Study Title: A Phase 2, Open-Label, Multi-Center Study to Evaluate the Safety, Pharmacokinetics and Pharmacodynamics of Lixivaptan in Subjects With Autosomal Dominant Polycystic Kidney Disease

ClinicalTrials.gov ID: NCT03487913

Clinical Study Protocol Version 5.0, dated March 21, 2019

Summary of Changes in the Conduct of the Study (from Section 9.8.1 of the Clinical Study Report, dated September 22, 2020

Clinical Study Protocol Version 5.0, dated March 21, 2019, Summary of Changes

Clinical Study Protocol Version 4.0, dated January 9, 2019, Summary of Changes

CLINICAL STUDY PROTOCOL

A Phase 2, Open-Label, Multi-Center Study to Evaluate the Safety, Pharmacokinetics and Pharmacodynamics of Lixivaptan in Subjects with Autosomal Dominant Polycystic Kidney Disease

Protocol Number: PA-102
IND Number: 136,419

Sponsor:	Palladio Biosciences, Inc. 12 Penns Trail Unit A Newtown, PA 18940
Sponsor Contact:	Elaine Richardson Vice President, Clinical Operations Palladio Biosciences, Inc. 12 Penns Trail, Unit A Newtown, PA 18940 ERichardson@palladiobio.com
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Version of Protocol:	5
Date of Protocol:	March 21, 2019

CONFIDENTIAL

All financial and nonfinancial support for this study will be provided by Palladio Biosciences. The concepts and information contained in this document or generated during the study are considered proprietary and may not be disclosed in whole or in part without the expressed, written consent of Palladio Biosciences. The study will be conducted according to the International Council for Harmonisation (ICH) harmonized tripartite guideline E6 (R1): Good Clinical Practice (GCP).

Protocol Approval - Sponsor Signature

Protocol Title	A Phase 2, Open-Label, Multi-Center Study to Evaluate the Safety, Pharmacokinetics and Pharmacodynamics of Lixivaptan in Subjects with Autosomal Dominant Polycystic Kidney Disease
Protocol Number	PA-102
Protocol Version	5
Protocol Date	March 21, 2019

Protocol accepted and approved by:

Neil H. Shusterman, M.D.
Chief Medical Officer
Palladio Biosciences, Inc.
12 Penns Trail, Unit A
Newtown, PA 18940

Signature

March 21, 2019
Date

Declaration of Investigator

I have read and understood all sections of the protocol titled "A Phase 2, Open-Label, Multi-Center Study to Evaluate the Safety, Pharmacokinetics and Pharmacodynamics of Lixivaptan in Subjects with Autosomal Dominant Polycystic Kidney Disease" and the accompanying current version of the Investigator Brochure (IB).

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 (R1): GCP, 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) 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

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PROTOCOL SYNOPSIS

Protocol Number:	PA-102
Protocol Title:	A Phase 2, Open-Label, Multi-Center Study to Evaluate the Safety, Pharmacokinetics and Pharmacodynamics of Lixivaptan in Subjects with Autosomal Dominant Polycystic Kidney Disease
Sponsor:	Palladio Biosciences 12 Penns Trail Unit A Newtown, PA 18940
Study Phase:	Phase 2
Study Sites:	Approximately 15 sites
Indication:	Autosomal Dominant Polycystic Kidney Disease
Rationale:	Therapeutic interventions aimed at counterbalancing the effect of vasopressin may be effective in delaying disease progression in autosomal dominant polycystic kidney disease (ADPKD).
Objectives:	The primary objectives of this study are: • To characterize the safety and tolerability of lixivaptan following multiple doses in ADPKD subjects with relatively preserved kidney function (chronic kidney disease CKD1 and CKD2) and moderately impaired renal function (CKD3). • To characterize the PK profile of lixivaptan and its major metabolites (WAY-141624, WAY-138451, and WAY-138758) following multiple doses of lixivaptan in ADPKD subjects with relatively preserved kidney function (CKD1 and CKD2) and moderately impaired renal function (CKD3). The secondary objectives of this study are: • To characterize the effect of lixivaptan on urine osmolality over a 24-hour period following multiple doses of lixivaptan in ADPKD subjects. • To characterize the time course of the hemodynamic effect of lixivaptan on urine output, total kidney volume, liver volume, circulating vasopressin, and serum creatinine in ADPKD subjects.
Subject Population:	The subjects will be male or female, between 18 and 65 years of age (inclusive) at the time of Screening, with a body mass index (BMI) between 18 and 35 kg/m^2 (inclusive) at the time of Screening. The subjects will have an estimated glomerular filtration rate (eGFR) $\geq$ 60 mL/min/1.73 m^2 (Cohort 1 and 3), or eGFR $\geq$ 30 to < 60 mL/min/1.73 m^2 (Cohort 2 and 4), with eGFR calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. Subjects will have been diagnosed with ADPKD based on modified Ravine criteria.
Study Design:	This is a Phase 2, open-label, parallel-group, multiple dose study designed to evaluate the PK, PD, safety, and tolerability of multiple BID doses of 50 and 200 mg lixivaptan in ADPKD subjects with chronic kidney disease in stages CKD1, CKD2 or CKD3.

Estimated Study Duration:	Screening Duration: 42 days (Day -44 through Day -3) Confinement Period: Subjects will be confined from Check in (Day -2 or Day-1) until Day 2 in the morning. Subjects will be confined from Day 6 in the afternoon through Day 8 in the morning. Outpatient: Subjects will return to the clinical research unit (CRU) on an outpatient basis on Day 4 in the morning and Day 6 in the morning for a trough PK assessment. End of Study Visit: Subjects will return on Day 35 (±2) for end of study assessment. Total Study Duration: Up to 80 days
Study Drug, Dosage, and Route of Administration:	The Sponsor (Palladio Biosciences, Inc.) will provide adequate supplies of lixivaptan 50 mg capsules for use during the study. Enrolled subjects will be assigned according to CKD classification to take one of 2 lixivaptan oral dose regimens for 7 days as shown below. Each dose regimen includes morning (AM) and evening (PM) oral administration of lixivaptan. Cohort / CKD stage / Dose / N 1 / CKD1 or CKD2 / 200 mg BID / 8 subjects 2 / CKD3 / 200 mg BID / 8 subjects 3 / CKD1 or CKD2 / 50 mg BID / 8 subjects 4 / CKD3 / 50 mg BID / 8 subjects Doses of lixivaptan will be administered both at the CRU and at home.
Study Assessments:	**Pharmacokinetic:** The following PK parameters will be calculated using noncompartmental methods, whenever possible, based on lixivaptan and lixivaptan metabolite concentrations: the maximum observed plasma drug concentration (C_{max}), the time to reach maximum plasma concentration (t_{max}), the area under the concentration-time curve (AUC) from time 0 until the last quantifiable concentration (AUC_{0-t}), AUC from time 0 until 14 hours postdose (AUC_{0-14}), AUC extrapolated to infinity (AUC_{0-inf}), the apparent terminal elimination rate constant (λz), the terminal elimination phase half-life ($t_{1/2}$), total body clearance (CL/F), and the volume of distribution (Vz/F). **Pharmacodynamic:** The pharmacodynamic (PD) analysis will include assessments of urine osmolality and urine output, total kidney volume, liver volume, plasma copeptin and serum creatinine. **Safety:** The safety analysis will include clinical laboratory findings (hematology, clinical chemistry, and urinalysis), 12-lead electrocardiograms (ECGs), vital signs, physical examination findings, assessments of adverse events (AE) and the questionnaire of tolerability.
Sample Size:	Approximately 32 subjects (8 subjects in CKD1/2 and 8 subjects in CKD3 per dose level).

Statistical Methods:	**Pharmacokinetic:** Lixivaptan and lixivaptan metabolite (WAY-141624, WAY-138451, and WAY-138758) plasma concentrations will be tabulated and summarized using descriptive statistics (including sample size, arithmetic and geometric mean, standard deviation [SD], coefficient of variation [CV%], median, minimum, and maximum) for each Cohort. The PK parameters for lixivaptan and lixivaptan metabolites will be summarized for each Cohort. Plasma concentration data for lixivaptan and lixivaptan metabolites will also be displayed graphically on linear and semi-logarithmic scales. The following plots will be presented for the concentration-time data: • Individual subject plasma concentration profile versus time, stratified by Cohort. • Arithmetic-mean concentration (±SD) versus time, stratified by Cohort. **Pharmacodynamic:** The change from baseline in urine osmolality, serum creatinine, plasma copeptin, total kidney volume (TKV) and liver volume (LV) after multiple doses of lixivaptan will be summarized by renal function group (CDK1/2 and CDK3), and at each scheduled time point using descriptive statistics (n, mean, SD, median, minimum, and maximum). **Safety:** Safety assessments, including clinical laboratory findings (hematology, clinical chemistry, and urinalysis), 12-lead electrocardiograms (ECGs), vital signs, physical examination findings, assessments of adverse events (AEs) and the questionnaire of tolerability will be analyzed descriptively.
Date of Protocol:	March 21, 2019

LIST OF ABBREVIATIONS

Abbreviation	Definition
ADPKD	autosomal dominant polycystic kidney disease
AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the curve
$AUC_{0\text{-}inf}$	area under the concentration-time curve extrapolated to infinity
$AUC_{0\text{-}t}$	area under the concentration-time curve from time 0 until the last quantifiable concentration
BID	twice per day
BLQ	below the limit of quantitation
BMI	body mass index
BUN	blood urea nitrogen
cAMP	cyclic adenosine 3',5'-monophosphate
CDER	Center for Drug Evaluation and Research
CDISC	Clinical Data Interchange Standards Consortium
CFR	Code of Federal Regulations
CKD	chronic kidney disease
CL/F	total body clearance
C_{max}	maximum observed plasma drug concentration
CRU	Clinical Research Unit
CS	clinically significant
CT	computerized tomography
CV	coefficient of variation
ECG	electrocardiogram
eCRF	electronic Case Report Form
EDC	electronic data capture
eGFR	estimated glomerular filtration rate
EOS	end of study
ET	early termination
FDA	Food and Drug Administration
FSH	follicle-stimulating hormone
GCP	Good Clinical Practice
HBsAg	hepatitis B surface antigen
hCG	human chorionic gonadotropin

Abbreviation	Definition
HIV	human immunodeficiency virus
I/E	inclusion/exclusion
IB	Investigator Brochure
ICF	Informed Consent Form
ICH	International Council for Harmonisation
IND	Investigational New Drug
IRB	Institutional Review Board
LV	liver volume
MedDRA	Medical Dictionary for Regulatory Activities
MRI	magnetic resonance imaging
n	number of non-missing observations
N	number of subjects
NCS	not clinically significant
OTC	over-the-counter
PD	pharmacodynamic
PK	pharmacokinetic
PKD	polycystic kidney disease
PT	preferred term
QTcF	QT interval corrected for heart rate according to Fridericia's formula
REMS	Risk Evaluation and Mitigation Strategy
SAE	serious adverse event
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System
Scr	serum creatinine
SD	standard deviation
SOC	system organ class
S_{osm}	serum osmolality
$t_{1/2}$	terminal elimination phase half-life
TEAE	treatment-emergent adverse event
TKV	total kidney volume
t_{max}	time to reach maximum plasma concentration
ULN	upper limit of normal
U_{osm}	urine osmolality
US	United States
Vz/F	volume of distribution

Abbreviation	Definition
λz	apparent terminal elimination rate constant

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.[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).[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 (HF) and syndrome of inappropriate anti-diuretic hormone secretion (SIADH).

Additional background information for lixivaptan can be found in the Investigator's Brochure.

1.2 Overview of ADPKD

Definition

Autosomal dominant polycystic kidney disease (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 proteins PC1 (polycystin 1) and PC2 (polycystin 2), 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.[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 accompanied 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.[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 function is normal. However, in some cases the increased liver volume may lead to hepatomegaly as a result of the continuous cyst enlargement.[5] This may cause symptoms of extrinsic compression, such as abdominal pain, early satiety, and obstruction of the hepatic veins or bile duct; and 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.[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 response of vasopressin to sodium challenge as compared to normal individuals.[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

In April 2018, the vasopressin V_2 receptor antagonist tolvaptan (JYNARQUE®) was approved in the 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 (REMS).

The European Medicines Agency (EMA) granted a positive opinion for the vasopressin V_2 receptor antagonist 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 (CHMP) recommended additional monitoring of the risk of liver damage with tolvaptan. Similar approvals were granted in Japan, Canada, and Australia/New Zealand. The US Food and Drug Administration (FDA) did not grant approval to tolvaptan for the treatment of ADPKD primarily due to concerns regarding liver safety.

1.4 Rationale for Lixivaptan Therapy for ADPKD

Given that elevated renal cAMP plays a pivotal role in the complex phenotypical manifestation of ADPKD,[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,[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[9] and tolvaptan[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.[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 largest placebo-controlled Phase 3 study ever conducted in ADPKD. 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.[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.[14] Because complete vasopressin suppression is ensured if urinary osmolality is lowered to < 300 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.[14]

More recently, the results of REPRISE, a second pivotal Phase 3 study with tolvaptan for the treatment of ADPKD, were published.[15] This trial randomized 1370 patients with later stage ADPKD, which was defined as having a baseline estimated 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 an animal model of 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 the PCK rat model of PKD

The PCK rat, an orthologous model of human polycystic kidney disease (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 human disease.[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. Additional background information on the effect of lixivaptan in the PCK rat can be found in the Investigator Brochure (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 and various patient populations (patients with hypervolemic and euvolemic hyponatremia and patients with ESRD) demonstrated that treatment with lixivaptan readily suppressed 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.[14]

Additional background information on the effect of lixivaptan on urine osmolality can be found in the Investigator Brochure (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 increased urinary volume output about 3 to 4 times more than furosemide or hydrochlorothiazide at comparable doses and decreased 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 genetically crossed to produce offspring with no circulating serum vasopressin, and that harbor a renal cyst inducing PKD mutation, do not develop cysts.[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.[9,10] Similarly, the vasopressin V_2 receptor antagonist lixivaptan reduces renal cAMP levels in a rodent model 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 Investigator Brochure.

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. Lixivaptan was generally safe and well-tolerated in this patient population. The most common adverse events (AE) were headaches, dizziness, thirst, orthostatic hypotension, and tachycardia events.

