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

Statistical Analysis Plan Version 2.0, dated 06-Sep-2022

Note: a summary of changes implemented from Version 1.0 (dated 10-Dec-2020) is provided in the Revision History.

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 Version, Date	4.0 / 17NOV2021
ICON ID:	5016/0003
Document Version, Date:	Final Version 2.0, 06Sep2022

Prepared by:
ICON Clinical Research Services

On behalf of:
Palladio Biosciences, Inc.,
5 Walnut Grove Drive,
Suite 120,
Horsham, PA 19044

DocUUID : 32136f7c-3683-49fd-b477-f395f9955d92

SIGNATURE PAGE

Prepared at ICON Clinical Research by:

David Raj
06 Sep 2022 11:20:41 PDT (-07:00)

REASON: I approve this document as author.

a05dfa7f-b536-4636-ad10-1bb0fd2ce580

David Raj, Senior Statistician II — Date

Reviewed at ICON Clinical Research by:

Gloria Crispino
06 Sep 2022 09:43:05 PDT (-07:00)

REASON: I approve this document

ea17d882-1f03-487b-bd6f-fff7db59e073

Gloria Crispino, Director of Biostatistics
(StatisticaMedica Ltd.) — Date

Reviewed at Palladio Biosciences, Inc. by:

Genzhou Liu
Genzhou Liu
06 Sep 2022 09:46:56 PDT (-07:00)

REASON: I have reviewed this document and have no comments that require addressing.

b23914c8-cfaf-496f-8d18-9a094689fd8a

Genzhou Liu, Head of Biometrics and Data Management — Date

Approved at Palladio Biosciences, Inc. by:

Neil H. Shusterman, MD
Neil Shusterman
06 Sep 2022 10:38:46 PDT (-07:00)

REASON: I approve this document

40af71ac-d834-444d-981a-9ead798865a8

Neil H. Shusterman, MD FACP, Chief Medical Officer — Date

Genzhou Liu
Genzhou Liu
06 Sep 2022 09:46:57 PDT (-07:00)

REASON: I approve this document

b23914c8-cfaf-496f-8d18-9a094689fd8a

Genzhou Liu, Head of Biometrics and Data Management — Date

REVISION HISTORY

Version/Date	Version name	Section	Changes implemented
Version 1.0/10Dec2020	Final V1.0	NA	NA
Version 2.0/06Sep2022	V2.0	1.0 9.0 11.4 Signature Page	- Text added denoting early termination of study - Text added to describe the changes to planned analyses and actual outputs - Added list of tables, listings and Figures to be actually produced due to the early termination of the study - Signature page is updated

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

LIST OF ABBREVIATIONS

Abbreviation or special term	Explanation
ADPKD	Autosomal Dominant Polycystic Kidney Disease
AE	Adverse Event
AESI	Adverse Event of Special Interest
ALP	Alkaline Phosphate
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical
BID	Twice per day
BLQ	Below limit of quantification
BMI	Body Mass Index
CI	Confidence Interval
CRF	Case Report Form
CS	Clinically Significant
CSR	Clinical Study Report
CV	Coefficient of Variation
DILI	Drug Induced Liver Injury
DILIN	Drug Induced Liver Injury Network
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
eDISH	evaluation of Drug-Induced Serious Hepatotoxicity
eGFR	estimated Glomerular Filtration Rate
HBsAg	Hepatitis B surface antigen
HCV	Hepatic C Antibody
HERC	Hepatic Events Review Committee
HPF	High Power Field
ICF	Informed Consent Form
ICH	International Conference on Harmonisation

Abbreviation or special term	Explanation
IRT	Interactive Response Technology
MedDRA	Medical Dictionary for Regulatory Activities
NCS	Not Clinically Significant
PD	Pharmacodynamic
PK	Pharmacokinetic
PT	Preferred Term
QTcF	QT interval corrected for heart rate using Fridericia's formula
RBC	Red Blood Cell
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOC	System Organ Class
TBL	Total Bilirubin
TEAE	Treatment Emergent Adverse Event
TE-AESI	Treatment Emergent – Adverse Event of Special Interest
TFLs	Tables, Figures and Listings
U_{osm}	Urine Osmolality
ULN	Upper Limit of Normal
ULQ	Upper Limit of Quantification
WHODrug	World Health Organisation Drug Dictionary
WOCBP	Women of childbearing potential

1 INTRODUCTION

The purpose of this Statistical Analysis Plan (SAP) is to provide detailed descriptions of the statistical methods, data derivations and data displays for study protocol PA-ADPKD-303 Version 3.0 "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" dated 02Sep2020 for final analysis. The table of contents and templates for the Tables, Figures and Listings (TFLs) will be produced in a separate document.

Any deviations from this SAP will be described and justified in the Clinical Study Report (CSR).

The preparation of this SAP is based on International Conference on Harmonization (ICH) E9 and E3 guidelines.

All data analyses and generation of TFLs will be performed using SAS version 9.4® or higher.

NOTE: Please see Section 9 for an explanation of the impact of early termination of this study on the actual output produced.

