Changes.

Administrative Changes

This section lists administrative changes, including changes of names or roles of companies or personnel and/or contact information. No changes listed here affect clinical decision making or consent.

Page	Section	Version 2 dated June 18, 2020	Version 3	Explanation
1 2 3 10	Title Page Protocol approval – Sponsor Signature Declaration of Investigator Protocol Synopsis	An Open-label Study of Lixivaptan in Subjects with Autosomal Dominant Polycystic Kidney Disease Who Previously Experienced Abnormal Liver Chemistry Test Results While Receiving Tolvaptan Protocol Number: PA-ADPKD-303: The ALERT Study	An Open-label Study of Lixivaptan in Subjects with Autosomal Dominant Polycystic Kidney Disease Who Previously Experienced Abnormal Liver Chemistry Test Results While Receiving Tolvaptan: The ALERT Study Protocol Number: PA-ADPKD-303~~: The ALERT Study~~	Corrects a placement error of the acronym “The ALERT Study”
1	Title Page	Sponsor Contact – no email address	Email: NShusterman@palladiobio.com	Added email address
1	Title Page	Medical Monitor – no affiliation	David Frid, MD ICON Clinical Research	Added medical monitor’s employment affiliation
1	Title page	Version of Protocol: 2	Version of Protocol: ~~2~~3	Updates protocol version
1	Title page	Date of protocol: June 18, 2020	Date of protocol: ~~June 18, 2020~~September 2, 2020	Updates protocol date

Page	Section	Version 2 dated June 18, 2020	Version 3	Explanation
2	Protocol approval – Sponsor signature	Protocol Version 2	Protocol Version: ~~2~~3	Updates protocol version
2	Protocol approval – Sponsor signature	Protocol date: June 18, 2020	Protocol date: ~~June 18, 2020~~September 2, 2020	Updates protocol date
2	Protocol approval – Sponsor signature	Signature date: June 18, 2020	Signature date: ~~June 18, 2020~~September 2, 2020	Updates protocol date
84	8.8.1. Data Management	Clinical data management will be performed in accordance with applicable standards and data cleaning procedures of the CRO)…	Clinical data management will be performed in accordance with applicable standards and data cleaning procedures of the contract research organization (CRO)…	Defines “CRO” at its first appearance in document.
All	Footer	18-Jun-2020	~~18-Jun-2020~~02-Sep-2020	Updates protocol date

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(s)	Section	Version 2 dated June 18, 2020	Version 3	Explanation
4-9	Tables of Contents, Listings, and Figures	N/A	N/A	Updated page numbers (change not tracked)
11	Synopsis – Subject Population	An eligibility review and discussion between the Investigator and the medical monitor will be conducted before the first subject is enrolled at each site	At Visits 1 and 2, subjects must meet all of the Inclusion criteria and none of the Exclusion criteria. An eligibility review and discussion between the Investigator and the medical monitor will be conducted before ~~the first~~each subject is enrolled (prior to Visit 3) at each site.	Clarifies text
14 80	Synopsis – Study design 8.1 Sample Size	Up to 50 subjects will be enrolled.	Up to 50 subjects will be enrolled and treated.	Clarifies text
15 39	Synopsis - Study Period Description and Estimated Duration 3.1.2 Detailed Study Design	Subjects […] and then will return for Visit 2.	Subjects […] and then will be scheduled ~~return~~ for Visit 2.	Clarifies text
16	Synopsis - Study Period Description and Estimated Duration	After successful completion of the Baseline Period, subjects will begin study drug and will enter the Titration Period.	After successful completion of Visit 5 assessments in the clinic~~the Baseline Period~~, subjects will be dispensed study drug and begin ~~taking study drug~~treatment. This completes the Baseline Period and subjects~~, begin~~	Clarifies procedures

Page(s)	Section	Version 2 dated June 18, 2020	Version 3	Explanation
			~~taking study drug and will~~ enter the Titration Period.	
16 38	Synopsis - Study Period Description and Estimated Duration 3.1.2 Detailed Study Design	Liver chemistry tests, urine specific gravity and U_{osm} will be measured weekly throughout the Titration Period.	Liver chemistry tests will be assessed weekly during the Titration Period. ~~u~~Urine specific gravity and U_{osm} will be measured ~~weekly throughout the Titration Period~~ at all titration visits that occur except Visit 11. All titration visits may be conducted remotely by a HHC with telemedicine.	Clarification of other testing during titration and use of remote visits.
16	Synopsis - Study Period Description and Estimated Duration	Subjects will continue to receive lixivaptan at the dose level achieved at the end of the Titration Period for up to 52 weeks (12 months).	Following successful completion of the Titration P~~p~~eriod, ~~S~~subjects will continue to receive lixivaptan in the Maintenance Period at the dose level achieved at the end of the Titration Period for up to 52 weeks (12 months).	Clarifies procedures
17	Synopsis - Study Period Description and Estimated Duration	Serum creatinine to determine final eGFR, will be obtained starting the 8th day after the final dose of study drug and continuing through the 28th day of the Follow-up Period.	~~Three (3) visits to assess S~~serum creatinine, to determine final eGFR, will be obtained over three (3) visits starting the 8th day after the final dose of study drug and continuing through the 28th day of the Follow-up Period.	Clarifies text
20	Synopsis – Statistical Methods	Up to 50 subjects will be enrolled based on the design of a similar study with a different drug.	Up to 50 subjects will be enrolled and treated based on the design of a similar study with a different drug.	Clarifies text
20 83	Synopsis – Statistical Methods 8.7 Statistical Analysis Methodology	[…] descriptive statistics and upper 95% confidence interval […]	[…] descriptive statistics (n and percentage) and ~~upper~~ 95% confidence interval […]	Rectifies error (the entire 95% confidence interval will be calculated)
26	List of abbreviations	--	ART: anti-retroviral therapy HHC: home healthcare clinician	Added abbreviations

Page(s)	Section	Version 2 dated June 18, 2020	Version 3	Explanation
32	1.6 Summary of Clinical Studies	the most common AEs were dry mouth, headache, and nausea	the most common AEs reported in 3 or more subjects were dry mouth, headache, and nausea.	Clarifies text
32 38	1.7 Study rationale 3.1.1 Overview of Study Design	Up to 50 subjects will be enrolled.	Up to 50 subjects will be enrolled and treated.	Clarifies text
34	1.7 Study rationale	Analysis of the Phase 2 ADPKD study also did not show any hepatic AEs.	~~An interim a~~Analysis of the Phase 2 ADPKD study also did not show any hepatic AEs.	Updates information
34	1.7 Study rationale	At the time of this writing, there has been no evidence of increased ALT values in this subject after 7.5 months of therapy with lixivaptan (**Error! Reference source not found.**)	At the time of this writing, there has been no evidence of increased ALT values in this subject after ~~7.5~~14 months of therapy with lixivaptan (**Error! Reference source not found.**)	Updates information from version 2
35	Figure 3	--	Figure 3 updated with recent data	Updates information from version 2
38	3.1.1 Overview of Study Design	A DNA sample to assess genetic variants associated with hepatic abnormalities (if and when feasible) will be obtained under a separate biobanking informed consent at the Baseline Period or later in the study.	A DNA sample to assess genetic variants associated with hepatic abnormalities (if and when feasible) will be obtained under a separate biobanking informed consent at ~~the end of the Baseline Period~~Visit 5 or later in the study.	Adds some more precision
42	4.1 Selection of Study Population	Approximately 100-200 subjects will be screened in order to enroll up to approximately 50 subjects at up to approximately 25 sites globally. An eligibility review and discussion between the Investigator and the medical monitor will be conducted before the first subject is enrolled at each site. Subjects will be	Approximately 100-200 subjects will be screened in order to enroll and treat up to approximately 50 subjects at up to approximately 25 sites globally. An eligibility review and discussion between the Investigator and the medical monitor will be conducted before ~~the first~~each subject is enrolled (completes Visit 3) at	Clarifies enrollment of subjects and rescreening procedures