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

Even though lixivaptan was never tested in ADPKD patients, proof of concept for the potential utility of lixivaptan for the treatment of ADPKD is provided by the vasopressin V_2 receptor antagonist tolvaptan. Results from the TEMPO 3:4 Phase 3 trial led to approval in Europe for the use of tolvaptan to slow the progression of cyst development and renal insufficiency of ADPKD in adults with CKD stage 1 to 3. The TEMPO 3:4 trial showed that tolvaptan slowed the progression of kidney enlargement and delayed the worsening of kidney function.[12] The study also established suppression of urine osmolality to < 300 mOsm/kg as a predictive pharmacodynamic marker of clinical efficacy for a vasopressin V_2 antagonist for the treatment of ADPKD. More recently, 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.[15]

1.7 Study Rationale

This study is being conducted to directly characterize the pharmacokinetic (PK), safety, and pharmacodynamic (PD) profiles of lixivaptan following administration of twice per day (BID) oral doses of 50 and 200 mg for 7 days in subjects with both autosomal dominant polycystic kidney disease (ADPKD) and chronic kidney disease (CKD) stage 1 (CKD1), stage 2 (CKD2) or stage 3 (CKD3). These proposed PK, safety, and PD assessments will be used to guide appropriate lixivaptan dosing recommendations for subjects with ADPKD and mild or moderate CKD.

1.8 Dose Rationale

The human equivalent exposure range of lixivaptan proposed by this study has been shown to be safe and tolerated in nonclinical animal studies and previous clinical studies conducted in healthy subjects and in subjects with hyponatremia of various etiologies.

As noted above, the selected doses are based on expected effect on urine osmolality. These doses were considered appropriate for the PK/PD evaluation of lixivaptan in subjects with ADPKD and mild or moderate CKD.

2 STUDY OBJECTIVES AND ENDPOINTS

2.1 Study Objectives

2.1.1 Primary Objectives

The primary objectives of this study are:

- To characterize the safety and tolerability of lixivaptan following multiple doses in ADPKD subjects with relatively preserved kidney function (CKD1 and CKD2) and moderately impaired renal function (CKD3); and
- To characterize the PK profile of lixivaptan and its major metabolites (WAY-141624, WAY-138451, and WAY-138758) following multiple doses of lixivaptan in ADPKD subjects with relatively preserved kidney function (CKD1 and CKD2) and moderately impaired renal function (CKD3).

2.1.2 Secondary Objectives

The secondary objectives of this study are:

- To characterize the effect of lixivaptan on urine osmolality over a 24-hour period following multiple doses of lixivaptan in ADPKD subjects; and
- To characterize the time course of the pharmacodynamic effect of lixivaptan on urine output, total kidney volume, liver volume, plasma copeptin and serum creatinine in ADPKD subjects.

2.2 Study Endpoints

2.2.1 Primary Endpoints

- The following PK parameters will be calculated using noncompartmental methods, whenever possible, based on the plasma concentrations of lixivaptan and its major metabolites (WAY-141624, WAY-138451, and WAY-138758), for lixivaptan and each metabolite: maximum observed plasma drug concentration (C_{max}), time to reach maximum plasma concentration (t_{max}), area under the plasma concentration-time curve (AUC) from time 0 until the last quantifiable concentration (AUC_{0-t}), AUC extrapolated to infinity (AUC_{0-inf}), terminal elimination phase half-life ($t_{1/2}$), apparent terminal elimination rate constant (λz), total body clearance (CL/F; lixivaptan only), and volume of distribution (Vz/F; lixivaptan only).
- The safety and tolerability of lixivaptan will be assessed through evaluation of physical examination findings, vital signs, 12-lead electrocardiograms (ECG), clinical laboratory findings (clinical chemistry, hematology and urinalysis), assessments of adverse events (AE) and a questionnaire assessing aquaretic tolerability (symptom burden of nocturia, urgency, frequency).

2.2.2 Secondary Endpoints

The effect of lixivaptan on urine osmolality by lixivaptan will be determined through evaluation of:

- Spot urine osmolality and 24h urine output.

The pharmacodynamic effects of lixivaptan will be determined through evaluation of:

- Serum creatinine, blood urea nitrogen (BUN), and estimated glomerular filtration rate (eGFR);
- Total kidney volume (TKV) and liver volume (LV) measured by abdominal magnetic resonance imaging (MRI); and
- Plasma copeptin, as a marker for circulating vasopressin.

3 INVESTIGATIONAL PLAN

3.1 Study Design

This is a Phase 2, open-label, parallel-group, multiple dose study designed to evaluate the PK, PD, safety, and tolerability of multiple BID doses of 50 and 200 mg lixivaptan in ADPKD subjects with chronic kidney disease in stages CKD1, CKD2 or CKD3. The study will be conducted at multiple Clinical Research Units (CRUs) in the United States (US).

For all subjects, this study includes a Screening period of 42 days (Day -44 through Day -3), i.e. up to 44 days prior to dosing on Day 1. Subjects will be confined to the CRU on two separate occasions, with a total period of confinement of up to 7 days and 5 nights. The first confinement period will last up to 4 days (up to 3 nights). Subjects will be admitted to the CRU in the afternoon of Day -2 or, depending on travel arrangements, in the morning of Day -1, and will be domiciled until discharge from the CRU in the morning of Day 2. The second confinement period will last 3 days (2 nights). Subjects will return to the CRU to be admitted in the afternoon of Day 6 and will be discharged from the CRU in the morning of Day 8. Alternatively, subjects may be offered the option to remain admitted to the CRU for the entire duration of the dosing period, i.e. from the initial admission on Day -2 (or Day -1 where applicable) until discharge on Day 8.

Except for the AM and PM doses on Days 1, 6, and 7, and the AM doses on Days 2, 4 and 6, all other doses will be self-administered by the subject. Subjects will be required to record self-administered doses in a Study Dosing Diary (Section 5.7.1).

Enrolled subjects will be assigned according to CKD classification to take one of 2 dose regimens (Table 5-1) for 7 days. Each dose regimen includes morning (AM) and evening (PM) oral administration of lixivaptan. Doses will be administered with approximately 10 hours separation between the AM and PM doses as follows. When subjects are confined to the CRU, the PM dose will be administered 10 ± 0.25 hours after the AM dose. When lixivaptan is self-administered, the PM dose will be administered 10 ± 1.0 hours after the AM dose. Subjects will maintain adequate hydration throughout the study by adhering to a predetermined daily fluid intake regimen (Section 5.9).

To be enrolled, subjects must meet the following diagnostic criteria for ADPKD (modified Pei-Ravine criteria):

- For subjects with family history of ADPKD, a minimum of 3 cysts per kidney by sonography or 5 cysts by computerized tomography (CT) or magnetic resonance imaging (MRI); or
- For subjects without family history of ADPKD, a minimum of 10 cysts per kidney by any radiologic method and exclusion of other cystic kidney diseases.

Subjects may be enrolled if they meet the modified Pei-Ravine criteria, have a confirmed CKD Classification of CKD1, CKD2 or CKD3 (Table 3-1 and see Appendix 1), and meet the other inclusion/exclusion (I/E) criteria (Section 4.1).

Table 3-1 Chronic Kidney Disease Classification Summary

CKD Classification	Cohorts	N	eGFR*
CKD1 or CKD2**	1 and 3	16 (8 per cohort)	eGFR ≥ 60 mL/min/1.73 m^2
CKD3	2 and 4	16 (8 per cohort)	eGFR ≥ 30 to < 60 mL/min/1.73 m^2

Abbreviations: CKD = chronic kidney disease, eGFR = estimated glomerular filtration rate, N = number of subjects.

*eGFR is calculated by the 2009 Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (Appendix 1).

**At least 2 subjects with CKD1 or CKD2 will be included in 8 subjects assigned to Cohorts 1 and 3.

Subjects may be allowed to re-screen or have repeat testing after agreement between the investigator and medical monitor. Lab tests that need to be repeated during Screening may be obtained through the services of an at-home nursing service provided by the Sponsor.

Subjects who withdraw early from the study may be replaced at the discretion of the Investigator and of the Sponsor to ensure that approximately 32 subjects (8 subjects in CKD1/2 and 8 subjects in CKD3 per dose level, see Table 5-1) complete the study.

Study assessments including PK blood sampling, urine collection, PD blood sampling, physical examination findings, vital signs, ECGs, clinical laboratory tests, and monitoring of AEs will be performed at the timepoints presented in Table 12-1. Adverse events and serious AEs (SAE) that are related to study participation (e.g., protocol-mandated intervention) will be recorded from the time the subject signs the Informed Consent Form (ICF) until the start of lixivaptan dosing. All other AEs and SAEs will be recorded from the start of lixivaptan dosing until exit from the study. Assessment windows are presented in Table 12-2.

3.1.1 Rationale of Study Design

The I/E criteria in Section 4.1 were chosen to recruit an adequate number of subjects representative of the ADPKD population to achieve the study's primary objectives as presented in Section 2.1.1.

Based on existing clinical evidence with lixivaptan and the related vasopressin antagonist tolvaptan, the PK and PD properties of lixivaptan are not expected to differ in ADPKD subjects with CKD1 and CKD2 stages, therefore these subjects with relatively preserved kidney function will be combined into one study group. This approach decreases the total number of subjects that need to be recruited without compromising the study objectives. Conversely, subjects with moderately impaired renal function (CKD3 stage) may show distinct PK and PD responses to lixivaptan and will be enrolled into a separate group.

The PK profile of lixivaptan and its metabolites will be monitored for 3-5 half-lives after the last dose to define terminal PK parameters. Therefore, PK blood draws are included up to 96 hours, i.e. approximately 4 half-lives of the longest-lived metabolite of lixivaptan, after the last dose of lixivaptan (Day 7 PM).

Treatment with lixivaptan is associated with transient and reversible increases in serum creatinine and circulating vasopressin. In addition, based on clinical evidence with the related vasopressin antagonist tolvaptan,[12] lixivaptan may be associated with transient and reversible decreases in Total Kidney Volume (TKV). In order to characterize the time course and magnitude of these pharmacodynamic effects of lixivaptan in ADPKD subjects, the study design includes measurements of serum creatinine, plasma copeptin as a marker for circulating vasopressin, and TKV at three time points: at baseline, after completion of the scheduled treatment period and 4 weeks after the last dose of lixivaptan.

The sample size of 32 subjects in total, with 16 subjects per Cohort, is typical for this type of study and is considered adequate to meet the study objectives.

4 SUBJECT SELECTION AND WITHDRAWAL CRITERIA

4.1 Selection of Study Population

Approximately 32 subjects will be enrolled at approximately 15 sites (or more as necessary to recruit subjects) in the US. Subjects will be assigned to study treatment only if they meet all of the inclusion criteria and none of the exclusion criteria.

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. Capable of understanding the written informed consent, provides signed and witnessed written informed consent, and agrees to comply with protocol requirements and study-related procedures.
2. Male or female, between 18 and 65 years of age (inclusive) at the time of Screening.
3. Body mass index (BMI) between 18 and 35 kg/m^2 (inclusive) at the time of Screening.
4. Estimated glomerular filtration rate (eGFR) $\geq$ 60 mL/min/1.73 m^2 (Cohort 1 and 3), or eGFR $\geq$ 30 to < 60 mL/min/1.73 m^2 (Cohort 2 and 4), with eGFR calculated by the CKD-EPI equation (Section 12.1).
5. Subject has been diagnosed with ADPKD by modified Ravine criteria:
   - For subjects with family history of ADPKD, a minimum of 3 cysts per kidney by sonography or 5 cysts by CT or MRI; or
   - For subjects without family history of ADPKD, a minimum of 10 cysts per kidney by any radiologic method and exclusion of other cystic kidney diseases.

   To verify the cyst count, medical records may be used in association with the Screening visit. Alternatively, in the absence of sonography or radiography evidence from medical records, subjects may be included at the discretion of the Investigator until a modified Ravine diagnosis can be confirmed through the baseline MRI assessment (see section 6.1.6).
6. Considered by the Investigator to be in good health relative to underlying CKD status and clinically stable with respect to underlying CKD, based on medical evaluation that includes medical and surgical history, as well as a complete physical examination including vital signs, ECG, and laboratory test results. A single repeat assessment is permitted for any laboratory, ECG, or vital sign parameter required for enrollment.
7. Female subjects must:
   - be non-pregnant and non-lactating;

- be either postmenopausal (defined as amenorrhea for ≥ 12 months and, if confirmation is necessary based on Investigator discretion, a confirmed follicle stimulating hormone [FSH] ≥ 40 mIU/mL), surgically sterile (defined as having undergone hysterectomy and/or bilateral oophorectomy), practice total abstinence from sexual intercourse as the preferred lifestyle (periodic abstinence is not acceptable), or agree to use an appropriate method of birth control consistently throughout the study and continue to use this method for 30 days after study drug administration.

  Double barrier methods of non-hormonal contraception are permitted in this study. Acceptable forms of contraception include the following:

  - intrauterine device, including Mirena®
  - 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 a male condom in addition to female subject's use of spermicide (cream, spray, gel, suppository, contraceptive sponge, or polymer film)
  - 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

  Estrogen-based hormonal contraception is not permitted in this pharmacokinetic study due to the potential for interaction with lixivaptan.

8. Male subjects who are sexually active with a partner of child-bearing potential must either be sterile (vasectomy with history of a negative sperm count following the procedure); practice total abstinence from sexual intercourse as the preferred lifestyle (periodic abstinence is not acceptable); use a male condom with any sexual activity; or agree to use a birth control method considered to be appropriate by the Investigator (such as hormonal contraception or one of the methods identified above for female subjects of childbearing potential) from the time of Screening until 90 days after study drug administration. Male subjects must agree not to donate sperm for a period of 90 days after study drug administration.
9. Patients who are smokers will be allowed to smoke ≤ 10 cigarettes per day during the study.
10. Subjects must be willing to be confined to the CRU for the entire duration required by the protocol, able to comply with all study-related requirements and able to adhere to study restrictions and visit schedules.

