Clinical Protocol REN-004

A Phase 2, Double-Blind, Randomized, Placebo-Controlled Study to Evaluate the Effect of RBT-1 on Preconditioning Response Biomarkers in Subjects Undergoing Coronary Artery Bypass Graft (CABG) and/or Cardiac Valve Surgery
(The START Study)

IND 138109

SPONSOR

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Protocol Version 5: 25 Aug 2022

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1 STUDY TITLE

A Phase 2, Double-Blind, Randomized, Placebo-Controlled Study to Evaluate the Effect of RBT-1 on Preconditioning Response Biomarkers in Subjects Undergoing Coronary Artery Bypass Graft (CABG) and/or Cardiac Valve Surgery (The START Study)

2 SPONSOR INFORMATION

2.1 Sponsor:

Name:	Renibus Therapeutics, Inc.
Address:	181 Grand Ave, Suite 225 Southlake, TX 76092

2.2 Sponsor Representative:

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Medical Monitor
Renibus Therapeutics, Inc.

Date

3 LIST OF ABBREVIATIONS

Abbreviation	Definition
AKI	acute kidney injury
CABG	coronary artery bypass graft
CFR	Code of Federal Regulations
CPB	cardiopulmonary bypass
eCRF	electronic case report form
eGFR	estimated glomerular filtration rate
FDA	Food and Drug Administration
FeS	iron sucrose
GCP	Good Clinical Practice
HO-1	heme oxygenase-1
ICF	informed consent form
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
IL-10	interleukin-10
IND	Investigational New Drug
IRB	Institutional Review Board
IV	intravenous
IWRS	Interactive Web Response System
KDIGO	Kidney Disease Improving Global Outcomes
KIM-1	kidney injury molecule-1
MAKE	Major Adverse Kidney Events
MAKE30	Major Adverse Kidney Events through Day 30 post-cardiac surgery
MAKE90	Major Adverse Kidney Events through Day 90 post-cardiac surgery
NGAL	neutrophil gelatinase-associated lipocalin
RBT-1	stannous protoporphyrin and iron sucrose
SAP	statistical analysis plan
SnPP	stannous protoporphyrin

Abbreviations used only in tables and figures are defined in the table and figure footnotes and are not included in the List of Abbreviations.

4 STUDY SYNOPSIS

Renibus Therapeutics, Inc.	**Protocol No. REN-004**
Name of Drug: RBT-1	**Phase of Development:** 2
Name of Active Ingredients: Stannous protoporphyrin (SnPP) and iron sucrose (FeS)	**Date of Study Synopsis:** 25 Aug 2022
Protocol Title: A Phase 2, Double-Blind, Randomized, Placebo-Controlled Study to Evaluate the Effect of RBT-1 on Preconditioning Response Biomarkers in Subjects Undergoing Coronary Artery Bypass Graft (CABG) and/or Cardiac Valve Surgery (The START Study)	
Investigational Sites: Up to 20 sites in the United States, Canada, Australia, and New Zealand.	

Study Objectives:

The overall objective is to evaluate the effect of RBT-1 on preconditioning response biomarkers in subjects undergoing CABG and/or cardiac valve surgery.

Primary Objective

The primary objective is to evaluate the efficacy of RBT-1 in generating a preconditioning response as measured by plasma biomarkers (heme oxygenase-1 [HO-1], ferritin, and interleukin-10 [IL-10]) from Baseline (pre-dose) through Day 1 pre-op.

Secondary Objectives

The secondary objectives are to evaluate the efficacy of RBT-1 on the following:

- Change in renal tubular injury biomarkers post-cardiac surgery through Day 3.
- Reduction in urine output (documented oliguria of <0.5 mL/kg/hour, not due to urinary tract obstruction or hypotension) for more than 6 hours post-cardiac surgery through Day 3.
- Incidence of acute kidney injury (AKI) (defined using the modified Kidney Disease Improving Global Outcomes [KDIGO] criteria) post-cardiac surgery through Day 5.

Exploratory Objectives

The exploratory objectives are to evaluate the following:

- Severity of AKI post-cardiac surgery through Day 7.
- Mean increase in serum creatinine post-cardiac surgery through Day 7 or until Discharge if prior to Day 7.
- Occurrence of Major Adverse Kidney Events (MAKE) – death, need for dialysis, and persistent renal dysfunction – post-cardiac surgery through Day 30 (MAKE30) and Day 90 (MAKE90) post-cardiac surgery.
- Proportion of subjects experiencing MAKE30.
- Proportion of subjects experiencing MAKE90.
- Days on ventilator.
- Days in intensive care unit (ICU).
- Hospital length of stay.
- Readmission rate.
- Changes in biomarkers of RBT-1 activity and/or kidney function.

Study Design:

This is a Phase 2, multicenter, double-blind, randomized, placebo-controlled study to evaluate the effect of RBT-1 on preconditioning response biomarkers in subjects who are at risk for AKI following cardiac surgery. Eligible subjects will be randomized to receive a single dose of low-dose RBT-1 (45 mg SnPP/240 mg FeS), high-dose RBT-1 (90 mg SnPP/240 mg FeS), or placebo (normal saline) via intravenous (IV) infusion over a 120-minute period ≥24 but ≤48 hours prior to scheduled cardiac surgery.

All subjects will be assessed through Day 90 post-cardiac surgery. Subjects will be evaluated at Screening, 48 and/or 24 hours prior to surgery depending on the time of dosing, on Day 1 (day of surgery), and daily through Day 7 or Discharge if earlier, as well as on Days 30, 60, and 90, as described in the Event Schedule (see Section 5). Assessments include biomarkers of preconditioning response and tubular injury, urine output, incidence and severity of AKI, serum creatinine, days on ventilator, days in intensive care unit, hospital length of stay, readmission rate, and safety (physical examination, clinical laboratory assessment, vital signs, concomitant medications, and adverse events). Evaluations for MAKE (death, need for dialysis, and change in kidney function) and safety will be performed through Day 90, as described in the Event Schedule (see Section 5).

Number of Subjects to be Enrolled:

Approximately 126 subjects are planned to be enrolled and randomized 1:1:1 to low dose RBT-1, high-dose RBT-1, and placebo (n=42/group).

Inclusion Criteria:

Eligible subjects must meet all the following criteria:

1. Male or female subjects ≥18 years of age at Screening.
2. Able and willing to comply with all study procedures.
3. Stable kidney function per Investigator assessment and no known episodes of AKI during the preceding 4 weeks.
4. Scheduled to undergo non-emergent cardiac surgery requiring cardiopulmonary bypass (CPB), which must include one of the following:
   - CABG alone;
   - Combined CABG and cardiac valve(s) replacement or repair;
   - Cardiac valve(s) replacement or repair alone.
5. Female and male subjects of childbearing potential must agree to use 2 forms of contraception, with at least 1 being a barrier method, or abstain from sexual activity for 30 days following study drug administration.
6. Male subjects must agree not to donate or sell sperm for 30 days following study drug administration.