<table>
<tr><th>Page(s)</th><th>Section</th><th>Version 2 dated June 18, 2020</th><th>Version 3</th><th>Explanation</th></tr>
<tr><td></td><td></td><td>enrolled only if they meet all the inclusion criteria and none of the exclusion criteria. With the approval of the medical monitor and sponsor, subjects who fail inclusion/exclusion criteria due to temporary or correctable reasons may be re-screened up to 2 times.</td><td>each site. Subjects will be enrolled only if they meet all the inclusion criteria and none of the exclusion criteria. With the approval of the medical monitor and sponsor, subjects who fail inclusion/exclusion criteria due to temporary or correctable reasons may be re-screened up to 2 times after re-consenting each time.</td><td></td></tr>
<tr><td>45</td><td>4.1.2 Exclusion criteria</td><td>20. Any malignancy within 5 years prior to Screening except for basal cell carcinoma successfully treated with local therapy.</td><td>20. Any malignancy within the 5 years prior to Screening except for basal cell carcinoma successfully treated with local therapy.</td><td>Corrects grammar</td></tr>
<tr><td>46</td><td>4.2.3 Subject Withdrawal Criteria</td><td>At a minimum, subjects should be encouraged to complete the Early Termination Visit (Visit 24) at the time of […]</td><td>At a minimum, subjects should be encouraged to complete the Early Termination Visit (Visit 24 – in the clinic) at the time of […]</td><td>Adds details on Visit 24</td></tr>
<tr><td>47</td><td>4.2.7 Withdrawals Due to Abnormal Liver Chemistry Test Results</td><td>In certain circumstances described in Section Error! Reference source not found., restarting study drug should only occur when the HERC believes the relationship between the abnormal liver chemistry test results and the study drug is 50% or less according to the DILIN criteria, modified, Section Error! Reference source not found.</td><td>In certain circumstances described in Section Error! Reference source not found., restarting study drug should only occur when the HERC believes the relationship between the abnormal liver chemistry test results and the study drug is less than 50% ~~or less~~ according to the DILIN criteria, modified, Section Error! Reference source not found.</td><td>Corrects error</td></tr>
<tr><td>48</td><td>5.1.3 Study Drug Packaging, Labeling, and Storage – Table 3</td><td><table><tr><th></th><th>Lixivaptan</th></tr><tr><td>Strength</td><td>50 mg</td></tr><tr><td>Formulation</td><td>Capsules, packaged 30 per bottle</td></tr></table></td><td><table><tr><th></th><th>Lixivaptan</th></tr><tr><td>Strength</td><td>50 mg</td></tr><tr><td>Formulation</td><td>Capsules, packaged 30 per bottle</td></tr></table></td><td>Clarifies that Palladio does not have a role in the manufacturing of the study drug. Palladio as sponsor of the study is well</td></tr>
</table>

Page(s)	Section	Version 2 dated June 18, 2020	Version 3	Explanation
		Inactive ingredients: Polyethylene glycol 400, NF/EP; Polyethylene glycol 1000, NF/EP; Povidone (K-17), USP/EP; Polysorbate 80, NF/EP; Butylated hydroxytoluene; Butylated hydroxyanisole **Supplier/ manufacturer**: Palladio Biosciences 5 Walnut Grove Drive Suite 120 Horsham PA 19044 PMRS, Inc. 202 Precision Road Horsham, PA 19044 **Packager**: PCI Pharma Services 4545 Assembly Drive Rockford, IL 61109	**Inactive ingredients**: Polyethylene glycol 400, NF/EP; Polyethylene glycol 1000, NF/EP; Povidone (K-17), USP/EP; Polysorbate 80, NF/EP; Butylated hydroxytoluene; Butylated hydroxyanisole **~~Supplier/ m~~Manufacturer**: ~~Palladio Biosciences 5 Walnut Grove Drive Suite 120 Horsham PA 19044~~ PMRS, Inc. 202 Precision Road Horsham, PA 19044 **Packager**: PCI Pharma Services 4545 Assembly Drive Rockford, IL 61109	documented in the protocol.
49	5.1.3 Study Drug Packaging, Labeling, and Storage	All study drug supplies should be stored in its original packaging in a secure, locked area under the responsibility of the Investigator or other authorized individual.	All study drug supplies should be stored at the study site in its original packaging in a secure, locked area under the responsibility of the Investigator or other authorized individual.	Clarifies that this instruction applies to storage at clinical sites.
56	6.1.1 Visit 1	Assign the subject an identification number and contact the IRT. The Investigator or designee will contact the IRT and enter the subject identification number as described in Section **Error! Reference source not found.**	Contact the IRT to Aassign the subject an identification number ~~and contact the IRT. The Investigator or designee will contact the IRT and enter the subject identification number~~ as described in Section **Error! Reference source not found.**	Simplifies the text
57 57 58 58 60	6.1.2 Visit 2 6.1.3 Visit 3 6.2.2 Visit 4 6.2.3 Visit 5	• Collect and review concomitant medication information and update the CRF	• Collect and review concomitant medication information ~~and update the eCRF~~	Remove useless text (CRF is always updated)

Page(s)	Section	Version 2 dated June 18, 2020	Version 3	Explanation
62	6.3.1 Visits 6 to 10 6.3.2 Visit 11/Last Titration Visit			
57	6.2 Baseline Period	The 3-week Baseline Period beginning with Visit 3 is designed to collect baseline data […]	The 3-week Baseline Period begins at the conclusion of Visit 2 and consists of 3 visits – Visits 3, 4, and 5. ~~beginning with Visit 3~~It is designed to collect baseline data […]	Clarifies the Baseline Period
57 59	6.2.1 Visit 3 6.2.3 Visit 5	• Schedule the subject to return for Visit […] to occur in 1 week.	• Schedule the subject ~~to return~~ for Visit […] to occur in 1 week.	Remove useless text
58	6.2.2 Visit 4	• Schedule the subject to return for Visit 5 to occur in 1 week	• Schedule the subject to return to the clinic for Visit 5 to occur in 1 week	Clarifies text
58	6.2.3 Visit 5	• Collect a plasma sample for measurement of lixivaptan. Note date and time of sample on the requisition form and the CRF.	• Collect a plasma sample for measurement of lixivaptan. Note date and time of sample on the requisition form ~~and the eCRF~~.	Rectifies text
63	6.3.2 Visit 11/Last Titration Visit	• Investigator or designee contact the IRT […]	• The ~~Investigator or designee~~site will c~~C~~ontact the IRT […]	Clarifies text
63	6.3.2 Visit 11/Last Titration Visit	• Schedule the subject to return for their next visit to occur in 4 weeks and remind the subject to take adequate fluids. Provide the subject with an appointment reminder card and any written instructions. Note that the next visit may be conducted remotely (if available and when necessary).	• Schedule the subject ~~to return~~ for their next visit to occur in 4 weeks and remind the subject to take adequate fluids. Provide the subject with an appointment reminder card and any written instructions. Note that the next visit may be conducted remotely (if available ~~and when necessary~~).	Clarifies text
63 65	6.4.1 Visits 12 to 23 6.5.1 Visit 25	• Contact the IRT […] • For remote visits, the IRT should be contacted sufficiently […]	• The site will ~~C~~contact the IRT […]	Clarifies text

