STATISTICAL ANALYSIS PLAN

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

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List of ACRONYMS/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
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
Hp	Haptoglobin
ICF	informed consent form
ICH	International Conference on Harmonisation
ICSR	Individual Case Safety Report
IEC	Independent Ethics Committee
IL-6	Interleukin 6
IL-10	Interleukin 10
IND	investigational new drug
IRB	Institutional Review Board
ITT	Intent-to-Treat
KIM-1	Kidney Injury Molecule-1
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
POC	Proof of Concept
PK	Pharmacokinetic(s)
PP	Per Protocol
SAS	Statistical Analysis System
SnPP	Stannous Protoporphyrin
TEAE	treatment emergent adverse event
$T_{½}$	elimination half-life

Abbreviation	Definition
TFL	Tables, Figures, and Listings
Tmax	time to maximum serum concentration
Vz	volume of distribution

1 INTRODUCTION

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.

The purpose of this statistical analysis plan (SAP) is to specifying the statistical approaches for the data analysis prior to database lock. This SAP covers the planned analyses of all data collected on the electronic case report forms (eCRFs). This SAP supersedes the statistical methods described in the clinical protocol. Deviations/changes from the planned analyses described in this SAP will be identified, with justification, in the appropriate section of the clinical study report. This SAP is developed based on the clinical study protocol REN-003, dated 29 March 2019.

The reader of this SAP is encouraged to also read the clinical protocol and other related documents for details on the planned conduct of this study. Operational aspects related to the collection and timing of planned clinical assessments are not repeated in this SAP unless relevant to the planned analyses.

2 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.

3 STUDY OVERVIEW

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.

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.
- If subjects meet eligibility criteria, they will receive SnPP by slow intravenous injection over a 120-minutes on study day1.
- Subjects will stay in the clinic for the initial 24 hours after administration of SnPP and will be discharged at the end of study day 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..Up to a total of 42 subjects consisting of 18 healthy volunteers and 24 chronic kidney disease (stage 3-4) subjects will receive single escalating doses of SnPP.

For a complete list of assessments performed throughout the study, see Time and Events Schedule (Section 10).

4 EFFICACY, PHARMOCOKINETIC, AND SAFETY VARIABLES

Efficacy Variables

- Percent change from baseline in biomarkers at each postbaseline time point
- Change from baseline in biomarkers at each postbaseline time point

The biomarkers include the following pharmacodynamic variables

- Haptoglobin (Hp)
- Ferritin
- Bilirubin
- Hemopexin
- IL-10
- Heme Oxygenase 1 (HO-1)
- P21

The urine biomarkers include the following pharmacodynamic variables

- Hemopexin
- Ferritin
- P21
- Heme-Oxygenase 1 (HO-1)

Pharmacokinetic (PK) Variables

- Maximum serum concentration (Cmax)
- Time to maximum serum concentration (Tmax)
- Area under the concentration-time curve from 0 to last time point (AUC_{0-t})
- Area under the concentration-time curve from 0 to infinity (AUC_{0-inf})
- Terminal elimination rate constant (λ_z)
- Terminal elimination half life ($T_{1/2}$)
- Clearance (CL)
- Volumn of distribution (V_z)

- Renal excretion and renal clearance (CL) of tin

Safety Variables

- Treatment-emergent adverse events
- Infusion-related reactions
- Clinical laboratory tests
- ECG
- Vital signs
- Physical exams
- Concomitant medications

5 DEFINITIONS

Terminology	Definitions
Enrolled	Subjects who signed the informed consent, meet all inclusion criterian and none of the exclusion criteria are entered into the study
Completer	Subjects who complete the End of Study (EOS) visit
Study Day	Day 1 is defined as the date subject took the study medication. Study Day for event post Day 1 is calculated as Event Date – Day 1 Date +1. Study Day for event prior to Day 1 is calculated as Event Date – Day 1 Date. Note: There is no Day 0.
Baseline	Baseline is defined as the non-missing value collected most recent to and before the study of the study medication infusion
Prior Medication	Medication started and ended prior to the study drug infusion
Concomitant Medication	Medication taken on/after the study drug infusion. Concomitant medication may start prior to study drug infusion
Treatment-emergent Adverse Event	Adverse event with onset date/time on or after the start of study drug infusion.

6 ANALYSIS POPULATIONS

The **Safety Analysis Population** will include all subjects who received study medication. This population will be used for all demographics, baseline characteristics, and safety summaries.