Exclusion Criteria:

Subjects will be ineligible for study participation if they meet any of the following criteria:

1. Presence of AKI at the time of Screening, as determined by the Investigator.
2. Surgery to be performed without cardiopulmonary bypass (CPB).
3. A significant portion of the surgery to be performed under conditions of circulatory arrest or hypothermia with rectal temperature <28°C (82.4°F), as determined by the Investigator in consultation with the Medical Monitor.
4. eGFR ≤20 mL/min/1.73m^2 or need for dialysis.
5. Surgery for aortic dissection or to correct a major congenital heart defect.
6. Administration of iodinated contrast media within 24 hours prior to cardiac surgery or evidence of contrast-induced nephropathy prior to cardiac surgery.
7. Cardiogenic shock or hemodynamic instability within the 24 hours prior to surgery and requirement for inotropes or vasopressors or other mechanical devices, such as intra-aortic balloon counter-pulsation.
8. Requirement for any of the following within 7 days prior to cardiac surgery:
   - Defibrillator or permanent pacemaker;
   - Mechanical ventilation;
   - Intra-aortic balloon counter-pulsation;
   - Left ventricular assist device;
   - Other forms of mechanical circulatory support.
9. Known history of cancer within the past 2 years, except for carcinoma in situ of the cervix or adequately treated non-melanoma cancer of the skin.
10. Known or suspected sepsis at time of Screening or confirmed or treated endocarditis within 30 days prior to cardiac surgery.
11. Other current active infection requiring systemic antibiotic treatment.
12. Abnormal hepatic function, defined as total bilirubin or alanine aminotransferase or aspartate aminotransferase >2X the upper limit of normal at time of Screening or Child Pugh Class C liver disease or higher.
13. Congenital coagulation disorder associated with a high risk of excessive bleeding or thrombosis per Investigator assessment.
14. Asplenia (anatomic or functional).
15. History of photosensitivity or active skin disease that, in the opinion of the Investigator, could be worsened by RBT-1.
16. Known hypersensitivity or previous anaphylaxis to SnPP or any tin-based product.
17. Serum ferritin >500 ng/mL or those who have received IV iron within 14 days of Screening.
18. Pregnancy or lactation.
19. Treatment with an investigational drug or participation in an interventional study within 30 days prior to administration of study drug.
20. In the opinion of the Investigator, any disease processes or confounding variables that would inappropriately alter the outcome of the study.
21. Inability to comply with the requirements of the study protocol.

Drug, Dose and Mode of Administration:

RBT-1 will be supplied as a sterile liquid (in separate vials of SnPP and FeS) for IV injection and is intended for single use. Instructions for dose preparation will be specified in the Pharmacy Manual.

Commercially available 0.9% sodium chloride (normal saline) for injection will be used for placebo administration.

Study drug (low-dose RBT-1 [45 mg SnPP/240 mg FeS], high-dose RBT-1 [90 mg SnPP/240 mg FeS], or placebo [normal saline]) will be administered via IV infusion over a 120-minute period at ≥24 but ≤48 hours prior to scheduled cardiac surgery.

Study Duration:

Study duration is approximately 90 days per subject.

Criteria for Evaluation:

Efficacy assessments include the following:

- Change in preconditioning plasma biomarkers through Day 1 pre-op:
  - HO-1, ferritin, and IL-10.
- Change in renal tubular injury biomarkers post-cardiac surgery through Day 3:
  - Kidney injury molecule-1 (KIM-1), cystatin C, and neutrophil gelatinase-associated lipocalin (NGAL)
- Reduction in urine output (documented oliguria of <0.5 mL/kg/hour, not due to urinary tract obstruction or hypotension) for more than 6 hours post-cardiac surgery through Day 3.
- Incidence of AKI post-cardiac surgery through Day 5, defined as:
  - An absolute increase in serum creatinine by 0.5 mg/dL, which is present over 2 laboratory measurements at least 12 hours apart; or
  - An increase in serum creatinine ≥1.5X Baseline.
- Severity of AKI post-cardiac surgery through Day 7.
- Mean increase in serum creatinine post-cardiac surgery through Day 7 or until Discharge if prior to Day 7.
- Occurrence of MAKE30 and MAKE90 post-cardiac surgery.
- Proportion of subjects experiencing MAKE30.
- Proportion of subjects experiencing MAKE90.
- Days on ventilator.
- Days in ICU.
- Hospital length of stay.
- Readmission rate.
- Changes in biomarkers of RBT-1 activity and/or kidney function.

Safety assessments include the following:

- Adverse events
- Laboratory data and vital signs at regular intervals.

Statistics: Details of the efficacy analysis and safety analysis will be defined in the statistical analysis plan (SAP).

Analysis Populations: The efficacy analyses will be conducted using the Intent-to-Treat population (all randomized subjects) and Per Protocol population (all randomized subjects who had no major protocol deviations). Safety analyses will be conducted using all subjects receiving any amount of study drug.

Data Analysis: 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 study drug.

Sample Size Estimate: Assuming an increase of 30% in the primary biomarkers from baseline in the RBT-1 treated groups versus a 10% reduction in the placebo group and a standard deviation of 10%, 6 subjects per group would provide 80% power to detect a difference between treatment and placebo groups with a significance level of 0.05. From a safety perspective, 40 subjects per group is consistent with the sample size typically used in early-stage clinical trials.

