Clinical Protocol REN-003

A Phase 1b dose-escalating study with Stannous Protoporphyrin (SnPP), in healthy volunteers and subjects with chronic kidney disease stage 3-4

IND 138109

SPONSOR

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Appendices

1 STUDY TITLE

A Phase 1b dose-escalating study with Stannous Protoporphyrin (SnPP), in healthy volunteers and subjects with chronic kidney disease stage 3-4

2 SPONSOR INFORMATION

2.1 Sponsor:

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_____ _____

Bhupinder Singh, MD, FASN, FNKF, FCRS Date
Chief Scientific Officer and Medical Monitor
Renibus Therapeutics, Inc.

3 LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	Adverse event
AKI	acute kidney injury
ALT	alanine aminotransferase
ARB	angiotensin-receptor blockers
AST	aspartate aminotransferase
$AUC_{(0-t)}$	area under the concentration-time curve from time 0 to t
$AUC_{(0-inf)}$	area under the concentration-time curve from time 0 to infinity
BUN	blood urea nitrogen
CFR	Code of Federal Regulations
CIOMS	Council for International Organizations of Medical Sciences
CKD	chronic kidney disease
CL	clearance
C_{max}	maximum serum or plasma concentration
Cr	creatinine
CRO	contract research organization
ECG	electrocardiogram
eCRF	electronic case report form
EDC	electronic data capture
FDA	Food and Drug Administration
FeS	Iron sucrose
GCP	Good Clinical Practice
GFR	glomerular filtration rate
GLP	Good Laboratory Practice
HO-1	Heme oxygenase
ICF	informed consent form
ICH	International Conference on Harmonisation
ICSR	Individual Case Safety Report
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IV	intravenous
Ll-6	Interleukin 6
IL-10	Interleukin 10
IND	investigational new drug
IRB	Institutional Review Board
ITT	Intent-to-Treat
KDIGO	Kidney Disease: Improving Global Outcomes
KIM-1	Kidney Injury Molecule-1

Abbreviation	Definition
λz	elimination rate constant
MedDRA	Medical Dictionary for Regulatory Activities
MITT	Modified Intent-to-Treat
NAG	N acetyl-beta-D-glucosaminidase
NGAL	Neutrophil Gelatinase-Associated Lipocalin
NOAEL	no-observed-adverse-effect level
PK	Pharmacokinetic(s)
POC	Proof of Concept
PP	Per Protocol
SnPP	Stannous Protoporphyrin
TEAE	treatment emergent adverse event
$T_{½}$	elimination half-life
Tmax	time to maximum serum concentration
Vz	volume of distribution

4 STUDY SYNOPSIS

Renibus Therapeutics, Inc.	**Protocol No. REN-003**
Name of Drug: Stannous Protoporphyrin (SnPP)	**Phase of Development:** 1b
Name of Active Ingredient: Stannous protoporphyrin (SnPP)	**Date of Study Synopsis** 25 March 2019
Protocol Title: A Phase 1b dose-escalating study with Stannous Protoporphyrin (SnPP), in healthy volunteers and subjects with chronic kidney disease stage 3-4.	
Investigational Sites: One contract research organization in the US.	
Study Objectives: The primary objectives of the study are to evaluate: 1. The safety and tolerability of SnPP in healthy volunteers and in subjects with stage 3-4 chronic kidney disease (CKD). 2. The effect of SnPP on biomarkers which are potential surrogates for cytoprotective activity in healthy volunteers and in subjects with stage 3-4 (CKD). 3. To establish the optimal dose that could potentially be used in combination with iron sucrose for testing in subjects at risk for developing acute kidney injury (AKI). 4. To study the Pharmacokinetics of SnPP	
Number Subjects to be Enrolled: Up to a total of 42 subjects consisting of 18 healthy volunteers and 24 subjects with chronic kidney disease will receive single escalating doses of SnPP. 42 subjects consisting of 18 healthy and 24 subjects with stage 3-4 CKD will be enrolled in one of three dose levels starting at the lowest dose of 9 mg SnPP for the Healthy volunteers and starting at the lowest dose of 27 mg SnPP for 3-4 CKD patients.	
Study Design: This is a Phase 1b, single-center, dose-escalating study to evaluate the safety, tolerability, PK and pharmacodynamic effect of SnPP in healthy volunteers and in subjects with stage 3-4 CKD. The following biomarkers will be used as surrogate measures of cytoprotective activity: Haptoglobin, Ferritin, Bilirubin, Hemopexin, IL-10, and Heme Oxygenase-1. Additionally, the P21 biomarker will be monitored at various points of the study as well as urine biomarker response to SnPP. Screening data will be reviewed to determine subject eligibility. Subjects who meet all inclusion criteria and none of the exclusion criteria are eligible to be enrolled into the study. The study is designed as follows: • A screening period with baseline evaluations for study eligibility. • Baseline for various biomarkers will be established by three different measurements in blood and urine prior to dosing. • If subjects meet eligibility criteria, they will receive a single dose of SnPP by slow intravenous injection over a 120-minutes on study day (SD) 1.	

- Subjects will stay in the clinic for the initial 24 hours after administration of SnPP and will be discharged at the end of SD 2 if no safety issues appear.
- The study will initially be dose-escalating in 3 groups of 6 healthy volunteers each, for a total of 18 healthy volunteers, evaluating escalating single dose of SnPP starting with a dose of 9 mg SnPP, followed by 27 mg SnPP, and 90 mg SnPP. Dose-escalation from one dose level to the next will only be allowed after a careful review of the safety, tolerability and PK data from the prior dose group as determined by the Data and Safety Monitoring Board (DSMB).
- After a careful review of the safety, tolerability and PK data from all dose groups from the healthy volunteer cohorts, the Data and Safety Monitoring Board (DSMB) will determine whether to proceed to the CKD cohorts. If the determination by the DSMB allows to proceed to the CKD cohorts, subjects with stage 3 CKD will be in 2 groups of 6 each, for a total of 12 subjects, evaluating escalating single dose of SnPP starting with a dose of 27 mg SnPP followed by 90 mg SnPP. Dose-escalation from one dose level to the next will only be allowed after a careful review of the safety, tolerability and PK data from the prior dose group as determined by the Data and Safety Monitoring Board (DSMB).
- After a careful review of the safety, tolerability and PK data from all dose groups from the stage 3 CKD cohorts, the Data and Safety Monitoring Board (DSMB) will determine whether to proceed to the stage 4 CKD cohorts. If the determination by the DSMB allows to proceed to the stage 4 CKD cohorts, subjects with stage 4 CKD will be in 2 groups of 6 each, for a total of 12 subjects, evaluating escalating single dose of SnPP starting with a dose of 27 mg SnPP followed by 90 mg SnPP. Dose-escalation from one dose level to the next will only be allowed after a careful review of the safety, tolerability and PK data from the prior dose group as determined by the Data and Safety Monitoring Board (DSMB).
- Subjects will be followed for 28 days after the dose of SnPP. Response biomarkers will be assessed at baseline and 2, 4, 8, 12, 18, 24, 48, 72, 96 and 168 hours after study drug administration. Safety will be assessed for up to 28 days after study drug administration.
- Blood samples for SnPP pharmacokinetic analysis will be assessed at baseline and 1, 2, 3, 4, 6, 8, 12, 18, and 24 hours after study drug administration. These data will be used in the dose escalation decision making review process along with the safety and tolerability of the previous dose. Urine samples will be collected and centrifuged and analyzed for subject responsiveness to SnPP therapy.
- Urinary Clearance of SnPP will be assessed by the measurement of tin in a 24-hour urine sample
- Total study duration will be 29 days for each subject, in addition to a screening period of up to 14 days.

Endpoints:

Pharmacodynamic/Safety Assessment:

The safety and tolerability of SnPP will be assessed by determining the incidence, relationship to study drug, and severity of treatment-emergent adverse events (TEAEs), withdrawals due to adverse events (AEs), and changes in vital signs, laboratory tests, changes in concomitant medication use due to AEs, and other standard safety parameters. In addition, Blood Urea Nitrogen (BUN), creatinine, Cystatin-C, Kidney Injury Molecule-1 (KIM-1), Neutrophil Gelatinase-Associated Lipocalin (NGAL), albuminuria, N acetyl-beta-D-glucosaminidase (NAG), liver enzymes, and troponin I.