4.1.2 Exclusion Criteria

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

1. Subjects with known sensitivity or idiosyncratic reaction to any compound present in lixivaptan, its related compounds such as benzazepines (e.g., tolvaptan, conivaptan, benazepril, fenoldopam, or mirtazapine), or any compound listed as being present in the study formulation.
2. Women who are pregnant or breast feeding.
3. Subjects who have taken any investigational drug or used an investigational device within 30 days or 5 half-lives, whichever is longer, prior to Day 1.
4. Subjects who have taken tolvaptan, conivaptan, somatostatin analogs (e.g. lanreotide, pasireotide, octreotide, etc.), mTOR kinase inhibitors (e.g. everolimus, sirolimus, etc.), or oral or intravenous antibiotics within 30 days or 5 half-lives, whichever is longer, prior to Day 1.
5. Subjects will be allowed to take their chronic medications unless excluded by the protocol (Section 5.8.2) and provided that their chronic medication therapy meets the conditions outlined in Section 5.8.1, including remaining constant throughout the duration of the study.
6. Subjects who have taken, or are expected to be taking, strong or moderate CYP3A4 or CYP2C8 inhibitors (e.g. 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, ritonavir and ritonavir-containing products, telaprevir, teriflunomide, tofisopam, troleandomycin, verapamil, voriconazole) within 14 days or 5 half-lives, whichever is longer, of dosing; or weak CYP3A4 or CYP2C8 inhibitors (e.g.chlorzoxazone, cilostazol, fosaprepitant, istradefylline, ivacaftor, lomitapide, ranitidine, ranolazine, tacrolimus, ticagrelor, trimethoprim) within 7 days or 5 half-lives, whichever is longer, from dosing. For weak CYP3A4 or CYP2C8 inhibitors, medications may be allowed in consultation with the Medical Monitor (MM).
7. Subjects who have taken, or are expected to be taking, strong or moderate CYP3A4 or CYP2C8 inducers (e.g. barbiturates, bosentan, carbamazepine, efavirenz, enzalutamide, etravirine, modafinil, mitotane, nevirapine, oxcarbazepine, phenytoin, pioglitazone, rifabutin, rifampin, St. John's wort) within 14 days or 5 half-lives, whichever is longer, of dosing; or weak CYP3A4 or CYP2C8 inducers (e.g. armodafinil, rufinamide) within 7 days or 5 half-lives, whichever is longer, from dosing. For weak CYP3A4 or CYP2C8 inducers, medications may be allowed in consultation with the Medical Monitor (MM).
8. Subject who have a transplanted kidney, or absence of a kidney.

9. Subjects with a history of clinically significant drug or alcohol abuse within the past 5 years.
10. Subjects with a history of testing positive for hepatitis B surface antigen (HBsAg), hepatitis C (HCV), or human immunodeficiency virus (HIV).
11. Subjects who have consumed grapefruit or Seville oranges (or their juices, or foods containing their extract) from 7 days prior to the first dose of study medication and until after the final dose.
12. Subjects with clinically significant incontinence, overactive bladder, or urinary retention (e.g., benign prostatic hyperplasia).
13. Subjects with clinically significant nocturia/urgency at Screening outside of the 2 to 4 times awakening per night expected for ADPKD patients.
14. Subjects with clinically significant liver disease, or clinically significant liver function abnormalities or serology other than that expected for ADPKD with cystic liver disease at baseline.
15. Subjects with aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP) or total bilirubin ≥ 1.5 × upper limit of normal (ULN) at Screening. Subjects with elevated bilirubin due to Gilbert's Syndrome may be allowed at the discretion of the Investigator.
16. Subjects with any history of significant bleeding or hemorrhagic tendencies.
17. Subjects with contraindications to or interference with MRI assessments (ferromagnetic metal prostheses, aneurysm clips, severe claustrophobia, or large abdominal/back tattoos) will be excused from this procedure, but may participate in the study providing that all other eligibility criteria are met. Investigator should seek MRI safety guidance from the local MRI facility.
18. Subjects who have experienced an illness that is considered by the Investigator to be clinically significant within 2 weeks of study drug administration on Day 1.
19. Subjects with a history of difficulty in donating blood, or who have donated or lost a significant volume (> 450 mL) of blood within 56 days, or plasma within 7 days, prior to Day -1.
20. Subjects who are unable to swallow medication.
21. Subjects who participated in strenuous exercise within 48 hours prior to Check-in (Day -1).
22. Subjects with New York Heart Association Functional Class 3 or 4 heart failure.
23. Exclusion criteria for Screening ECG (a single repeat is allowed for eligibility determination):
    - Sinus pauses > 3 seconds.
    - Any significant arrhythmia or conduction abnormality, which, in the opinion of the Investigators and Medical Monitor, could interfere with the safety for the individual subject.

- Non-sustained or sustained ventricular tachycardia, or ≥ 3 consecutive ventricular ectopic beats.

24. Subjects with any clinically significant concomitant disease or condition other than ADPKD (including treatment for such conditions) that, in the opinion of the Investigator, could either interfere with the study drug or pose an unacceptable risk to the subject.
25. Subjects with any other clinically significant abnormalities in laboratory test results at Screening that would, in the opinion of the Investigator, increase the subject's risk of participation, jeopardize complete participation in the study, or compromise interpretation of study data.
26. Subjects who have experienced elevated AST or ALT (defined as ≥ 2.0 × ULN) while taking tolvaptan.
27. Any other reason, e.g. serious mental disorders, that would render the subject unsuitable for study enrollment at the discretion of the Investigator.

4.2 Withdrawal of Subjects from the Study

The duration of the study is defined for each subject as the date signed written informed consent is provided through the End of Study assessment, which will be on Day 35 or the date of Early Termination (ET) for subjects who are withdrawn prior to the End of Study assessment.

4.2.1 Reasons for Withdrawal/Discontinuation

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. Every effort should be made to keep subjects in the study and, in the case of voluntary withdrawal, to inquire as to the reason(s) for withdrawal. The reasons for subjects not completing the study will be recorded.

If a subject withdraws for any reason after having received study drug, the subject will be asked to complete ET procedures, which will be the same as those scheduled to be performed prior to discharge from the CRU on Day 8. If a subject voluntarily withdraws before receiving the study drug, no additional safety assessments or testing will be required.

A subject may be withdrawn from the study for any of the following reasons:

1. Does not meet the protocol inclusion or exclusion criteria.
2. Noncompliance with the protocol.

3. 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 or an intercurrent illness that justifies withdrawal. Mild elevations in serum creatinine or serum sodium are known pharmacodynamic effects of vasopressin antagonists like lixivaptan and are transient and reversible. Therefore, patients should not be withdrawn 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.
4. Lost to follow-up.
5. Other (e.g., pregnancy, development of contraindications of use of study drug).
6. The subject withdraws consent, or the Investigator or Sponsor decide to discontinue the subject's participation in the study.
7. The Investigator will also withdraw a subject if the Sponsor terminates the study.

Upon occurrence of a serious or intolerable AE, the Investigator will confer with the Sponsor. If a subject is discontinued because of an AE, the event will be followed until it is resolved or stable as determined by the Investigator. Any subject may withdraw his or her consent at any time.

Subject safety will be closely monitored throughout the study and the study will be conducted following GCP. The entire study may be stopped at any time at the discretion of the Investigator in consultation with the Sponsor.

4.2.2 Handling of Withdrawals

Subjects are free to withdraw from the study or study treatment at any time. Subject participation in the study may be stopped at any time at the discretion of the Investigator or at the request of the Sponsor.

Subjects who discontinue study treatment or active participation in the study will no longer receive study drug. When a subject withdraws from the study, the reason(s) for withdrawal shall be recorded by the Investigator on the relevant page of the electronic case report form (eCRF). Whenever possible, all subjects who discontinue study treatment or withdraw from the study prematurely will undergo all end-of-study assessments at the time of ET. Subjects who fail to return for final assessments will be contacted by the site in an attempt to have them comply with the protocol. Methods for follow up will consist of 2 documented phone calls followed by 1 registered letter.

It is vital to obtain follow-up data on any subject withdrawn because of an AE or SAE. In every case, efforts must be made to undertake protocol-specified, safety, follow-up procedures.

4.2.3 Replacements

If the Investigator withdraws a subject for a reason related to the study drug (in the opinion of the Investigator), then the subject is considered discontinued from the study. Discontinued subjects will be replaced if deemed necessary by the Sponsor.

If a subject does not complete the study for a reason that is unrelated to the study drug, the subject may be replaced if the Sponsor instructs the site to do so. The decision regarding the replacement of subjects will be documented.

5 STUDY TREATMENTS

5.1 Method of Assigning Subjects to Treatment Groups

Each subject will be assigned a screening number. Once enrolled into the study, subjects will be assigned a unique subject number starting with a 2 digit site number, followed by a unique 2 digit subject number.

Subjects who replace discontinuing subjects after the first study drug administration has taken place will be assigned their own unique subject number and same cohort assignment as the subject being replaced.

5.2 Blinding and Randomization

This is an open-label study and no blinding procedures will be used. Following enrollment, subjects will be centrally assigned to one of 4 cohorts depending on CKD status (Table 5-1) and based on a predetermined algorithm. The 200 mg BID dose cohorts will be enrolled first. At least 2 males or females will be included in the 8 subjects (i.e., can be up to a 25%/75% split of male/female or 75%/25% split of male/female) assigned to each cohort. Similarly, at least 2 subjects with CKD1 or CKD2 will be included in 8 subjects each assigned to Cohorts 1 and 3.

5.3 Treatments Administered

Subjects assigned to one of the 2 dose levels of lixivaptan (Table 5-1) will receive oral lixivaptan BID (AM and PM) from Day 1 through Day 7. The PM dose will be administered approximately 10 hours after the AM dose.

Table 5-1 Study Cohorts and Lixivaptan Dose Levels

Cohort	CKD stage	Dose*	N	
1	CKD1 or CKD2	200 mg BID	8 subjects	Candidate maximum tolerated dose
2	CKD3	200 mg BID	8 subjects	
3	CKD1 or CKD2	50 mg BID	8 subjects	Candidate lowest effective dose
4	CKD3	50 mg BID	8 subjects	

*PM dose to be administered approximately 10 hours after the AM dose

Prior to the morning doses on Days 1 and 7, subjects will fast overnight for ≥ 8 hours prior to study medication administration. Water will be allowed *ad libitum* during the overnight fasting. Liquids (other than water) are considered part of the ≥ 8 hour overnight fast.

In the morning of Days 1 and 7, subjects will receive lixivaptan with room temperature water (approximately 240 mL [8 ounces]) and will remain fasted for 2 hours after dosing. Water

will be allowed *ad libitum* during the 2 hours fasting, however subjects will be required to abstain from consuming other liquids until 2 hours after dosing.

The evening doses on Days 1 and 7 will be administered in the CRU under fasting conditions, i.e. 2 hours before and 2 hours after meals. Liquids (other than water) are considered part of the 2 hour fast.

Study drug will be administered orally to subjects. Administration of each dose of study drug while the subject is confined at the CRU will be supervised, verified, and documented according to the CRU's standard operating procedures. A mouth and hand check will be performed immediately after study drug administration.

The actual date and time of each dose administration will be entered in the eCRF.

When not confined to the CRU, subjects will self-administer lixivaptan with water (approximately 240 mL) and without fasting. Subjects will continue to adhere to the recommended fluid intake regimen outlined in Section 5.9.

5.4 Identity of Investigational Product

The Sponsor will provide adequate supplies of lixivaptan for use during the study as shown in Table 5-2.

Table 5-2 Investigational Product

	Lixivaptan	
Strength	50 mg	
Formulation	Capsules	
Batch Number	B17121A1	
Supplier/ manufacturer	Palladio Biosciences 12 Penns Trail, Unit A Newtown, PA 18940	PMRS, Inc. 202 Precision Road Horsham, PA 19044

5.5 Management of Clinical Supplies

5.5.1 Study Drug Packaging and Storage

Lixivaptan 50 mg capsules are packaged in HDPE bottles with desiccant. Each bottle contains thirty 50 mg capsules and is labeled with protocol number and necessary regulatory statements.

All investigational drug supplies should be stored in a secure, locked area under the responsibility of the Investigator or other authorized individual. Lixivaptan 50 mg capsules must be stored under refrigerated conditions between 2-8°C (36-46°F) in tightly closed containers and protected from extreme conditions of temperature, light and humidity. While subjects are confined to the CRU, doses of lixivaptan will be prepared and dispensed by the Investigator or a qualified designee. When subjects are not confined to the CRU, they should

be instructed to store study drug in its original packaging under refrigerated conditions until ready to take.

Unused study medication dispensed to and returned by a study subject should be stored under refrigerated conditions until returned to the Sponsor or designee or destroyed according to applicable regulations.

5.5.2 Test Article 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 test article is dispensed and used by each subject in the study. Reasons for departure from the expected dispensing regimen must also be recorded. At the completion of the study, to satisfy regulatory requirements regarding drug accountability, all unused study drugs will be reconciled and retained, returned to the Sponsor or designee, or destroyed according to applicable regulations.

5.6 Overdose Management

An overdose is any dose of study treatment 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.

5.6.1 Treatment of Overdose

In the event of suspected overdose, the appropriate supportive clinical care should be instituted at the discretion of the Investigator or as dictated by the subject's clinical status.

5.7 Treatment Compliance

When subjects are confined at the CRU, each dose of study drug will be administered by the Investigator or qualified designee. A hand and mouth check will be performed immediately after each study drug administration to verify that the study drug was swallowed. The determination of plasma concentrations of lixivaptan during the analytical phase will provide further confirmation of treatment compliance.

Subjects will maintain a diary of outpatient administration of lixivaptan as described below.

5.7.1 Study Dosing Diary

Subjects will maintain a Study Dosing Diary for the duration of the outpatient treatment period (Day 2 to Day 6) and during the week immediately following the last dose of lixivaptan (PM dose on Day 7). Subjects will be asked to record in the Study Dosing Diary each self-administered dose of lixivaptan, including date and time of dose and number of capsules taken, any subject-reported AEs and any other medications taken, including over-the-counter medications.

Subjects will be asked to bring the Study Dosing Diary together with their unused study product at each scheduled visit to the CRU.

Information from the diary will be entered into the eCRF.

5.8 Prior and Concomitant Therapy

Use of all prior medications within 1 month prior to study drug administration will be recorded in the subject's eCRF. The minimum requirement is that drug name and the dates of administration are recorded. This will include all prescription drugs, herbal products, vitamins, minerals, and 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 and the potential for masking symptoms of a more significant underlying event (Section 5.8.1). The Investigator is responsible for ensuring that details regarding concomitant medication use are recorded in the eCRF. For each concomitant medication administered after admission to the CRU on Day -2 or Day -1, as the case may be, the following details will be documented and recorded in the subject's eCRF: name of medication, dose administered, date 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 OTC medications) without consulting the Investigator.