Page(s)	Section	Version 2 dated June 18, 2020	Version 3	Explanation
66	6.5.2 Visit 26		• For remote visits, the site must contact the IRT ~~should be contacted~~ sufficiently […]	
65	6.4.2 Visit 24/Early Termination	• Collect a plasma sample […] on the requisition form and the CRF. • […] • Schedule the subject to return […] as a remote visit if available and when necessary.	• Collect a plasma sample […] on the requisition form ~~and the eCRF~~. • […] Schedule the subject to return […] as a remote visit if available ~~and when necessary~~.	Rectifies text
65	6.5 Follow-up Period	The Follow-up Period will last 28 days and include 3 visits (Visit 25, Visit 26, and Visit 27). If available and when necessary, Visits 25 and 26 may be done remotely.	The Follow-up Period will last 28 days and include 3 visits (Visit 25, Visit 26, and Visit 27). If available ~~and when necessary~~, Visits 25 and 26 may be done remotely by a HHC.	Clarifies and rectifies text
68	7.3.3 Physical Examination	Brief physical examination will subsequently be measured at the timepoints specified	Brief physical examination will subsequently be ~~measured~~ performed at the timepoints specified	Corrects grammar
71	7.3.7 Assessment of Liver Symptoms, Signs, or Test Abnormalities	Management of hepatic and liver chemistry test abnormalities are discussed below. *The appearance of any suspicious symptoms or signs of liver dysfunction in a subject at any time during the study should trigger prompt testing of liver chemistry tests (i.e., within 48 hours).*	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 4 (Section* ***Error! Reference source not found.****) in a subject at any time during the study should trigger prompt testing of liver chemistry tests (i.e., within 48 hours).*	Provides link to list of symptoms and signs
73	7.3.7.3 Special Reporting of Liver Events	A liver disease eCRF will be utilized during the study. The purpose of the liver disease eCRF and additional testing is to facilitate review of each subject who develops liver chemistry test abnormalities during the study to	A liver dysfunction details~~isease~~ eCRF will be utilized during the study. The type of information needed to complete it is provided in Appendix 4 (Section**Error! Reference source not found.**). The purpose of the liver dysfunction details~~isease~~ eCRF and additional testing	(4 corrections) Clarifies text and describes use of a checklist

Page(s)	Section	Version 2 dated June 18, 2020	Version 3	Explanation
		determine the probable cause(s) of these abnormalities.	is to facilitate review of each subject who develops liver chemistry test abnormalities and/or signs or symptoms of liver dysfunction during the study to determine the probable cause(s) of these abnormalities.	
73	7.3.7.3 Special Reporting of Liver Events	The Investigator must complete a liver disease eCRF and submit it within 24 hours of awareness through the SAE reporting pathway…	The Investigator must complete ~~a~~ the liver ~~disease~~ dysfunction details eCRF and submit it within 24 hours of awareness through the SAE reporting pathway (described in Section **Error! Reference source not found.**)…	Clarifies how to submit liver dysfunction events
73	7.3.7.3 Special Reporting of Liver Events	• develops any signs or symptoms of hepatic dysfunction (e.g. nausea, vomiting, right upper quadrant pain, malaise, jaundice)	• develops any signs or symptoms of hepatic dysfunction (see Appendix 4 [Section **Error! Reference source not found.**]~~e.g. nausea, vomiting, right upper quadrant pain, malaise, jaundice~~)	Refers reader to complete list of signs and symptoms
73	7.3.7.3 Special Reporting of Liver Events	The liver eCRF and AESI report form should be updated as new information becomes available.	The ~~liver eCRF and~~ AESI report form should be updated as new information becomes available.	Clarifies the forms to use to report new information
75	7.3.8.3 Reporting AEs, SAEs, and AEs of Special Interest	For the purpose of this study, pregnancies and liver events are considered to be AESIs. Liver events will be reported directly from the EDC system; however, pregnancy events will need to be reported on special pregnancy forms to ICON PVSS. Any AE that meets SAE or AESI criteria will be reported to ICON Pharmacovigilance & Safety Services (PVSS) immediately (i.e., within 24 hours) after site personnel first learn	For the purpose of this study, ~~pregnancies and~~ liver events are considered to be AESIs. Liver events will be reported directly from the electronic data capture (EDC) system~~; however,~~ . P~~p~~regnancy events will need to be reported on special pregnancy forms to ICON PVSS. Any AE that meets SAE or AESI criteria will be reported to ICON Pharmacovigilance & Safety Services (PVSS) immediately (i.e., within 24 hours)	Clarifies that pregnancy is not an AE of Special Interest but will be reported prompted through ICON's pharmacovigilance department using a paper-based reporting form.

Page(s)	Section	Version 2 dated June 18, 2020	Version 3	Explanation
		about the event. Investigators should record all SAE/AESI details available, including Investigator causality assessment, on an Adverse Event eCRF or appropriate AESI forms and submit the report via the electronic data capture (EDC) system within 24 hours of becoming aware of the event. Notification of SAE/AESI entry will be generated and sent to ICON PVSS via the EDC system.	after site personnel first learn about the event. Investigators should record all SAE/AESI details available, including Investigator causality assessment, on an Adverse Event eCRF or appropriate AESI forms and submit the report via the electronic data capture (EDC) system within 24 hours of becoming aware of the event. Notification of SAE/AESI entry will be generated and sent to ICON PVSS via the EDC system. Pregnancy events will also be reported to ICON PVSS within 24 hours of recognition of the event, but on the special forms provided.	
78	7.3.10 HERC	This committee will independently determine the probable causality for liver chemistry test abnormalities of concern (Section **Error! Reference source not found.**) and of relatedness to lixivaptan.	This committee will independently determine the probable causality for instances of suspected liver dysfunction and liver chemistry test abnormalities of concern (Section **Error! Reference source not found.**) and of relatedness to lixivaptan.	Explicitly states that episodes of liver dysfunction as well as liver chemistry abnormalities will be reviewed by the HERC.
81	8.4.3 Additional Hepatic Safety Endpoints	>3 x, 5 x, >10 x, and 20 x ULN elevations for ALT	>3 x, 5 x, ~~>~~10 x, and 20 x ULN elevations for ALT	Correction of typographical error
84	8.8.1 Data Management	Investigative site personnel will enter subject data into eCRFs using the [EDC system name] program.	Investigative site personnel will enter subject data into eCRFs using the ~~[~~EDC system ~~name] program~~designated by the sponsor.	Correction of template language
86	10 Investigator's Obligations	10 INVESTIGATOR'S OBLIGATIONS	10 INVESTIGATOR'S OBLIGATIONS AND STUDY PERSONNEL	Clarifies sponsorship of study and identification of CRO.