Biomarker Monitoring

The following biomarkers will be evaluated: Haptoglobin (Hp), Ferritin and bilirubin, Hemopexin, IL-10, Heme Oxygenase 1 (HO-1) and P21, as well as urine biomarker response to SnPP. Biomarkers will be measured at baseline and 2, 4, 8, 12, 18, 24, 48, 72, 96 and 168 hours after administration of SnPP. All biomarkers will be measured at the same time points.

Healthy Volunteer Subjects

Inclusion Criteria:

1. Male and female subjects age 18 to 80 years (inclusive, at time of ICF).
2. Body weight <125 kg.
3. Able and willing to comply with all study procedures.
4. Female subjects must be either post-menopausal for at least 1 year or surgically sterile (tubal ligation, hysterectomy or bilateral oophorectomy) for at least 3 months, or if of childbearing potential, have a negative pregnancy test and agree to use dual methods of contraception, or abstain from sexual activity from Screening until 28 days after study drug administration.

   Male subjects with female partners of childbearing potential must agree to use a highly effective method of contraception from screening until 28 days after study drug administration. All fertile men with female partners of childbearing potential should be instructed to contact the Investigator immediately if they suspect their partner might be pregnant (e.g., missed or late menstrual period) at any time during study participation.

Exclusion Criteria:

1. History of malignancy except carcinoma in situ in the cervix, early stage prostate cancer or non-melanoma skin cancers. Cancer free for less than five years.
2. Use of investigational drugs or participation in another clinical trial within 30 days or <5 half-lives prior to screening, whichever is longer.
3. Serum ferritin > 500 ng/ml or who have received IV iron within 28 days of screening.
4. Women who are pregnant, breastfeeding, or planning to become pregnant while participating in the study.
5. Any significant acute or chronic diseases.
6. Subjects with abnormal baseline liver tests or hepatitis serologies that suggest active infection.
7. Regular use of drugs of abuse and/or positive findings on urinary drug screening.
8. Current tobacco use and/or positive findings on urinary cotinine screening.
9. Subjects who are severely physically or mentally incapacitated and who, in the opinion of investigator, are unable to perform the subjects' tasks associated with the protocol.
10. Presence of any condition which, in the opinion of the investigator, places the subject at undue risk or potentially jeopardizes the quality of the data to be generated.
11. Subjects with history of photosensitivity or active skin disease, which in the opinion of the investigator could increase the risk of photosensitivity.
12. Known hypersensitivity or previous anaphylaxis to SnPP or tin based product.

CKD Subjects

Inclusion Criteria:

1. Male and female subjects age 18 to 80 years (inclusive, at time of ICF).
2. Body weight <125 kg.
3. Able and willing to comply with all study procedures.
4. Female subjects must be either post-menopausal for at least 1 year or surgically sterile (tubal ligation, hysterectomy or bilateral oophorectomy) for at least 3 months, or if of childbearing potential, have a negative pregnancy test and agree to use dual methods of contraception, or abstain from sexual activity from Screening until 28 days after study drug administration.

   Male subjects with female partners of childbearing potential must agree to use a highly effective method of contraception from screening until 28 days after study drug administration. All fertile men with female partners of childbearing potential should be instructed to contact the Investigator immediately if they suspect their partner might be pregnant (e.g., missed or late menstrual period) at any time during study participation.
5. CKD stage 3 as determined by a GFR between 30-59 ml/min as estimated using the CKD-EPI equation.
6. CKD stage 4 as determined by a GFR between 15-29 ml/min as estimated using the CKD-EPI equation.

Exclusion Criteria:

1. History of malignancy except carcinoma in situ in the cervix, early stage prostate cancer or non-melanoma skin cancers. Cancer free for less than 5 years.
2. Use of investigational drugs or participation in another clinical trial within 30 days or 5 half-lives prior to screening, whichever is longer.
3. Serum ferritin > 500 ng/ml or who have received IV iron within 28 days of screening, or currently being treated with oral iron.
4. Women who are pregnant, breastfeeding, or planning to become pregnant while participating in the study.
5. Regular use of drugs of abuse and/or positive findings on urinary drug screening.
6. Current tobacco use and/or positive findings on urinary cotinine screening.
7. Subjects who are severely physically or mentally incapacitated and who, in the opinion of investigator, are unable to perform the subjects' tasks associated with the protocol.
8. Presence of any condition which, in the opinion of the investigator, places the subject at undue risk or potentially jeopardizes the quality of the data to be generated.
9. Subjects with history of photosensitivity or active skin disease, which, in the opinion of the investigator could increase the risk of photosensitivity.
10. Subjects with abnormal baseline liver tests or hepatitis serologies that suggest active infection.
11. Subjects with CKD and not at their baseline of renal function and have significant acute or chronic illnesses.
12. Known hypersensitivity or previous anaphylaxis to SnPP or to components thereof.

Drug, Dose and Mode of Administration:

SnPP is supplied as a sterile liquid in vials for intravenous (IV) injection and intended for single use. Glass vials are plugged with Teflon-coated rubber stoppers and sealed with aluminum seals. Vials of 5 mL will be used for each cohort.

SnPP at a dose of either 9 mg SnPP, 27 mg SnPP, or 90 mg SnPP will be administered on SD 1. The infusions will be given using a syringe pump over a 120-minute period.

Rationale for the Dose and Schedule Selection:

The proposed SnPP doses and administration schedules are based on previous human studies with SnPP and preclinical pharmacology and toxicology data. The proposed starting dose of 9 mg SnPP is 1/10 the NOAEL in rats and 1/36 the NOAEL in dogs based on body surface scaling in accordance with the conversion factors in Appendix A of the Guidance for Industry Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers (2005) and a human body weight of 60 kg, and has previously been administered safely to human subjects.

Study Duration:

Study duration is for 29 days per subject.

Criteria for Evaluation:

Biomarkers

- Key parameters: Haptoglobin, Ferritin, Hemopexin, IL-10, Heme Oxygenase 1 (HO-1) and Bilirubin. Urine samples will be collected and centrifuged and analyzed for subject responsiveness to SnPP therapy.
- Additional secondary parameters: P21

Pharmacodynamic/Safety parameters

- Blood Urea Nitrogen (BUN), creatinine, Cystatin-C, Kidney Injury Molecule-1 (KIM-1), Neutrophil Gelatinase-Associated Lipocalin (NGAL), albuminuria, N acetyl-beta-D-glucosaminidase (NAG), liver enzymes and troponin I at regular intervals
- Adverse events, serious adverse events, suspected adverse reactions, and serious unexpected suspected adverse reactions
- Laboratory safety data, vital signs, and temperature at regular intervals

Pharmacokinetics

- SnPP on SD 1

Study Hypothesis:

It is hypothesized that SnPP is safe and well tolerated up to the top dose of 90 mg SnPP. It is also hypothesized that SnPP will modulate biomarker levels in a dose-related fashion.

Statistical Considerations

Sample Size: No formal sample size calculation has been performed for this phase 1b study. However, it is believed that six subjects per dose group in the healthy volunteer cohorts (3) and the CKD stages 3 & 4 cohorts (2 each) will be adequate to establish POC based on biomarker response and identify an optimal dose for further testing, recognizing that a battery of different surrogate biomarkers will be evaluated

Analysis Populations and General Methodology: All subjects who receive study medication will be included in the Safety population for safety analysis. Subjects who receive study medication and have a post-baseline efficacy assessment will be included in the Modified Intent-

to-Treat (MITT) population for efficacy analysis. All subject data will be listed. When appropriate, summary statistics will be provided (number of non-missing values, mean, median, standard deviation, minimum, and maximum for continuous variables and number and percentage of subjects for categorical variables) by treatment group for all measures, including demographic and baseline assessments, safety, and efficacy endpoints. Baseline for all safety and efficacy endpoints is defined as the last observations before administration of the first IV infusion of study drug. No imputation will be used for missing data.

Safety Analysis: Safety measures including AEs, infusion-related reactions, clinical laboratory tests, vital signs, physical exams, and concomitant medication usage will be summarized descriptively by treatment group and study visit, where appropriate.

Biomarker Analysis: Biomarker response will be summarized by treatment group and study visit, comparing change from baseline at 2, 4, 8, 12, 18, 24, 48, 72, 96 and 168 hours after start of infusion.