The **Modified Intent-to-Treat (MITT) Population** will include all subjects who received study medication and have a post-baseline efficacy assessment. This population will be used for all efficacy analysis.

The **PK Analysis Population** will include all subjects who receive study medication and provided sufficient and valid serum SnPP concentration data to allow the determination of PK parameters. This population will be used for the PK parameter summaries.

7 STATISTICAL METHODS OF ANALYSIS

7.1 General Principles

The statistical analyses will be reported using summary tables, listings, and figures (TLFs). All analyses and tabulations will be performed using SAS® Version 9.4 or later. Continuous variables will be summarized using descriptive statistics (sample size [n], mean, standard deviation [SD], minimum, median, and maximum). Categorical variables will be tabulated with number and percentage of subjects. Unless otherwise noted, percentages will be based on the number of subjects from the population, as appropriate.

Individual subject data will be provided in listings. All listings will be sorted by dose group (1 = 9mg SnPP, 2 = 27mg SnPP, 3 = 90mg SnPP), stratum (CKD Stage 3 patients and CKD Stage 4 patients versus healthy volunteer subjects), and collection date and time, where applicable,

Unless otherwise noted, tabulations of categorical data will present only those categories appearing in the data.

7.1.1 *Handling Missing Values*

7.1.1.1 Study Drug Administration Date or Time

It is expected that all necessary information on study drug administration (start and stop date and time) will be complete. Any such information that is missing and cannot be obtained through query resolution may be imputed, on a case-by-case basis, in a conservative manner that minimizes bias.

7.1.1.2 Adverse Event or Concomitant Medications Dates or Times

For AEs or concomitant medications with missing or partially missing start/stop date/time, the following imputation rules will be applied:

For partial start date/time:

- If the year is unknown, then the date will be assigned as the date and time of first dose of study treatment.
- If the month is unknown, then:
  - i) If the year matches the year of study drug administration, then the month and day will be imputed to be the first month and day on study.
  - ii) Otherwise, ‘January’ will be assigned.

- If the day is unknown, then:
  - i) If the month and year match the month and year of the dose of the study drug administration date, then the day of the dose of earliest study drug administration date with matching month and year will be imputed.
  - ii) Otherwise, '01' will be assigned.
- If the time is unknown, then:
  - i) If the date (day, month, and year) matches the date of an administration of study drug, then the time of dose of study drug time from that date will be imputed.
  - ii) Otherwise, '00:00' will be assigned.

For partial stop date/time:

- If the year is unknown, then the date will be assigned as the date subject discontinued from study, and the time will be set to the last time of the day ('23:59').
- If the month is unknown, then the month subject discontinued from study will be assigned.
- If the day is unknown, then the last day of the month will be assigned.
- If the time is unknown, then the last time of the day will be assigned ('23:59').

7.1.1.3 Adverse Event Severity or Relationship to Study Drug

If severity of an AE is not reported, then for tables of AEs by severity, the event will be classified as 'Severe' and clearly footnoted. If relationship to study drug is not reported for an AE, then for tables of study-drug related AEs, the event will be assigned the relationship of 'definite'. Tables presenting related AEs will include all AEs with relationships of 'possible', 'probable' or 'definite' as assessed by the investigator or defined as 'definite' when no investigtor assessment is made.

7.1.2 ***Multiplicity Adjustments***

Not applicable.

7.1.3 ***By-Center Analyses***

This is a single-center study.

7.2 Subject Disposition

Subject disposition will be tabulated by dose group and overall and will present the number and, when appropriate, percentage of subjects who were:

- Enroled
  - Not treated
  - Treated
- In the safety analysis set
- In the PK analysis set
- In the MITT analysis set
- Completed the study as planned

- Discontinued from the study, and
- Reasons for discontinuation from the study

Percentages for all rows will use the number of subjects treated as the denominator.

The reasons for exclusion of subjects from the MITT and PK analysis set will be included in the data listing.

Data will be summarized for each dose group and overall as well as by stratum (CKD Stage 3 patients and CKD Stage 4 patients versus healthy volunteers).

Note that the reasons for exclusion of a subject from the MITT and PK analysis set are not mutually exclusive, thus a subject may be excluded for mutliple reasons.