2 STUDY OBJECTIVES

2.1 Primary Objective

The primary objective of this study is to assess the effect of lixivaptan on hepatic safety in subjects with Autosomal Dominant Polycystic Kidney Disease (ADPKD) who experienced abnormal liver chemistry test results on tolvaptan that resulted in permanent discontinuation of tolvaptan.

2.2 Secondary Objectives

The key secondary objectives of this study are:

1) 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.
2) To assess renal function (efficacy) in ADPKD subjects while on lixivaptan using change in estimated glomerular filtration rate (eGFR).

2.3 Exploratory Objectives

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

3 STUDY DESIGN

3.1 General 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, and urinalysis), eGFR, urine osmolality determinations, and trough serum concentrations of lixivaptan. A deoxyribonucleic acid (DNA) sample to assess genetic variants associated with hepatic abnormalities (if and when feasible) will be obtained under a separate bio banking 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 antihypertensive 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 twice per day (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). The study schematic is depicted in Figure 1.

Figure 1: Study Schematic

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 anti-

hypertensive medications 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 return to the clinic for end-of-screening assessments at Visit 2, 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 home healthcare clinician [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 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. 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 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]). 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 3). 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.

3.2 Randomization and Blinding

The study will not be randomized and will be an open-label study.

3.3 Study Treatments and Assessments

3.3.1 Study Duration

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.

3.3.2 Study Drug and Administration

The investigational product, lixivaptan capsule, is formulated as a white hard gelatin capsule with a clear band (without markings) containing 50 mg of lixivaptan.

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.

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 1. Two additional dose levels (Levels 3a and 4a) are provided that reduce the evening

(PM) dose by 50 mg (from 150 mg to 100 mg and from 200 mg to 150 mg) if aquaretic effects are limiting 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 Urine Osmolality (Uosm)) 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 1: 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 (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 Table 2). The most common dosing scenarios that can be advanced to the Maintenance Period are shown in Table 2. 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):

Follow the flowchart in Figure 2 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.

Table 2: 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 [*]	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 [**]	50 mg BID	100 mg BID	150 mg BID	200 mg BID	200 mg BID	200/150 mg (AM/PM)

[*] when 2nd week of Level 3 (150 mg BID) is not tolerated
[**] when 2nd week of Level 4 (200 mg BID) is not tolerated

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 allowed.

A detailed description of procedures and assessments to be conducted during this study is summarized in the Scheduled of Study Assessments in Table 3.

Figure 2: Titration Flowchart

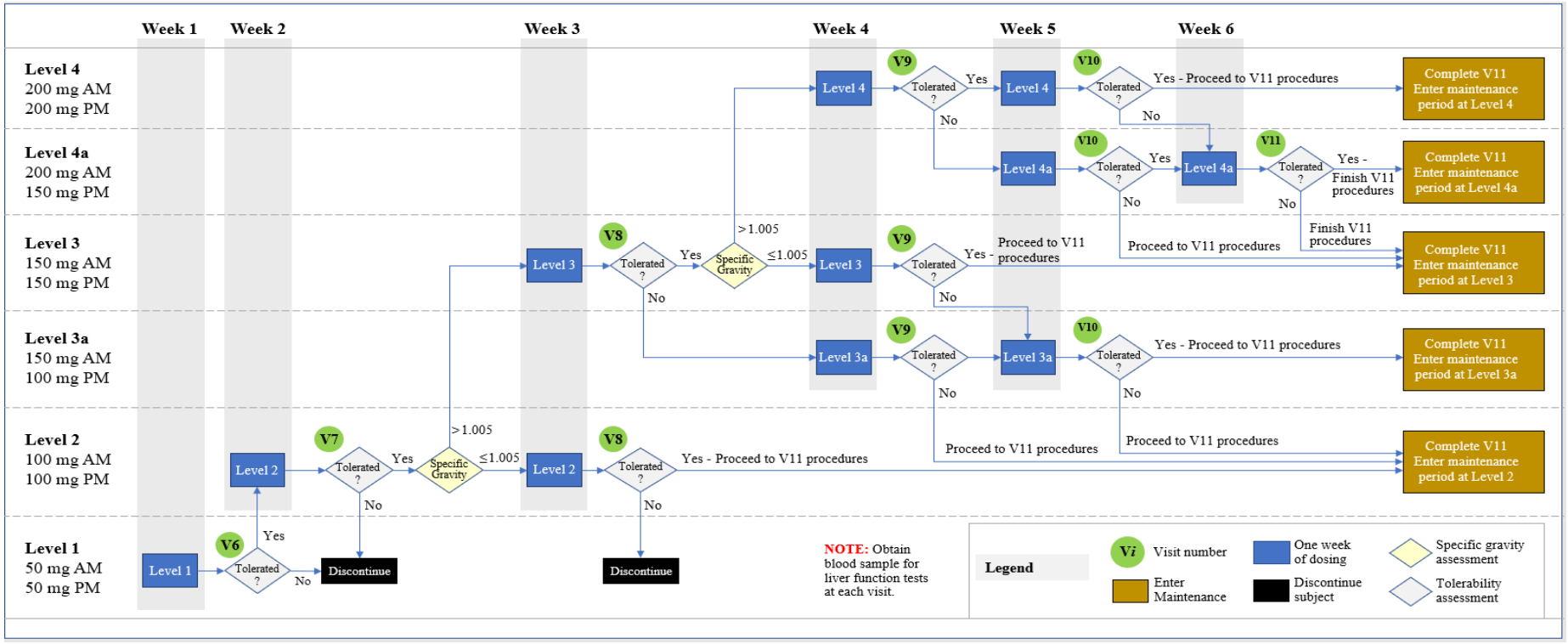

Table 3: Schedule of Procedures

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.