Percent change from baseline will be analyzed using the mixed model for repeated measures (MMRM). The between-group comparison will be performed using the simple contrast at 2, 4, 8, 12, 18, 24, 48, 72, 96 and 168 hours post-dosing

In addition to absolute and percent changes from baseline, a responder analysis will also be considered. The responder definition and its appropriate analysis methodology will be detailed in the statistical analysis plan (SAP) for the study. Exploratory analysis using Cochran-Mantel-Haenszel test stratified by baseline or primary diagnosis will be carried out.

Pharmacokinetic Analysis: Pharmacokinetic parameters of each treatment group will be estimated using a non-compartmental analysis following completion of each dose and prior to dose escalation. The maximum serum concentration (C_{max}) and time to maximum serum concentration (Tmax) will be obtained directly from the data. The elimination rate constant (λz), elimination half-life ($T_{½}$), area under the concentration-time curve from time 0 to time t ($AUC_{[0-t]}$) and area under the concentration-time curve from time 0 to infinity ($AUC_{[0-inf]}$), clearance (CL), and volume of distribution (Vz) will be calculated for each subject and summarized by treatment group. The renal excretion and renal clearance (CL) of tin will also be calculated.

A compartmental model may be fit to the data if appropriate and used to predict plasma concentration-time profiles for other doses and regimens

5 EVENT SCHEDULE

5.1 Event Schedule – All subjects

Study Day	**Screening**	**1**	**2,3,4,5,8[1]**	**14[**]**	**29/End of Study[1]**
Parameter	**Within 14 days prior to first dose**				
Written informed consent	X				
Eligibility criteria	X				
Demographics	X				
Medical history	X				
Physical examination and weight	X	X[2]			X
Electrocardiogram	X	X[2,3]			X
Vital signs	X	X[2]	X		X
Serum chemistry[8,9]	X	X[2]	X		X
Hematology[8,9]	X	X[2]	X		X
Baseline determination[13]	X	X			
Response biomarkers[8,11]		X[2,4]	X		
Safety biomarkers[8,12]		X[2]	X	X	X
SnPP PK[4,11]		X			
Urinalysis including sediment[9]	X	X[2]	X		X
Urine pregnancy test[10]	X	X	X at Day 8		X
Urine drug/alcohol/cotinine screen	X				
Confinement in clinical testing unit[5]		X[5]			
SnPP infusion		X[6]			
Discharge from clinical testing unit			X[7]		
Prior/Concomitant medications	X	X	X		X
Adverse events		X	X		X
Investigational product reconciliation					X

[1] Day 8 visit will occur (± 1) days and End of Study (EOS) visit will occur 28 (± 1) days after administration of SnPP.
[2] Baseline parameters performed prior to dosing on Study Day 1.
[3] Two ECG's will be performed on Study Day 1: the first at Baseline (prior to infusion) and the second within 10 minutes of infusion completion.
[4] Blood samples for SnPP pharmacokinetic analysis will be assessed at baseline and 1, 2, 3, 4, 6, 8, 12, 18, and 24, hours (±15 minutes) after start of infusion. Additional biomarker samples will also be collected at 2, 4, 8, 12, 18 hours (±15 minutes) after start of SnPP. Urinary tin excretion will be measured on a 24-hour urine sample.
[5] Subjects will be confined in the clinical testing unit a minimum of 24 hours from the morning of Study Day 1 until after collection of the 24-hour post-dose sample on Study Day 2.
[6] SnPP will be administered by infusion over 120-minutes after Baseline ECG, vital signs and blood and urine samples are collected.
[7] Discharge from unit following evaluations and sample collection required on Study Day 2
[8] Measured fasting (nothing by mouth except water, coffee or tea, with or without milk and/or sugar, and essential medications only for a minimum of 8 hours prior to sample collection at the clinic).
[9] Parameters to be measured are detailed in Appendix 1.
[10] For women of childbearing potential using kits supplied by the laboratory
[11] Haptoglobin, Ferritin, Bilirubin, Hemopexin, IL-10, P21 and HO-1.
[12] Cystatin-C, IL-6, SnPP, total porphyrin, Tin, Troponin I, albuminuria, Kidney Injury Molecule-1 (KIM-1), Neutrophil Gelatinase-Associated Lipocalin (NGAL), N acetyl-beta-D-glucosaminidase (NAG), (in addition to BUN/Cr which are part of the Serum Chemistry panel)
[13] Three measurements will be taken for the urinary safety biomarkers (albuminuria, Kidney Injury Molecule-1 (KIM-1), Neutrophil Gelatinase-Associated Lipocalin (NGAL),and N acetyl-beta-D-glucosaminidase (NAG)), eGFR, and response biomarkers prior to dosing.

** Day 14 safety evaluation will be conducted at the request of the DSMB after each cohort safety review

6 INTRODUCTION

6.1 Background Information

Acute kidney injury (AKI) is an abrupt loss of kidney function in which the body accumulates waste products and is unable to maintain electrolyte, acid-base, and water balance [Tögel 2014]. It is a major risk factor for morbidity, mortality, and the development of chronic kidney disease (CKD) [Goldberg 2009, Ishani 2009, Liangos 2006, Wald 2009, Xue 2006]. A meta-analysis of 154 studies conducted worldwide in over 3.5 million subjects reported pooled incidence and mortality rates of AKI in hospitalized adult subjects of 21.6% and 23.9%, respectively [Susantitaphong 2013]. In cardiac surgery subjects, the incidence of postoperative AKI is approximately 30% and significantly increases the risk of death after cardiac surgery [Rosner 2006]. Progression to advanced-stage CKD within 24 months occurs in approximately 15 to 20% of subjects who survive AKI [Chawla 2012].

Many different types of exposure may cause AKI; however, the chance of developing AKI after exposure depends on a number of susceptibility factors that vary widely among individual subjects [Kellum 2013]. Sepsis, critical illness, circulatory shock, burns, trauma, cardiac surgery (particularly with cardiopulmonary bypass), major non-cardiac surgery, nephrotoxic drugs, radiocontrast agents, and poisonous plants and animals are all recognized as causes of AKI. Susceptibility factors that increase a subject's risk of developing AKI include dehydration or volume depletion, advanced age, female gender, Black race, chronic kidney disease, chronic disease (heart, lung, liver), diabetes mellitus, cancer, and anemia. AKI can lead to a number of complications, including metabolic acidosis, hyperkalemia, uremia, changes in body fluid balance, and effects on other organ systems, including need for dialysis and possibly death. People who have experienced AKI have an increased risk of developing chronic kidney disease possibly requiring dialysis in the future. The annual cost to the US health care system is estimated at approximately $15B [Silver 2017].

The diagnosis of AKI is based on the following criteria introduced by the Kidney Disease: Improving Global Outcomes (KDIGO) AKI Work Group in 2012 [KDIGO 2012]: increase in serum creatinine $\geq$ 0.3 mg/dL within 48 hours; or increase in serum creatinine $\geq$ 1.5 times baseline, which is known or presumed to have occurred within the prior 7 days; or urine volume $<$ 0.5 mL/kg/hour for 6 hours. The management of AKI is purely symptomatic, with the aim of limiting damage and preventing further loss of function. No pharmacologic therapy is approved for the treatment or prevention of AKI.

It has long been recognized that after an initial ischemic or toxic injury, the kidney develops marked resistance to subsequent damage [Honda 1987, Zager 1984, Zager 1995a, Zager 1995b, Zager 1995c]. This phenomenon, which is mediated in part by an upregulation of renal cytoprotective and anti-inflammatory stress proteins (e.g., heme oxygenase 1 [HO-1], ferritin, haptoglobin, hemopexin, alpha 1 antitrypsin, interleukin 10 [IL-10]), has been referred to as "ischemic preconditioning" or "acquired cytoresistance." This phenomenon could be used in a clinical setting to preemptively protect organs, especially when a known insult is approaching.

6.2 Study Drug

A pharmacological intervention with the ability to stimulate the kidney's natural protective pathways to prevent AKI would represent a significant medical advance. SnPP is under development as a prophylactic agent to induce cytoresistance in organs when a known insult is imminent. The initial target for development is the kidneys and prevention of AKI. However, the phenomenon of acquired cytoresistance is also relevant to other bodily organs such as the heart and liver, and will be the focus of extended development of SnPP in combination with other agents, more specifically, Iron Sucrose (FeS.)