5 EVENT SCHEDULE

Visit	Visit 0 (Screening)†	Visit 1a	Visit 1b††	Visit 2		Visit 3	Visit 4	Visits 5-7	Visit 8*	Visit 9*	Visit 10*	Visit 11*/ Final Visit
Procedure / Time point	Within 30 Days Prior to Visit 1	≥24 h but ≤48 h Prior to Surgery	24 h (± 4 h) Prior to Surgery	D1 Pre-Op	D1 Post-Op	D2	D3	D4, 5, 6 (If Hospitalized)	D7 (± 3 days)	D30 (± 3 days)	D60 (± 3 days)	D90 (± 3 days)
Informed Consent Form	X											
Patient Eligibility (Inclusion/Exclusion Criteria)	X	X										
Demographics	X											
Medical History	X											
Prior/Concomitant Medication	X	X	X	X	X	X	X	X	X	X	X	X
Physical Exam	X	X		X		X[1]	X[1]	X[1]	X	X		X
Weight and BMI	X											X
Vital Signs	X	X		X		X	X	X	X	X	X	X
Serum Chemistry [2]	X[3]	X		X		X	X	X	X	X	X	X
Hematology [4]	X	X		X		X	X	X	X	X	X	X
Urinalysis [5]	X									X		X
Urine Output [6]					X	X	X					
Blood Biomarkers [7]		X	X	X[8]		X	X					
Urinary Biomarkers [9]		X	X	X[8]		X	X					
Urine Pregnancy Test [10]	X									X		
Study Drug Infusion [11]		X										
Adverse Events [12]		X	X	X	X	X	X	X	X	X	X	X

BMI = body mass index; D = Day; h = hour; Post-Op = post-operatively; Pre-Op = pre-operatively

† Screening labs will be analyzed at a local laboratory; for subjects who will be administered study drug on the same day of Screening, blood and urine samples will need to be collected for central laboratory analysis in accordance with Visit 1a; physical examinations and routine labs (serum chemistry, hematology, urinalysis) performed as part of standard of care within 1 month of dosing administration (Visit 1a) can be used for Screening assessments.

†† Visit 1b will be performed in subjects who receive study drug within 36 to 48 hours prior to surgery.

* Visits 8-11 (Days 7, 30, 60, and 90) may be conducted remotely as virtual visits if in-person visits are not possible; if virtual visits are conducted: 1) blood and urine samples may be collected by remote phlebotomy, such as through a mobile phlebotomist or other blood collection center and 2) physical examinations can be symptom-directed.

1 Physical examination can be symptom-directed on Days 2-6.

2 Serum chemistry parameters include total protein, albumin, bicarbonate, blood urea nitrogen, serum creatinine, total bilirubin, alkaline phosphatase, glucose, sodium, potassium, phosphate, calcium, magnesium, gamma-glutamyl transferase, alanine aminotransferase, aspartate aminotransferase, and cystatin C.
3 Serum ferritin is also to be assessed at Screening if not already included in local laboratory chemistry panel.
4 Hematology parameters include red blood cell count [RBC], hemoglobin, hematocrit, mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC], platelets, mean platelet volume [MPV], red cell distribution width [RDW], white blood cell count [WBC], and WBC differential (including neutrophils [absolute and percentage], lymphocytes [absolute and percentage], monocytes [absolute and percentage], eosinophils [absolute and percentage], and basophils [absolute and percentage]).
5 Urinalysis parameters include glucose, ketones, leukocytes, nitrite, blood, pH, protein, specific gravity, and reflex microscopic.
6 Urine output will be measured through Day 3.
7 Blood biomarkers include preconditioning response biomarkers (plasma heme oxygenase-1 [HO-1], ferritin, and interleukin-10 [IL-10]) and other biomarkers of RBT-1 activity and kidney function.
8 Blood and urinary biomarkers will be collected within 4 hours prior to cardiac surgery.
9 Urinary biomarkers include tubular injury biomarkers (kidney injury molecule-1 [KIM-1], cystatin C, and neutrophil gelatinase-associated lipocalin [NGAL]), and other biomarkers of RBT-1 activity and kidney function.
10 A pregnancy test will be performed in female subjects of childbearing potential.
11 RBT-1 and placebo will be administered via intravenous infusion over a 120-minute period.
12 Adverse events assessment will include photosensitivity assessment.

6 INTRODUCTION

6.1 Background Information

In cardiac surgery subjects, the incidence of postoperative acute kidney injury (AKI) is approximately 30% and significantly increases the risk of death after cardiac surgery [Rosner 2006]. 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 [Kellum 2013]. Progression to advanced-stage chronic kidney disease within 24 months occurs in approximately 15% to 20% of subjects who survive AKI [Chawla 2012].

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 ≥0.3 mg/dL within 48 hours; increase in serum creatinine ≥1.5X 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 (eg, heme oxygenase 1 [HO-1], ferritin, haptoglobin, hemopexin, hepcidin, 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.

RBT-1 is comprised of 2 different compounds, iron sucrose (FeS) and stannous protoporphyrin (SnPP). FeS is an intravenous (IV) iron replacement product that has been in use for more than 65 years and is currently registered in more than 90 countries worldwide. In the United States, FeS is approved for the treatment of iron deficiency anemia in pediatric and adult patients with chronic kidney disease [Venofer 2015], with expanded indications (ie, need for rapid iron supply, patients unable to tolerate or comply with oral iron therapy, patients with inflammatory bowel disease in whom oral iron therapy is ineffective) approved for use outside the United States [Venofer 2016]. Tin protoporphyrin, a potent competitive inhibitor of heme oxygenase activity, is not commercially available, but 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].

RBT-1 mediates preconditioning cytoresistance in the kidney by activating the body's major antioxidant response pathway, resulting in the upregulation of cytoprotective proteins, including but not limited to HO-1, ferritin, and IL-10.

RBT-1 is under development as a prophylactic agent to induce cytoresistance in organs when a known insult is imminent.

6.3 Preclinical Experience

RBT-1 has been shown to induce profound renal preconditioning and the emergence of a cytoprotective state in animal models that resulted in marked protection against diverse forms of AKI [Zager 2016]. In contrast to ischemic preconditioning, which induces local or remote ischemia, the acquired cytoresistance induced by RBT-1 was achieved without resultant damage to the kidney, liver, or heart. Thus, these data suggest that RBT-1 may provide a clinical prophylactic strategy for protecting against both renal and extrarenal injuries prior to an insult, such as exposure to surgery, balloon angioplasty, or administration of a radiocontrast agent, or nephrotoxic chemotherapy.

Initial non-clinical pharmacology and toxicology studies have been conducted and are detailed in the Investigator's Brochure. Single-dose acute toxicity studies of IV RBT-1 demonstrated no-adverse-effect-levels of 25 mg/kg FeS/10 mg/kg SnPP in the rat and 7.5 mg/kg FeS/3 mg/kg SnPP in the dog. Single-extended acute dose toxicity studies of IV SnPP demonstrated no-adverse-effect-levels of 10 mg/kg SnPP in the rat (human equivalent dose of 96 mg) and the dog (human equivalent dose of 324 mg).

6.4 Clinical Experience

Phase 1 studies in healthy volunteers and subjects with Stage 3/4 chronic kidney disease have demonstrated that the cytoprotective proteins ('response biomarkers') induced by RBT-1 and its components are upregulated in humans. RBT-1 is safe and well tolerated at the highest dose tested (90 mg SnPP and 240 mg FeS). Photosensitivity, which was expected, was the primary adverse event observed, but was not dose-limiting. Adverse events were generally mild to moderate in severity. No serious adverse events were reported.