<table>
<tr><th>Page(s)</th><th>Section</th><th>Version 2 dated June 18, 2020</th><th>Version 3</th><th>Explanation</th></tr>
<tr><td></td><td></td><td></td><td>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 and the CRO’s medical monitor are listed on the cover page of this protocol. Contact information for other sponsor personnel, ICON Clinical Research Limited, the designated CRO, and other vendors are listed in the Investigator Site File provided to each site</td><td></td></tr>
<tr><td>94</td><td>13.2 Appendix 2: DILI Network Causality Criteria, modified</td><td><table><tr><th>Causality score</th><th>Likelihood (%)</th><th>Description</th></tr><tr><td>4 = possible</td><td>25-49</td><td>The causality is not supported by ‘the preponderance of evidence’ as implicating the drug but the evidence cannot be considered definite or highly likely</td></tr></table></td><td><table><tr><th>Causality score</th><th>Likelihood (%)</th><th>Description</th></tr><tr><td>4 = possible</td><td>25-49</td><td>The causality is not supported by ‘the preponderance of evidence’; however, one cannot definitively exclude the possibility ~~as implicating the drug but the evidence cannot be considered definite or highly likely~~</td></tr></table></td><td>Cut-and-paste error from source document corrected</td></tr>
</table>

Clinically-relevant Changes

This section describes changes that affect the type or frequency of protocol procedures, or the addition of information to the protocol that influences clinical decision making or consent.

Page	Section	Version 2 dated June 18, 2020	Version 3	Explanation
14 44	Synopsis – Exclusion Criteria 4.1.2 Exclusion Criteria	16. History of infection with human immunodeficiency virus (HIV).	16. History of infection with human immunodeficiency virus (HIV) unless the subject is stable and doing well on a non-CYP interacting anti-retroviral therapy (ART) regimen and who has not required more than 2 changes in their ART regimen.	Allows subjects into the study with HIV infection under specified conditions.
15	Synopsis - Study Period Description and Estimated Duration	-	Certain visits may be conducted remotely with a home healthcare clinician (HHC), but Visits 1, 5, 17, 24, and 27 are to be conducted in the clinic (with only extraordinary exceptions at the discretion of the medical monitor and sponsor). Some of the remote visits will include a telemedicine component with the Investigator. Details are provided in **Error! Reference source not found.**.	Increases the number of remote visits with a HHC from 13 to 22
15	Synopsis - Study Period Description and Estimated Duration	Subjects who meet all the inclusion criteria and none of the exclusion criteria and achieve stability of background anti-hypertensive medications in the absence of diuretics will return to the clinic for end-of-screening assessments at Visit 2, including body weight, vital signs, and serum chemistry testing (if	Subjects who meet all the inclusion criteria and none of the exclusion criteria and achieve stability of background anti-hypertensive medications in the absence of diuretics will either return to the clinic or be scheduled for a remote visit with ~~the~~a HHC -for Visit 2 end-of-screening assessments ~~at Visit 2~~, including body weight, vital signs, and	Adds the use of a remote visit with a HCC and telemedicine

Page	Section	Version 2 dated June 18, 2020	Version 3	Explanation
		diuretics were discontinued or anti-hypertensive medications were adjusted).	serum chemistry testing (if diuretics were discontinued or anti-hypertensive medications were adjusted) and final eligibility determination (utilizing telemedicine if a remote visit).	
16	Synopsis - Study Period Description and Estimated Duration	Subjects who fail inclusion/exclusion criteria due to temporary or correctable reasons may be re-screened (up to 2 additional times) after consultation with the medical monitor and sponsor. Subjects will enter the Baseline Period.	Subjects 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 and sponsor. Immediately following successful completion of Visit 2, ~~S~~subjects ~~who successfully complete the Screening Period~~ will enter the Baseline Period.	Clarifies that reconsent is necessary if a subject re-screens (after previously screening failing)
16	Synopsis - Study Period Description and Estimated Duration	--	Visits 3 and 4 may be conducted remotely by a HHC.	Adds the use of remote visits with a HCC for Visits 3 and 4
16	Synopsis - Study Period Description and Estimated Duration	--	All titration visits may be conducted remotely by a HHC with telemedicine.	Adds the use of remote visits with a HCC for Visits 6-11
16-17	Synopsis - Study Period Description and Estimated Duration	--	Visits 12, 13, 14 , 15, 16, 18, 19, 20, 21, 22, and 23 may be conducted remotely by a HHC. If conducted remotely, Visits 14 and 20 will include telemedicine and all other remote maintenance visits may	Adds the use of remote visits with a HCC for Visits 12-23 and telemedicine for Visits 14 and 20

Page	Section	Version 2 dated June 18, 2020	Version 3	Explanation
			include a telemedicine component if deemed necessary by the Investigator.	
17	Synopsis - Study Period Description and Estimated Duration	--	Visits 25 and 26 may be conducted remotely by a HHC (using telemedicine if necessary).	Adds the use of remote visits with a HCC for Visits 25 and 26
19 50	Synopsis - Study Drug, Dosage, and Route of Administration 5.2 Dose Administered	The Investigator may temporarily hold the drug for up to 7 days, if necessary, to manage intercurrent illness, tolerability issues, planned or unplanned surgical procedures or life situations, e.g., airplane travel, etc.	The Investigator may temporarily hold the drug for up to 7 days, if necessary, to manage acute intercurrent illness, tolerability issues, planned or unplanned surgical procedures or life situations, e.g., airplane travel, etc. Additionally, subjects who have a longer interruption due to illness, including COVID-19, or other reasons may re-start study drug when medically stable after discussion with the medical monitor and sponsor.	Adds provisions for long interruption of study drug treatment
19 20 36	Synopsis – Criteria for Evaluation Synopsis – Analysis of Primary and Secondary Endpoints 2.2.1 Primary Endpoint	Proportion of subjects who develop ALT levels >3 x ULN during the Titration and Maintenance Periods that were assessed by the Investigator in consultation with the medical monitor and sponsor and adjudicated by the independent Hepatic Events Review Committee (HERC) to be related to lixivaptan and resulted in discontinuation of the study drug.	Proportion of subjects who develop ALT levels >3 x ULN during the Titration ~~and~~ or Maintenance Periods that were assessed ~~by the Investigator in consultation with the medical monitor and sponsor and adjudicated~~ by the independent Hepatic Events Review Committee (HERC) to be at least probably related to lixivaptan and resulted in discontinuation of the study drug.	Delegation of determining relationship to the independent HERC and clarification of the causality assessments that are clinically meaningful.
19	Synopsis – Criteria for Evaluation	Proportion of subjects who develop ALT levels >5 x ULN during the Titration and Maintenance Periods	Proportion of subjects who develop ALT levels >5 x ULN during the Titration ~~and~~ or Maintenance Periods that were	Delegation of determining relationship to the independent HERC and