4 STUDY ENDPOINTS

4.1 Primary Endpoint

The primary endpoint is to assess the proportion of subjects who develop alanine aminotransferase (ALT) levels >3 x Upper Limit of Normal (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.

4.2 Secondary Endpoints

4.2.1 Safety Endpoints

- 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 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.

4.2.2 Efficacy Endpoint

- 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).

4.3 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.

5 SAMPLE SIZE AND POWER

Up to 50 subjects will be enrolled. 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.

6 ANALYSIS POPULATIONS

6.1 Enrolled Population

The Enrolled Population is defined as all 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.

6.2 Safety Population

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

6.3 Efficacy Population

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 efficacy analyses.

6.4 Pharmacokinetic (PK) Population

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.

6.5 Pharmacodynamic (PD) Population

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.

6.6 Protocol deviations/violations and exclusions from analysis sets

A protocol deviation is defined as an unintended or unanticipated departure from the procedures or processes approved by the Sponsor and the Institutional Review Board (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 the following but not limited to:

- Not adhering to inclusion or exclusion criteria
- Enrollment of the subject without prior Sponsor approval
- Not adhering to Food and Drug Administration (FDA) regulations or ICH Good Clinical Practice (GCP) guidelines
- Subjects not ≥ 80% compliant with the prescribed study drug doses during the study

Protocol deviations will be identified and reviewed by the project team prior to database lock.

7 STATISTICAL CONSIDERATIONS AND ANALYSIS

7.1 Derived General Variables

The below table (Table 4) provide the list of derived variables for demographic and baseline characteristics, various duration derivations, drug compliance, baseline derivations and other important derivations applicable for this study.

Table 4: Derived Variables

Variables	Formula
Demographic characteristics	
Age at informed consent (in years)	Age = year of informed consent – year of birth
BMI (kg/m^2)	weight (kg)/[height (m)]^2
Derivation of Duration	
Study Duration (Days)	(Date of Last Visit – Date of informed consent) + 1
Study day at any visit	Date of interest – date of first dose of study drug. One day is added if this difference is ≥ 0
Adverse event duration (in days)	Event end date - Event onset date + 1.
Duration of Exposure	
Duration of Exposure (days) in Titration Period	(Date of last administration of study drug – Date of first administration of study drug) +1, during the 6 week titration period
Duration of Exposure (days) in Maintenance Period	(Date of last administration of study drug – Date of first administration of study drug) +1, during the 52 week maintenance period
Treatment Compliance	
Treatment Compliance	(Total No. of capsules dispensed – Total No. of capsules returned)*50/total assigned dose*100 where, total assigned dose will be derived by sum of (AM+PM dose)*duration of this dose level for all different dosing levels
eGFR Baseline and Final Assessment Derivation (**Appendix I**)	
Baseline eGFR	Mean of 3 eGFR assessments (Visit 3, Visit 4 & Visit 5) obtained during the baseline period
Final Assessment eGFR	Mean of 3 eGFR assessments (Visit 25, Visit 26 & Visit 27) obtained during the follow-up period
Baseline Derivations	

Baseline	Latest non-missing measurement prior to first dose of study drug administration
Change from baseline	Test Value at Post baseline value – Baseline Value
Geometric Coefficient of Variation (CV%) for Pharmacokinetic data	
Geometric CV%	CV (%) =$100\times\sqrt{\exp(\hat{\sigma}^2)-1}$, where $\hat{\sigma}^2$ denotes the variance of the natural log-transformed values.

7.2 Handling of Missing Data

7.2.1 Handling of Missing or Partial Dates

7.2.1.1 Imputation rules for missing or partial AE start date:

If only Day of AE start date is missing:

If the AE start year and month are the same as that for the first dose date (date of titration visit 6), then:

- If the full (or partial) AE end date is NOT before the first dose date (date of titration visit 6) or AE end date is missing, then impute the AE start day as the day of first dose date (titration visit 6); otherwise, impute the AE start day as 1.
- Otherwise, impute the AE start day as 1.

If Day and Month of AE start date are missing:

If AE start year = first dose year (year of titration visit 6), then:

- If the full (or partial) AE end date is NOT before the first dose date (date of titration visit 6) or AE end date is missing, then impute the AE start Month and Day as the Month and Day of first dose date (date of titration visit 6); otherwise, impute the AE start Month as January and the Day as 1.
- Otherwise, impute the AE start MONTH as January and the DAY as 1.

If Year of AE start date is missing:

If the year of AE start is missing or AE start date is completely missing then query site with no imputation. Also compare the full (or partial) AE end date to the first dose date (date of titration visit 6). If the AE end date is before the first dose date (date of titration visit 6) then the AE should be considered as a pre-treatment AE. Otherwise, the AE will be considered as TEAE.