The current study is a Phase 2, multicenter, double-blind, randomized, placebo-controlled study to evaluate the effect of RBT-1 on preconditioning response biomarkers in subjects who are at risk for AKI following cardiac surgery. It is hypothesized that treatment with RBT-1 prior to scheduled cardiac surgery will increase response biomarkers, reducing the incidence of AKI post-cardiac surgery.

7 STUDY OBJECTIVES

The overall objective is to evaluate the effect of RBT-1 on preconditioning response biomarkers in subjects undergoing CABG and/or cardiac valve surgery.

7.1 Primary Objective

The primary objective is to evaluate the efficacy of RBT-1 in generating a preconditioning response as measured by plasma biomarkers (HO-1, ferritin, and IL-10) from Baseline (pre-dose) through Day 1 pre-op.

7.2 Secondary Objective

The secondary objectives are to evaluate the efficacy of RBT-1 on the following:

- Change in renal tubular injury biomarkers post-cardiac surgery through Day 3.
- Reduction in urine output (documented oliguria of <0.5 mL/kg/hour, not due to urinary tract obstruction or hypotension) for more than 6 hours post-cardiac surgery through Day 3.
- Incidence of AKI (defined using the modified KDIGO criteria) post-cardiac surgery through Day 5.

7.3 Exploratory Objectives

The exploratory objectives are to evaluate the following:

- Severity of AKI post-cardiac surgery through Day 7.
- Mean increase in serum creatinine post-cardiac surgery through Day 7 or until Discharge if prior to Day 7.
- Occurrence of MAKE30 and MAKE90 post-cardiac surgery.
- Proportion of subjects experiencing MAKE30.
- Proportion of subjects experiencing MAKE90.
- Days on ventilator.
- Days in ICU.
- Hospital length of stay.
- Readmission rate.
- Changes in biomarkers of RBT-1 activity and/or kidney function.

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) or Independent Ethics Committee (IEC) will be used. If English is NOT the subject's primary language, the subject will be consented using an IRB/IEC-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.

9 STUDY POPULATION

9.1 Inclusion Criteria

Eligible subjects must meet all the following criteria:

1. Male or female subjects ≥18 years of age at Screening.
2. Able and willing to comply with all study procedures.
3. Stable kidney function per Investigator assessment and no known episodes of AKI during the preceding 4 weeks.
4. Scheduled to undergo non-emergent cardiac surgery requiring cardiopulmonary bypass (CPB), which must include one of the following:
    - CABG alone;
    - Combined CABG and cardiac valve(s) replacement or repair;
    - Cardiac valve(s) replacement or repair alone.
5. Female and male subjects of childbearing potential must agree to use 2 forms of contraception, with at least 1 being a barrier method, or abstain from sexual activity for 30 days following study drug administration.
6. Male subjects must agree not to donate or sell sperm for 30 days following study drug administration.

9.2 Exclusion Criteria

Subjects will be ineligible for study participation if they meet any of the following criteria:

1. Presence of AKI at the time of Screening, as determined by the Investigator.
2. Surgery to be performed without cardiopulmonary bypass (CPB).
3. A significant portion of the surgery to be performed under conditions of circulatory arrest or hypothermia with rectal temperature <28°C (82.4°F), as determined by the Investigator in consultation with the Medical Monitor.
4. eGFR ≤20 mL/min/1.73m^2 or need for dialysis.
5. Surgery for aortic dissection or to correct a major congenital heart defect.
6. Administration of iodinated contrast media within 24 hours prior to cardiac surgery or evidence of contrast-induced nephropathy prior to cardiac surgery.
7. Cardiogenic shock or hemodynamic instability within the 24 hours prior to surgery and requirement for inotropes or vasopressors or other mechanical devices, such as intra-aortic balloon counter-pulsation.
8. Requirement for any of the following within 7 days prior to cardiac surgery:
    - Defibrillator or permanent pacemaker;
    - Mechanical ventilation;

- Intra-aortic balloon counter-pulsation;
- Left ventricular assist device;
- Other forms of mechanical circulatory support.

9. Known history of cancer within the past 2 years, except for carcinoma in situ of the cervix or adequately treated non-melanoma cancer of the skin.
10. Known or suspected sepsis at time of Screening or confirmed or treated endocarditis within 30 days prior to cardiac surgery.
11. Other current active infection requiring systemic antibiotic treatment.
12. Abnormal hepatic function, defined as total bilirubin or alanine aminotransferase or aspartate aminotransferase >2X the upper limit of normal at time of Screening or Child Pugh Class C liver disease or higher.
13. Congenital coagulation disorder associated with a high risk of excessive bleeding or thrombosis per Investigator assessment.
14. Asplenia (anatomic or functional)
15. History of photosensitivity or active skin disease that, in the opinion of the Investigator, could be worsened by RBT-1.
16. Known hypersensitivity or previous anaphylaxis to SnPP or any tin-based product.
17. Serum ferritin >500 ng/mL or those who have received IV iron within 14 days of Screening.
18. Pregnancy or lactation.
19. Treatment with an investigational drug or participation in an interventional study within 30 days prior to administration of study drug.
20. In the opinion of the Investigator, any disease processes or confounding variables that would inappropriately alter the outcome of the study.
21. Inability to comply with the requirements of the study protocol.