Page	Section	Version 2 dated June 18, 2020	Version 3	Explanation
20-21 36-367	Synopsis – Analysis of Primary and Secondary Endpoints 2.2.2 Secondary Endpoint	that were assessed by the Investigator in consultation with the medical monitor and sponsor and adjudicated by the independent HERC to be related to lixivaptan and resulted in discontinuation of the study drug. Proportion of subjects who develop ALT levels >3 x ULN during the Titration and Maintenance Periods that were assessed by the Investigator in consultation with the medical monitor and sponsor and adjudicated by the HERC to be related to lixivaptan and resulted in dose reduction of the study drug.	assessed ~~by the Investigator in consultation with the medical monitor and sponsor and adjudicated~~ by the independent HERC to be at least probably related to lixivaptan and resulted in discontinuation of the study drug. Proportion of subjects who develop ALT levels >3 x ULN during the Titration ~~and~~ or Maintenance Periods that were assessed ~~by the Investigator in consultation with the medical monitor and sponsor and adjudicated by the~~ independent HERC to be at least probably related to lixivaptan and resulted in dose reduction of the study drug.	clarification of "relatedness" for the other secondary endpoints.
20 80	Synopsis – Statistical Methods 8.2 Populations for Analysis	**Populations for Analysis:** The Safety Population is defined as those subjects who received at least 1 dose of lixivaptan. This population will be utilized for all safety analyses. The Efficacy Population is defined as those subjects who received at least 1 dose of lixivaptan and have at least 1 eGFR determination during the Follow-up Period. This population will be used for all efficacy analyses.	**Populations for Analysis:** The Enrolled Population is defined as all subjects who meet inclusion/exclusion criteria during Screening Period and complete Visit 3. All general summaries, except safety and efficacy analyses are based on the Enrolled Population, unless specified otherwise. The Safety Population is a subset of the Enrolled Population defined as those subjects who received at least 1 dose of lixivaptan. This population will be utilized for all safety analyses. The Efficacy Population is a subset of the Safety Population defined as those subjects who ~~received at least 1 dose of lixivaptan~~ have at least 1 eGFR	Updates analysis populations definitions and defines 3 new populations based on discussions during the preparation of the statistical analysis plan (SAP)

Page	Section	Version 2 dated June 18, 2020	Version 3	Explanation
			determination during the Baseline Period and have at least 1 eGFR determination during the Follow-up Period. This population will be used for all efficacy analyses. The Pharmacokinetic (PK) Population is a subset of the Safety Population defined as those subjects who had at least 1 on-therapy evaluable PK measurement. The Pharmacodynamic (PD) Population is a subset of the Enrolled Population defined as those subjects who have at least 1 set of urine specific gravity and urine osmolality measurements from the same specimen.	
24	Table 1. Schedule of Procedures	Assess for liver dysfunction: shown from Visit 6 to Visit 24	Added assessment for liver dysfunction to Visit 25	Lab results from Visit 24 needed to be checked as Visit 25
24	Table 1. Schedule of procedures	-- Footnote d. A brief physical examination will be performed at V11 and V27.	ECGs and physical examination: Removed at V11, added at V17 Footnote d. A brief physical examination will be performed at ~~V11~~ V17 and V27.	Changes ECG and brief physical examination schedule to a visit that will be in the clinic
25	Table 1. Schedule of procedures footnotes (second bullet)	Remote visits (if available) may occur at V3, V4, V12, V13, V15, V16, V18, V19, V21, V22, V23, V25 and V26.	Remote visits (if available ~~and when necessary~~) may occur at V2 (with telemedicine), V3, V4, V6 – V11 (each with telemedicine), V12, V13, and V14 (with telemedicine), V15, V16, V18, V19, and V20 (with telemedicine), V21, V22, V23, V25 and V26. Any remote visit may have a telemedicine component added if needed. With permission from~~of~~ the	Reflects the increase in the number of remote visits

Page	Section	Version 2 dated June 18, 2020	Version 3	Explanation
			medical monitor and sponsor, other visits may be done remotely on a case-by-case extraordinary basis.	
38	3.1.1 Overview of Study Design	All assessments and the relative timings are listed in the Schedule of Study Procedures (Table 1).	All assessments and the relative timings are listed in the Schedule of Study Procedures (Table 1). Certain visits may be conducted remotely with a home healthcare clinician (HHC), but Visits 1, 5, 17, 24, and 27 are to be conducted in the clinic (with only extraordinary exceptions at the discretion of the medical monitor and sponsor). Some of the remote visits will include a telemedicine component with the Investigator while other remote visits may have that option if deemed necessary for a medical reason. Details are provided in **Error! Reference source not found.**.	Details remote visits with a HHC and telemedicine versus in-clinic visits
39	3.1.2 Detailed Study Design – Screening Period	Subjects who meet all the inclusion criteria and none of the exclusion criteria and achieve stability of background medications will return to the clinic for end-of-screening assessments including body weight, vital signs, and a Complete Chemistry Panel (only if blood pressure medications were adjusted) at Visit 2. Eligible subjects will then enter the Baseline Period at the completion of Visit 2. Subjects who fail inclusion/exclusion criteria because of temporary or correctable reasons may be re-screened up to 2 times with the	Subjects who meet all the inclusion criteria and none of the exclusion criteria and achieve stability of background medications will ~~return to the clinic~~be scheduled for end-of-screening assessments including body weight, vital signs, and a Complete Chemistry Panel (only if blood pressure medications were adjusted) at Visit 2 (which may be conducted remotely by a HHC with telemedicine to determine final eligibility). Eligible subjects will then enter the Baseline Period immediately at the completion of Visit 2. Subjects who fail inclusion/exclusion	Explains that Visit 2 can be done remotely Explains that new ICF is needed for re-screening

Page	Section	Version 2 dated June 18, 2020	Version 3	Explanation
		approval of the medical monitor and sponsor.	criteria because of temporary or correctable reasons may be re-screened up to 2 times after obtaining new informed consents with the approval of the medical monitor and sponsor.	
39	3.1.2 Detailed Study Design – Baseline Period	After completion of the Baseline Period, subjects will enter the Titration Period.	Visits 3 and 4 may be conducted remotely by a HHC. ~~After~~ At the successful completion of the Baseline Period (Visit 5), subjects will be dispensed study drug and begin treatment ~~enter the Titration Period~~. This completes the Baseline Period and subjects will enter the Titration Period.	Details transition between Baseline Period and Titration Period
39	3.1.2 Detailed Study Design – Titration Period	Liver chemistry tests, urine specific gravity, and U_{osm} will be measured weekly during the Titration Period.	Liver chemistry tests will be assessed weekly during the Titration Period~~,~~. ~~u~~Urine specific gravity and U_{osm} will be measured ~~weekly during the Titration Period~~at all titration visits that occur except Visit 11. All titration visits may be conducted remotely by a HHC with telemedicine.	Clarifies when certain labs are obtained and adds the use of remote visits with telemedicine for titration visits.
40	3.1.2 Detailed Study Design – Maintenance Period	Assessments, including liver chemistry tests, will be performed every 4 weeks during this period.	Assessments, including liver chemistry tests, will be performed every 4 weeks during this period. Visits 12, 13, 14, 15, 16, 18, 19, 20, 21, 22, and 23 may be conducted remotely by a HHC. If conducted remotely, Visits 14 and 20 will include telemedicine and the other visits may include telemedicine if deemed necessary by the Investigator..	Adds the use of remote visits with a HCC and telemedicine for Visits 12-23
40	3.1.2 Detailed Study Design – Follow-up Period	Three assessments of serum creatinine to determine eGFR will be obtained starting as early as the 8th	Three assessments of serum creatinine to determine eGFR will be obtained starting as early as the 8th day after the	Adds the use of remote visits with a HCC for Visits 25 and 26