Compare the imputed AE start date with the treatment-emergent period to determine whether the AE is pre-treatment AE, TEAE (Treatment Emergent Adverse Event) or post-treatment AE.

7.2.1.2 Imputation rules for missing or partial medication start/stop dates:

Missing or partial medication start date:

- If only DAY is missing, use the first day of the month.
- If DAY and Month are both missing, use the first day of the year.
- If DAY, Month and Year are all missing, use a date before the first dose date (date of titration visit 6).

Missing or partial medication stop date:

- If only DAY is missing, use the last day of the month.
- If DAY and Month are both missing, use the last day of the year.

If DAY, Month and year are all missing, assign 'continuing' status to stop date

7.2.2 Handling of Missing Efficacy or Safety Data

If any values of baseline or follow-up eGFR values are missing (i.e. for instance if only two measurements are available then the mean of two measurements will be considered and if in case more than two values are missing the last available measurement will be considered), the remaining available values will be used to determine the baseline or follow-up eGFR. Unscheduled visit values will not be considered for eGFR calculation.

No imputation will be done for any of the safety/PK/PD data.

8 STATISTICAL METHODS

8.1 General Statistical Conventions

All statistical procedures will be completed using SAS Grid version 9.4 or higher.

Unscheduled visit results will be included in date/time chronological order, within subject listings only. Unscheduled visit shall not be summarized in tables.

Categorical variables will be summarized using number (n) and percentage in each category including missing category if any. All percentages will be rounded to one digit after decimal point. The number and percentage will be presented in the form XX (XX.X %), where the percentage is in the parentheses. If the percentage of a category is "100", then display it as "100" (do not add decimal after 100). 95% confidence intervals will be presented along with frequency counts and percentages for primary and secondary safety endpoints.

Continuous variables will be summarized using number of subjects (n), number of missing subjects, mean, median, standard deviation (SD), minimum, and maximum for continuous variables. All mean and median values will be formatted to one more decimal place than the measured value. Standard deviation values will be formatted to two more decimal places than the measured value. The minimum and maximum will be displayed to the same number of decimal places as the original data. For pharmacokinetic data, geometric mean (with one decimal than the measured value) and geometric CV % (with 1 decimal place) will be presented

additionally.

All general summaries will be presented by Overall, having all the subjects, unless otherwise specified. For safety assessments, the summaries will be presented by titration and maintenance combined, follow-up and overall, unless otherwise specified.

All subject data will be presented in individual subject data listings. All listings will be sorted for presentation in order of dose level if applicable, subject number (including site ID), date/time and visit. The dose level as well as subject's sex, age and race will be stated on each listing. In general, no imputed dates or values will be presented in listings unless specified.

8.2 Subject Disposition

The following categories will be summarized only for "Overall" Column. The summary is based on all subjects.

- No. of subjects screened
- No. of subjects who screen failed
- No. of subjects who re-screened
- No. of subjects who complete visit 3 during baseline period (Enrolled Population)
- No. of baseline failure subjects
- No. of subjects who completed the baseline period

The following categories will be summarized overall. The summary is based on Enrolled population.

- No. of subjects who completed study
- No. of subjects who discontinued from study
- Primary reason for study discontinuation
- No. of subjects who completed treatment
- No. of subjects who discontinued from treatment
- Primary reason for treatment discontinuation
- No. of subjects who entered titration period
- No. of subjects who completed treatment during titration period
- No. of subjects who discontinued treatment during titration period
- No. of subjects who entered maintenance period
- No. of subjects who completed treatment during maintenance period

- No. of subjects who discontinued treatment during maintenance period

In addition, number of subjects in the safety population who completed or discontinued during titration and maintenance period will be summarized by last dose level assigned during the specified period.

A listing of subject disposition will be provided for all subjects, with the extent of their participation in the study and the reason for discontinuation from study or treatment.

Additionally, a listing of inclusion/exclusion criteria will be presented for all subjects.

8.3 Protocol Deviations

A summary of subjects excluded from safety population, efficacy population, pharmacokinetic and pharmacodynamic populations will be presented by enrolled population.

A summary of major (key) protocol deviations will be presented for Enrolled population. All protocol deviations will be listed.

8.4 Demographics and Baseline Characteristics

Subject characteristics and demographics will be summarized for the Enrolled population. The summary will include descriptive statistics such as the number of subjects (n), mean, SD, median, minimum (min), and maximum (max) for continuous measures and frequency counts and percentages for categorical measures. No formal statistical comparisons will be made among dose levels.

The following demographics will be summarized: Age (years), Height (cm), Weight (kg), Body Mass Index (BMI) (kg/m^2), Systolic Blood Pressure (mmHg), Diastolic Blood Pressure (mmHg), Sex, Child bearing potential for female population, Race (Primary, Secondary and subcategory) and Ethnicity.

Baseline disease characteristics include:

- Baseline eGFR
- Baseline AST or ALT levels
- Baseline Total Bilirubin levels
- History of ADPKD

Above summary of demographics and baseline characteristics will be repeated for safety and efficacy populations if any subject in the Enrolled population was excluded from these two populations.