7.3 Description of Demographics and Baseline Characteristics

7.3.1 *Demographics*

The summary of demographic data will present:

- Age (years) – descriptive statistics
- Sex – n (%)
- Ethnicity – n (%)
- Race – n (%)

Age is calculated from the date the subject signed the informed consent form (ICF) and birth. It is presented as the number of years, rounding down to the nearest integer year. For partial birthdates, impute the first of the month for missing day and January for missing month to calculate age. It is presumed that birth year is known.

The demographic summary will present the data for each dose group and overall as well as by stratum (CKD Stage 3 patients and CKD Stage 4 patients versus healthy volunteers). Summaries will be provided for all (safety, MITT and PK) analysis sets.

7.3.2 *Baseline Characteristics*

The summary of baseline characteristic data will present:

- Physical examination and weight
- Electrocardiogram

Descriptive statistics (n, mean, SD, median, minimum and maximum) will be provided by dose group for the Safety Analysis Set.

7.4 Prior and Concomitant Medications

Prior and concomitant medications will be coded using the World Health Organization Drug Dictionary (WHODD version March 2019) and will be classified according to the default anatomical therapeutic chemical (ATC) classification and preferred term.

Prior medications are defined as medications with a stop date and time prior to the start of study drug administration.

Concomitant medications are defined as medications taken after the start of study drug administration (i.e., started prior to the start of study drug administration and continued after or started after the start of study drug administration).

Prior and concomitant medications will be summarized separately using n (%) of subjects for each dose group by ATC class and preferred term for the safety analysis set.

Subjects may have more than one medication per ATC class and preferred term. At each level of subject summarization, a subject will be counted only once if one or more medications are reported by the subject at the same level.

7.5 Measurements of Treatment Compliance

Study treatment is administered by the site personnel other than the subject him/herself, therefore compliance is assured.

7.6 Efficacy Analysis

7.6.1 *Percent Chamge in Response Biomarkers*

The efficacy endpoint is the percent change (PCH_T) in each response biomarker relative to the Baseline period.

PCH_T will be calculated as 100 x (T – B)/B, where T and B are Response biomarker at 2, 4, 8, 12, 18, 24, 48, 72, 96 and 168 hours post dose (T) and baseline period (B).

PCH_T will be analyzed using a Mixed-Effect Model for Repeated Measure (MMRM) as follows:

- A 2-way MMRM pooling stage 3 and stage 4 CKD patients and healthy subjects together
- A 2-way MMRM pooling stage 3 and stage 4 CKD patients together
- A 2-way MMRM for Healthy subjects

The model will include dose level, visit (2, 4, 8, 12, 18, 24, 48, 72, 96 and 168 hours), and dose-by-visit interaction as factors and baseline response biomarker as covariate. The model parameters will be estimated using the restricted maximum likelihood method with the toeplitz variance-covariance matrix and Kenward-Roger estimate for the denominator degrees of freedom. The between dose comparison will be performed using constrast in the main effect and the simple contrast at each time point. Least squares mean (LSM) and the associated 95% confidence interval (CI) for each dose level will be presented. The difference in LSM between doses and the 95% CI for the difference will also be presented. Results will be presented for the pooled population, as well as for the CKD and healthy populations separately.

Additionally, LSM for each dose group will be plotted over time for the CKD patients and healthy volunteers pooled population as well as for each population separately. Dose response relationship will be examined graphically and may be analyzed where appropriate.

A sample SAS code is provided as follows. The code assumes that "visit" has 10 values (2, 4, 8, 12, 18, 24, 48, 72, 96 and 168 hours), "dose" is coded as 1 = 9 mg SnPP, 2 = 27 mg SnPP, and 3 = 90 mg SnPP, "ckd" is coded as 2= CKD patients and healthy volunteers pooled, 1 = patients with CKD, 0 = healthy colunteers, and "resp" = PCH_T, the % change from baseline being analyzed.

```
ODS OUTPUT LSMEANS=lsm DIFFS=diff ESTIMATE=estm;
PROC MIXED DATA=XXXX;
        BY ckd;
        CLASS dose usubjid visit;
        MODEL resp = base visit dose dose*visit / DDF=KR;
        REPEATED visit / SUBJECT=usubjid(dose) TYPE=UN;
        ESTIMATE "90 mg v 27 mg overall" dose  1 0 -1;
        LSMEANS dose / DIFF CL;
        ESTIMATE "90 mg v 27 mg @ 168 hours " dose 0 -1 1
             dose*visi 0 0 0 0 0 0 0 0 0 0
                                  0 0 0 0 0 0 0 0 0 -1
                                  0 0 0 0 0 0 0 0 0 1 / CL;
RUN;
ODS OUTPUT CLOSE;
```

Note: Depending on the convergence status in the computation, Toeplitz (toep), 1st order autoregressive AR(1), and compound symmetry (CV) variance-covariance matrix will be applied sequentially. The final analysis will be based on the first matrix leading to the convergence in the computation.