Page	Section	Version 2 dated June 18, 2020	Version 3	Explanation
		day after the final dose of study medication and continuing through the 28th day of the Follow-up Period.	final dose of study medication and continuing through the 28th day of the Follow-up Period. Visits 25 and 26 may be conducted remotely by a HHC (using telemedicine if deemed necessary by the Investigator).	
48	5.1.3 Study Drug Packaging, Labeling, and Storage	Once study drug is dispensed, the subject should be instructed to store it in its original packaging at temperatures between 2-25°C [35-77°F]), but protected from extreme conditions of temperature, light, and humidity.	Once study drug is dispensed, the subject should be instructed to store it in its original packaging at temperatures between 2-25°C [35-77°F]), but protected from extreme conditions of temperature, light, and humidity. For subjects who utilize remote visits, study drug will be dispensed and shipped from the study site to the subject's home or to an alternate location pre-specified by the subject, via an experienced courier approved by the Sponsor. Subjects 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 health clinician arrives.	Provides the ability to ship study drug to the subject using an approved, experienced courier when the subject elects to have remote visits at home.
51	5.6 Treatment Compliance	The dates of all study medication dosing, including interruptions, missed doses or overdose, must be recorded in the eCRF. If the subject 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	The dates of all study medication dosing, including interruptions, missed doses or overdose, must be recorded in the eCRF. If the subject 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 subject should be re-educated regarding the	Added text to handle situation of prolonged treatment interruption that does not represent non-compliance.

Page	Section	Version 2 dated June 18, 2020	Version 3	Explanation
		subject should be re-educated regarding the correct study drug doses to be administered.	correct study drug doses to be administered. However, if the Investigator, medical monitor, and sponsor have agreed to a treatment interruption (Section **Error! Reference source not found.**), then this will not be considered a protocol deviation.	
53	5.7.1.2 Prohibited Therapy	Strong or moderate CYP3A4 or CYP2C8 inhibitors, including […]	Strong or moderate CYP3A4 or CYP2C8 inhibitors, including […] remdesivir […]	Adds remdesivir (COVID-19 treatment which is a 3A4 inhibitor) to the list of prohibited medications
55	6 Timing of Study Procedures	Certain visits must occur in the clinic. Other visits may occur in the clinic or through an at-home visiting nurse (if available).	Certain visits must occur in the clinic. Other visits may occur in the clinic or remotely by a HHC ~~through an at-home visiting nurse~~ (if available).	Switches from at-home nurse visits to remote visits
55	6.1 Screening Period	Subjects […] will return for Visit 2. At Visit 2, screening test results will be reviewed […]	Subjects […] will ~~return~~ be scheduled for Visit 2. At Visit 2 (which may be conducted remotely by a HHC and telemedicine to determine eligibility) screening test results will be reviewed […]	Explains that Visit 2 can be conducted remotely
55	6.1 Screening Period	Subjects who successfully complete the Screening Period will enter the Baseline Period. Subjects who fail inclusion/exclusion criteria will be discontinued but may be re-screened with the approval […]	Subjects who successfully complete the Screening Period will immediately enter the Baseline Period. Subjects who fail inclusion/exclusion criteria will be discontinued but may be re-screened and new informed consents obtained with the approval […]	Explains the need to redo an informed consent if subjects are re-screened
55	6.11 Visit 1	Subjects will undergo informed consent…	Visit 1 will be conducted at the clinical site. Subjects will undergo informed consent…	Restates that Visits 1 is an in-clinic visit

Page	Section	Version 2 dated June 18, 2020	Version 3	Explanation
56	6.1.2 Visit 2	Subjects will report to the clinic for Visit 2 to review tests from Visit 1 and finalize screening assessments.	Subjects will either report to the clinic for Visit 2 or be scheduled for a remote visit with a HHC to review tests from Visit 1 and finalize screening assessments. Telemedicine will be used to determine final eligibility by the investigator.	Adds that Visit 2 can be in clinic or remote
57	6.1.2 Visit 2	Determine whether the subject is still eligible to participate in the study. ○ If the subject is not eligible to participate, record the subject as a Screen Failure and contact the IRT to discontinue the subject from the study and complete the appropriate eCRFs.	Determine whether the subject is still eligible to participate in the study. ○ If the subject is not eligible to participate, the site will record the subject as a Screen Failure and contact the IRT to discontinue the subject from the study and complete the appropriate eCRFs. Subjects 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 and sponsor.	Details re-screening procedures
57	6.2.1 Visit 3	--	Prior to Visit 3 the eligibility review by the medical monitor will be completed for each subject. Subjects will either report to the clinic for Visit 3 or be scheduled for a remote visit with a HHC.	Adds eligibility procedures before Visit 3 and states that Visit 3 can be conducted remotely
58	6.2.2 Visit 4	--	Subjects will either report to the clinic for Visit 4 or be scheduled for a remote visit with a HHC.	States that Visit 4 can be conducted remotely
59	6.2.3 Visit 5	Provide subject with a urine collection container […] to be brought in at Visit	Provide subject with a urine collection container […] to be brought in at Titration Period Visit 6 or provided to the	Explains how the urine sample is collected if the visit is done with an HCC

Page	Section	Version 2 dated June 18, 2020	Version 3	Explanation
		6 (titration Period) for specific gravity and U_{osm} determination.	HHC if the visit will be conducted remotely ~~(Titration Period)~~ for specific gravity and U_{osm} determination	
59	6.3.1 Visits 6 to 10	Visits 6, 7, 8, 9, and 10 will occur weekly ± 3 days *as needed* for titration. All titration visits will be conducted in the clinic. Subjects will report to the clinic in the morning for their visits (after a minimum 8-hour overnight fast except for water) to undergo Titration Period assessments. Subjects should bring in any remaining study drug with them.	Visits 6, 7, 8, 9, and 10 will occur weekly ± 3 days *as needed* for titration and may be conducted remotely by a HHC. ~~All titration visits will be conducted in the clinic.~~ Subjects will either report to the clinic or interact with a HHC in the morning for their visits (after a minimum 8-hour overnight fast except for water) to undergo Titration Period assessments. Note that telemedicine will be utilized at these visits for the Investigator to assess the subject's status and tolerability of the study drug and to make a titration decision. Any other issues may also be discussed with the subject. Subjects should bring into the clinic, or provide to the HHC, any remaining study drug ~~with them~~.	Changes conduct of Visits 6 to 10
60 62 63 64 65	6.3.1 Visits 6 to 10 6.3.2 Visit 11/Last Titration Visit 6.4.1 Visits 12 to 23 6.4.2 Visit 24/ Early Termination 6.5.1 Visit 25	• Assess for liver dysfunction (liver chemistry tests, symptoms, signs)	• Assess clinically for liver dysfunction (~~liver chemistry tests,~~ symptoms, signs [if a clinic visit]) in accordance with the checklist and instructions in Appendix 4 (Section **Error! Reference source not found.**).	Removes the liver chemistry tests from assessment of liver dysfunction (assessed separately) and adds the use of a new checklist.