5.8.1 Permitted Therapy

Allowed medications include those typically prescribed to treat ADPKD, CKD and their complications, with the exception of tolvaptan. These include angiotensin II receptor blockers (e.g., valsartan, candesartan, telmisartan, irbesartan) and angiotensin-converting enzyme inhibitors (e.g., enalapril, lisinopril).

Acetaminophen, at doses $\leq$ 1 g per day, is permitted for use any time during the study in subjects who usually take acetaminophen to control episodic pain. Higher daily doses of acetaminophen may be allowed on a case-by-case basis upon the Investigator's discretion and only in consultation with the Medical Monitor. The use of acetaminophen at any dose is not permitted in subjects who cannot recall taking acetaminophen at least once in the prior 12 months.

Non-sedating antihistamines (other than loratadine) and decongestants may be 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 5.8.2), on a case-by-case basis upon the Investigator's discretion in consultation with the Medical Monitor. All medications should be stable for 1 month prior to dosing, allowing for minor adjustments per the standard of care to treat medical conditions and avoiding, whenever possible, adjustments that will significantly impact serum creatinine levels. The stability of certain drugs, such as insulin and warfarin, should be based on the standard of care rather than on a stable dose.

Lixivaptan has the potential to inhibit the metabolism of CYP3A4 and CYP2C8 substrates, including atorvastatin, simvastatin, and amlodipine. Therefore, care should be exercised when administering lixivaptan in combination with these substrates. In order to minimize the potential for AEs due to drug-drug interactions, prior to enrollment in the study, the Investigator, in consultation with the Medical Monitor, should consider potential dose adjustments or alternative therapeutic options that are not CYP3A4 or CYP2C8 substrates (e.g. rosuvastatin to replace simvastatin or atorvastatin) 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 < 20 mg daily in subjects who are administered the 50 mg BID dose of lixivaptan and < 10 mg daily in subjects who are administered the 200 mg BID dose of lixivaptan. If amlodipine is needed for clinical care, the dose should not exceed 5 mg daily.

5.8.2 Prohibited Therapy

Appropriate medications may need to be administered at the discretion of the Investigator as described in Section 5.8 and Section 5.8.1 if required for proper care of the patient. However, subjects must abstain from taking certain prescription and non-prescription drugs or supplements during the period prior to Day 1, and until after the final assessment on Day 35.

The following medications are strictly prohibited for all subjects within 30 days prior to study drug administration on Day 1 (or 5 half-lives, whichever is longer) and until after the final assessments on Day 35:

- Treatment with tolvaptan or conivaptan;
- Treatment with any investigational drug or device;
- Treatment with somatostatin analogs (e.g. lanreotide, pasireotide, octreotide, etc.);
- Treatment with mTOR kinase inhibitors (e.g. everolimus, sirolimus, etc.);
- Treatment with diuretics.

Hormonal contraception is not permitted for female subjects of child-bearing potential.

The following medications are strictly prohibited for all subjects within 14 days prior to study drug administration on Day 1 (or 5 half-lives, whichever is longer) and until after the final PK assessment:

- Treatment with 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, ritonavir and ritonavir-containing products, telaprevir, teriflunomide, tofisopam, troleandomycin, verapamil, voriconazole;
- Treatment with strong or moderate CYP3A4 or CYP2C8 inducers, including barbiturates, bosentan, carbamazepine, efavirenz, enzalutamide, etravirine, modafinil,

mitotane, nevirapine, oxcarbazepine, phenytoin, pioglitazone, rifabutin, rifampin, St. John's wort.

The following medications are prohibited for all subjects within 7 days prior to study drug administration on Day 1 (or 5 half-lives, whichever is longer) and until after the final PK assessment:

- Treatment with weak CYP3A4 or CYP2C8 inhibitors, including, chlorzoxazone, cilostazol, fosaprepitant, istradefylline, ivacaftor, lomitapide, ranitidine, ranolazine, tacrolimus, ticagrelor, trimethoprim. For weak CYP3A4 or CYP2C8 inhibitors, medications may be allowed in consultation with the Medical Monitor;
- Treatment with weak CYP3A4 or CYP2C8 inducers, including armodafinil and rufinamide. For weak CYP3A4 or CYP2C8 inducers, medications may be allowed in consultation with the Medical Monitor.

The use of CYP3A4 or CYP2C8 substrates is not prohibited, however care should be exercised when administering lixivaptan in combination with these substrates (Section 5.8.1) due to the potential for drug-drug interactions.

5.9 Diet, Fluid, and Activity Control

During confinement at the CRU, subjects will receive standardized meals (breakfast, lunch, dinner, and snack) at scheduled times that do not conflict with other study-related activities. Meals or snacks may be provided to subjects per the Investigator's discretion. Patients will be advised to maintain their normal diet upon discharge from the CRU.

During Days -2 and -1 of CRU confinement, subjects should continue consuming their usual amount of fluids. Due to the expected aquaretic effects of lixivaptan, subjects should be reminded to maintain adequate fluid intake and be mindful of their thirst status at each study visit starting with Day 1 of CRU confinement. Subjects should be instructed to replenish fluids with each void and aim to drink 4-5 liters of fluid per day, or as needed to maintain adequate hydration, while taking lixivaptan. Additionally, subjects will be instructed to drink 1-2 glasses of fluid before bedtime, regardless of perceived thirst, and replenish fluids overnight with each episode of nocturia. During CRU confinement, water consumption will be freely available to all subjects and daily fluid intake will be recorded. Fluid intake will not be recorded outside of the CRU. The Investigator will assess ongoing fluid needs (e.g., by monitoring daily body weight, urine output, laboratory testing, etc.) as necessary in order to maintain adequate hydration of the subject throughout the study. Special care must be taken if subjects are at an increased risk of water loss, e.g. in case of vomiting or diarrhea.

Patients will refrain from consumption of grapefruit or Seville oranges (or their juices) from 7 days prior to the first dose of study medication until after the final PK assessment. Consumption of caffeinated beverages (coffee, tea, sodas, etc.) will be permitted during the study, however subjects must be advised to limit their consumption to two caffeinated drinks (12-16 fl. oz. each) per day. Energy drinks with caffeine are prohibited.

Patients will be advised not to consume alcohol from 72 hours before admission to the CRU on Day -1 or Day -2, as the case may be, until discharge from the CRU on Day 8, and for 72 hours before the final end of study assessment.

Subjects will refrain from strenuous exercise from 48 hours prior to Check-in (Day -1) and during the study through the final end of study assessment.

Patients who are smokers will be allowed to smoke ≤ 10 cigarettes per day during the study.

6 STUDY ASSESSMENTS AND PROCEDURES

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

The study assessments to be performed at each visit and timepoint are specified in the study schedule of assessments (Table 12-1). Assessment windows are presented in Table 12-2.

6.1 Safety and Tolerability Assessments

The safety and tolerability of lixivaptan will be assessed by evaluation of AEs, physical examinations, vital sign measurements, ECGs, clinical laboratory parameters (hematology, clinical chemistry including liver function tests, and urinalysis), and the questionnaire for tolerability. Additional safety assessments may be performed as needed at the discretion of the Investigator. Safety assessments will be performed at scheduled intervals from Day -2 through the end of study assessment as presented in the study schedule of assessments (Table 12-1). Assessment windows are presented in Table 12-2.

6.1.1 Body Height, Weight, and BMI

Body height (centimeters) and weight (kilograms) will be measured to the nearest tenth, and BMI will be calculated (BMI [kg/m^2] = body weight [kg] / height^2 [m^2]). Assessment windows are presented in Table 12-2.

6.1.2 Physical Examinations and Medical History

Complete physical examinations will include, at a minimum, 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. A licensed physician or qualified designee will conduct the examinations.

Brief physical examinations include, at a minimum, assessment of the following systems: skin, head, eyes, and throat, chest and heart auscultation, abdominal examination, neurologic condition (i.e., alert and oriented x3; not including cranial nerves), and edema and hydration assessment. A licensed physician or qualified designee will conduct the examinations.

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.

Medical history and demographic data, including initials, sex, age, race, and ethnicity will be recorded at the timepoints specified in the study schedule of assessments (Table 12-1).

6.1.3 Vital Sign Measurements

Vital sign measurements will include systolic blood pressure, diastolic blood pressure, pulse rate, respiration rate, and body temperature and will be performed at the timepoints specified

in the study schedule of assessments (Table 12-1). Assessment windows are presented in Table 12-2.

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 using an automated sphygmomanometer. A confirmatory repeat vital sign measurement may be performed at the discretion of the Investigator. If other procedures are scheduled at the same timepoint, vital signs will be obtained first, before an ECG and/or blood draw.

6.1.4 12-Lead Electrocardiograms

Electrocardiogram parameters of heart rate, ventricular rate, PR interval, QRS duration, QT interval (uncorrected), and QT interval corrected for heart rate according to Fridericia's formula (QTcF) will be performed at the timepoints specified in the study schedule of assessments (Table 12-1). Assessment windows are presented in Table 12-2.

Electrocardiograms will be obtained with the subject remaining in a supine or semi-recumbent position following 5 minutes of rest. All electrocardiograms 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 one of three categories: normal, abnormal but not clinically significant (NCS), or abnormal and CS. All CS findings will be reported as AEs.

6.1.5 Clinical Laboratory Tests

Blood and urine specimens will be collected for the clinical laboratory (hematology, clinical chemistry, and urinalysis), pregnancy tests (all female subjects) and FSH tests (if necessary for postmenopausal female subjects) at the timepoints specified in the study schedule of assessments (Table 12-1). A confirmatory assessment should be obtained as soon as possible for any clinically significant abnormal laboratory parameter. 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.

Assessment windows are presented in Table 12-2. Clinical laboratory parameters for analysis are presented in Table 6-1.

Table 6-1 Clinical Laboratory Parameters

Chemistry Panel	Hematology	Urinalysis
Alanine aminotransferase*	Hematocrit	pH
Albumin	Hemoglobin	Specific gravity
Alkaline phosphatase*	Red blood cell count	Protein
Aspartate aminotransferase*	Quantitative platelet count	Glucose
Bilirubin Total and Direct*	White blood cell count	Ketones
Blood urea nitrogen	with differential (total and %):	Bilirubin
Calcium	Neutrophils	Blood
Chloride	Lymphocytes	Nitrite
CO_2	Monocytes	Urobilinogen
Creatinine	Eosinophils	Leukocyte esterase
Gamma-glutamyl transferase*	Basophils	Microscopic examination (if
Glucose		positive blood, nitrite,
Phosphorous	**Other Tests**	leukocyte esterase, or protein)
Potassium	Urine or serum pregnancy test	Urea
Protein	(female subjects)	Creatinine
Sodium	FSH (if necessary for	Sodium
Urea	postmenopausal female subjects)	
Uric acid		

Abbreviations: CO_2 = carbon dioxide; FSH = follicle-stimulating hormone.
*Liver function testing

6.1.6 MRI Assessment

In order to investigate lixivaptan's pharmacodynamic effect on kidney and liver volume, patients will undergo a standardized abdominal MRI assessment without use of intravenous contrast for the determination of combined renal volume of both kidneys (total kidney volume or TKV) and liver volume (LV) at 3 timepoints as specified in Table 12-1:

1) at baseline (any time from Screening until CRU admission);
2) immediately after completion of the 7-day treatment period (Day 8 or 9); and
3) at the final End of Study assessment (Day 35 ± 2).

Given the importance of performing the second MRI assessment within a short time interval after the last dose of study medication (PM dose on Day 7), it is recommended that all MRI assessments be scheduled for each subject at the time of the Screening visit. Assessment windows are presented in Table 12-2.

Occasionally, patients may be unable to continue into the study after completing their Screening visit and baseline MRI assessment (for example, due to changed personal circumstances or for not meeting laboratory inclusion criteria). These patients may subsequently qualify for the study and be rescreened at a later time. In this case, their prior baseline MRI assessment may be used instead of obtaining a new baseline MRI scan;

provided, however, that no more than 8 weeks have elapsed from the time of the initial MRI assessment until admission into the CRU.

The MRI acquisition protocol will be detailed in the study imaging manual. MRI images will be sent to a central reading facility for quality control and measurement of TKV and LV. Depending on technical feasibility, combined renal cyst volume (total cyst volume of both kidneys) and combined renal parenchyma volume (total parenchyma volume of both kidneys) may be determined for a subset of subjects at a later date.

In the absence of sonography or radiography evidence at the time of the Screening visit, the baseline MRI assessment could be used to confirm a subject's diagnosis of ADPKD according to modified Ravine criteria.

Subjects with ferro-magnetic prostheses, aneurysm clips, severe claustrophobia or other contraindications or exclusions interfering with MRI measurements will be excused from this procedure, but may participate in the study providing that all other eligibility criteria are met. Investigator should seek MRI safety guidance from local MRI facility.

6.1.7 Urine Osmolality and Specific Gravity

Spot urine collections will be obtained at the timepoints specified in the study schedule of assessments (Table 12-1). Subjects will be asked to completely empty their bladders 15 minutes prior to each scheduled spot urine collection, after which each subject will have 30 minutes to provide a spot urine specimen for the determination of urine osmolality and specific gravity. The goal is to provide a spot urine sample within 30 minutes. Subjects who will not be able provide a sample within the initial 30 minutes after voiding will be given an additional 30 minutes to do so and the different sampling time will be recorded.

Spot urine samples will be used to construct the time course of urine osmolality and specific gravity upon dosing. Urine osmolality measurements will be performed at the central laboratory, whereas the determination of urine specific gravity will be performed in the CRU in two manners: using dipsticks provided by the Sponsor and using existing CRU equipment, where available.

6.1.8 Questionnaire for Tolerability

A questionnaire will be provided in the study reference manual that will assess how well patients tolerate aquaresis-associated events due to the planned lixivaptan doses. The questionnaire will be completed at 2 timepoints as specified in the study schedule of assessments (Table 12-1).

6.1.8.1 Sample Collections

Instructions regarding the collection, processing, and shipment of laboratory samples is detailed in a separate laboratory manual. All samples will be given a unique identifier. The exact clock time of dosing, as well as actual sample collection date and time will be entered on the eCRF.