7.6.2 *Absolute Chamge*

In addition to the percent changes from baseline, absolute change from baseline will also be analyzed using MMRM. The method of analysis will be identical after replacing variable "resp" with "T-B"in Section 7.6.1.

7.6.3 *Responder Analysis*

Repeated measures responder analyses will be fitted using the generalized estimating equations (GEE) with PROC GENMOD in SAS for the proportion of patients reporting a response (defined as a 50% or greater reduction in each response biomarker from baseline) at each Visit.

The model set up is similar to MMRM (Section 7.6.1). A logit link function will be used for this binary response variable. The unstructured within-subject variance-covariance matrix will be assumed. If computational convergence is an issue, other types of variance-covariance matrices will be explored. Odds ratios and 95% confidence intervals will be presented comparing proportion of responders between dose levels at each assessment timepoint. Results will be presented for the pooled population, as well as for the CKD and healthy populations separately.

Sample SAS code is given below. Variable "resp" is coded as 1 = Responder and 0 = Non-Responder.

```
ODS OUTPUT LSMEANS=lsm DIFFS=diff ESTIMATE=estm;
 PROC GENMOD DATA=dataset;
  BY ckd;
  CLASS visit dose usubjid;
     MODEL resp(EVENT='1') = base visit dose dose*visit / LINK=LOGIT DIST=BIN;
  REPEATED SUBJECT = usubjid /TYPE=UN;
  LSMEANS dose / DIFF CL;
 RUN;
 ODS OUTPUT CLOSE;
```

7.6.4 *Exploratory analysis*

Percent change and change in each response biomarker will be analyzed using the Cochran-Mantel-Haensel (CMH) test stratified by baseline. In the stratification, baseline will be divided into quintiles based on the pooled CKD and Healthy subjects in the MITT population. Between group comparison will be carried out at each time point using the CMH test for row mean score difference (RMS) with the modified ridit scores. Analysis will compare the 90 mg group to the 27 mg group at each timepoint overall and for CKD patients and healthy volunteers separately.

Sample SAS code is as follows:

```
PROC FREQ;
        BY visit;
        TABLE base*dose*resp / SCORE=MODRIDIT;
RUN;
```

7.7 Safety Analyses

No inferential statistics are planned for any safety assessment.

7.7.1 *Adverse Events*

Safety assessments will be based mainly on the nature, frequency, relationship, and severity of adverse events (AEs). AEs will be coded by primary system organ class (SOC) and preferred term (PT) according to the Medical Dictionary for Regulatory Activities (MedDRA) version 22.0. The treatment-emergent adverse events (TEAEs) will be summarized by the number and percentage (n and %) of subjects in each SOC and PT. For summaries by relationship to study drug, "possibly related" will be combined with "related", and "unlikely/remotely related" will be combined with "not related." When multiple AEs are reported with the same preferred term, the AE of the strongest relation will be included in the summary by relationship, and the AE of the most severe grade will be included in the summary by severity table.

The following AE incidence tables will be presented.

- Overview of TEAEs to include

  - Number (%) of subjects who reported at least one TEAE
  - Number (%) of subjects who reported at least one treatment-related TEAE
  - Number (%) of subjects who reported at least one severe TEAE
  - Number (%) of subjects who reported at least one serious TEAE
  - Number (%) of subjects who reported at least one TEAE leading to treatment discontinuation
- TEAEs by PT sorted by decreasing order of subject incidence in the combined dose groups
- TEAEs by SOC and PT sorted alphabetically
- Study Drug-Related TEAEs by SOC and PT
- TEAEs with Grade 2+ Severity by SOC and PT

All AEs will be listed with onset/stop day, relationship to study drug, severity, action taken, and outcome. Pertinent subject information including dose group and demographics will also be included. Separate listings will be provided for TEAEs leading to study discontinuation, treatment-emergent serious AEs (TESAEs), and infusion-related TEAEs.

7.7.2 *Laboratory Test*

Hematology, biochemistry, and urinalysis test results will be summarized by dose group and visit using descriptive statistics. For quantitative laboratory parameters, both actual values and change from baseline values will be summarized.