Stannous protoporphyrin, a potent competitive inhibitor of heme oxygenase activity, is not commercially available, a 45 mg dose has been safely administered to healthy volunteers [Anderson 1986, Berglund 1988, Emtestam 1993], term newborns with hyperbilirubinemia [Kappas 1988], and adult subjects with hepatic dysfunction affecting heme metabolism or bilirubin conjugation [Anderson 1986, Berglund 1990] or porphyria [Dover 1993, Galbraith 1989].

6.3 Preclinical Experience

Initial non-clinical pharmacology and toxicology studies have been conducted and are detailed in the Investigator's Brochure. Single-extended acute dose toxicity studies of intravenous SnPP demonstrated no-adverse-effect-levels of 10 mg/kg SnPP in the rat (human equivalent does of 96 mg) and the dog (human equivalent dose of 324 mg).

6.4 Clinical Experience

Safety data from intravenous SnPP administration at doses up to 3 mg/kg has shown photosensitivity in subjects when exposed to sunlight or UV-A light following dosing [Anderson 1986, Berglund 1990, Dover 1993, Galbraith 1989, Kappas 1988]. No other adverse effects of SnPP administration have been consistently reported in the literature.

7 STUDY OBJECTIVES

7.1 Primary Objectives

The primary objectives of the study are to evaluate:

1. The safety and tolerability of SnPP in healthy volunteers and in subjects with stage 3-4 CKD.
2. The effect of SnPP on biomarkers which are potential surrogates for cytoprotective activity in healthy volunteers and in subjects with stage 3-4 CKD.
3. To establish the optimal dose for testing in subjects at risk for developing AKI.
4. To study the Pharmacokinetics of SnPP

8 INFORMED CONSENT

The investigator or designee will be responsible for obtaining a signed, written informed consent form (ICF) and providing a copy to each subject, legally authorized guardian, or a person with legal responsibility for the subject's health care decisions prior to the performance of any clinical activities or procedures pursuant to this protocol. Subjects who are vision impaired may have the ICF read to them and their witnessed consent documented. Only the consent form approved by the Institutional Review Board (IRB)/Independent Ethics Committee (IEC) will be used. If English is NOT the subject's primary language, the subject will be consented using an IRB-approved ICF in the requisite language. This consent will be conducted by a member of the research team who is fluent in the language and thus able to answer any scientific or procedural questions raised by a non-English speaking subject.

Subject identity should be confirmed by the presentation of a photo identification to ensure the correct individual is consented, screened, and enrolled (if eligible).

Only the PID, subject initials, and demographics will be recorded in the eCRF. If the subject name appears on any source document collected (e.g., hospital discharge summary), it must be removed from the document if the document will be viewed by the Sponsor or a sponsor-contracted study vendor not permitted access to subject identifying information. All study findings will be stored in electronic databases. The subjects will give explicit written permission for representatives of the Sponsor, regulatory authorities, and the IRB to inspect their medical records to verify the information collected. Subjects will be informed that all personal information made available for inspection will be kept confidential to the extent permitted by all applicable state, local, and federal data protection/privacy laws and/or regulations and will not be made publicly available. If the results of the trial are published, the subject's identity will remain confidential.

At study check-in to the study site, subjects will be advised not to share their study information with other subjects.

9 STUDY POPULATION

9.1 Healthy Volunteer (HV)

9.1.1 HV Inclusion Criteria

1. Male and female subjects age 18 to 80 years (inclusive, at time of signing the informed consent form [ICF]).
2. Body weight <125 kg.
3. Able and willing to comply with all study procedures.
4. Female subjects must be either post-menopausal for at least 1 year or surgically sterile (tubal ligation, hysterectomy or bilateral oophorectomy) for at least 3 months, or if of childbearing potential, have a negative pregnancy test and agree to use dual methods of contraception, or abstain from sexual activity from Screening until 28 days after study drug administration.

Male subjects with female partners of childbearing potential must agree to use a highly effective method of contraception from screening until 28 days after study drug administration. All fertile men with female partners of childbearing potential should be instructed to contact the Investigator immediately if they suspect their partner might be pregnant (e.g., missed or late menstrual period) at any time during study participation.

9.1.2 HV Exclusion Criteria

1. History of malignancy except carcinoma in situ in the cervix, early stage prostate cancer or non-melanoma skin cancers. Cancer free for less than 5 years
2. Use of investigational drugs or participation in another clinical trial within 30 days or 5 half-lives prior to screening, whichever is longer.
3. Serum ferritin > 500 ng/ml or who have received IV iron within 28 days of screening.
4. Women who are pregnant, breastfeeding, or planning to become pregnant while participating in the study.
5. Any significant acute or chronic diseases.
6. Subjects with abnormal baseline liver tests or hepatitis serologies that suggest active infection.
7. Regular use of drugs of abuse and/or positive findings on urinary drug screening.
8. Current tobacco use and/or positive findings on urinary cotinine screening.
9. Subjects who are severely physically or mentally incapacitated and who, in the opinion of investigator, are unable to perform the subjects' tasks associated with the protocol.
10. Presence of any condition which, in the opinion of the investigator, places the subject at undue risk or potentially jeopardizes the quality of the data to be generated.
11. Subjects with history of photosensitivity or active skin disease
12. Known hypersensitivity or previous anaphylaxis to SnPP.

9.2 CKD Subjects

9.2.1 CKD Inclusion Criteria

1. Male and female subjects age 18 to 80 years (inclusive, at time of ICF).
2. Body weight <125 kg.
3. Able and willing to comply with all study procedures.
4. Female subjects must be either post-menopausal for at least 1 year or surgically sterile (tubal ligation, hysterectomy or bilateral oophorectomy) for at least 3 months, or if of childbearing potential, have a negative pregnancy test and agree to use dual methods of contraception, or abstain from sexual activity from Screening until 28 days after study drug administration.
   Male subjects with female partners of childbearing potential must agree to use a highly effective method of contraception from screening until 28 days after study drug administration. All fertile men with female partners of childbearing potential should be

instructed to contact the Investigator immediately if they suspect their partner might be pregnant (e.g., missed or late menstrual period) at any time during study participation.

5. CKD stage 3 as determined by a GFR between 30-59 ml/min as estimated using the CKD-EPI equation.
6. CKD stage 4 as determined by a GFR between 15-29 ml/min as estimated using the CKD-EPI equation.

9.2.2 CKD Exclusion Criteria

1. History of malignancy except carcinoma in situ in the cervix, early stage prostate cancer or non-melanoma skin cancers. Cancer free for less than 5 years.
2. Use of investigational drugs or participation in another clinical trial within 30 days or 5 half-lives prior to screening, whichever is longer.
3. Serum ferritin > 500 ng/ml or who have received IV iron within 28 days of screening.
4. Women who are pregnant, breastfeeding, or planning to become pregnant while participating in the study.
5. Regular use of drugs of abuse and/or positive findings on urinary drug screening.
6. Current tobacco use and/or positive findings on urinary cotinine screening.
7. Subjects who are severely physically or mentally incapacitated and who, in the opinion of investigator, are unable to perform the subjects' tasks associated with the protocol
8. Presence of any condition which, in the opinion of the investigator, places the subject at undue risk or potentially jeopardizes the quality of the data to be generated.
9. Subjects with abnormal baseline liver tests or hepatitis serologies that suggest active infection.
10. Subjects with CKD and not at their baseline of renal function and have significant acute or chronic illnesses.
11. Subjects with history of photosensitivity or active skin disease
12. Known hypersensitivity or previous anaphylaxis to SnPP.

9.3 Study Design

This is a Phase 1b, single-center, dose-escalating study to evaluate the safety, tolerability, PK and pharmacodynamic effect of SnPP in healthy volunteers and in subjects with stage 3-4 CKD. The following biomarkers will be used as surrogate measures of protective activity: Haptoglobin, Ferritin, Bilirubin, Hemopexin, IL-10, P21 and HO-1.

Screening data will be reviewed to determine subject eligibility. Subjects who meet all inclusion criteria and none of the exclusion criteria are eligible to be enrolled into the study. A baseline for safety and response biomarkers will be established by taking three measurements between screening and prior to dosing.

The study is designed as follows:

- A screening period with baseline evaluations for study eligibility.