A listing including demographic and baseline characteristics will be presented separately by subject for all subjects.

8.4.1 Medical History

A summary of medical history by medical history code (as per the case report form (CRF)) will be presented by system organ class (SOC) and preferred term (PT) using Medical Dictionary for Regulatory Affairs® (MedDRA) Version 23.0 or higher will be presented for the safety population.

A by subject listing including medical history will be presented for all subjects.

8.4.2 Prior and Concomitant Medications

Subject's CRF captured all medications within 1 month prior to study drug administration. However, additional medications specified in Exclusion Criteria 7, 8, and 9 in protocol Section 4.1.2 will also be recorded within the prior 3 months.

Prior medications are defined as medications where dosing ended prior to the first dose of study drug administration (exclusive). Concomitant medications are defined as medications that were taken between the first dose (inclusive) and last dose of study drug (inclusive). This also includes medications started prior and still ongoing at the start of the study drug. Details for imputing missing or partial start and/or stop dates of medication are described in section 7.2.1.

Separate summaries showing the number and percentage of subjects who took prior or concomitant medications will be presented by Anatomical Therapeutic Chemical (ATC) coding scheme of the World Health Organisation Drug Dictionary (WHO Drug) Global B3 March 2019 version or later on Level 2 term and preferred term for the Safety population.

A listing of prior/concomitant medications will be presented for all subjects. Prohibited medications/therapies if any will be highlighted by marking asterisk symbol (*) in the ATC column of the table and listing.

8.4.3 Concomitant Procedures

The Concomitant Procedures during the study will be coded using MedDRA Version 23.0 or higher. A by-subject listing including details of concomitant procedures will be presented for all subjects.

8.5 Treatment Exposure and Compliance

8.5.1 Study Treatment Exposure

Total dose and duration of exposure for study drug (section 7.1) for titration period (period of 6 weeks) and maintenance period (period of 52 weeks), will be summarized using descriptive statistics by titration and maintenance period based on safety population.

A by subject listing of exposure will be presented based for the safety population.

8.5.2 Treatment Compliance

Treatment Compliance will be derived using the formula below:

$$\frac{(Total\ No.\ of\ capsules\ dispensed - Total\ No.of\ capsules\ returned) \times 50}{Total\ assigned\ dose} \times 100$$

Where total assigned dose (in mg) will be derived by sum of (AM+PM dose) × duration of this dose level for all different dosing levels.

Study drug compliance will be summarized using standard summary statistics based on safety population. Study drug compliance will also be summarized in "<80%", 80% – 120%" and ">120%" using frequency tables.

A complete listing of drug accountability will be presented based for the safety population.

8.5.3 Drug Tolerability and Modifications

Summary of subjects with intolerable doses and dose modifications will be presented using frequency and percentages for each visit based on safety population.

A complete listing of drug tolerability/modification by visits will be presented based for the safety population.

8.6 Safety Analyses

Safety assessments will be conducted throughout the entire study period. Analyses will be performed using the safety population, unless otherwise specified. All safety data will be summarized by period. No formal statistical hypothesis testing will be conducted.

The safety evaluations will include primary hepatic and secondary hepatic safety analysis. Analyses of adverse events, clinical laboratory tests (clinical chemistry/serology, hematology, urinalysis, other tests, additional liver testing & other testing), vital signs, physical examination, 12-lead electrocardiogram (ECG) and serum/urine pregnancy results will also be presented. The safety analyses involving changes from baseline to a specific time point in safety variables (e.g., laboratory parameters, vital signs, and ECG) will only include subjects from the safety population who have data available for both the baseline and the time point under consideration unless otherwise specified.

8.6.1 Analysis of Primary Safety Endpoint

Descriptive statistics (n and percentage) along with exact binomial 95% Confidence Interval (CI)) will be presented to summarize the proportion of subjects who develop ALT levels >3 x ULN that were assessed by the independent HERC to be related to lixivaptan and resulted in discontinuation of the study drug during the Titration and Maintenance Periods.

The above summary will be presented additionally based on the investigator decision alone as documented in the CRF.

8.6.2 Analysis of Secondary Safety Endpoint

Similarly, descriptive statistics (n and percentage) along with exact binomial 95% CI will be presented to summarize the following 2 secondary endpoints:

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

The above summaries will be presented additionally based on the investigator decision alone as documented in the CRF.

8.6.3 Analysis of Additional Hepatic Safety Endpoints

The proportion of subjects along with the exact binomial 95% CI 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 Aspartate aminotransferase (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) will be evaluated using Drug-Induced Serious Hepatotoxicity (eDISH) plots
- Elevation of aminotransferase in temporal association with nausea, vomiting, anorexia, abdominal pain, or fatigue will be explored graphically using swimmer plots.
- Possible liver-related deaths and liver-related treatment discontinuations.

The liver dysfunction occurring during the titration and maintenance period judged using the Drug Induced Liver Injury Network (DILIN) (Appendix II) probability criteria independently by investigator and HERC will be listed separately.

8.6.4 Analysis of Additional Potential Hepatic Safety Endpoints

The summary of additional hepatic safety assessments will be presented based on potential hepatic testing (Appendix III) based on liver chemistry abnormalities. The assessments will be

presented tabulated using descriptive statistics or frequency and percentages based on the data type. These data will further be listed separately based on all subjects.