Laboratory test result will be classified as low, normal, high (LNH) according to whether the value is below (L), within (N), or above (H) the reference range provided by the laboratory. Within-treatment comparisons for hematology and chemistry will be based on the 3x3 shift tables that, for a particular laboratory test, showing how the LNH classification at baseline shifts to the LNH classification at postbaseline visit.

A complete lab data listing, including hematology, biochemistry, and urinalysis will be provided for all subjects.

7.7.3 *Vital Signs*

Vitals signs are resting pulse (beats per minute), oral body temperature (°C), systolic blood pressure (mmHg), and diastolic blood pressure (mmHg). Vital signs will be summarized by dose group at each scheduled timepoint. Summaries will present both actual and change-from-baseline results.

7.7.4 *ECG*

The investigator evaluation for the overall ECG findings by normal, abnormal not clinically significant (NCS), and abnormal clinically significant (CS) will be tabulated with number and % of subjects at each time point.

Actual and change from baseline in PR, QRS, QT, RR, QTc, and HR will be summarized with descriptive statistics at each time point.

Data listings will be presented for ECG parameters by time point. QTc meeting the criteria of >450 but ≤480, >480 but ≤500 or >500 msec for the actual value, or >30 msec or >60 msec increase from baseline will be identified.

7.8 Pharmacokinetic Analysis

7.8.1 *Sample collections for Pharmacokinetic Analysis*

Blood samples will be obtained for PK analysis at baseline, before dosing and again at 1, 2, 3, 4, 6, 8, 12, 18, and 24 hours post-dosing.

7.8.2 *Pharmacokinetic Parameter Calculation Methods*

Pharmacokinetic parameters will be calculated by the noncompartmental analysis method from concentration-time data following these guidelines:

- Actual sampling times relative to the start time of the study drug infusion will be used for all calculations of the PK parameters. If the actual time is not available, the scheduled time will be used.
- Concentrations from unscheduled PK blood samples will be used in the derivation of the parameters.
- There will be no imputation of missing data.

For the calculation of the non-compartmental parameters,

- Pre-dose concentration ≥5% of the corresponding C_{max} will be excluded from analysis.
- Concentration below the lower limit of quantification (LLOQ) occurring from the predose to the first concentration ≥LLOQ will be set to zero.
- All remaining concentration below LLOQ (BLOQ) will be set to missing.

Pharmacokinetic parameters will be estimated according to the following guidelines:

- The maximum observed plasma concentration (C_{max}) will be obtained directly from the concentration-time data.
- Time to maximum concentration (T_{max}) is the time at which C_{max} is observed.
- The apparent terminal elimination rate constant (λ_z) will be estimated at terminal phase by linear regression after log-transformation of the concentrations:
    - Only those data points that are judged to describe the terminal log-linear decline will be used in the regression.
    - A minimum number of three data points in the terminal phase will be used in calculating λ_z with the line of regression starting at post-C_{max} data point (C_{max} will not be part of the regression slope) and including C_{last} and t_{last}.
    - The adjusted correlation coefficient (R^2 adjusted) in general should be greater than 0.90. Any value less than 0.90 may be used according to the pharmacokineticist's best knowledge and judgment.
    - An appropriate number of decimal places should be used for λ_z to enable the reported value of half-life ($t_{½}$) to be calculated.
- Half-life ($t_{1/2}$) will be calculated as $Log_e(2)/\lambda_z$.

- AUC will be calculated as follows:
    - The linear trapezoidal method will be employed for all incremental trapezoids arising from increasing concentrations and the logarithmic trapezoidal method will be used for those arising from decreasing concentrations.
    - $AUC_{(0-t)} = \int_0^t C(t)dt$
    - $AUC_{(O-\infty)} = \int_0^t C(t)dt + \int_t^\infty C(t)dt = AUC_{(0-t)} + C_t / \lambda_z$
- C_t is the last observed quantifiable concentration.
- Volume of distribution (V_d) will be estimated based on the teriminal phase as Dose/($\lambda_z \cdot AUC_{0-\infty}$).
- Apparent clearance CL will be estimated as Dose/$AUC_{0-\infty}$.

7.8.3 *Pharmacokinetic Concentrations and Variables*

The analysis of the PK data will be based on the PK analysis set.