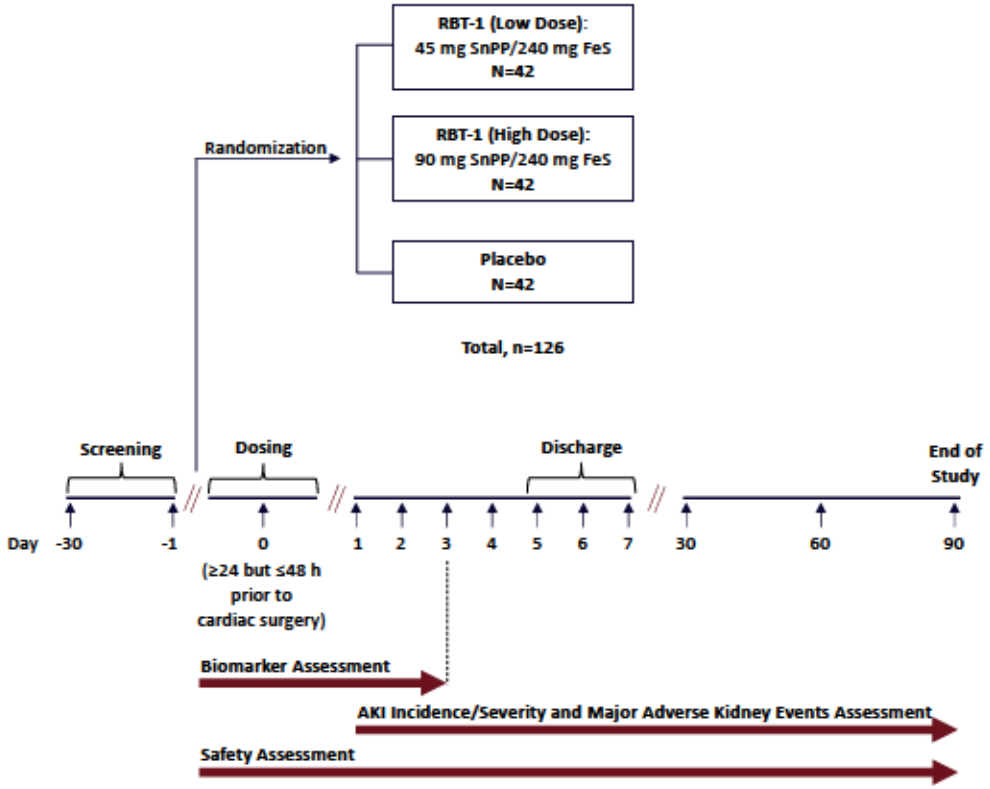
9.3 Study Design

This is a Phase 2, multicenter, double-blind, randomized, placebo-controlled study to evaluate the effect of RBT-1 on preconditioning response biomarkers in subjects who are at risk for AKI following cardiac surgery. Eligible subjects will be randomized to receive a single dose of low-dose RBT-1 (45 mg SnPP/240 mg FeS), high-dose RBT-1 (90 mg SnPP/240 mg FeS), or placebo (normal saline) via IV infusion over a 120-minute period ≥24 but ≤48 hours prior to scheduled cardiac surgery.

All subjects will be assessed through Day 90 post-cardiac surgery. Subjects will be evaluated at Screening, 48 and/or 24 hours prior to surgery depending on the time of dosing, on Day 1 (day of surgery) and daily through Day 7 or Discharge if earlier, as well as on Days 30, 60, and 90, as described in the Event Schedule (see Section 5). Assessments include biomarkers of preconditioning response and tubular injury, urine

output, incidence and severity of AKI, serum creatinine, days on ventilator, days in ICU, hospital length of stay, readmission rate, and safety (physical examination, clinical laboratory assessment, vital signs, concomitant medications, and adverse events). Evaluations for MAKE (death, need for dialysis, and change in kidney function) and safety will be performed through Day 90, as described in the Event Schedule (see Section 5).

The duration of the study will be approximately 90 days per subject. A schematic of the study design is presented below:



AKI = acute kidney injury; FeS = iron sucrose; h = hour; SnPP = stannous protoporphyrin

9.3.1 Stopping Rules

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

9.4 Recordkeeping and Monitoring

All subject data will be reported in the electronic data capture system, except for central laboratory reports, which will be uploaded by electronic transfer by the central 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 eCRFs. The supporting documentation will be maintained at the site for a minimum of either:

1. Two (2) years following the approval of RBT-1 for this indication by the Food and Drug Administration (FDA)

or

2. Two (2) years following notification by the Sponsor 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 IRB/IEC-approved protocol and ICF and the FDA and International Council for 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/IEC.

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/IEC and FDA or other governmental agency with oversight or compliance responsibilities for assuring subject rights and welfare.

9.5 Investigational Product

Subjects will be randomized to receive a single dose of double-blind RBT-1 (low-dose RBT-1 [45 mg SnPP/240 mg FeS] or high-dose RBT-1 [90 mg SnPP/240 mg FeS]) or placebo (normal saline).

9.5.1 Study Drug Description

The investigational product (RBT-1) is a combination of SnPP in a solution of sodium hydroxide and of FeS in a saline solution buffered with sodium bicarbonate.

Commercially available 0.9% sodium chloride (normal saline) for injection will be used for placebo administration at study sites.

9.5.2 Packaging

RBT-1 will be supplied as a sterile liquid (in separate vials of SnPP and FeS) for IV injection and is intended for single use. Glass vials of 5 mL are plugged with Teflon-coated rubber stoppers and sealed with aluminum seals.

9.5.3 Investigational Product Dispensing

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

9.5.4 Dosage Preparation and Administration

Detailed instructions for dose preparation will be specified in the Pharmacy Manual.

Study drug (RBT-1 or placebo) will be administered via IV infusion over a 120-minute period ≥24 but ≤48 hours prior to scheduled cardiac surgery. Study drug administration may be performed in an outpatient setting.

Subjects will be observed for signs and symptoms of hypersensitivity during study drug administration and for at least 30 minutes after administration. Subjects should be

observed until they are deemed clinically stable. RBT-1 will only be administered when personnel and therapies are available for the treatment of any infusion-related reactions.

9.5.5 Storage Requirements

RBT-1 should be stored at controlled room temperature conditions (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 the Sponsor.

9.5.7 Retrieval and Destruction

Periodically throughout the study, sites will perform investigational product reconciliation on a per-subject basis. The Sponsor's 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 Randomization and Blinding

Each subject will be assigned an identification number by the site after the subject provides written informed consent.

The identification number will consist of a 7-digit number:

- The first 4 digits will designate the site number as assigned by the Sponsor.
- The last 3 digits will designate the order of the subject at that site.

The first subject who signs the ICF at the site is assigned the identification number 001, the second subject who signs the ICF is assigned 002, the third is assigned 003, etc. The subject will maintain the same identification number throughout the entire study. If a subject signs the ICF but does not meet the inclusion/exclusion criteria, the subject will be marked as a screen failure on the Screening and Enrollment Log provided by the Sponsor and will be entered in the electronic data capture system as a screen failure.

If the subject qualifies for study entry after Screening, the site staff will use the Interactive Web Response System (IWRS) to obtain the subject's randomized, double-blinded treatment assignment.

9.7 Study Visits

9.7.1 Screening (Within 30 Days Prior to Visit 1)

Study candidates will be evaluated for study entry according to the stated inclusion and exclusion criteria (Sections 9.1 and 9.2, respectively). The following procedures will be performed during Screening to establish each candidate's eligibility for enrollment into the study:

- Obtain signed, written informed consent. The informed consent must be signed before any procedures are performed.

- Record demographic information.
- Record medical history.
- Record concomitant medications.
- Perform physical examination, including weight and body mass index.
- Record vital signs.
- Obtain urine sample for pregnancy testing of female subjects of childbearing potential.
- Obtain blood samples for serum chemistry and hematology testing.
- Assess serum ferritin if not already included in the local laboratory chemistry panel.
- Obtain urine sample for urinalysis.