Blood samples for the PK analysis of lixivaptan will be collected at the timepoints specified in the study schedule of assessments (Table 12-1). Assessment windows are presented in Table 12-2.

6.1.9 Adverse Events

6.1.9.1 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 6.1.9.2 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.

6.1.9.2 Eliciting and Documenting Adverse Events and Serious Adverse Events

AEs and SAEs that are related to study participation (e.g., protocol-mandated intervention) will be recorded from the time the subject signs the ICF until the start of study treatment. All other AEs and SAEs will be recorded from the start of study treatment until exit from the study. Other changes in health or new symptoms occurring during the screening period will be recorded as medical history.

At every study visit, subjects will be asked a standard nonleading question to elicit any medically related changes in their well-being. They will also be asked if they have been hospitalized, had any accidents, used any new medications, or changed concomitant medication regimens (both prescription and over-the-counter medications).

In addition to subject observations, AEs will be documented from any data collected that is deemed clinically significant by the Investigator (e.g., laboratory values, physical examination findings, vital signs, or ECG changes) or identified from review of other documents (e.g., subject diaries) that are relevant to subject safety.

6.1.9.3 Reporting Adverse Events

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, time of onset, Investigator-specified assessment of severity and relationship to study drug, time of resolution of the event, seriousness, any required treatment or evaluations, and outcome. Adverse events 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 that the subject is screened 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.

Any abnormal 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, deemed 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.

Any AE that meets SAE criteria (Section 6.1.9.1) must be reported to the Medical Monitor and Sponsor (or designee) immediately (i.e. within 24 hours) after the time site personnel first learn about the event. Investigators should record all SAE details available, including investigator causality assessment, on an Adverse Event eCRF and submit the report via the EDC system within 24 hours of becoming aware of the event. Notification of SAE entry will be generated and sent to Palladio Drug Safety and its designee by the EDC system. In the event the EDC system is unavailable, a completed SAE Report Form should be submitted to IQVIA drug safety via email at PHV_PA-102@quintiles.com or via fax at 1-866-599-1342.

If questions arise regarding SAE report submission, please contact the IQVIA Lifecycle Safety SAE hotline for assistance at 1-866-599-1341.

6.1.9.4 Assessment of Severity

AEs should be graded as mild, moderate, severe, life-threatening, or death using the following definitions:

- Mild (Grade 1): 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 (Grade 2): 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 (Grade 3): Events interrupt the participant's normal daily activities and generally require systemic drug therapy or other treatment; they are usually incapacitating.
- Life-threatening (Grade 4): Events that place the participant at immediate risk of death or are disabling.
- Death (Grade 5): Events that result in death.

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. Adverse events characterized as intermittent do not require documentation of onset and duration of each episode.

6.1.9.5 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 6-2.

Table 6-2 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).

6.1.9.6 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.

6.2 Laboratory Analyses

Any abnormal 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.

6.3 Pregnancy

Pregnancy is not regarded as an AE unless there is a suspicion that an investigational product may have interfered with the effectiveness of a contraceptive medication. Any pregnancy that occurs during study participation must be reported using a clinical study pregnancy reporting form. To ensure subject safety, each pregnancy must be reported within 24 hours of learning of its occurrence to the Medical Monitor and to IQVIA Lifecycle Safety on the paper pregnancy reporting form to PHV_PA-102@quintiles.com.

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 the Medical Monitor and the Sponsor.

7 STATISTICAL AND ANALYTICAL PLAN

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

7.1 Pharmacokinetic Parameters

The following PK parameters will be calculated using noncompartmental methods, whenever possible, based on lixivaptan and lixivaptan metabolite (WAY-141624, WAY-138451, and WAY-138758) concentrations, and at the timepoints shown in Table 12-1:

PK Day 1 (AM Dose)

PK Parameter	Definition
C_{max}	maximum observed plasma drug concentration
t_{max}	time to reach maximum plasma concentration
AUC_{0-t}	area under the concentration-time curve from time 0 until the last quantifiable concentration, calculated using the linear trapezoidal rule for increasing values, and the log trapezoidal rule for decreasing values
AUC_{0-inf}	area under the concentration-time curve extrapolated to infinity, calculated as: $AUC_{0-inf} = AUC_{0-t} + C_t/\lambda z$
Λz	apparent terminal elimination rate constant
$t_{1/2}$	terminal elimination phase half-life, calculated as $\ln(2)/\lambda z$
CL/F	total body clearance, calculated as $Dose/AUC_{0-inf}$ (lixivaptan only)
Vz/F	volume of distribution, calculated as $CL/F/\lambda z$ (lixivaptan only)

PK Day 1 (PM Dose) – Study Day 1 - 2

PK Parameter	Definition
C_{max}	maximum observed plasma drug concentration
t_{max}	time to reach maximum plasma concentration
AUC_{0-t}	area under the concentration-time curve from time 0 until the last quantifiable concentration, calculated using the linear trapezoidal rule for increasing values, and the log trapezoidal rule for decreasing values

Day 7 (AM Dose)

PK Parameter	Definition
C_{max}	maximum observed plasma drug concentration
t_{max}	time to reach maximum plasma concentration
AUC_{0-t}	area under the concentration-time curve from time 0 until the last quantifiable concentration, calculated using the linear trapezoidal rule for increasing values, and the log trapezoidal rule for decreasing values
CL/F	total body clearance, calculated as Dose/AUC_{0-last} (lixivaptan only)
Vz/F	volume of distribution, calculated as CL/F/λz (lixivaptan only)

Day 7 (PM Dose)

PK Parameter	Definition
C_{max}	maximum observed plasma drug concentration
t_{max}	time to reach maximum plasma concentration
AUC_{0-14}	area under the concentration-time curve from time 0 until 14 hours postdose
AUC_{0-t}	area under the concentration-time curve from time 0 until the last quantifiable concentration, calculated using the linear trapezoidal rule for increasing values, and the log trapezoidal rule for decreasing values
Λz	apparent terminal elimination rate constant
$t_{1/2}$	terminal elimination phase half-life, calculated as ln(2)/λz

Pharmacokinetic parameters will be calculated using validated software and using actual sampling times. The calculation of PK summary statistics will be performed using validated software.

Calculation of accumulation ratios (Day 7/Day 1), metabolite to parent ratios for each metabolite, an assessment of dose proportionality, or additional calculations may be made as appropriate to fully characterize available data. Additional details regarding the PK parameter calculations are presented in the SAP.

7.2 Pharmacodynamic Parameters

The PD analysis will include assessments of urine osmolality and urine output, total kidney volume, liver volume, plasma copeptin, and serum creatinine in ADPKD subjects (see Table 12-1). Pharmacodynamic data will be listed and summarized as detailed in the SAP.

7.3 Safety Variables

The safety analysis will include clinical laboratory findings (hematology, clinical chemistry, and urinalysis), 12-lead ECGs, vital signs, physical examination findings, assessments of AEs and the questionnaire of tolerability. Safety data will be listed and summarized as detailed in the SAP.

7.4 Sample Size Calculations

Calculations were performed assuming a coefficient of variation (CV) of 0.30 and 0.41 for lixivaptan C_{max} and AUC, respectively. Assuming lognormal distribution, this implies that the variances for the logarithm of C_{max} and AUC are 0.086 and 0.155, respectively. Under these assumptions and a random sample of 8 observations, the probability is 0.90 that the point estimate of the population central value (observed geometric mean) for lixivaptan C_{max} and AUC will be within (84.3%, 118.6%) and (79.5%, 125.7%) of the population central value (the median), respectively.

From the perspective of tolerability assessment for multiple dose regimens, for the true population incidence rates of 0.10, 0.20, 0.30, 0.40 and 0.50 for a given AE, the probabilities that the AE would not be observed in a group of 8 subjects administered lixivaptan are given in Table 7-1.

Table 7-1 Probability of Not Observing an Adverse Event for Various True Incidence Rates

True Incidence Rate	Probability of Not Observing an AE in Group of 8 Subjects
0.1	0.43
0.2	0.168
0.3	0.058
0.4	0.017
0.5	0.004

Abbreviation: AE = adverse event.

7.5 Analysis Sets

The following analysis sets will be used in the statistical analyses.

Safety Analysis Set: The Safety Analysis Set will consist of all subjects who receive any study drug. This analysis set will be used for the safety analyses and for summarization of baseline/demographic characteristics.

Pharmacokinetic Analysis Set: The PK Analysis Set will consist of all subjects who undergo plasma PK sampling and have evaluable PK assay results. This analysis set will be used for the PK analysis and for summarization of concentration/parameter data.

Pharmacodynamic Analysis Set: The PD Analysis Set will consist of all subjects who receive study drug and have at least 1 postdose PD assessments of urine osmolality. This analysis set will be used for the PD analysis and for summarization of PD biomarker data.

7.6 Description of Subgroups to be Analyzed

No subgroup analyses are planned.

7.7 Statistical Analysis Methodology

Statistical analysis will be performed using SAS® software Version 9.2 or higher. All continuous variables will be summarized using the following descriptive statistics: number of non-missing observations (n), mean, standard deviation (SD), median, minimum, and maximum. Categorical variables will be summarized using the following descriptive statistics: frequency counts and percentages. All data will be listed in data listings. Missing data will not be imputed but will be analyzed as missing.

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.

7.7.1 Pharmacokinetic Analysis

The parameters describing the PK will be derived from plasma concentrations of lixivaptan and its metabolites and actual sample draw times using noncompartmental analysis. For summary of plasma and urine concentrations, concentrations below the limit of quantification (BLQ) will be treated as zero for the calculation of all summary statistics except for the calculation of geometric mean and CV% of geometric mean, where they are treated as missing.

Plasma concentration data for lixivaptan and its metabolites will also be displayed graphically on linear and semi-logarithmic scales. The following plots will be presented for the concentration-time data:

- Individual subject plasma concentration profile versus time, stratified by renal function group.
- Arithmetic-mean concentration (±SD) versus time, stratified by renal function group.

Additional details regarding the PK parameter calculations are presented in the SAP.

7.7.2 Pharmacodynamic Analyses

The change from baseline in urine osmolality, serum creatinine, plasma copeptin, TKV and LV after multiple doses of lixivaptan will be summarized by renal function group (CDK1/2 and CDK3), and at each scheduled time point using descriptive statistics (n, mean, SD, median, minimum, and maximum).

Except for the case of urine osmolality, the baseline value is the last value observed prior to first administration of study drug and any values after first administration of study drug are regarded as post-baseline values. The change-from-baseline variables will be calculated as the post-baseline value minus the value at baseline.

For urine osmolality assessments, the baseline value for each time point after first administration of study drug is the value observed at the corresponding time point on Day -1 (or Day 1 for the AM predose assessment only). Any values after first administration of

study drug are regarded as post-baseline values. The change-from-baseline variables will be calculated as the post-baseline value minus the value at baseline. The number of subjects who show suppressed urine osmolality, defined as spot urine osmolality < 300 mOsm/kg for 24h, on Day 1 (AM predose) compared with Day 8 (AM predose) will be determined.

7.7.3 Safety Analyses

Clinical Laboratory Tests

All laboratory data will be summarized by renal function group and at each scheduled timepoint using descriptive statistics (n, mean, SD, median, minimum, and maximum).

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 by renal function group. For categorical data, change-from-baseline will be summarized using frequency and proportion at each scheduled timepoint by renal function group.

Individual data listings of laboratory results will be presented for each subject. Values outside of the laboratory's reference range (i.e., those with low or high values) will be flagged in the laboratory listings.

For all continuous 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 by renal function group (using number of subjects with results of negative, trace, or positive).

ECGs

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

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.

Continuous ECG parameters including heart rate, ventricular rate, PR interval, QRS duration, QT interval (uncorrected), and QTcF interval will be summarized for renal function group and over each scheduled timepoint in terms of absolute values using descriptive statistics (n, mean, SD, median, minimum, and maximum).

Vital Signs

Changes from baseline in vital signs at each scheduled timepoint will be summarized by renal function group for the Safety Analysis Set 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. There will not be any imputation for missing values. All vital sign data will be listed individually by each subject based on the Safety Analysis Set.

Physical Examinations

Changes in baseline in physical examination findings (Normal, Abnormal-NCS, Abnormal-CS) will be summarized using counts and percentages for renal function group and will also be listed individually for each scheduled timepoint.

Adverse Events

An overall summary of the number and percentage of subjects in each category will be presented, as will an overall summary of the number of events in each category. The number and percentage of subjects reporting AEs in each category above will be summarized by renal function group according to the system organ class (SOC) and preferred term (PT) assigned to the event using MedDRA.

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. A summary of deaths (for all deaths, AE outcome, not AE term, is death) will be provided by number and percentage of subjects by renal function group.

Questionnaire for Tolerability

Tolerability data obtained from the questionnaire will be listed and summarized as detailed in the SAP.

7.7.4 Other Analyses

Listings will be provided for subject disposition, demographics, medical history, prior and concomitant medications, and summary tables will be prepared for disposition and demographics.

7.8 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.

7.8.1 Data Management

As part of the responsibilities assumed by participating in the study, the Investigator agrees to maintain adequate case histories for the subjects treated as part of the research under this protocol. 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 Oracle Clinical Remote Data Capture program. 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 IQVIA standards and data cleaning procedures to ensure the integrity of the data, e.g. removing errors and inconsistencies in the data. Adverse events and concomitant medication terms will be coded using the MedDRA and World Health Organization Drug Dictionaries, respectively.

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

8 ETHICS

8.1 Institutional Review Board

Federal regulations and the ICH guidelines require that approval be obtained from an IRB before participation of human subjects in research studies. Before study onset, the protocol, informed consent, advertisements to be used for the recruitment of study subjects, and any other written information regarding this study to be provided to the subject or the subject's legal guardian must be approved by the IRB. Documentation of all IRB approvals and of the IRB compliance with ICH harmonized tripartite guideline E6 (R1): GCP will be maintained by the site and will be available for review by the Sponsor or its designee.

All IRB approvals should be signed by the IRB chairman or designee and must identify the IRB 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. The Investigator must promptly supply the Sponsor or its designee, the IRB, and, where applicable, the institution, with written reports on any changes significantly affecting the conduct of the study or increasing the risk to subjects.