8.6.5 Adverse Event (AE)

All AE verbatim descriptions will be coded using the MedDRA, version 23.0 or higher.

A 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. For this study, pregnancies and liver events are considered to be AESIs (Adverse Events of Special Interest). All AEs will be summarized by period (titration and maintenance period, follow-up period, and overall).

If a subject experiences multiple AEs under the same preferred term (system organ class), then the subject will be counted only once for that preferred term (system organ class). If a subject experiences the same AE more than once with different severity, then the event with the maximum severity will be tabulated in "by maximum severity" tables. In addition, AEs with a missing intensity will be presented in the summary table as an intensity category of "Missing."

If a subject, experiences the same AE more than once with different relationship, then the event with the strongest relationship will be tabulated in "by strongest relationship" tables. TEAEs with a missing relationship to study medication will be regarded as "Possible" to study medication. Adverse events with a relationship to study drug considered possibly, probably, or definitely related will be considered related.

An overview table will also be presented with the number and percentage of subjects as well as number of events for the following categories:

- At least one AE
- At least one TEAE
- At least one Serious TEAE
- Related TEAE
- Related Serious TEAE
- Any TE-AESI
- TEAEs by maximum severity
- TEAEs by strongest relationship to the study drug
- TEAEs leading to dose modifications (i.e. dose reduced or dose interrupted)
- TEAEs leading to study drug withdrawal
- TEAEs leading to death.

All TEAEs will be summarized as below:

- TEAEs by SOC and PT
- Serious TEAEs by SOC and PT
- Related TEAEs by SOC and PT
- TE-AESI by SOC and PT
- TEAEs leading to deaths by SOC and PT
- TEAEs leading to drug modification by SOC and PT
- TEAEs leading to study drug withdrawal by SOC and PT
- TEAEs by SOC, PT, and severity
- TEAEs by SOC, PT, and maximum severity
- TEAEs by SOC, PT, and relationship
- TEAEs by SOC, PT, and strongest relationship
- TEAEs by PT
- Serious TEAEs by PT
- Related TEAEs by PT

Adverse events will be presented in data listings including dose level, subject number, start/end dates/times of event, MedDRA system organ class, preferred term, duration of the event, seriousness, severity, action taken, relationship to study drug and outcome.

The following by subject listings will be provided for all subjects:

- AEs
- Serious AEs
- TE-AESIs
- AEs leading to Deaths
- AEs leading to dose modification (including interruption, reduction and discontinuation)

8.6.6 Clinical Laboratory Evaluations

Laboratory safety assessments include complete chemistry, hematology, liver chemistry, urinalysis and other tests. Additionally, assessment also includes serum creatinine, HBsAg and Anti-HCV.

The specific clinical laboratory evaluations and additional live chemistry testing are as below in Table 4.

Table 4: Clinical Laboratory Evaluations

Chemistry	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 with differential (total and %) only if WBC count is abnormal: Neutrophils Lymphocytes Monocytes Eosinophils Basophils	Ketones
Creatinine		Bilirubin
Glucose		Blood
Phosphorous		Urobilinogen
Potassium		Leukocytes
Protein		Nitrite
Sodium		Microscopic examination (if positive for blood, protein, nitrite, or leukocytes)
Uric acid		
Liver Chemistry Panel	**Other Tests**	
Alanine aminotransferase	Urine specific gravity (local to guide titration)	
Alkaline phosphatase	Urine osmolality	
Aspartate aminotransferase	Urine or serum pregnancy test (female subjects)	
Bilirubin Total and Direct	Specimen for potential DNA analysis	
Glutamate dehydrogenase (GLDH)		

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

All laboratory data will be summarized in International System of Units (SI). Quantitative laboratory measurements reported as "< X", i.e. below the lower limit of quantification (BLQ), or "> X", i.e. above the upper limit of quantification (ULQ), will be converted to X for the purpose of quantitative summaries, but will be presented as recorded, i.e. as "< X" or "> X" in the listings.

Descriptive statistics of absolute value and change from baseline (mean, SD, median, min, and max) for clinical laboratory data will be presented for each scheduled time point. 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 categorical data, the laboratory results will be summarized using frequency and proportion at each scheduled time point.

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).

Shift table from baseline to worst post-baseline value according to abnormality assessed by investigators (normal, Clinically Significant (CS) and Not Clinically Significant (NCS)) will be presented for all applicable laboratory evaluations.

Potentially clinically significant results of clinical laboratory evaluations identified using prospectively defined criteria (Table 5) will also be summarized descriptively.