The Investigator will evaluate the results of all examinations, including clinical laboratory tests, and determine each candidate's suitability for the study according to the eligibility criteria.

Screening and dosing can occur on the same day if all pre-dose assessments completed and evaluated prior to dosing. However, if Screening and dosing occur on the same day, blood and urine samples will need to be collected for central laboratory analysis prior to dosing, as would be done on Visit 1a.

Physical examinations and routine labs (serum chemistry, hematology, urinalysis) performed as part of standard of care within 1 month of dosing administration (Visit 1a) can be used for Screening assessments.

9.7.2 Visit 1a

The following procedures will be performed prior to dosing:

- Re-confirm subject's eligibility to participate in the study.
- Record concomitant medications.
- Perform physical examination.
- Record vital signs.
- Obtain blood samples for serum chemistry, hematology, and blood biomarker testing.
- Obtain urine sample for urinary biomarker testing.
- Access the IWRS to obtain the subject's randomized, blinded treatment assignment (see Section 9.6).

Once all pre-dose procedures have been completed:

- Study drug (RBT-1 or placebo) will be administered via IV infusion over a 120-minute period under the supervision of the Investigator/designee. The date,

rate of infusion, total volume of infusion, and start/stop times of dosing will be recorded.

- Solicit any adverse events.

9.7.3 Visit 1b

Visit 1b will be conducted in subjects who are administered study drug within 36 to 48 hours prior to cardiac surgery. The following procedures will be performed:

- Record concomitant medications.
- Obtain blood samples for blood biomarker testing.
- Obtain urine sample for urinary biomarker testing.
- Solicit any adverse events.

9.7.4 Visit 2 (Day 1)

9.7.4.1 Prior to Cardiac Surgery

The following procedures will be performed prior to the scheduled cardiac surgery on Day 1:

- Record changes in concomitant medications.
- Perform physical examination.
- Record vital signs.
- Obtain blood samples for serum chemistry, hematology, and blood biomarker testing.
- Obtain urine sample for urinary biomarker testing.
- Solicit any adverse events.

9.7.4.2 Post-Cardiac Surgery

The following procedures will be performed post-cardiac surgery on Day 1:

- Record changes in concomitant medications.
- Measure urine output.
- Solicit any adverse events.

9.7.5 Visits 3 – 8 (Days 2 – 7) or Discharge, If Earlier

The following procedures will be performed daily on Days 2 – 7 or discharge, if earlier, from the hospital:

- Record changes in concomitant medications.
- Perform physical examination; on Days 2 – 6, physical examination can be symptom-directed.
- Record vital signs.

- Obtain blood samples for serum chemistry and hematology.
- Measure urine output (Days 2 and 3 only).
- Obtain blood samples for blood biomarker testing (Days 2 and 3 only).
- Obtain urine sample for urinary biomarker testing (Days 2 and 3 only).
- Solicit any adverse events.

Note that the Day 7 visit may be conducted remotely as a virtual visit if an in-person visit is not possible. If a virtual visit is conducted: 1) blood and urine samples may be collected by remote phlebotomy, such as through a mobile phlebotomist or other blood collection center, and 2) the physical examination can be symptom-directed.

9.7.6 Visit 9 (Day 30)

Subjects will return to the site on Day 30 for the following procedures:

- Record changes in concomitant medications.
- Perform physical examination.
- Record vital signs.
- Obtain blood samples for serum chemistry and hematology.
- Obtain urine sample for urinalysis.
- Obtain urine sample for pregnancy testing in female subjects of childbearing potential.
- Solicit any adverse events.

Note that the Day 30 visit may be conducted remotely as a virtual visit if an in-person visit is not possible. If a virtual visit is conducted: 1) blood and urine samples may be collected by remote phlebotomy, such as through a mobile phlebotomist or other blood collection center, and 2) the physical examination can be symptom-directed.

9.7.7 Visit 10 (Day 60)

Subjects will return to the site on Day 60 for the following procedures:

- Record changes in concomitant medications.
- Record vital signs.
- Obtain blood samples for serum chemistry and hematology.
- Solicit any adverse events.

Note that the Day 60 visit may be conducted remotely as a virtual visit if an in-person visit is not possible. If a virtual visit is conducted, blood and urine samples may be collected by remote phlebotomy, such as through a mobile phlebotomist or other blood collection center.

9.7.8 Visit 11 (Day 90)/Final Visit

Subjects will return to the site on Day 90 for the following procedures:

- Record changes in concomitant medications.
- Perform physical examination, including weight and body mass index.
- Record vital signs.
- Obtain blood samples for serum chemistry and hematology.
- Obtain urine sample for urinalysis.
- Solicit any adverse events.

Note that the Day 90 visit may be conducted remotely as a virtual visit if an in-person visit is not possible. If a virtual visit is conducted: 1) blood and urine samples may be collected by remote phlebotomy, such as through a mobile phlebotomist or other blood collection center, and 2) the physical examination can be symptom-directed.

9.8 Clinical Laboratory Evaluations

Screening laboratory samples will be processed at a local laboratory. All other laboratory samples will be processed at a central laboratory. Each of the laboratory parameters to be evaluated during the study and collection times including serum chemistry, hematology, urinalysis, pregnancy testing, and biomarkers are detailed in the Event Schedule (see Section 5). Cystatin C will only be measured at Baseline (Visit 1a) and on Days 30 (Visit 9) and 90 (Visit 11).

Routine labs (serum chemistry, hematology, urinalysis) performed as part of standard of care within 1 month of dosing administration (Visit 1a) can be used for Screening assessments.

Blood and urine samples may be collected by remote phlebotomy, such as through a mobile phlebotomist or other blood collection center, for a visit conducted virtually on Day 7, 30, 60, or 90. Detailed instructions regarding sample collection and processing are provided in the laboratory manual.

9.9 Clinical Procedures and Observations

Investigators/study staff will regularly monitor subjects throughout study participation. At each post-cardiac surgery visit, the Investigator/study staff will record whether or not the subject is in the ICU, on a ventilator, has been discharged from the hospital, or readmitted to the hospital.

9.9.1 Medical History

A complete medical history will be obtained at Screening.

9.9.2 Concomitant Medications

All concomitant medications taken by the subject from the time of Screening through Day 90 will be recorded.

Concomitant medications that can cause photosensitivity should be avoided, if possible, during the study.

9.9.3 Physical Examination

Physical examinations will be performed by the Investigator or designee whose license permits the performing of physical examinations at the timepoints defined in the Event Schedule (see Section 5). Physical examinations should be performed at Screening, prior to dosing, prior to cardiac surgery on Day 1, Day 7, Day 30, and Day 90.