8.2 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, ICH GCP, and all applicable regulations.

8.3 Subject Information and Consent

A written informed consent in compliance with US Title 21 Code of Federal Regulations (CFR) Part 50 shall be obtained from each subject before entering the study or performing any unusual or non-routine procedure that involves risk to the subject. An informed consent template 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 consent should be reviewed by the Sponsor or its designee or both before IRB submission. Once reviewed, the consent will be submitted by the Investigator to his or her IRB 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.

9 INVESTIGATOR'S OBLIGATIONS

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.

9.1 Confidentiality

All laboratory specimens, evaluation forms, reports, and other records will be identified in a manner designed to maintain subject confidentiality. All records will be kept in a secure storage area with limited access. Clinical information will not be released without the written permission of the subject (or the subject's legal guardian), except as necessary for monitoring and auditing by the Sponsor, its designee, the FDA, or the IRB.

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.

9.2 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 IQVIA 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 IQVIA is financially responsible for further treatment of the subject's disease.

9.3 Investigator Documentation

Before beginning the study, the Investigator will be asked to comply with ICH E6 (R1) Section 8.2 and Title 21 of the CFR by providing the following essential documents, including but not limited to:

- IRB approval
- 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-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

9.4 Study Conduct

The Investigator agrees that the study will be conducted according to the principles of ICH E6 (R1). 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.

9.5 Adherence to Protocol

The Investigator agrees to conduct the study as outlined in this protocol in accordance with ICH E6 (R1) and all applicable guidelines and regulations.

9.6 Adverse Events and Study Report Requirements

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

9.7 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.

9.8 Records Retention

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.

9.9 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.

10 STUDY MANAGEMENT

10.1 Monitoring

10.1.1 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, in addition to maintaining necessary telephone 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.

10.1.2 Inspection of Records

Investigators and institutions involved in the study will permit study-related monitoring, audits, IRB review, and regulatory inspections by providing direct access to all study records. In the event of an audit, 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.

The Investigator should promptly notify the Sponsor and IQVIA of any audits scheduled by any regulatory authorities and promptly forward copies of any audit reports received to the Sponsor.

10.2 Management of Protocol Amendments and Deviations

10.2.1 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 for approval before subjects can be enrolled into an amended protocol, and before the changes can be implemented.

10.2.2 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 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 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 1).

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 should be notified of all protocol deviations in a timely manner.

10.3 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.

10.4 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.

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12 APPENDICES

12.1 Appendix 1: Chronic Kidney Disease Classification Criteria

This study uses the 2009 CKD-EPI creatinine equation,[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/κ or 1.

12.2 Appendix 2: Schedule of Assessments

Table 12-1 Schedule of Assessments

Visit type:	Screening[17]	Admit[17]	In-CRU	In-CRU	Discharge after AM Assessments		PK Visit in AM		PK Visit in AM; Admit for PM dose	In-CRU	Discharge after PK Collection and Assessments	PK Collection[18]	End of Study Visit
Procedure: **Study Day:**	**-44 to -3 or -2**	**-2 or -1**	**-1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9-11**	**35 ±2**
Informed Consent	X												
Demographics	X												
Medical History	X												
Serum/Urine Pregnancy Test[1,2]	X	X											X
FSH[3]	X												
Height/Weight/BMI	X												X
Inclusion/Exclusion	X												
Previous Medications[1]	X	X											
Concomitant Medications[4]	X		X	X	X		X		X	X	X	X	X
Clinical Lab Evaluations[5]	X		X	X	X					X	X		X
Serum Creatinine[6]	X		X	X	X					X	X		X
Electrocardiogram[7]	X			X							X		
Vital Signs[8]	X		X	X	X		X		X	X	X		X
Physical Examinations[1,9]	X	X									X		X
Adverse Events[10]	X												
Daily Fluid Intake[11]			X	X	X (AM only)					X	X (AM only)		
Administer Dose in-CRU[12]				AM PM	AM		AM		AM PM	AM PM			
Self-Administer Dose[12]					PM	AM PM	PM	AM PM					
PK Blood Samples[13]				X	X		X		X	X	X	X	
Urine Osmolality[14]			X	X	X					X	X		
Daily Urine Output[15]			X	X	X (AM only)					X	X (AM only)		
Tolerability Questionnaire					X					X			
Plasma Copeptin[16]			X		X					X			X
TKV and LV (by MRI)			X							X			X

Abbreviations: BMI= body mass index, CRU = clinical research unit, EOS = end of study, FSH = follicle-stimulating hormone, MRI = magnetic resonance imaging, PK = pharmacokinetic, Preg. = pregnancy, TKV = total kidney volume, LV = liver volume.

[1] Assessments will be performed on Day -1 or Day -2 for subjects admitted to CRU on Day -2.

[2] Serum pregnancy test to be performed at Screening; Urine pregnancy test to be performed at Day -2 or Day -1 and at End of Study.

[3] Postmenopausal subjects with a urine pregnancy result outside the post-menopausal range or an indeterminate pregnancy test will undergo additional testing with FSH if necessary based on Investigator discretion to confirm postmenopausal status prior to study enrollment.

[4] Includes over the counter medications.

[5] Clinical labs: hematology, clinical chemistry (including liver function), and urinalysis at approximately the same time of day (in the morning and, on dosing days, before the lixivaptan dose). The list of analytes is shown in Table 6-1. Lab tests that need to be repeated during Screening may be obtained through the services of an at-home nursing service provided by the Sponsor.

[6] Serum creatinine will be measured as part of the clinical laboratory assessment.

[7] 12-lead ECG at Screening, Day 1 pre-dose, and prior to discharge from clinic on Day 8. Electrocardiograms to be obtained following 5 minutes rest in supine or semi-recumbent position. The same position must be used throughout the study for a given subject.

[8] Vital signs will be measured following 5 minutes rest in a seated position. Systolic and diastolic blood pressure, pulse rate, respiratory rate, body temperature will be measured at the following time points: prior to Day -1 procedures, prior to discharge from CRU on Day 8, and at the End of Study visit. In addition, blood pressure and pulse rate will also be measured before each administration of study medication in the CRU.

[9] Complete physical examination at Screening, and prior to discharge on Day 8. Brief physical examination on Day -2 or Day -1. Symptom-directed physical examinations as needed.

[10] Adverse events and SAEs that are related to study participation (e.g., protocol-mandated intervention) will be recorded from the time the subject signs the informed consent form until exit from the study. All other AEs and SAEs will be recorded from the first dose of lixivaptan until exit from the study.

[11] Record daily fluid intake on Day -1, Day 1 and Day 7. On Day -1, measurement will begin 24 hours before the expected time of first study medication on Day 1 AM. On Day 1 and Day 7, measurements will begin immediately before the AM dose and will continue for 24 hours into the morning of Day 2 and Day 8, respectively.

[12] Subjects will take study medication in the AM and PM for 7 days. Subjects will be asked to record each dose of the study product taken on an outpatient basis in the Study Dosing Diary. Subjects will be asked to bring the Study Dosing Diary together with their unused study product at each visit to the CRU.

[13] PK blood collection timepoints:
Day 1: 0, 1, 2, 4, 6, 9, 10, 11, 12, 14, and 16 hours post AM dose. The collections at 0 and 10 hours are immediately before the AM and PM doses, respectively;
Day 2, 4 and 6: 0 hours (immediately before AM dose);
Day 7: 0, 1, 2, 4, 6, 9, 10, 11, 12, 14, and 16 hours post AM dose. The collections at 0 and 10 hours are immediately before the AM and PM doses, respectively;
Day 8: 0 hours (corresponding to 24 hours after AM dose on Day 7) and 10 hours (corresponding to 24 hours after PM dose on Day 7);
Day 9: 48 hours after PM dose on Day 7;
Day 11: 96 hours after PM dose on Day 7.

[14] Urine will be collected for determination of spot urine osmolality and urine specific gravity at the following timepoints:
Day -1: 0 (start), 1, 2, 4, 6, 9, 10, 11, 12, and 14 hours.

Day 1: 0, 1, 2, 4, 6, 9, 10, 11, 12, and 14 hours post AM dose. The collections at 0 and 10 hours are immediately before the AM and PM doses, respectively;
Day 2: 0 hours (immediately before AM dose on Day 2, corresponding to 24 hours after AM dose on Day 1).
Day 7: 0, 1, 2, 4, 6, 9, 10, 11, 12, and 14 hours post AM dose. The collections at 0 and 10 hours are immediately before the AM and PM doses, respectively;
Day 8: 0 hours (corresponding to 24 hours after AM dose on Day 7).
Note: Subject may not be able to void at all timepoints - see Section 6.1.7. For all voids, the volume (mL) and the date and time of void will be recorded.

[15] Record daily urine output on Day -1, Day 1 and Day 7. On Day -1, measurement will begin 24 hours before the expected time of first study medication on Day 1 AM. On Day 1 and Day 7, measurements will begin immediately before the AM dose and will continue for 24 hours into the morning of Day 2 and Day 8, respectively.

[16] Blood samples for determination of plasma copeptin will be taken at approximately the same time of day (in the morning and, on dosing days, before the lixivaptan dose).

[17] Subjects will be admitted to the CRU in the evening of Day -2 or in the morning of Day -1. For subjects admitted to the CRU on Day -2, the Screening period will be until Day -3. For subjects admitted to the CRU on Day -1, the Screening period will be until Day -2.

[18] Three PK sampling points are contemplated after patients are discharged from the CRU on Day 8 (Day 8 PM, Day 9 PM, Day 11 PM). Patients may return to the site for these PK sample collections. Alternatively, if patients are not able to return to the CRU after discharge, they may utilize an at-home nursing service provided by the Sponsor. If a patient does not return to the site for PK sample collection after discharge on Day 8, but has the blood drawn by an at-home nursing service, this will not be considered a protocol deviation.

12.3 Appendix 3: Assessment Windows

Table 12-2 Assessment Windows

Procedure	Dosing Day	Window (units)
Lixivaptan PM dose	Day 1 – Day 7	In CRU: 10 hr ± 15 minutes after AM dose Self-Administered: 10 hr ± 1 hr after AM dose
	Assessment Day	
PK Blood Samples	Day 1, Day 2, Day 4, Day 6, Day 7, Day 8, Day 9, Day 11	- 60 minutes (predose Day 1) - 10 minutes (predose Day 7) ± 3 min (≤4 hr) ± 5 min (> 4 hr but ≤ 12 hr) ± 10 min (> 12 hr but ≤ 24 hr) ± 1 hr (> 24 hr but ≤ 48 hr) ± 2 hr (> 48)
Urine Osmolality and Urine Specific Gravity	Day 1-2, Day 7-8	Subjects will empty their bladders completely 15 ± 5 minutes prior to each scheduled collection, after which they will have 30 minutes to provide a spot urine sample. Subjects who will not be able to provide a sample within 30 minutes will be given an additional 30 minutes to do so and the different sampling time will be recorded.
Electrocardiogram	Screening, Day 1, Day 8	- 60 minutes (predose), ± 30 minutes (postdose)
Vital Signs	Screening, Day -1, Day 1, Day 2, Day 4, Day 6, Day 7, Day 8, Day 35	- 60 minutes (predose), ± 30 minutes (postdose)
Clinical Laboratory Samples	Screening, Day -1, Day 2, Day 7, Day 35	- 60 minutes (predose Day 2 and Day 7) In the morning on Day -1 and Day 35
Tolerability Questionnaire	Day 2, Day 7	Subjects will complete the questionnaire in the morning of Day 2, before discharge from the CRU, and in the morning of Day 7.
TKV and LV (MRI)	Baseline End of dosing End of study	Baseline: between Screening and Day -1 End of dosing: Day 7 or 8 or 9 End of study: Day 35 ± 2

Table abbreviations: hr = hours, PK= pharmacokinetic

Summary of Changes in the Conduct of the Study (from Section 9.8.1 of the Clinical Study Report, dated September 22, 2020

Changes in the Conduct of the Study

There were 2 protocol versions that were not implemented or submitted to the IRB. Protocol versions 1 and 2 were marked final and dated 19 March 2018 but the version number 1.0 remained unchanged in the footer of both versions. Refer to Note to File (dated 23 February 2020) in Appendix 16.1.1 to distinguish between the 2 documents.

Protocol version 3 (dated 24 April 2018) was the first protocol version submitted to the IRBs and amended. There were 2 subsequent protocol amendments, versions 4 and 5, that were implemented after the first subject was enrolled. Copies of the protocol and protocol amendments are provided in Appendix 16.1.1. Brief summaries of the changes are outlined below.

Protocol version 4 (dated 09 January 2019) implemented the following changes:

- Clinically relevant changes that affected the type or frequency of protocol procedures, or the addition of information to the protocol that influenced clinical decision making or consent:
    - Increased eligible age to 65 years for inclusion in the study;
    - Provided flexibility in screening procedures;
    - Allowed the use of the low progesterone-releasing intrauterine device Mirena;
    - Limited hormonal contraception exclusion to estrogen-containing contraceptives. Progesterone-only contraceptives were allowed.
    - Allowed inclusion of subjects with elevated bilirubin due to Gilbert's Syndrome at the discretion of the investigator.
    - Allowed inclusion of subjects with contraindications to or interference with MRI assessments provided they met all other inclusion criteria. Clarified that no MRI tests would be conducted on these subjects.
    - Added exclusion of subjects who showed signs of liver toxicity on tolvaptan therapy.
    - Clarified batch number of investigational product.
    - Clarified fluid intake recommendations before the first dose of study drug and while taking study drug.
    - Allowed supine position for ECGs provided that the same position was maintained for the same subject throughout the study.
    - Clarified nature of pregnancy test at EOS visit.
    - Added Day 11 to the Assessment Window table.
    - Clarified the appropriate time window for time values that were on the extremes of each time interval.
- Clarified text which included corrections of typos, text changes for consistency or other clarifications which did not affect clinical decision making or consent. These included deletion of mesylate, the counter-ion of fenoldopam from the exclusion criteria.

- Administrative changes, including changes of names or roles of companies or personnel and/or contact information. These changes did not affect clinical decision making or consent.