Concentrations will be listed by dose group, subject, nominal time, and actual time. Concentrations that are below LLOQ will be indicated by BLOQ in this listing.

Concentrations will be summarized by dose group at each time point. The following descriptive statistics will be presented at each nominal time point: n, arithmetic mean, SD, geometric mean, %CV, median, minimum and maximum.

PK parameters will be summarized. Descriptive statistics for calculated PK parameters will include: n, arithmetic mean, SD, geometric mean, %CV, median, minimum and maximum values. The geometric mean and %CV will not be presented for T_{max}. Values of %AUC extrapolated > 20% will be flagged in the listings.

Individual plasma concentration versus actual times will be plotted by dose in linear and semi-logarithmic scale. Mean plasma concentration at the scheduled timepoints will be plotted by dose group in linear and semi-logarithmic scale with the associated standard errors (SE only for the linear scale) at each scheduled timepoint.

7.9 Interim Analysis

No interim analyses are planned for this study.

8 SAMPLE SIZE CALCULATIONS

No formal sample size calculation has been performed for the study. However, it is believed that 6 subjects per dose group will be adequate to establish differences in biomarker response between groups.

9 REFERENCES

10 TIME AND EVENTS SCHEDULE OF STUDY PROCEDURES

10.1 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

11 CONVENTIONS FOR THE PRESENTATIONS OF TABLES, FIGURES AND LISTINGS

The specification for the tables, Figures and Listings are provided in a separate document.

12 LIST OF TABLES

Note, all tables will be repeated for CKD Patients Population and Healthy Subjects Population with Table numbers.

CliniOps

STATISTICAL ANALYSIS PLAN (AMENDMENT # 1) of REN003

Version 1.0

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1. Statistical Analysis Plan Detail

Client Name:	Renibus Therapeutics, Inc.
Study Name:	A Phase 1b dose-escalating study with Stannous Protoporphyrin (SnPP), in healthy volunteers and subjects with chronic kidney disease stage 3-4
Protocol:	REN003
Protocol No.:	IND 138109
Protocol Date/Version:	25 March 2019
IND No.:	138109
Study Phase:	1b
Study Drug:	SnPP
Statistical Analysis Plan Date/Version	28 July 2019 Version.1.0
Prepared by:	Annetta Krebs, MS
Sponsor:	Renibus Therapeutics, Inc. 950 E State Highway 114, Suite 160 Southlake, TX 76092 Tel: (480) 220-7084

2. Purpose of Amendment

The purpose of this amendment is to adjust the section on the SAP to reflect the following changes:

- Define the baseline

3. Amendment Checklist

1. UPDATE the table in “SECTION 5: DEFINITIONS” to reflect the updated definition of ‘Baseline’:

Terminology	**Definitions**
Enrolled	Subjects who signed the informed consent, meet all inclusion criterian and none of the exclusion criteria are entered into the study
Completer	Subjects who complete the End of Study (EOS) visit

Terminology	Definitions
Study Day	Day 1 is defined as the date subject took the study medication. Study Day for event post Day 1 is calculated as Event Date – Day 1 Date +1. Study Day for event prior to Day 1 is calculated as Event Date – Day 1 Date. Note: There is no Day 0.
Baseline	Baseline is defined as the mean of three values from screening, study day -1 (one day before study day) and study day 1 prior to dosing
Prior Medication	Medication started and ended prior to the study drug infusion
Concomitant Medication	Medication taken on/after the study drug infusion. Concomitant medication may start prior to study drug infusion
Treatment-emergent Adverse Event	Adverse event with onset date/time on or after the start of study drug infusion.

2. Update the Table 14.3-2.1.1.1, Table 14.3-2.1.1.2, Table 14.3-2.1.1.3, Table 14.3-2.2.1.1 , Table 14.3-2.2.1.2, Table 14.3-2.2.1.3, Table 14.3-2.3.1.1, Table 14.3-2.3.1.2, Table 14.3-2.3.1.3, Table 14.3-3.1, Table 14.3-3.2 and Table 14.3-3.3 based on updated definition of 'Baseline'

All other contents remain the same

4. Signature

The following signature confirms that the activity mentioned above are completed by the responsible parties.

Donald Jeffrey Keyser Jeffrey Keyser President and COO Renibus Therapeutics, Inc	10/02/2020 Date
Anuradha Ghosh Anuradha Ghosh Data Science Lead and Project Manager CliniOps, Inc.	October 1, 2020 Date
	Date