Table 5: Potentially Clinically Important Criteria (PCS): Laboratory

Lab Test Category	PCS Criteria
Chemistry	Sodium: <132 mmol/L
	Sodium: >149 mmol/L
	Potassium:<2.5 mmol/L
	Potassium: >6.5 mmol/L
	Calcium: <1.62 mmol/L
	Calcium:>3.49 mmol/L
	Uric acid:>715 µmol/L
	Albumin: <25 g/L
	eGFR: Decrease from baseline of ≥25%
	Glucose: ≤2.5 mmol/L
	Glucose:≥14.0 mmol/L
Hematology	Leukocytes: ≤ 2.5 x 10^9/L
	Leukocytes: ≥ 15.0 x 10^9/L
	Neutrophils: ≤ 1.5 x 10^9/L
	Platelets: ≤ 50.0 x 10^9/L
	Hemoglobin: Decrease from baseline of ≥ 20 g/L
	Hematocrit: Decrease from baseline of ≥ 10% (absolute change)
Urinalysis	Glucose: ≥2+
	RBC/HPF: >10
	Protein: >2+
	Ketones: > 2 +
	Leukocytes: >10

Abbreviations: RBC=Red Blood Cell; HPF=high power field.

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.

Abnormal laboratory results will be listed separately by all subjects.

8.6.7 Vital Signs

Vital sign assessments are performed in order to characterize basic body function. Absolute and changes from baseline in vital signs at each scheduled time point will be summarized using descriptive statistics (n, mean, SD, median, minimum, and maximum). The parameters collected are the following: systolic blood pressure (mmHg), diastolic blood pressure (mmHg), heart rate (beats/min), respiratory rate (breaths/min), body temperature (°C), weight (kg) and BMI (kg/m^2). Vital signs summary will be based on safety population.

Potentially clinically significant results of vital signs (Table 6) identified using prospectively defined criteria will also be summarized descriptively.

Table 6: Potentially Clinically Important Criteria: Vital Signs

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

Vital sign assessments will be presented subject in a data listing for all subjects.

8.6.8 Physical Examinations

A complete physical examination will be performed during Screening Visit 1, Visit 17 and the final Maintenance/Early Termination Visit (Visit 24) and Follow-up period (Visit 27). Brief physical examination will subsequently be measured at the time points specified in study schedule of procedures (Table 3).

Complete physical examinations will include 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 incidence of abnormality in physical examination will be tabulated separately for each visit based on the safety population.

Abnormal physical examination will be presented for each subject in a data listing for all subjects.

8.6.9 Electrocardiograms (ECG)

Observed values and changes from baseline for continuous ECG parameters including heart rate, ventricular rate, PR interval, QRS duration, QT interval (uncorrected), RR interval and QT interval corrected for heart rate according to Fridericia's formula (QTcF) interval will be

summarized over each scheduled time point in terms of absolute values using descriptive statistics (n, mean, SD, median, minimum, and maximum).

Overall evaluation of ECG 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. QTcF will be summarized using frequency and percentage for the following categories:

Subjects with the highest QTcF post-baseline value

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

Subjects with the highest QTcF change value

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

Potentially clinically important criteria (Sponsor defined)

- ≥450 msec

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

ECG results will be listed by dose levels and subjects for all subjects. Additionally, a listing will be provided for Investigator-identified ECG abnormalities.

8.6.10 Pregnancy Test

Serum pregnancy test for Women of childbearing potential (WOCP) 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.

Results of serum pregnancy test will be presented for each subject by dose level in a data listing for all female subjects.

8.7 Efficacy Analyses

The efficacy analyses will be based on the efficacy population.

8.7.1 Analysis of Efficacy Endpoints

8.7.1.1 Change from Baseline in eGFR

eGFR will be calculated and reported whenever serum creatinine (Appendix I) is obtained either as part of the Complete Chemistry Panel or when serum creatinine alone is measured.

Descriptive statistics will be presented for baseline (mean of 3 determinations obtained during the baseline period) and final assessment (eGFR determination obtained during the follow-up period). Change from baseline to final assessment will also be summarized using descriptive statistics.

A listing of serum creatinine including eGFR data by subject will be presented for all subjects.

No formal statistical analysis is planned for evaluation of efficacy endpoint.

8.7.2 Exploratory Analysis

8.7.2.1 Relationship of Liver Chemistry Test Abnormalities to Drug Levels

Descriptive statistics (mean, SD, CV %, median, geometric mean, geometric CV %, min and max,) will be presented for plasma lixivaptan trough concentrations at each scheduled time point for PK population.

Any possible relationships between liver chemistry test abnormalities and trough levels of lixivaptan on PK population collected during the study will be explored if sufficient data exist. A boxplot at scheduled trough collection timepoints by liver chemistry abnormality on trough levels of plasma lixivaptan concentration will be generated. A scatterplot with regression line will be presented with X-axis as plasma trough concentration and Y-axis with liver function test (ALT, ALP, TBL and ALP, respectively). The individual abnormal liver function test values will be labeled or highlighted. In addition, an individual plot will be generated for trough level (Y-axis) and time after dosing (X-axis) with liver function test as second Y-axis (in x ULN for ALT, AST, TBL, or ALP).

The following liver chemistry test abnormality will be assessed:

- >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
- TBL >2 x ULN
- ALP >2 x ULN

8.7.2.2 Assessment of Genetic Variants

A descriptive summary for safety population including frequency and percentages will be presented by different genetic variants by associated hepatic abnormalities if in the event that specific genetic variants confirming increased or decreased risk to hepatic abnormalities in subjects with drug induced liver injury (DILI) become known during the time of the study.