Page	Section	Version 2 dated June 18, 2020	Version 3	Explanation
60	6.3.1 Visits 6 to 10	• Follow the flowchart in **Error! Reference source not found.** […]. ○ Based on the decision made from the flowchart, contact the IRT to receive the kit assignment for the next period of time ○ Dispense study drug and instruct subject how many capsules to take based on the dose level guide in **Error! Reference source not found.**	• Follow the flowchart in **Error! Reference source not found.** […]. For remote visits, the HHC will call the Investigator to receive direction on this decision. The Investigator may want to utilize telemedicine to interact with the subject. ○ Based on the decision made from the flowchart, contact the IRT to receive the kit assignment for the next period of time ○ Dispense study drug and instruct subject how many capsules to take based on the dose level guide in **Error! Reference source not found.** ○ For remote visits, the site will contact the IRT sufficiently in advance of the scheduled visit day to obtain kit assignment and to allow sufficient time for drug to be shipped to arrive at subject's home prior to the visit date.	Updates procedures for titration decisions
60	6.3.1 Visits 6 to 10	• At each visit, remind the subject […] urine specimen on the morning of their next visit, which they will bring with them.	• At each visit, remind the subject […] urine specimen on the morning of their next visit, which they will bring with them (if a clinic visit) or provide to the HHC if a remote visit is conducted.	Explains procedure to collect urine specimen for remote visits
62	6.3.2 Visit 11/Last Titration Visit	The procedures specified for Visit 11 […] enter the Maintenance Period. Subjects will report to the clinic in the morning to undergo the assessments for the last visit of the Titration Period.	The procedures specified for Visit 11 […] enter the Maintenance Period at that time. The last titration visit may be in the office or conducted remotely by a HHC. If conducted remotely,	Explains the changes that ensue if Visit 11 is conducted remotely

Page	Section	Version 2 dated June 18, 2020	Version 3	Explanation
		Subjects should bring in any remaining study drug with them.	telemedicine will be used for the Investigator to interact with the subject and confirm that the subject can continue into the Maintenance Period. Subjects will either report to the clinic in the morning or interact with the HHC, if a remote visit is conducted, to undergo the assessments for the last visit of the Titration Period. Subjects should bring any remaining study drug into the clinic or provide to the HHC ~~any remaining study drug with them~~.	
62	6.3.2 Visit 11/Last Titration Visit	• Perform a brief physical exam • Perform a 12-lead ECG	• ~~Perform a brief physical exam~~ • ~~Perform a 12-lead ECG~~	Removes the brief physical exam and ECG at Visit 11 and moves them to Visit 17
63	6.3.2 Visit 11/Last Titration Visit	--	○ For remote visits, the site will contact the IRT sufficiently in advance of the scheduled visit day to obtain kit assignment and to allow sufficient time for study drug to be shipped to arrive at subject's home prior to the visit date.	Explains the changes that ensue if visits are conducted remotely
63	6.4 Maintenance Period	The Maintenance Period will last 52 weeks (Visit 12 to Visit 24). If available and when necessary, the following visits may be done remotely: Visits 12, 13, 15, 16, 18, 19, 21, 22, and 23.	The Maintenance Period will last 52 weeks (Visit 12 to Visit 24). If available ~~and when necessary~~, the following visits may be done remotely: Visits 12, 13, 14 (with telemedicine), 15, 16, 18, 19, 20 (with telemedicine), 21, 22, and 23. Note that telemedicine may be added to any other visit if the Investigator judges that additional interaction or information is needed to assess the subject's status.	Adds remote visits

Page	Section	Version 2 dated June 18, 2020	Version 3	Explanation
63	6.4.1 Visits 12 to 23	Subjects should bring in any remaining study drug with them.	Subjects should bring any remaining study drug into the clinic or provide to the HHC. ~~any remaining study drug with them.~~	Explains the changes that ensue if visits are conducted remotely
63	6.4.1 Visits 12 to 23	• Check AEs and SAEs and inquire about tolerability of the study drug • Assess for liver dysfunction (liver chemistry tests, symptoms, signs). • Review study drug compliance/ reconciliation • Collect and review concomitant medication information and update the CRF	• Review lab results from prior visit ○ For subjects exhibiting an increase in ALT or AST during the Maintenance Period (>3 x ULN) or total bilirubin (>2 x ULN), follow the instructions in Section **Error! Reference source not found.**. • Check AEs and SAEs and inquire about tolerability of the study drug • Assess clinically for liver dysfunction (~~liver chemistry tests,~~ symptoms, signs [if a clinic visit]) as directed by the checklist in Appendix 4 (Section **Error! Reference source not found.**) • Review study drug compliance/ reconciliation • Collect and review concomitant medication information ~~and update the eCRF~~ • Perform a brief physical exam (Visit 17) • Perform a 12-lead ECG (Visit 17)	Details changes to procedures for Visits 12 to 23 including moving the brief physical exam and 12-lead ECG to Visit 17
64	6.4.2 Visit 24/Early Termination	• Check AEs and SAEs • Assess for liver dysfunction (liver chemistry tests, symptoms, signs). A	• Review lab results from prior visit ○ For subjects exhibiting an increase in ALT or AST during the Maintenance Period (>3 x ULN) or	Details new procedures and changes to procedures for Visit 24

Page	Section	Version 2 dated June 18, 2020	Version 3	Explanation
		checklist will be provided is the visit is conducted remotely.	total bilirubin (>2 x ULN), follow the instructions in Section **Error! Reference source not found.**. • Check AEs and SAEs • Assess clinically for liver dysfunction (~~liver chemistry tests,~~ symptoms, signs) as directed by the checklist in Appendix 4 (Section **Error! Reference source not found.**).	
65	6.5 Follow-up Period	Visits 25 and 26 may be done remotely	Visits 25 and 26 may be done remotely by a HHC (with telemedicine if necessary).	Allows for telemedicine
65	6.5.1 Visit 25	--	• Review lab results from prior visit ○ For subjects exhibiting an increase in ALT or AST (>3 x ULN) or total bilirubin (>2 x ULN) at the prior visit, follow the instructions in Section **Error! Reference source not found.**. • Assess clinically for liver dysfunction (symptoms, signs [if a clinic visit]) as directed by the checklist in Appendix 4 (Section **Error! Reference source not found.**).	Details new procedures for Visit 25
66	6.5.3 Visit 27 (End of Study Visit)	Visit 27 is the last visit in this study and will occur 28±3 days after the last dose of study drug (Visit 24).	Visit 27 is the last visit in this study and will occur 28±3 days after the last dose of study drug (Visit 24). The visit will be conducted in the clinic.	Adds that this visit is in-clinic
67	7.2.1 Specific Gravity and Urine Osmolality	They will bring that specimen to their clinic visit where specific gravity will be measured by dipstick using an automated device in the clinic.	They will either bring that specimen to their clinic visit or provide to a HHC if a remote visit is conducted, where specific gravity will be measured by dipstick using an automated device ~~in the clinic~~.	Updates procedures for specific gravity