Physical examinations performed as part of standard of care within 1 month of dosing administration (Visit 1a) can be used for Screening assessments.

Symptom-directed physical examinations may be performed on Days 2 – 6. Symptom-directed physical examinations may also be performed on Days 7, 30, and 90 if those visits are conducted virtually.

Weight and body mass index will be collected at Screening and on Day 90.

9.9.4 Vital Signs

Vital signs (blood pressure, pulse rate, body temperature) will be collected throughout the study at the timepoints defined in the Event Schedule (see Section 5). During hospitalization, vital signs will be recorded once per day.

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 (Day 90). Adverse events will be followed by the Investigator until event resolution, the subject is lost to follow-up, or the adverse event is otherwise explained or not considered clinically significant by the Investigator. Adverse events not resolved by Day 90 will be considered ongoing.

Adverse events assessments will include photosensitivity assessment. Because safety data from IV SnPP administration at doses up to 3 mg/kg has shown photosensitivity in subjects exposed to sunlight or ultraviolet-A light following dosing [Anderson 1986, Berglund 1990, Dover 1993, Galbraith 1989, Kappas 1988], subjects will be advised to do the following for 30 days following treatment administration:

- Wear a broad-spectrum sunscreen that is at least SPF (sun protection factor) 30, even on cloudy or cool days. Sunscreen should be applied 30 minutes before going outside, giving the skin time to absorb it. Because sunscreen tends to be broken down over time by the sun and rubbed or washed off with sweating and water exposure, it should be reapplied at least every 2 hours outdoors and immediately after swimming or heavy sweating. At least 1 ounce (2 tablespoons) is needed to cover the entire body surface.
- Avoid direct sunlight, especially during the times of day when sun rays are strongest; sit in the shade.
- Avoid tanning beds.
- Wear long sleeves, pants, or long skirts when in the sun.
- Wear a hat with a wide brim all the way around to shade the face, ears, and neck.

- Wear sunglasses.

10.1 Investigator Reporting Requirements

The Investigator must immediately report (within 24 hours by a written report sent by fax or e-mail) all serious adverse events to the Sponsor, 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, FCRS
Medical Monitor
Renibus Therapeutics, Inc.

E-mail: SAE_REN004@renibus.com
Fax: 1-817-887-5046

Because the Investigator is knowledgeable about the human subject (eg, medical history, concomitant medications), administers the study drug, monitors the subject's response to the study 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 (ie, whether there is a reasonable possibility that the study drug caused the event) in the report to the Sponsor. Copies of each report to the Sponsor will be kept in the Investigator's study file.

The Investigator is responsible for complying with their IRB/IEC's requirements for reporting serious adverse events. Any expedited safety report (ie, serious unexpected suspected adverse reaction) received from the Sponsor should be submitted to the IRB/IEC. Copies of each report and documentation of IRB/IEC 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 the Sponsor according to the timetable for reporting (eg, end of study).

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 (eg, 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" (ie, 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 (eg, 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 (eg, tendon rupture)
- An aggregate analysis of specific events observed in a clinical study (eg, 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's 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's 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 Investigational New Drug (IND) Safety Reports and to other appropriate regulatory authorities in countries other than the United States in an Individual Case Safety Report or using a Council for International Organizations of Medical Sciences 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.
- Inpatient 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 the Sponsor, 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's 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 (eg, IND Safety Report or Individual Case Safety Report) of potentially serious risks from clinical studies or any other source (ie, a serious unexpected suspected adverse reaction), as soon as possible, but no later than 15 calendar days after the Sponsor 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 (ie, 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 the Sponsor receives the safety information.

10.2 Assessment of Relationship to Study Drug

The following criteria must be used to by the Investigator to characterize the relationship or association of study drug in causing or contributing to the adverse event:

Unrelated: Relationship suggests no association of the event to study drug; the event is related to other etiologies, such as the subject's clinical state or concomitant medications

Unlikely: Relationship suggests an unlikely association of the event to study drug given the subject's current disease state

Possible: Relationship suggests treatment with study drug caused or contributed to the event; the event follows a reasonable temporal sequence from administration of study drug and/or follows a known response pattern to the study drug; the event might have been produced by other factors (eg, the subject's clinical state or other modes of therapy administered to the subject)

Related: Relationship suggests a definite causal relationship to study drug; the event follows a known response pattern to the study drug and cannot be reasonably explained by the known characteristics of the subject's clinical state or other modes of therapy administered to the subject

10.3 Assessment of Severity

The following criteria must be used by the Investigator to rate the intensity of the adverse event:

Mild: Awareness of signs or symptoms, but easily tolerated and are of minor irritant type causing no loss of time from normal activities; symptoms do not require therapy or a medical evaluation; signs and symptoms are transient

Moderate: Events introduce a low level of inconvenience or concern to the subject and may interfere with daily activities, but are usually improved by simple therapeutic measures; moderate experiences may cause some interference with functioning

Severe: Events interrupt the subject's normal daily activities and generally require systemic drug therapy or other treatment; they are usually incapacitating

10.4 Breaking the Blind

This is a double-blind study. Breaking the blind in a clinical trial on an emergency basis should only occur when knowledge of the treatment to which a subject was allocated would have implications for the emergency medical management of the subject or if required for regulatory reporting. If necessary, emergency breaking of the blind can be conducted through the Interactive Web Response System by the Medical Monitor. The reason for unblinding, person conducting the unblinding, person(s) who know the unblinded treatment, and date/time of unblinding will be recorded.

11 STATISTICAL CONSIDERATIONS

This section outlines the statistical considerations to be used in the analysis of data from the study. A separate SAP will describe data handling and statistical techniques.

11.1 Criteria for Evaluation

Efficacy assessments include the following:

- Change in preconditioning plasma biomarkers from Baseline (pre-dose) through Day 1 pre-op:
    - HO-1, ferritin, and IL-10
- Change in renal tubular injury biomarkers post-cardiac surgery through Day 3:
    - KIM-1, cystatin C, and NGAL

- Reduction in urine output (documented oliguria of <0.5 mL/kg/hour, not due to urinary tract obstruction) for more than 6 hours post-cardiac surgery through Day 3.
- Incidence of AKI post-cardiac surgery through Day 5, defined as:
    - An absolute increase in serum creatinine by 0.5 mg/dL, which is present over 2 laboratory measurements at least 12 hours apart; or
    - An increase in serum creatinine ≥1.5X Baseline.
- Severity of AKI post-cardiac surgery through Day 7.
- Average increase in serum creatinine post-cardiac surgery through Day 7 or until Discharge if prior to Day 7.
- Occurrence of MAKE30 and MAKE90 post-cardiac surgery.
- Proportion of subjects experiencing MAKE30.
- Proportion of subjects experiencing MAKE90.
- Days on ventilator.
- Days in intensive care unit.
- Hospital length of stay.
- Readmission rate.
- Changes in biomarkers of RBT-1 activity and/or kidney function.