Protocol version 5 (dated 21 March 2019) implemented the following changes:

- Clinically relevant changes that affected the type or frequency of protocol procedures, or the addition of information to the protocol that influenced clinical decision making or consent:
  - Extended screening duration from 4 weeks to 6 weeks.
  - Increased study duration by the addition of 14 days to Screening.
  - Updated information on the status of the vasopressin V2 receptor antagonist tolvaptan (JYNARQUE®) in the US.
  - Provided flexibility to stop enrollment of CKD1 subjects in Cohorts 1 and 3 if sufficient experience with CKD2 subjects had not occurred.
  - Provided the use of a home nursing service to draw blood if repeat lab tests were needed during Screening.
  - Deleted amlodipine from the exclusion criteria and prohibited therapy as it was a CYP3A4 substrate and not an inhibitor.
  - As inhibition of CYP3A4 by lixivaptan might reduce metabolism of amlodipine, a dosing limit was added.
  - Increased the allowable upper limit of liver function test (LFT) results at Screening to 1.5 × ULN based on the observation in the first 8 subjects treated with lixivaptan at 200 mg BID (highest dose) in this study that no significant LFT abnormalities had been detected. The highest values were 1.2 × ULN for transaminases with no increase in total bilirubin.
  - Clarified that if a subject did not come back for visits after Day 8, it was not a protocol deviation if the blood was drawn only by the at-home nursing service.
- Clarified text which included corrections of typos, text changes for consistency or other clarifications. These changes did not affect clinical decision making or consent.
- Administrative changes, including changes of names or roles of companies or personnel and/or contact information. These changes did not affect clinical decision making or consent.

Study PA-102
Clinical Study Protocol version 5 dated March 21, 2019
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.

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 4 dated January 9, 2019	Version 5	Explanation
1	Title page	Version of Protocol: 4	Version of Protocol: **5**	Clarifies protocol version
1	Title page	Date of protocol: January 9, 2019	Date of protocol: **March 21, 2019**	Updates protocol date
1	Title page	Sponsor Contact: Ruth Ann Subach, PharmD, BCPS Vice President, Clinical Development and Operations Design Space Inpharmatics, LLC Harleysville, PA 19438 rsubach@dsipharmatics.com	Sponsor Contact: **Elaine Richardson** **Vice President, Clinical Operations** **Palladio Biosciences, Inc.** **12 Penns Trail, Unit A** **Newtown, PA 18940** **ERichardson@palladiobio.com**	Updates Sponsor Contact name and contact information.
3	Protocol approval – Sponsor signature	Version of Protocol: 4	Version of Protocol: **5**	Updates protocol version

Page	Section	Version 4 dated January 9, 2019	Version 5	Explanation
3	Protocol approval – Sponsor signature	Protocol date January 9, 2019	Protocol date **March 21, 2019**	Updates protocol date
3	Protocol approval – Sponsor signature	Lorenzo Pellegrini, Ph.D. President & CEO Palladio Biosciences, Inc. 12 Penns Trail, Unit A Newtown, PA 18940	**Neil H. Shusterman, M.D.** **Chief Medical Officer** Palladio Biosciences, Inc. 12 Penns Trail, Unit A Newtown, PA 18940	Change of responsibility to Chief Medical Officer
3	Protocol approval – Sponsor signature	Signature date January 9, 2019	Signature date **March 21, 2019**	Updates protocol date
11	Synopsis	Date of protocol: January 9, 2019	Date of protocol: **March 21, 2019**	Updates protocol date
All	Footnote	09-Jan-2019	**21-Mar-2019**	Updates protocol date
All	Footnote	Version 4	Version 5	Updates protocol version

Text Clarifications

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

Page	Section	Version 4 dated January 9, 2019	Version 5	Explanation
11	Synopsis	**Pharmacokinetic:** Lixivaptan and lixivaptan metabolite (WAY-141624, WAY-138451, and WAY-138758) plasma concentrations will be tabulated and summarized using descriptive statistics (including sample size, arithmetic and geometric mean, standard deviation [SD], coefficient of variation [CV%], median, minimum, and maximum) for each Cohort. The PK parameters for lixivaptan and lixivaptan metabolites will be will be summarized for each Cohort.	**Pharmacokinetic:** Lixivaptan and lixivaptan metabolite (WAY-141624, WAY-138451, and WAY-138758) plasma concentrations will be tabulated and summarized using descriptive statistics (including sample size, arithmetic and geometric mean, standard deviation [SD], coefficient of variation [CV%], median, minimum, and maximum) for each Cohort. The PK parameters for lixivaptan and lixivaptan metabolites **will be** summarized for each Cohort.	Deletion of duplicate words
13	List of Abbreviations		REMS Risk Evaluation and Mitigation Strategy	Adds new abbreviation
35	5.8.1 Permitted Therapy	Allowed medications include those typically prescribed to treat ADPKD, CKD and their complications, with the exception of tolvaptan. These include angiotensin II receptor blockers (valsartan, candesartan, telmisartan, irbesartan) and angiotensin-converting enzyme inhibitors (enalapril, lisinopril).	Allowed medications include those typically prescribed to treat ADPKD, CKD and their complications, with the exception of tolvaptan. These include angiotensin II receptor blockers (**e.g.,** valsartan, candesartan, telmisartan, irbesartan) and angiotensin-converting enzyme inhibitors (**e.g.,** enalapril, lisinopril).	Added “e.g.”, to show these are examples and not a complete list.

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 4 dated January 9, 2019	Version 5	Comments
10	Synopsis	Screening Duration: 28 days (Day -30 through Day -3)	Screening Duration: **42** days (Day **-44** through Day -3)	Extends the duration of Screening from 4 weeks to 6 weeks
10	Synopsis	Total Study Duration: 66 days	Total Study Duration: **Up to 80** days	Study duration is increased by the addition of 14 days to Screening.
16	1.3 Overview of Available Therapies for ADPKD	As of the time of this document there are no drug therapies specifically approved in the US for the treatment of ADPKD.	**In April 2018, the vasopressin V_2 receptor antagonist tolvaptan (JYNARQUE®) was approved in the 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 (REMS).**	Updates information on the status of Jynarque in the United States.
22	3.1 Study Design	For all subjects, this study includes a Screening period of 28 days (Day -30 through Day -3), i.e. up to 30 days prior to dosing on Day 1.	For all subjects, this study includes a Screening period of **42** days (Day **-44** through Day -3), i.e. up to 44 days prior to dosing on Day 1.	Extends the duration of Screening from 4 weeks to 6 weeks.

<table>
<tr><th>Page</th><th>Section</th><th>Version 4 dated January 9, 2019</th><th>Version 5</th><th>Comments</th></tr>
<tr><td>23</td><td>3.1 Study Design, Table 3-1, footnote</td><td>Table 3-1 Chronic Kidney Disease Classification Summary
<table><tr><th>CKD Classification</th><th>Cohorts</th><th>N</th><th>eGFR*</th></tr><tr><td>CKD1 or CKD2**</td><td>1 and 3</td><td>16 (8 per cohort)</td><td>eGFR ≥ 60 mL/min/1.73 m^2</td></tr><tr><td>CKD3</td><td>2 and 4</td><td>16 (8 per cohort)</td><td>eGFR ≥ 30 to < 60 mL/min/1.73 m^2</td></tr></table>Abbreviations: CKD = chronic kidney disease, eGFR = estimated glomerular filtration rate, N = number of subjects.
*eGFR is calculated by the 2009 Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (Appendix 1).</td><td>Table 3-2 Chronic Kidney Disease Classification Summary
<table><tr><th>CKD Classification</th><th>Cohorts</th><th>N</th><th>eGFR*</th></tr><tr><td>CKD1 or CKD2**</td><td>1 and 3</td><td>16 (8 per cohort)</td><td>eGFR ≥ 60 mL/min/1.73 m^2</td></tr><tr><td>CKD3</td><td>2 and 4</td><td>16 (8 per cohort)</td><td>eGFR ≥ 30 to < 60 mL/min/1.73 m^2</td></tr></table>Abbreviations: CKD = chronic kidney disease, eGFR = estimated glomerular filtration rate, N = number of subjects.
*eGFR is calculated by the 2009 Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (Appendix 1).
**At least 2 subjects with CKD1 or CKD2 will be included in 8 subjects assigned to Cohorts 1 and 3.</td><td>Provides for flexibility to stop enrollment of CKD1 subjects in Cohorts 1 and 3 if sufficient experience with CKD2 subjects has not occurred.</td></tr>
<tr><td>23</td><td>3.1 Study Design</td><td>Subjects may be allowed to re-screen or have repeat testing after agreement between the investigator and medical monitor.</td><td>Subjects may be allowed to re-screen or have repeat testing after agreement between the investigator and medical monitor. Lab tests that need to be repeated during Screening may be obtained through the services of an at-home nursing service provided by the Sponsor.</td><td>Provides for the use of a home nursing service to draw blood if repeat lab tests are needed during Screening.</td></tr>
<tr><td>27</td><td>4.1.2 = Exclusion criteria</td><td>6.Subjects who have taken, or are expected to be taking, strong or moderate CYP3A4 or CYP2C8 inhibitors (e.g. aprepitant, boceprevir, clarithromycin, chloramphenicol (not eye</td><td>6.Subjects who have taken, or are expected to be taking, strong or moderate CYP3A4 or CYP2C8 inhibitors (e.g. aprepitant, boceprevir, clarithromycin, chloramphenicol (not eye</td><td>Amlodipine is a CYP3A4 substrate and not an inhibitor. Thus, it has been deleted from this section.</td></tr>
</table>

Page	Section	Version 4 dated January 9, 2019	Version 5	Comments
		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, ritonavir and ritonavir-containing products, telaprevir, teriflunomide, tofisopam, troleandomycin, verapamil, voriconazole) within 14 days or 5 half-lives, whichever is longer, of dosing; or weak CYP3A4 or CYP2C8 inhibitors (e.g., amlodipine, chlorzoxazone, cilostazol, fosaprepitant, istradefylline, ivacaftor, lomitapide, ranitidine, ranolazine, tacrolimus, ticagrelor, trimethoprim) within 7 days or 5 half-lives, whichever is longer, from dosing. For weak CYP3A4 or CYP2C8 inhibitors, medications may be allowed in consultation with the Medical Monitor (MM).	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, ritonavir and ritonavir-containing products, telaprevir, teriflunomide, tofisopam, troleandomycin, verapamil, voriconazole) within 14 days or 5 half-lives, whichever is longer, of dosing; or weak CYP3A4 or CYP2C8 inhibitors (e.g., **~~amlodipine,~~** chlorzoxazone, cilostazol, fosaprepitant, istradefylline, ivacaftor, lomitapide, ranitidine, ranolazine, tacrolimus, ticagrelor, trimethoprim) within 7 days or 5 half-lives, whichever is longer, from dosing. For weak CYP3A4 or CYP2C8 inhibitors, medications may be allowed in consultation with the Medical Monitor (MM).	
28	4.1.2. – Exclusion criteria	15. Subjects with aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP) or total bilirubin ≥ 1.0 × upper limit of normal (ULN) at Screening. Subjects with elevated	15. Subjects with aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP) or total bilirubin **> 1.5** × upper limit of normal (ULN) at Screening. Subjects with elevated	Increases the allowable upper limit of liver function test (LFT) results at Screening to 1.5 × the upper limit of normal based on the observation in the first 8 subjects treated with

Page	Section	Version 4 dated January 9, 2019	Version 5	Comments
		bilirubin due to Gilbert's Syndrome may be allowed at the discretion of the Investigator.	bilirubin due to Gilbert's Syndrome may be allowed at the discretion of the Investigator.	lixivaptan at 200 mg BID (highest dose) in this study that no significant LFT abnormalities have been detected. The highest values were 1.2 times the upper limit of normal for transaminases with no increase in total bilirubin.
32	5.2 Blinding and Randomization	This is an open-label study and no blinding procedures will be used. Following enrollment, subjects will be centrally assigned to one of 4 cohorts depending on CKD status (Table 5 1) and based on a predetermined algorithm. The 200 mg BID dose cohorts will be enrolled first. At least 2 males or females will be included in the 8 subjects (i.e., can be up to a 25%/75% split of male/female or 75%/25% split of male/female) assigned to each cohort.	This is an open-label study and no blinding procedures will be used. Following enrollment, subjects will be centrally assigned to one of 4 cohorts depending on CKD status (Table 5 1) and based on a predetermined algorithm. The 200 mg BID dose cohorts will be enrolled first. At least 2 males or females will be included in the 8 subjects (i.e., can be up to a 25%/75% split of male/female or 75%/25% split of male/female) assigned to each cohort. **Similarly, at least 2 subjects with CKD1 or CKD2 will be included in 8 subjects each assigned to Cohorts 1 and 3.**	Provides for flexibility to stop enrollment of CKD1 subjects in Cohorts 1 and 3 if sufficient experience with CKD2 subjects has not occurred.
36	5.8.1 – Permitted therapy	Lixivaptan has the potential to inhibit the metabolism of CYP3A4 and CYP2C8 substrates, including atorvastatin and simvastatin, therefore care should be exercised when administering lixivaptan in combination with these substrates. In order to minimize the potential for AEs	Lixivaptan has the potential to inhibit the metabolism of CYP3A4 and CYP2C8 substrates, including atorvastatin, ~~and~~ simvastatin, **and amlodipine.** Therefore, care should be exercised when administering lixivaptan in combination with these substrates. In	Because inhibition of CYP3A4 by lixivaptan may reduce metabolism of amlodipine, a dosing limit has been added.

Page	Section	Version 4 dated January 9, 2019	Version 5	Comments
		due to drug-drug interactions, prior to enrollment in the study, the Investigator, in consultation with the Medical Monitor, should consider potential dose adjustments or alternative therapeutic options that are not CYP3A4 or CYP2C8 substrates (e.g. rosuvastatin to replace simvastatin or atorvastatin) 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 < 20 mg daily in subjects who are administered the 50 mg BID dose of lixivaptan and < 10 mg daily in subjects who are administered the 200 mg BID dose of lixivaptan.	order to minimize the potential for AEs due to drug-drug interactions, prior to enrollment in the study, the Investigator, in consultation with the Medical Monitor, should consider potential dose adjustments or alternative therapeutic options that are not CYP3A4 or CYP2C8 substrates (e.g. rosuvastatin to replace simvastatin or atorvastatin) 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 < 20 mg daily in subjects who are administered the 50 mg BID dose of lixivaptan and < 10 mg daily in subjects who are administered the 200 mg BID dose of lixivaptan. **If amlodipine is needed for clinical care, the dose should not exceed 5 mg daily.**	
37	5.8.2 Prohibited Therapy	• Treatment with weak CYP3A4 or CYP2C8 inhibitors, including amlodipine, chlorzoxazone, cilostazol, fosaprepitant, istradefylline, ivacaftor, lomitapide, ranitidine, ranolazine, tacrolimus, ticagrelor, trimethoprim. For weak CYP3A4 or CYP2C8 inhibitors, medications may be allowed in consultation with the Medical Monitor;	• Treatment with weak CYP3A4 or CYP2C8 inhibitors, including ~~**amlodipine,**~~ chlorzoxazone, cilostazol, fosaprepitant, istradefylline, ivacaftor, lomitapide, ranitidine, ranolazine, tacrolimus, ticagrelor, trimethoprim. For weak CYP3A4 or CYP2C8 inhibitors, medications may be allowed in consultation with the Medical Monitor;	Amlodipine is a CYP3A4 substrate and not an inhibitor. Thus, it has been deleted from this section.