Page	Section	Version 2 dated June 18, 2020	Version 3	Explanation
69	7.3.4 Vital Sign Measurements	Subjects will remain at rest in a seated position for…	Subjects will remain at rest in a seated position for… 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.	Allows for positions other than seated for vital signs under certain circumstances.
69	7.3.4 Vital Sign Measurements	…Any confirmed, clinically significant vital sign measurements must be recorded as an AE.	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.	Specifies when CS vital signs should be recorded as AEs
70	7.3.5 12-Lead ECGs	All CS findings will be reported as AEs.	All CS findings will be reported as Medical History during the Screening and Baseline Periods and as AEs after the start of study drug.	Specifies when CS ECGs should be recorded as AEs
73	7.3.7.2 Changes in Study Drug Dosing if Liver Chemistry Test Abnormalities Occur…	-	Liver chemistry test increases of a lesser extent should be discussed with the medical monitor. They may be related to the underlying variability of such tests in ADPKD patients. Generally, dosing should continue, but the frequency of laboratory testing should increase, particularly during the Maintenance Period. Slowing of titration may also be considered if the abnormalities occur during the Titration Period.	Additional instructions for handling liver chemistry test increases that are below the threshold of >3 x ULN for transaminases or >2 x ULN for total bilirubin.
74	7.3.8.2 Eliciting and Documenting AEs and SAEs	Other changes in health or new symptoms occurring during the	Other changes in health or new symptoms occurring during the	Adds the Baseline Period to record AEs and SAEs

Page	Section	Version 2 dated June 18, 2020	Version 3	Explanation
		screening period will be recorded as medical history.	~~s~~Screening or Baseline ~~p~~Periods will be recorded as medical history.	
75	7.3.8.3 Reporting AEs, SAEs, and AEs of Special Interest	Any medical condition that is present at the time the subject is screened, i.e., medical history, but does not deteriorate should not be reported as an AE.	Any medical condition that is present at the time the subject is screened or during baseline, i.e., medical history, but does not deteriorate should not be reported as an AE.	Adds the Baseline Period to statement
77	7.3.8.8 Pregnancy	Pregnancy is not regarded as an AE unless there is a medical/surgical complication. Any pregnancy that occurs during study participation must be reported as an AESI using a clinical study pregnancy reporting form. To ensure subject safety, each pregnancy must be reported through this mechanism to ICON PVSS within 24 hours of learning of its occurrence.	Pregnancy is not regarded as an AE unless there is a medical/surgical complication. Any pregnancy of a subject or the partner of a subject that occurs during study participation must be reported ~~as an AESI~~ using a clinical study pregnancy reporting form. To ensure subject safety, each pregnancy must be reported through this mechanism to ICON PVSS within 24 hours of learning of its occurrence.	Adds that pregnancies in partners of subjects will be followed like pregnancies in subjects.
78	7.3.10 HERC	-	The HERC will also assess the relationship to tolvaptan of prior instances of liver dysfunction and liver chemistry test abnormalities that occurred while enrolled subjects were previously receiving tolvaptan.	Additional assessment by HERC
80	8.4.1 Primary Hepatic Safety Endpoint	Descriptive statistics (n, percentage, 95% confidence interval) will be used to summarize the proportion of subjects who develop ALT levels >3 x ULN that were assessed by the Investigator in consultation with the medical monitor and sponsor and adjudicated by the independent HERC to be related to lixivaptan and resulted	Descriptive statistics (n, percentage, 95% confidence interval) will be used to summarize the proportion of subjects who develop ALT levels >3 x ULN that were assessed ~~by the Investigator in consultation with the medical monitor and sponsor and adjudicated~~ by the independent HERC to be at least probably related to lixivaptan and	Updates primary endpoint to match other instances in the protocol.

Page	Section	Version 2 dated June 18, 2020	Version 3	Explanation
		in discontinuation of the study drug during the Titration and Maintenance Periods.	resulted in discontinuation of the study drug during the Titration ~~and~~ or Maintenance Periods.	
80-81	8.4.2 Secondary Hepatic Safety Endpoints	Similarly, descriptive statistics (n, percentage, 95% confidence interval) will be utilized to present the 2 secondary endpoints: The proportion of subjects who develop ALT levels >5 x ULN that were assessed by the Investigator in consultation with the medical monitor and sponsor and adjudicated by the independent HERC to be related to lixivaptan and resulted in discontinuation of the study drug during the Titration and Maintenance Periods; and The proportion of subjects who develop ALT levels >3 x ULN that were assessed by the Investigator in consultation with the medical monitor and sponsor and adjudicated by the independent HERC to be related to lixivaptan and resulted in lixivaptan dose reduction during the Titration and Maintenance Periods	Similarly, descriptive statistics (n, percentage, 95% confidence interval) will be utilized to present the 2 secondary endpoints: The proportion of subjects who develop ALT levels >5 x ULN that were assessed ~~by the Investigator in consultation with the medical monitor and sponsor and adjudicated~~ by the independent HERC to be at least probably related to lixivaptan and resulted in discontinuation of the study drug during the Titration ~~and~~ or Maintenance Periods; and The proportion of subjects who develop ALT levels >3 x ULN that were assessed ~~by the Investigator in consultation with the medical monitor and sponsor and adjudicated~~ by the independent HERC to be at least possibly related to lixivaptan and resulted in lixivaptan dose reduction during the Titration ~~and~~ or Maintenance Periods	Updates the secondary endpoints to match other instances in the protocol.

Page	Section	Version 2 dated June 18, 2020	Version 3	Explanation
85	9.3 Subject Information and Consent	A written informed consent […] shall be obtained from each subject before entering the study or performing any unusual or non-routine procedure […]. Informed consent templates may […]	~~A~~ Ww~~w~~ritten informed consents […] shall be obtained from each subject before entering the study, upon re-screening, and when ~~or~~ performing any unusual or non-routine procedure […]. ~~An~~ Iinformed consent templates may […]	Adds the need to have a new ICF for re-screening
89	11.1.1 Monitoring of the Study	In doing so, the Monitor will visit the Investigator and study site at periodic intervals, in addition to maintaining necessary telephone and letter contact.	In doing so, the Monitor 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.	Changes the study monitoring procedures due to remote visits
95	13.4 Appendix 3: Additional Potential Hepatic Testing…	--	• Clinical chemistry: ○ Glutamate dehydrogenase (GLDH)	Addition of newly described liver chemistry test
96-97	13.4 Appendix 4: Liver Dysfunction Checklist	--	Adds Appendix 4	Adds Liver Dysfunction Checklist, a form to be completed for both remote and clinic visits with associated instructions.