- If subjects meet eligibility criteria, they will receive SnPP by direct "push" infusion over a 120-minute period on SD 1.
- Subjects will stay in the clinic for the initial 24 hours after administration of SnPP and will be discharged on SD 2 if no safety issues appear.
- The study will initially be dose-escalating in 3 groups of 6 healthy volunteers each, for a total of 18 healthy volunteers, evaluating escalating single dose of SnPP starting with a dose of 9 mg SnPP, followed by 27 mg SnPP, and 90 mg SnPP. Dose-escalation from one dose level to the next will only be allowed after a careful review of the safety, tolerability and PK data from the prior dose group as determined by the Data and Safety Monitoring Board (DSMB).
- After a careful review of the safety, tolerability and PK data from all dose groups from the healthy volunteer cohorts, the Data and Safety Monitoring Board (DSMB) will determine whether to proceed to the CKD cohorts. If the determination by the DSMB allows to proceed to the CKD cohorts, subjects with stage 3 CKD will be in 2 groups of 6 each, for a total of 12 subjects, evaluating escalating single dose of SnPP starting with a dose of 27 mg SnPP followed by 90 mg SnPP. Dose-escalation from one dose level to the next will only be allowed after a careful review of the safety, tolerability and PK data from the prior dose group as determined by the Data & Safety Monitoring Board (DSMB).
- After a careful review of the safety, tolerability and PK data from all dose groups from the stage 3 CKD cohorts, the Data and Safety Monitoring Board (DSMB) will determine whether to proceed to the stage 4 CKD cohorts. If the determination by the DSMB allows to proceed to the stage 4 CKD cohorts, subjects with stage 4 CKD will be in 2 groups of 6 each, for a total of 12 subjects, evaluating escalating single dose of SnPP starting with a dose of 27 mg SnPP followed by 90 mg SnPP. Dose-escalation from one dose level to the next will only be allowed after a careful review of the safety, tolerability and PK data from the prior dose group as determined by the Data and Safety Monitoring Board (DSMB).
- The infusions will be given over a 120-minute period using a syringe pump.
- Subjects will be followed for 28 days after the dose of SnPP. Response and Safety biomarkers will be assessed at baseline and (2, 4, 8, 12, 18, response biomarkers only) 24, 48, 72, 96 and 168 hours after study drug administration. Safety will be assessed for up to 28 days after study drug administration. Urine samples will be collected and centrifuged and analyzed for subject responsiveness to SnPP therapy.
- Blood samples for pharmacokinetic analysis will be collected at baseline and 1, 2, 3, 4, 6, 8, 12, 18 and 24 h post start of infusion.
- Urinary excretion of SnPP will be assessed in a 24-hour urine sample by measuring tin in urine
- Total study duration will be up to 29 days per subject.

9.3.1 Stopping Rules

There are no pre-established stopping rules for the study.

9.4 Recordkeeping and Monitoring

All subject data will be reported in the electronic data capture (EDC) system with the exception of the laboratory reports, which will be uploaded by electronic transfer by the laboratory. All original source documents should be available for periodic monitoring and/or retrieval by a

Sponsor representative designee. The investigator is responsible for the accuracy of all data entered in the electronic case report forms (eCRFs) and for the timely completion of the electronic case report forms (eCRFs). The supporting documentation will be maintained at the site for a minimum of either:

1. Two (2) years following notification by Renibus Therapeutics, Inc. to the FDA of the termination of the entire investigation.

The Sponsor must be contacted and give written authorization prior to any study records being destroyed at investigative sites.

This study will be conducted in compliance with Good Clinical Practice (GCP), which includes the Sponsor and Institutional Review Board (IRB) -approved protocol and ICF and the FDA and International Conference on Harmonisation (ICH) regulatory guidelines and requirements. No changes will be made without prior approval unless it is imperative for subject safety. Any such departures from the protocol will be reported immediately to the Sponsor and relevant IRB.

All subjects' medical records and study-related documents will be made available to the Sponsor for regular monitoring and audits as well as to the IRB and FDA or other governmental agency with oversight or compliance responsibilities for assuring subject rights and welfare.

9.5 Investigational Supplies

Subjects will be dosed with open-label SnPP.

9.5.1 Study Drug Description

The investigational product is a mixture of Stannous protoporphyrin IX dichloride (SnPP) in a solution of sodium hydroxide

The term investigational product refers to SnPP.

9.5.2 Packaging

SnPP will be packaged and labeled in filled vials of 5 ml at Patheon, Inc.. SnPP will be packaged in a clear vial protected from light.

Following receipt of Form FDA 1572 and IRB approval for a given site, investigational product will be sent to the study site.

9.5.3 Investigational Product Dispensing

All investigational product will be dispensed by designated and trained site pharmacy staff.

9.5.4 Dosage Preparation and Administration

The vials of SnPP will be directly infused over a 120-minute period, further instructions will be included in the Pharmacy Manual.

Total infusion time will be 120 (± 5) minutes as detailed in the Pharmacy Manual.

Subjects will be observed for signs and symptoms of hypersensitivity during and after administration for at least 30 minutes and until clinically stable following completion of each administration. SnPP will only be administered when personnel and therapies are immediately available for the treatment of serious hypersensitivity reactions.

9.5.5 Storage Requirements

The investigational product should be stored at controlled room temperature conditions (defined as 20 - 25°C/68 - 77°F with excursions of less than 24 consecutive hours permitted between 15 - 30°C/59 - 86°F) and protected from light.

9.5.6 Investigational Product Accountability

The investigator or designee is responsible for maintaining accurate records accounting for the receipt, dispensing, and final disposition of all investigational products using the appropriate investigational product logs provided by Renibus Therapeutics, Inc.

9.5.7 Retrieval and Destruction

Periodically throughout the study, sites will perform investigational product reconciliation on a per-subject basis. The Renibus site monitor will review the log and once any discrepancies have been resolved, authorize destruction/disposition of the supplies associated with a given subject by signing the reconciliation log and will witness the destruction/disposition.

9.6 Study Visits

9.6.1 General Information

- All recorded clock times should utilize a 24-hour clock.
- In accordance with the clinical site's standard operating procedures, an intravenous catheter or a butterfly needle (of a sufficient gauge to avoid sample hemolysis) may be inserted for the purpose of collection of blood samples. Alternatively, the site preference may be to use direct venipuncture for the purpose of collection of blood samples. Either method is acceptable.
- There are no dietary restrictions in this study. Subjects should continue to eat their normal diet.
- Subjects will be encouraged to remain well hydrated during the study to promote sample collection.
- Blood samples for Chemistry, Hematology, biomarkers and pharmacokinetic analysis will be collected into the appropriate collection tubes and processed in accordance with the instructions specified in the laboratory manual.
- The ICF must be signed before any of the Screening or Study procedures are performed.

9.6.2 Screening (Within 14 Days Prior to Study Day 1)

1. Subjects will arrive at the clinical testing unit in the morning after fasting (defined as nothing by mouth except water) for at least 8 hours prior to the collection of any blood samples.
2. Eligibility will be confirmed.
3. Demographic characteristics and medical history will be obtained.
4. Physical examination (including height and weight), vital signs (after sitting ≥ 5 minutes), and 12-lead ECG (after sitting ≥ 5 minutes) will be performed.

5. Blood and urine will be collected for clinical laboratory tests (Appendix 1) and drug/alcohol/cotinine screen.
6. For women of childbearing potential, urine pregnancy test will be performed.
7. Eligible subjects will return to the clinical testing unit within 14 days for confinement and dosing.
8. An additional blood and urine sample will be taken between screening and prior to dosing on Study Day 1 in order to establish a baseline measurement for safety and efficacy biomarkers (a total of three measurements).

9.6.3 Study Day 1

1. Subjects will arrive at the clinic at 8:00am (± 60 minutes).
2. All eligibility criteria will be assessed.

   For CKD subjects, subjects whose GFR value does not meet the inclusion criteria may be re-screened 2 more times during the trial. If the ICF has been signed within 30 days and there are no new revisions, a new ICF is not necessary. If a subject is re-screened, the subject will be given a new screening number in the eCRF system and source documents.
3. If all eligibility criteria are met, the following Baseline samples will be collected (Appendix 1):

   - Blood samples for standard assessment of hematology and serum chemistry.
   - Blood samples for measurement of response biomarkers (Haptoglobin, Ferritin, Bilirubin, Hemopexin, IL-10, P21 and HO-1).
   - Blood samples for measurement of Safety biomarkers (Tin, Total Porphyrins, IL-6, Troponin I and Cystatin-C)**.**
   - Blood samples for measurement of SnPP.
   - Urine for standard assessment of urinalysis parameters and safety biomarkers (albuminuria, Cystatin-C, Kidney Injury Molecule-1 (KIM-1), Neutrophil Gelatinase-Associated Lipocalin (NGAL), and NAG).
   - Urine samples for measurement of response biomarkers (Hemopexin, Ferritin, P 21 and HO-1), and for urinary clearance of SnPP. Urine samples will be collected and centrifuged and analyzed for subject responsiveness to SnPP therapy.