Safety assessments include the following:

- Adverse events.
- Laboratory data and vital signs at regular intervals.

11.2 Study Populations

The efficacy analyses will be conducted using the Intent-to-Treat population (all randomized subjects) and Per Protocol population (all randomized subjects who had no major protocol deviations). Safety analyses will be conducted using all subjects receiving any amount of study drug.

11.3 Data Analysis

Details of the efficacy analysis and safety analysis will be defined in the SAP. 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 study drug.

11.4 Sample Size Calculations

Assuming an increase of 30% in the primary biomarkers from baseline in the RBT-1 treated groups versus a 10% reduction in the placebo group and a standard deviation of 10%, 6 subjects per group would provide 80% power to detect a difference between treatment and placebo groups with a significance level of 0.05. From a safety perspective, 40 subjects per group is consistent with the sample size typically used in early-stage clinical trials.

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 (ie, 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 will be included in the 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/IEC will be notified of termination and reason(s). Procedures for follow-up of research subjects will be developed by the study physician in consultation with the Sponsor and IRB/IEC.

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. A comprehensive Data Management Plan will be developed and approved by a representative of the Data Management contract research organization 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 adverse events are recorded to the satisfaction of the Chief Medical Officer;
- All data queries have been resolved to the satisfaction of the Lead Biostatistician;
- All decisions have been made regarding all protocol violators and population exclusions;

- Written authorizations to lock the database are obtained from Clinical Data Management and the Chief Medical Officer.

14 ETHICAL CONSIDERATIONS

14.1 Ethical Conduct of the Study

The study will be conducted in accordance with US Title 21 CFR and the ICH E6 (R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1) Guidance for Industry. The Declaration of Helsinki and its most recent updates (Fortaleza, Brazil; 2013) will be observed.

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

The Investigator will supply the following to the IRB/IEC:

- 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/IEC original approval of the protocol and the informed consent, and re-approval of the study (annual or semi-annual, per IRB/IEC guidelines)
- The IRB/IEC 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/IEC 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/IEC 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 will monitor safety data throughout the course of the study. The Sponsor's Drug Safety Department, 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 Guidance 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/IEC 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 the 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 Clinical Research Organization during the study. I will adhere to all FDA, ICH, revised Declaration of Helsinki (2013) 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-004

A Phase 2, Double-Blind, Randomized, Placebo-Controlled Study to Evaluate the Effect of RBT-1 on Preconditioning Response Biomarkers in Subjects Undergoing Coronary Artery Bypass Graft (CABG) and/or Cardiac Valve Surgery (The START Study)

Protocol – Version 5: 25 Aug 2022

17 REFERENCES

Anderson KE, Simionatto CS, Drummond GS, Kappas A. Disposition of tin-protoporphyrin and suppression of hyperbilirubinemia in humans. *Clin Pharmacol Ther*. 1986;39(5):510-20.

Berglund L, Angelin B, Blomstrand R, Drummond G, Kappas A. Sn-protoporphyrin lowers serum bilirubin levels, decreases biliary bilirubin output, enhances biliary heme excretion and potently inhibits hepatic heme oxygenase activity in normal human subjects. *Hepatology*. 1988;8(3):625-31.

Berglund L, Angelin B, Hultcrantz R, et al. Studies with the heme oxygenase inhibitor Sn-protoporphyrin in subjects with primary biliary cirrhosis and idiopathic haemochromatosis. *Gut*. 1990;31(8):899-904.

Chawla L, Kimmel P. Acute kidney injury and chronic kidney disease: an integrated clinical syndrome. *Kidney Int*. 2012;82(5):516-24.

Dover SB, Moore MR, Fitzsimmons EJ, Graham A, McColl KE. Tin protoporphyrin prolongs the biochemical remission produced by heme arginate in acute hepatic porphyria. *Gastroenterology*. 1993;105(2):500-6.

Emtestam L, Angelin B, Berglund L, Drummond GS, Kappas A. Photodynamic properties of Sn-protoporphyrin: clinical investigations and phototesting in human subjects. *Acta Derm Venereol*. 1993;73(1):26-30.

Galbraith RA, Kappas A. Pharmacokinetics of tin-mesoporphyrin in man and the effects of tin-chelated porphyrins on hyperexcretion of heme pathway precursors in subjects with acute inducible porphyria. *Hepatology*. 1989(9):882-8.

Honda IK, Hishida A, Ikuma K, Yonemura K. Acquired resistance to acute renal failure: editorial review. *Kidney Int.* 1987;31(6):1233-8.

Kappas A, Drummond GS, Manola T, Petmezaki S, Valaes T. Sn-protoporphyrin use in the management of hyperbilirubinemia in term newborns with direct Coombs-positive ABO incompatibility. *Pediatrics*. 1988;81(4):485-97.

KDIGO AKI Work Group. KDIGO clinical practice guideline for acute kidney injury. *Kidney Int Supp*. 2012;17:1-138.

Kellum J, Lameire N. Diagnosis, evaluation, and management of acute kidney injury: A KIDGO summary (part 1). *Crit Care*. 2013;17(1):204.

Rosner MH, Okusa MD. Acute kidney injury associated with cardiac surgery. *Clin J Am Soc Nephrol*. 2006;1(1):19-32.

Venofer® [package insert]. American Regent, Inc., Shirley, NY; August 2015.

Venofer® [summary of product characteristics]. Vifor Pharma UK Limited, Surrey, UK; November 2016.

Zager RA, Baltes LA, Sharma HM, Jurkowitz MS. Responses of the ischemic acute renal failure kidney to additional ischemic events. *Kidney Int.* 1984;26(5):689-700.

Zager RA, Burkhart KM, Gmur DJ. Postischemic proximal tubular resistance to oxidant stress and Ca2+ ionophone-induced attack. Implications for reperfusion injury. *Lab Invest.* 1995a;72(5):592-600.

Zager RA. Heme protein-induced tubular cytoresistance: expression at the plasma membrane level. *Kidney Int.* 1995b;47(5):1336-45.

Zager RA. Obstruction of proximal tubules initiates cytoresistance against hypoxic damage. *Kidney Int.* 1995c;47(2):628-37.

Zager RA, Johnson AC, Frostad KB. Combined iron sucrose and protoporphyrin treatment protects against ischemic and toxin-mediated acute renal failure. *Kidney Int.* 2016;90(1):67-76.