Page	Section	Version 4 dated January 9, 2019	Version 5	Comments
63	Table 21-1 Schedule of Assessments	Screening -30 to -3 or -2	Screening **-44** to -3 or -2	Increased duration of Screening from 4 weeks to 6 weeks
64	Footnote 5 to Table 12-1 - Schedule of Assessments	5. Clinical labs: hematology, clinical chemistry (including liver function), and urinalysis at approximately the same time of day (in the morning and, on dosing days, before the lixivaptan dose). The list of analytes is shown in Table 6-1. .	5. Clinical labs: hematology, clinical chemistry (including liver function), and urinalysis at approximately the same time of day (in the morning and, on dosing days, before the lixivaptan dose). The list of analytes is shown in Table 6-1. **Lab tests that need to be repeated during Screening may be obtained through the services of an at-home nursing service provided by the Sponsor.**	Provides for the use of a home nursing service to draw blood if repeat lab tests are needed during Screening.
65	Footnote 18 to Table 12-1 - Schedule of Assessments	If a patient does not return to the site for PK sample collection after discharge on Day 8, this will not be considered a protocol deviation.	If a patient does not return to the site for PK sample collection after discharge on Day 8, **but has the blood drawn by an at-home nursing service,** this will not be considered a protocol deviation.	Indicates that if a subject does not come back for visits after Day 8, then it is not a protocol deviation *only* if the blood is drawn by the at-home nursing service.

Study PA-102
Clinical Study Protocol version 4 dated January 9, 2019
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.

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 April 24, 2018	Version 4	Explanation
1	Title page	Version of Protocol: 3	Version of Protocol: **4**	Clarifies protocol version
1	Title page	Date of protocol: April 24, 2018	Date of protocol: **January 9, 2019**	Updates protocol date
1	Title page	Medical Monitor: Hooman Hajian, MD, MPH Medical Director, IQVIA 10188 Telesis Court, Suite 400 San Diego, CA 92121 Mobile: (425) 205 0946	Medical Monitor: **Andrew Lowy, MD, FRACP** **Medical Director, IQVIA** **Direct: (984) 201-6550 (including urgent medical issues)** **Mobile: (310) 431-7666** **Fax: +1 (520) 829-9405** **Email: Andrew.Lowy@quintiles.com** **IQVIA 24/7 Medical Emergency Contact Center (MECC):** **(973) 659-6677;** **(512) 652-0191 (back-up)**	Updates Medical Monitor name and contact information. Adds contact information for Medical Emergency Contact Center.

Page	Section	Version 3 dated April 24, 2018	Version 4	Explanation
All	Footnote	24-Apr-2018	**09-Jan-2019**	Updates protocol date
All	Footnote	Version 3	Version **4**	Clarifies protocol version
3	Protocol approval – Sponsor signature	Version of Protocol: 3	Version of Protocol: **4**	Clarifies protocol version
3	Protocol approval – Sponsor signature	Protocol date April 24, 2018	Protocol date **January 9, 2019**	Updates protocol date
3	Protocol approval – Sponsor signature	Signature date April 24, 2018	Signature date **January 9, 2019**	Updates protocol date
4	Declaration of investigator	…the accompanying Investigator Brochure (IB), version 15, dated March 12, 2018.	…the accompanying current version of the Investigator Brochure (IB).	Updates version of Investigator Brochure by referring to the current version, which is documented in the trial master file.
11	Synopsis	Date of protocol: April 24, 2018	Date of protocol: **January 9, 2019**	Updates protocol date
28	4.1.2. – Exclusion criteria			Updated exclusion criteria numbering to reflect insertion of one additional exclusion criterion at position 26 in the list

Text Clarifications

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

Page	Section	Version 3 dated April 24, 2018	Version 4	Explanation
26	4.1.2 – Exclusion criteria	1. Subjects with known sensitivity or idiosyncratic reaction to any compound present in lixivaptan, its related compounds such as benzazepines (e.g., tolvaptan, conivaptan, benazepril, fenoldopam, **mesylate,** or mirtazapine), or any compound listed as being present in the study formulation.	1. Subjects with known sensitivity or idiosyncratic reaction to any compound present in lixivaptan, its related compounds such as benzazepines (e.g., tolvaptan, conivaptan, benazepril, fenoldopam, or mirtazapine), or any compound listed as being present in the study formulation.	Deletion of *mesylate*, the counter-ion of fenoldopam
27	4.1.2 – Exclusion criteria	18. Subjects have experienced an illness that is considered by the Investigator to be clinically significant within 2 weeks of study drug administration on Day 1.	19. Subjects **who** have experienced an illness that is considered by the Investigator to be clinically significant within 2 weeks of study drug administration on Day 1.	
39	6.1.6 – MRI Assessment	Given the importance of performing the second MRI assessment within a short time interval after the last dose of study medication (PM dose on Day 7), all MRI assessments will be scheduled for each subject in conjunction with the Screening visit.	Given the importance of performing the second MRI assessment within a short time interval after the last dose of study medication (PM dose on Day 7), **it is recommended that** all MRI assessments be scheduled for each subject **at the time of** the Screening visit.	
61	12 - APPENDICES	This study uses the 2009 CKD-EPI creatinine equation,[16] which is recommended by the KDIGO Clinical Practice Guidelines for Management of Chronic Kidney Disease[3].	This study uses the 2009 CKD-EPI creatinine equation,16 which is recommended by the KDIGO Clinical Practice Guidelines for Management of Chronic Kidney Disease.	Deletion of reference at end of sentence.

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 April 24, 2018	Version 4	Comments
9	Synopsis	The subjects will be male or female, between 18 and 60 years of age.	The subjects will be male or female, between 18 and **65** years of age.	Increases the eligible age for inclusion into the study to 65 years.
22	3.1 - Study Design	Subjects will be allowed to re-screen for the study for up to two times.	Subjects **may be allowed to re-screen or have repeat testing after agreement between the investigator and medical monitor.**	Introduces more flexibility in screening procedures.
24	4.1.1 – Inclusion Criteria	2. Male or female, between 18 and 60 years of age (inclusive) at the time of Screening.	2. Male or female, between 18 and **65** years of age (inclusive) at the time of Screening.	Increases the eligible age for inclusion into the study to 65 years.
25	4.1.1 – Inclusion Criteria	Double barrier methods of non-hormonal contraception are permitted in this study. Acceptable forms of contraception include the following: • intrauterine device	Double barrier methods of non-hormonal contraception are permitted in this study. Acceptable forms of contraception include the following: • intrauterine device**, including Mirena®**	Clarifies that use of the low progesterone-releasing intrauterine device Mirena is allowed.
25	4.1.1 – Inclusion Criteria	Hormonal contraception is not permitted in this pharmacokinetic study due to the potential for	**Estrogen-based** hormonal contraception is not permitted in this pharmacokinetic study due to	Limits hormonal contraception exclusion to estrogen-containing contraceptives. Progesterone-only

Page	Section	Version 3 dated April 24, 2018	Version 4	Comments
		interaction with lixivaptan.	the potential for interaction with lixivaptan.	contraceptives are allowed.
27	4.1.2. – Exclusion criteria	15. Subjects with aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP) or total bilirubin ≥ 1.0 × upper limit of normal (ULN) at Screening.	15. Subjects with aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP) or total bilirubin ≥ 1.0 × upper limit of normal (ULN) at Screening. **Subjects with elevated bilirubin due to Gilbert's Syndrome may be allowed at the discretion of the Investigator.**	Allows inclusion of subjects with elevated bilirubin due to Gilbert's Syndrome at the discretion of the Investigator.
27	4.1.2. – Exclusion criteria	17. Subjects with contraindications to or interference with MRI assessments (ferromagnetic metal prostheses, aneurysm clips, severe claustrophobia, or large abdominal/back tattoos. Investigator should seek MRI safety guidance from local MRI facility).	18. Subjects with contraindications to or interference with MRI assessments (ferromagnetic metal prostheses, aneurysm clips, severe claustrophobia, or large abdominal/back tattoos) **will be excused from this procedure, but may participate in the study providing that all other eligibility criteria are met.** Investigator should seek MRI safety guidance from local MRI facility.	Allows inclusion of subjects with contraindications to or interference with MRI assessments provided they meet all other inclusion criteria. Clarifies that no MRI tests will be conducted on these subjects.
28	4.1.2. – Exclusion criteria		**26. Subjects who have experienced elevated AST or ALT (defined as ≥ 2.0 × ULN) while taking tolvaptan.**	Adds exclusion of subjects who have shown signs of liver toxicity on tolvaptan therapy.

Page	Section	Version 3 dated April 24, 2018	Version 4	Comments
32	5.4 – Identity of Investigational Product	Batch 17120A	Batch **17121A1**	Clarifies batch number of investigational product.
37	5.9 - Diet, Fluid, and Activity Control	Due to the expected aquaretic effects of lixivaptan, subjects should be reminded to maintain adequate fluid intake and be mindful of their thirst status at each study visit. Subjects should be instructed to replenish fluids with each void and aim to drink 4-5 liters of fluid per day, or as needed to maintain adequate hydration.	**During Days -2 and -1 of CRU confinement, subjects should continue consuming their usual amount of fluids.** Due to the expected aquaretic effects of lixivaptan, subjects should be reminded to maintain adequate fluid intake and be mindful of their thirst status at each study visit **starting with Day 1 of CRU confinement**. Subjects should be instructed to replenish fluids with each void and aim to drink 4-5 liters of fluid per day, or as needed to maintain adequate hydration, **while taking lixivaptan**.	Clarifies fluid intake recommendations before the first dose of study drug and while taking study drug.
39	6.1.4 - 12-Lead Electrocardiograms	Electrocardiograms will be obtained with the subject remaining in a semi-recumbent position following 5 minutes of rest.	Electrocardiograms will be obtained with the subject remaining in a **supine or** semi-recumbent position following 5 minutes of rest. **All electrocardiograms 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.**	Allows supine position for electrocardiograms provided that the same position is maintained for the same subject throughout the study.

Page	Section	Version 3 dated April 24, 2018	Version 4	Comments
40	6.1.6 – MRI Assessment		**Occasionally, patients may be unable to continue into the study after completing their Screening visit and baseline MRI assessment (for example, due to personal circumstances or for not meeting laboratory inclusion criteria). These patients may subsequently qualify for the study and be rescreened at a later time. In this case, their prior baseline MRI assessment may be used instead of obtaining a new baseline MRI scan; provided, however, that no more than 8 weeks have elapsed from the time of the initial MRI assessment until admission into the CRU.**	Clarifies that the baseline MRI assessment completed by patients who subsequently screen fail or are otherwise unable to continue into the study is valid for a period of 8 weeks. Patients who successfully rescreen must be admitted into the CRU within 8 weeks from the original MRI assessment date for such MRI to be considered valid.
41	6.1.6 – MRI Assessment	Subjects with ferro-magnetic prostheses, aneurysm clips, severe claustrophobia or other contraindications or exclusions interfering with MRI measurements will be excluded from study participation.	Subjects with ferro-magnetic prostheses, aneurysm clips, severe claustrophobia or other contraindications or exclusions interfering with MRI measurements **will be excused from this procedure, but may participate in the study providing that all other eligibility criteria are met. Investigator should seek MRI safety guidance from the local MRI facility**.	Allows inclusion of subjects with contraindications to or interference with MRI assessments provided they meet all other inclusion criteria. Clarifies that no MRI tests will be conducted on these subjects.

Page	Section	Version 3 dated April 24, 2018	Version 4	Comments
62	Footnote 2 to Table 12-1 - Schedule of Assessments	2. Serum pregnancy test to be performed at Screening; Urine pregnancy test to be performed at Day -2 or Day -1.	2. Serum pregnancy test to be performed at Screening; Urine pregnancy test to be performed at Day -2 or Day -1 **and at End of Study**.	Clarifies nature of pregnancy test at End of Study visit.
63	Footnote 7 to Table 12-1 – Schedule of Assessments	Electrocardiograms to be obtained following 5 minutes rest in semi-recumbent position.	Electrocardiograms to be obtained following 5 minutes rest in **supine or** semi-recumbent position. **The same position must be used throughout the study for a given subject.**	Allows supine position for electrocardiograms provided that the same position is maintained for the same subject throughout the study.
63	Table 12-2 - Assessment Windows. PK Blood Samples – Assessment Day	Day 1, Day 2, Day 4, Day 6, Day 7, Day 8, Day 9	Day 1, Day 2, Day 4, Day 6, Day 7, Day 8, Day 9, **Day 11**	Adds Day 11 to the Assessment Window table.
65	Table 12-2 - Assessment Windows. PK Blood Samples – Window (units)	- 60 minutes (predose Day 1) - 10 minutes (predose Day 7) ± 3 min (< 1-4 hr) ± 5 min (> 4-12 hr) ± 10 min (> 12-24 hr) ± 1 hr (> 24-48 hr) ± 2 hr (> 48-96 hr)	- 60 minutes (predose Day 1) - 10 minutes (predose Day 7) ± 3 min **(≤ 4 hr)** ± 5 min **(> 4 hr but ≤ 12 hr)** ± 10 min **(> 12 hr but ≤ 24 hr)** ± 1 hr **(> 24 hr but ≤ 48 hr)** ± 2 hr **(> 48)**	Clarifies the appropriate time window for time values that lie on the extremes of each time interval.