2. A Baseline ECG will be performed (Section 9.8.1).
3. A full physical examination including height, weight, and vital signs will be performed (Sections 9.8.3 and 9.8.4).
4. Medical history and prior/concomitant medication information will be collected. (Section 9.8.2)
5. Subjects who meet all inclusion/exclusion criteria will be enrolled into the Study and the following procedures will take place:

   - The dose of SnPP will be administered in the clinic as a “push,” direct infusion over a 120 ± 5-minute period. Food may be given after the infusion.

Observe for signs and symptoms of hypersensitivity during and after administration for at least 30 minutes and until clinically stable following completion of each administration. Only administer when personnel and therapies are immediately available for the treatment of serious hypersensitivity reactions.

Should a subject experience nausea during or after infusion, the investigator may order Ondansetron (Zofran®) per its label instructions. Zofran may also be given prophylactically if at lower doses nausea is observed in subjects.

- **Patients will be advised on other interventions necessary to mitigate the risk of phototoxicity such as wearing a hat, sunglasses, long sleeve shirts and sunscreen with an SPF≥30, when appropriate,.**
- A post-infusion ECG will be performed 10 minutes (± 15 minutes) after the end of the infusion.
- The clinic staff will solicit any adverse events.
- Response biomarker samples will be collected at 2, 4, 8, 12, and 18 hours (± 15 minutes) after the start of the infusion.
- Blood samples for pharmacokinetic analysis will be collected at 1, 2, 3, 4, 6, 8, 12, 18 and 24 hours (± 15 minutes) after the start of the infusion.
- Lunch and dinner will be provided by the clinic.

9.6.4 Study Day 2

The following procedures will be done at 24 hours (± 15 minutes) after dosing:

1. Blood samples for standard assessment of clinical chemistry, hematology, and safety and response biomarkers will be collected (Appendix 1).
2. Blood samples for pharmacokinetic analysis will be collected.
3. Urine samples for standard assessment of urinalysis parameters and response biomarkers will be collected (Appendix 1).
4. The clinic staff will solicit any adverse events and note any changes in concomitant medications.
5. The subject will be discharged from the clinic per site discharge procedures.

9.6.5 Study Days 3,4,5,8 (Day 8 ± 1 day)

1. Subjects will arrive at the clinic at 8:00am (± 60 minutes).
2. Blood samples for standard assessment of clinical chemistry, hematology, and safety and response biomarkers will be collected (Appendix 1).
3. Urine samples for standard assessment of urinalysis parameters and response biomarkers will be collected (Appendix 1).
4. A urine pregnancy test will be performed if the subject is a woman of childbearing potential at Day 8.

5. The clinic staff will solicit any adverse events and note any changes in concomitant medications since the last visit.

9.6.6 Study Day 14

Study Day 14 safety review will be conducted at the request of the DSMB after the data assessment from each cohort.

9.6.7 Study Day 29/End of Study Visit (28 ± 1 days following the SnPP dose)

1. Subjects will arrive at the clinic at 8:00am (± 60 minutes).
2. Blood samples for standard assessment of clinical chemistry, hematology, and safety biomarkers will be collected (Appendix 1).
3. Urine samples for standard assessment of urinalysis parameters and safety biomarkers will be collected (Appendix 1).
4. A full physical examination including weight and vital signs will be performed (Sections 9.8.3 and 9.8.4).
5. The clinic staff will solicit any adverse events and note any changes in concomitant medications since the last visit.
6. A urine pregnancy test will be performed if the subject is a woman of childbearing potential.

9.7 Clinical Laboratory Evaluations

All samples for clinical laboratory evaluations will be collected under fasting conditions, defined as nothing by mouth except water, and essential medications only (for CKD patients) for a minimum of 8 hours prior to collection of the samples.

9.7.1 Blood Chemistry, Hematology, Pharmacokinetic and Response biomarker Samples

The chemistry, hematology and response biomarker parameters to be evaluated during the study are presented in Appendix 1. Detailed instructions regarding sample collection and processing are provided in the laboratory manual. Collection times are summarized in the Events Schedules (Section 5 and Section 9.6).

9.7.2 Urine Samples

The urine parameters, including SnPP urinary excretion, and response biomarkers to be evaluated during the Study are presented in Appendix 1. Detailed instructions regarding sample collection and processing are provided in the laboratory manual. Collection times are found in the Events Schedules (Section 5 and Section 9.6). All urine samples will be analyzed by the laboratory, except for urine pregnancy tests for women of childbearing potential.

All women of childbearing potential (defined as not surgically sterile nor postmenopausal for at least 2 years) will have a urine pregnancy test performed at the clinic as part of the screening procedure prior to any investigational product administration and at an End of Study using the pregnancy kit supplied by the laboratory.

9.8 Clinical Procedures and Observations

The research clinic will follow its own standard operating procedures for the daily ongoing monitoring of subjects while subjects are in the clinic (e.g. pulse, O_2 saturation, etc.). Subjects will be monitored for any adverse events occurring at any time throughout the study.

9.8.1 ECG

ECGs will utilize a 12-lead recording. Collection times are summarized in the Events Schedules (Section 5 and Section 9.6). ECG results will be assessed by the investigator/sub-investigator.

9.8.2 Concomitant Medications

All concomitant medications taken by the subject from 30 days prior to Study Day 1 through the end of study (28 [± 1] days after the dose of investigational product) will be recorded.

9.8.3 Physical Examination

A full physical examination, including height (Study Day 1 only) and weight (weighed on the same scale in the same state of dress), will be conducted at Day 1 and at end of the study. Collection times are summarized in the Events Schedules (Section 5 and Section 9.6).

All physical examinations are to be performed by the investigator/sub-investigator or designee whose license permits the performing of physical examinations.

9.8.4 Vital Signs

Blood pressure (systolic and diastolic), pulse, and body temperature will be measured before investigational product is administered and after the subject has been in a sitting position for ≥ 5 minutes. Collection times are summarized in the Events Schedules (Section 5 and Section 9.6).

9.8.5 Meals and Dose Administration

There are no dietary restrictions in this study. Subjects should continue to eat their normal diet. Breakfast may be eaten before or after the infusion of SnPP on Study Day 1.

9.8.6 Prohibited Medications

Medications which can cause photosensitivity are prohibited for subjects in the Study.

Oral iron medication may be taken prior to screening but must be discontinued upon enrollment.

10 ADVERSE EVENT REPORTING

All adverse events will be recorded on the designated study eCRF for each subject beginning with the first administration of investigational product and ending with the date of the end of study treatment follow-up. Any unresolved adverse events will be followed by the investigator until event resolution, the subject is lost to follow-up, the adverse event is otherwise explained or not considered clinically significant by the investigator.

10.1 Investigator Reporting Requirements

The investigator must immediately report (within 24 hours by telephone and followed by a written report sent by fax or e-mail) all serious adverse events to Renibus Therapeutics, Inc. regardless of whether the investigator believes that they are drug related, including those events

listed in the protocol as anticipated to occur in the study population independent of drug exposure (see Section 10.2).

These serious adverse events must be reported to:

Bhupinder Singh**,** MD, FASN, FNKF

Medical Monitor

Renibus Therapeutics, Inc
950 E State Highway 114, Suite 160
Southlake, TX 76092

Cell:	(480) 220-7084
Fax	(817) 549-1254
E-mail:	medicalmonitor@renibus.com

Because the investigator is knowledgeable about the human subject (e.g., medical history, concomitant medications), administers the investigational drug, monitors the subject's response to the drug, is aware of the subject's clinical state and thus may be sensitive to distinctions between events due to an underlying disease process versus events that may be drug-related, and may have observed the event, the investigator must include an assessment of causality (i.e., whether there is a reasonable possibility that the drug caused the event) in the report to Renibus Therapeutics, Inc Copies of each report to Renibus Therapeutics, Inc. will be kept in the investigator's study file.