8.7.2.3 Pharmacodynamic (PD) Assessments

A descriptive summary based on PD population will be presented for urine specific gravity and urine osmolality for all protocol schedule visits. Number and percentages will be presented for urine specific gravity ≤ 1.005 and urine osmolality ≤ 250 for all the applicable visits in titration, maintenance and follow-up period. The Relationship between urine specific gravity and urine osmolality (Uosm) in the titration and maintenance period will be explored using Pearson's correlation coefficient for PD population. Scatter plots will be presented to show the strength of the correlation between urine specific gravity and Uosm with X-axis as urine specific gravity and Y-axis as Uosm.

8.8 Interim Analysis

There will be no interim analysis during this study.

9 CHANGES TO PLANNED ANALYSIS FROM STUDY PROTOCOL

This study has been early terminated by the Sponsor. Due to only 6 participants being enrolled into treatment maintenance period in the study and most of them being prematurely terminated from the study, no statistical analyses will be performed and only a limited set of summary tables will be generated along with the study data listings to facilitate the creation of a synoptic clinical study report.

Please see Appendix IV for the TLFs to be produced.

10 REFERENCES

1. ICH Topic E3: Structure and Content of Clinical Study Reports (CPMP/ICH/137/95-adopted December 1995).
2. ICH Topic E9: Statistical Principles for Clinical Trials (CPMP/ICH/363/96 – adopted March 1998).
3. Fontana RJ, Watkins PB, Bonkovsky HL, Chalasani N, Davern T, Serrano J, Rochon J; DILIN Study Group. 2009. Drug-Induced Liver Injury Network (DILIN) prospective study: rationale, design and conduct. Drug Saf. 32(1):55-68.

11 APPENDICES

11.1 Appendix I: Chronic Kidney Disease Classification Criteria

This study uses the 2009 CKD-EPI creatinine equation (Levey A, 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.

11.2 Appendix II: DILI Network Causality Criteria, modified

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

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.

11.3 Appendix III: 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

11.4 Appendix IV: List of Tables, Listings, and Figures

List of Tables, Listings, Figures
Listing 16.2.1.1 Subject Disposition All Subjects
Listing 16.2.2 Protocol Deviations All Subjects
Listing 16.2.4.1 Demographic Characteristics All Subjects
Listing 16.2.4.2.1 Baseline Characteristics - eGFR All Subjects
Listing 16.2.4.2.2 Baseline Characteristics - ALT, AST and Total Bilirubin All Subjects
Listing 16.2.4.3 Medical History All Subjects
Listing 16.2.4.4 Concomitant Procedures All Subjects
Listing 16.2.4.5 Medical History of Autosomal Dominant Polycystic Kidney Disease (ADPKD) All Subjects
Listing 16.2.4.6 Prior and Concomitant Medications All Subjects
Listing 16.2.5.1 Drug Accountability Safety Population
Listing 16.2.5.2 Drug Tolerability/Modification Safety Population
Listing 16.2.6.1 Listing of Serum Creatinine and estimated Glomerular Filtration Rate (eGFR) All Subjects
Listing 16.2.7.1.1 Liver Dysfunction Details All Subjects
Listing 16.2.7.1.2 Potential Hepatic Events All Subjects
Listing 16.2.7.2 Criteria for Primary and Secondary Hepatic Safety Endpoints All Subjects
Listing 16.2.7.3 Adverse Events All Subjects
Listing 16.2.8.1.1 Laboratory Test Results - Hematology All Subjects
Listing 16.2.8.1.2.1 Laboratory Test Results - Chemistry All Subjects
Listing 16.2.8.1.3 Laboratory Test Results - Liver Chemistry All Subjects
Listing 16.2.8.1.4 Laboratory Test Results - Urinalysis All Subjects
Listing 16.2.8.1.6 Additional Potential Hepatic Testing All Subjects
Listing 16.2.9.1 Vital Signs Results All Subjects
Listing 16.2.10 .1 Electrocardiogram (ECG) Results All Subjects
Listing 16.2.12 Pregnancy Results All Female Subject
Table 14.1.1.1 Summary of Subject Disposition All Subjects
Table 14.1.1.4 Summary of Protocol Deviation Enrolled Population
Table 14.1.2.1.1 Summary of Demographic Characteristics Enrolled Population
Table 14.1.2.2.1 Summary of Baseline Disease Characteristics Enrolled Population
Table 14.1.5.1 Summary of Study Drug Exposure Safety Population
Table 14.3.1.1.1 Overall Summary of Treatment Emergent Adverse Events (TEAEs) Safety Population
Table 14.3.1.2.1 Summary of TEAEs by System Organ Class and Preferred Term Safety Population
Table 14.3.1.2.2.1 Summary of Serious TEAEs by System Organ Class and Preferred Term Safety Population
Table 14.3.1.2.2.2 Summary of Non-serious TEAEs by System Organ Class and Preferred Term Safety Population
Table 14.3.1.2.4 Summary of Adverse Event of Special Interest by System Organ Class and Preferred Term Safety Population
Table 14.3.2.1 Summary of Liver Chemistry Abnormalities Defined as Primary and Secondary Hepatic Safety Endpoints Safety Population
Table 14.3.5.4 Summary of Post-Baseline QTcF Safety Population