The investigator is responsible for complying with their IRB's requirements for reporting serious adverse events. Any expedited safety report (i.e., serious unexpected suspected adverse reaction) received from Renibus Therapeutics, Inc. should be submitted to the IRB. Copies of each report and documentation of IRB notification will be kept in the investigator's study file.

The investigator must record non-serious adverse events in the eCRF and report them to Sponsor according to the timetable for reporting (e.g., end of study). The investigator's assessment of causality is not required for non-serious adverse events.

10.2 Definitions

Adverse Event: Adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An adverse event can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, without any judgment about causality.

Adverse Reaction: Adverse reaction means any adverse event definitely caused by the drug.

Suspected Adverse Reaction: Suspected adverse reaction means any adverse event for which there is a "reasonable possibility" (i.e., evidence indefinite but suggests a causal relationship between the drug and the adverse event) that the drug caused the adverse event. By definition, a suspected adverse reaction is identical to the definition of adverse drug reaction per ICH E2A.

Examples that would suggest a causal relationship:

- A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, Stevens-Johnson Syndrome)
- One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug (e.g., tendon rupture)
- An aggregate analysis of specific events observed in a clinical trial (e.g., known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.

Unexpected Adverse Event or Unexpected Adverse Reaction: An adverse event or adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed.

Adverse events or adverse reactions that are mentioned as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug will be considered "unexpected" if they are not specifically mentioned as occurring with the particular drug under investigation.

Until the investigator brochure is updated to include a new serious suspected adverse reaction, subsequent occurrences of similar serious suspected adverse reactions must be submitted expeditiously to FDA in IND Safety Reports and to other appropriate regulatory authorities in countries other than the US in an Individual Case Safety Report (ICSR) or using a Council for International Organizations of Medical Sciences (CIOMS) I form, as appropriate.

Serious Adverse Event or Serious Adverse Reaction: An adverse event or adverse reaction is considered "serious" if, in the view of either the investigator or Sponsor, it results in any of the following outcomes:

- Death
- A life-threatening adverse event
- In subject hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

If either the investigator or Sponsor believes that the event is serious, it must be evaluated by the Sponsor for expedited reporting to regulatory authorities.

Life-Threatening Adverse Event or Life-Threatening Adverse Reaction: An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or Renibus, its occurrence places the subject at immediate risk of death.

Serious Unexpected Suspected Adverse Reaction: An adverse event for which there is a reasonable possibility that the drug caused the adverse event, and it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed, and it results in any of the serious outcomes listed above. This criterion is consistent with the concepts of FDA 21 Code of Federal Regulation (CFR) 312 and the ICH E2A Guideline for expedited safety reports.

The Sponsor must notify the appropriate regulatory authority(ies) and all participating investigators in an expedited safety report (e.g., IND Safety Report or ICRS) of potentially serious risks from clinical trials or any other source (i.e., a serious unexpected suspected adverse reaction), as soon as possible, but no later than 15 calendar days after Renibus Therapeutics, Inc. receives the safety information and determines that the information qualifies for reporting.

During the course of drug development, the Sponsor may become aware of new safety information from a variety of sources and will decide if an individual case of a serious and unexpected adverse event meets the criteria for reporting to regulatory authorities.

If the adverse event does not meet all criteria (i.e., reasonable possibility of causality, serious and unexpected), it should not be submitted as an expedited safety report.

Any unexpected fatal or life-threatening suspected adverse reaction must be reported to regulatory authorities no later than 7 calendar days after Renibus Therapeutics, Inc. receives the safety information.

10.3 Breaking the Blind

Not applicable as this is an open label study.

11 STATISTICAL CONSIDERATIONS

This section outlines the nature and rationale for the statistical methods to be used for the analysis of the data from the study. A separate Statistical Analysis Plan (SAP) will describe data handling and statistical techniques in full detail.

11.1 Study Populations

All subjects who receive study medication will be included in the safety and PD analyses. All subjects who receive study medication and provided sufficient and valid serum SnPP concentration data to allow the determination of PK parameters will be considered as potential PK evaluable subjects. No imputation will be used for missing data.

11.2 Demographic and Baseline Characteristics

Demographic and baseline variables that will be summarized by dose group include:

- Demographics
- Medical history
- Complete PE
- Efficacy and Safety variables including vital signs and laboratory tests, including chemistry, hematology, and urinalysis obtained at Screening.

11.3 Analysis of Efficacy Endpoints

Descriptive statistics will be used to summarize Response biomarker results at baseline, each visit, and the change from baseline for each visit.

Percent change from baseline will be analyzed using the mixed model for repeated measures (MMRM). In this analysis, percent change from baseline at each post treatment time point will be the response variable; treatment, visit, and treatment-by-visit interaction as fixed factors, patient as repeated measure unit over visits, and baseline as covariate. The between-group comparison will be performed using the simple contrast at 2, 4, 8, 12, 18, 24, 48, 72, 96 and 168 hours post-dosing.

In addition to the percent changes from baseline, absolute change from baseline will also be analyzed using MMRM. Moreover, a responder analysis will also be considered. The responder definition and its appropriate analysis methodology will be detailed in the statistical analysis plan (SAP) for the study. Exploratory analysis using Cochran-Mantel-Haenszel test stratified by baseline or primary diagnosis will be carried out.

11.4 Analysis of Pharmacodynamic/Safety Endpoints

The evaluation of safety will include adverse events, clinical laboratory parameters, vital signs, physical examinations, and ECGs.

The principle of treatment emergence will be employed for the analysis of adverse event data. A Treatment emergent adverse event (TEAE) is defined to be any event that occurs during the observation period of the study and was not present at baseline, or exacerbation of a condition present at baseline.

TEAEs will be classified by the Medical Dictionary for Regulatory Activities (MedDRA). The type, incidence, timing (onset, duration), relationship, and severity of adverse events will be reported for treatment-emergent and serious adverse events. Reasons for withdrawal due to adverse events will also be reported.

Descriptive statistics, including mean, mean change from baseline, standard deviation, minimum, maximum, and median, will be presented for laboratory parameters, vital signs, and ECG parameters. The numbers and percentages of subjects with treatment-emergent laboratory, vital sign, and ECG values that meet Sponsor-defined potentially clinically significant criteria will be summarized. Categorical (low, normal, and high relative to the normal range) shifts from baseline will be summarized with the number and percentage of subjects for laboratory parameters.

All safety data will be displayed in listings.

11.5 Analysis of Pharmacokinetic (PK) Data

Pharmacokinetic (PK) parameters will be calculated for each subject and summarized by treatment group. PK profiles in which the pre-dose concentration is > 5% of the corresponding C_{max} (and therefore greater than the lower limit of quantification [LLOQ]) will be excluded from all descriptive statistics and statistical analyses.

For the PK analysis performed, actual blood sampling times will be used in all PK analyses. Protocol-specified times will be used to calculate mean plasma or serum concentrations for

tabular and graphical displays. All PK calculations and generation of individual subject plasma or serum concentration versus time graphs will be performed using SAS®.

PK parameters will be calculated using non-compartmental analysis. The maximum serum concentration (C_{max}) and time to maximum serum concentration (Tmax) will be obtained directly from the data. The elimination rate constant (λz), elimination half-life ($T_{½}$), area under the concentration-time curve from time 0 to time t ($AUC_{[0-t]}$) and area under the concentration-time curve from time 0 to infinity ($AUC_{[0-inf]}$), clearance (CL), and volume of distribution (Vz) will be calculated for each subject and summarized by treatment group.. Only plasma samples with concentrations ≥ LLOQ will be used in the PK analysis. Plasma concentrations < LLOQ will be taken as 0 for the calculation of the descriptive statistics at each sampling time. For the PK analysis, plasma concentrations < LLOQ that occur from pre-dose to the first concentration ≥ LLOQ will be taken as 0 and those that occur thereafter will be taken as missing.

The first dose escalation, from 9 mg to 27 mg, will be based on assuming a dose-proportional increase in Cmax and AUC and will use the maximum values across the first cohort. The subsequent decisions to escalate to the next dose level will be based on the available pharmacokinetic exposure data from the previous dose cohort(s) and compared with the exposure levels at the NOAEL established in the acute dose toxicity study in male and female Sprague Dawley rats. The dose escalation decision will utilize best available modelling practices, i.e. fitting the power model to the individual subject Cmax and AUC vs. dose. A dose will not be administered that would result in an upper 95th percentile for the model-predicted Cmax and AUC values exceeding 39,250 ng/mL and 209,500 hr.ng/mL (mean of males and females), respectively. These values were the observed values at the NOAEL of 10 mg/kg/day in the GLP toxicology study performed in Sprague Dawley rats.

11.6 Sample Size Calculations

No formal sample size calculation has been performed for the study. However, it is believed that 4 subjects per dose group will be adequate to establish POC based on biomarker response and identify an optimal dose for further testing, recognizing that a battery of different surrogate biomarkers will be evaluated.

11.7 Interim Analysis

No interim analysis is planned.

12 WITHDRAWAL FROM STUDY

Every reasonable effort should be made to maintain protocol compliance and participation in the study. Should a subject withdraw or be prematurely terminated from the study for any reason, the reason for early study withdrawal will be recorded. If withdrawal is the result of a serious

adverse reaction, the subject will be followed until the condition has resolved, as determined by the investigator.

The investigator or Sponsor may withdraw any subject at any time for medical reasons or for administrative reasons (i.e., subjects unable or unwilling to comply with the protocol). If so, the subject will be censored at time of withdrawal and, if possible, a final evaluation (End of Study procedures) will be made. All treated subjects (those who have received at least 1 dose of investigational product) will be included in the ITT and safety analyses.

In the unlikely event the Sponsor or FDA should determine it is appropriate to terminate the study early, every effort will be made for transitioning subjects with minimal disruption to the subject and investigator. The IRB will be notified of termination, and reason(s) and procedures for follow-up of research subjects will be developed by the study physician in consultation with the Sponsor and IRB.

13 DATA MANAGEMENT

The standard procedures for handling and processing records will be followed as per GCP and the data management standard operating procedures of the contract research organization (CRO). A comprehensive Data Management Plan will be developed and approved by a representative of the Data Management CRO and the Sponsor.

13.1 Database Lock

The database will be locked in order to protect write access after the following preconditions are fulfilled:

- All data are entered in the database
- All data queries have been resolved to the satisfaction of the Lead Biostatistician
- All decisions have been made regarding all protocol violators and ITT population exclusions
- The eCRF will be signed off/approved by the Site Investigator
- Written authorizations to lock the database are obtained from Renibus Clinical Data Management and the Chief Scientific Officer.

14 ETHICAL CONSIDERATIONS

14.1 Ethical Conduct of the Study

The study will be conducted in accordance with United States Title 21 CFR and the ICH E6 (R1) Guidelines of Good Clinical Practice. The Declaration of Helsinki and its most recent updates (Seoul, 2008) will be observed.

The investigator will provide the Sponsor/designee with documentation of IRB approval of the protocol and the sample informed consent document before the study may begin at the investigative site. The IRB will review the protocol as required.

The investigator will supply the following to the IRB:

- The current Investigator's Brochure and updates
- Study protocol and amendments
- Informed consent and assent document and updates
- Relevant curricula vitae
- Safety alerts
- Serious adverse reaction reports

The investigator must provide the following documentation to the Sponsor or designee:

- The IRB original approval of the protocol and the informed consent, and re-approval of the study (annual or semi-annual, per IRB guidelines)
- The IRB approvals of any revisions to the informed consent document or amendments to the protocol
- All other documents that are required by local regulatory authorities

14.2 Regulatory Considerations

After reading the protocol, each investigator/sub-investigator will sign a protocol signature page and return a copy of the signed page to the Sponsor/designee, while maintaining the original at the site.

14.3 Protocol Amendments and Study Termination

The IRB must be informed and give approval for any amendments likely to affect the safety of the subjects or the conduct of the study.

The IRB must be advised in writing of the study's completion or early termination and a copy of the notification must be provided to the Sponsor.

14.4 Safety Monitoring

The Sponsor's Medical Monitor and will monitor safety data throughout the course of the study. Renibus Drug Safety, or their designee, will expedite to the regulatory authorities only the suspected adverse reactions that are product-related and unexpected in accordance with FDA 21 CFR 312, FDA draft guidance (Sept 2010) on Safety Reporting Requirements for INDs, and the ICH E2A guideline.

14.5 Quality Control and Quality Assurance

By signing this protocol, the Sponsor agrees to be responsible for implementing and maintaining quality control and quality assurance systems with written standard operating procedures to ensure that the study is conducted and data are generated, documented, and reported in compliance with the protocol, accepted standards of GCPs, and all applicable federal, state, and local laws, rules and regulations relating to the conduct of the clinical study.

By signing this protocol, the investigator agrees to conduct the study in an efficient and diligent manner and in conformance with this protocol, generally accepted standards of GCP, and all applicable federal, state, and local laws, rules and regulations relating to the conduct of the clinical study.

The investigator also agrees to allow monitoring, audits, IRB review and regulatory agency inspection of study-related documents and procedures and provide for direct access to all study-related source data and documents. Investigators will be given notice before a quality assurance audit occurs.

The investigator shall prepare and maintain complete and accurate study documentation in compliance with GCP standards and applicable federal, state, and local laws, rules and regulations, and promptly submit to the Sponsor all forms and reports required by this protocol following completion or termination of the clinical study or as otherwise required due to any agreement with the Sponsor.

15 GENERAL CONSIDERATIONS

15.1 Discontinuation of the Study

The Sponsor reserves the right to discontinue this study or investigator's participation in this study for safety or administrative reasons at any time.

16 AGREEMENT WITH PROTOCOL

I have read this protocol and agree to conduct this clinical study as outlined herein. I will ensure that all sub-investigators and other study staff members have read and understand all aspects of this protocol. I agree to cooperate fully with Renibus Therapeutics, Inc. and its appointed Contract Research Organizations (CROs) during the study. I will adhere to all Food and Drug Administration (FDA), International Conference on Harmonisation (ICH), revised Declaration of Helsinki (2008) and other applicable regulations and guidelines regarding clinical trials on a study drug during and after study completion.

Principal Investigator:

Printed Name: _____

Signature: _____

Date: _____

Protocol REN-003

A Phase 1b dose-escalating study with Stannous Protoporphyrin (SnPP), in healthy volunteers and subjects with chronic kidney disease stage 3-4.

Original Protocol: 25 March 2019

17 REFERENCES

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Appendix 1 Safety Clinical Chemistry, Hematology, and Urinalysis Tests Performed by the Laboratory at Screening, During the Study, and at Follow-up (EOS)

Clinical Chemistry[1]
Total protein
Albumin
Bicarbonate
Blood urea nitrogen (BUN) [5]
Creatinine[5]
Total bilirubin
Alkaline phosphatase
Glucose
Sodium
Potassium
Inorganic phosphate
Calcium (total and ionized)
Magnesium
Gamma-glutamyl transferase
Alanine aminotransferase (ALT)
Aspartate aminotransferase (AST)
Cystatin-C[5]
Tin[5]
Tin Protoporphyrin
IL-6
Total porphyrins

Hematology[2]
Hemoglobin
Hematocrit
Erythrocyte count (red blood cells)
Differential leukocytes
Total leukocytes (white blood cells)
Platelets

Urinalysis[3]
pH
Specific gravity
Glucose
Ketones
Bilirubin
Urobilinogen
Blood
P21
HO-1
Hemopexin
Ferritin
Urine sediment
Urine samples to test for SnPP response
SnPP Urinary excretion[3']
N acetyl-beta-D-glucosaminidase (NAG)
Kidney Injury Molecule-1 (KIM-1)
Neutrophil Gelatinase-Associated Lipocalin (NGAL)
Cystatin-C
Albuminuria
Urine pregnancy test[4]

Response Biomarkers
Haptoglobin
Ferritin
Hemopexin
IL-10
P21
HO-1
Bilirubin[5]

1 Collect into serum separator tubes.
2 Collect into K2 EDTA tubes.
3 Approximately 10 mL of urine required for urinalysis including sediment.
3' Urinary excretion to be tested in a 24-hour volume sample
4 Urine dipstick: all women of childbearing potential prior to the first dose of SnPP on Study Day 1 and End of Study. Perform in the clinic using the pregnancy kit supplied by the laboratory.
5 Biomarkers run with standard Chemistry samples