
Statistical Analysis Plan

1. TITLE PAGE

Study Title: *Phase 2 Open Label Single Arm Repeat Dose Study to Assess the Effect of SNF472 on Wound Healing in Uraemic Calciphylaxis Patients*

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2. LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AE	Adverse event
BWAT	Bates Jensen Wound Assessment Tool
C _{max}	Maximum observed plasma concentration
CS	Clinically significant
CUA	Calciphic Uremic Arteriopathy
CRF	Case Report Form
ECG	Electrocardiogram
EOS	End of Study
FU	Follow-Up
ICF	Informed Consent Form
ICH	International Council on Harmonisation
IMP	Investigational medicinal product
IV	Intravenous
N	Number of subjects
NSAID	non-steroidal anti-inflammatory drugs
L	Litre
PK	Pharmacokinetic(s)
PTH	Parathyroid Hormone
QT	QT interval
QTc	Corrected QT interval
QTcB	Corrected QT interval using Bazett's formula
QTcF	Corrected QT interval using Fridericia's formula
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SCR	Screening
SOC	System Organ Class
VAS	Visual analog scale

3. VERSION HISTORY

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APPROVAL

A signature below confirms that this statistical analysis plan meets the analysis requirements for Protocol SNFCT2015-04.

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4. INTRODUCTION

This document describes the statistical methods and data presentations to be used in summarizing and analyzing the data for Protocol SNFCT2015-04. Background information is provided for the overall study design and objectives. The reader is referred to the study protocol and case report forms (CRFs) for details of study conduct and data collection.

5. STUDY OBJECTIVES AND ENDPOINTS

5.1 Study objectives

The objective of this study is to evaluate the safety and efficacy of SNF472 on top of standard of care on promoting wound healing and other parameters of therapeutic response in haemodialysis subjects with calciphylaxis (calcific uraemic arteriopathy, CUA).

5.2 Endpoints

5.2.1 Primary endpoint

- The primary endpoint is the absolute change in BWAT total score between baseline (Week 1) and Week 12 for the primary lesion (the largest one).

5.2.2 Secondary endpoints

Wound healing:

- Absolute change from baseline in BWAT total and component scores by visit for the primary lesion, secondary lesion, and tertiary lesions.
- Qualitative change from baseline in imaging score by visit for the primary lesion, secondary lesion, and tertiary lesions.

Pain:

- Absolute change from baseline by visit in the Pain Visual Analog Scale (VAS).

Quality of Life:

- Absolute change from baseline at Weeks 6 and 12 in global score and subscales of wound QoL score.

Wound and disease complications:

- Presence/absence of new wound infection at any time post baseline
- Presence/absence of new systemic infection at any time post baseline
- Surgical treatment of the wound at any time post baseline
- Requires or prolongs hospitalization
- Death

6. STUDY DESIGN

6.1 General study design and plan

It is an open-label single-arm clinical trial. Up to 15 CUA subjects will be enrolled, treated, and followed over a 12-week period to assess the safety and efficacy of SNF472 for the treatment of CUA.

6.2 Visits schedule

Table 001 Visits Schedule

	SCR	TREATMENT PERIOD																											FU									
Week	-2, -1	1			2			3			4			5			6			7			8			9			10			11			12			13
Day		1	3	5	1	3	5	1	3	5	1	3	5	1	3	5	1	3	5	1	3	5	1	3	5	1	3	5	1	3	5	1	3	5	1	3	5	
Informed Consent	X																																					
Demographics	X																																					
Lesions Overview	X																																					
Images Collection	X	X			X						X						X						X													X	X	
Visit	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Body Weight	X																X																			X		
Biopsy (if performed by site) ¹	X																																					
Physical Examination	X																																					X
ECG	X																																					X
ECG (pre dose & end of infusion)		X															X																			X		
Safety Laboratory Assessments ²	X	X															X																			X	X	
PTH		X																																			X	
Potassium, Magnesium, Iron (ferritin/transferrin) and Zinc	X																																					X
Potassium, Magnesium, Iron (ferritin/transferrin) and Zinc (predose & end of infusion)*		X																																			X	
Ionised Calcium	X																																					X

¹ Biopsy is not an inclusion criterion, but if performed the report and analysis will be documented

² These data sets will be used from the routine monthly blood analysis performed on each patient

	SCR	TREATMENT PERIOD																																				FU																				
Week	-2, -1	1			2			3			4			5			6			7			8			9			10			11			12			13																				
Day		1	3	5	1	3	5	1	3	5	1	3	5	1	3	5	1	3	5	1	3	5	1	3	5	1	3	5	1	3	5	1	3	5	1	3	5	1	3	5																		
Ionised Calcium (predose & end of infusion)		X			X						X						X						X						X						X						X			X														
Pregnancy Test ³	X										X															X																					X											
Treatment History (prior medication) ⁴	X																																																									
Medical History	X																																																									
Eligibility ⁵	X																																																									
CUA Diagnosis	X																																																									
Treatment (Intravenous SNF472) ⁶		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X									
Wound QoL Score		X															X																																									
Pain Score (VAS scale)	X	X			X						X						X						X						X																		X	X										
Lesion Score (BJWAT)		X			X						X						X						X						X																		X	X										
Biomarkers		X																																																			X					
Plasma PK and PD sampling (predose & end of infusion)*		X																																																X	X							
Concomitant Medication		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
End of Study Visit																																																								X		

³ Pregnancy test is to be serum at baseline and urine monthly thereafter

⁴ Prior medication includes medications within 6 months of study start

⁵ The inclusion/exclusion criteria will be assessed for each patient at Screening – final entry into the study depends on confirmation by the PI, Dr VB

⁶ A Treatment Period includes 3 dialysis sessions/week x 12 weeks (36 treatments)

6.3 Study population

6.3.1 Inclusion Criteria

Patients who meet the following criteria will be considered eligible to participate in the clinical trial:

1. Patients with either newly diagnosed CUA OR recurrent CUA that has been dormant with no skin lesion involvement for at least 90 days from study start (new or recurrent diagnosis must be made within 5 weeks of study start)
2. Patients who signed the written informed consent to participate in this clinical trial (prior to any clinical trial-related procedures being performed), after reading the Patient Information Sheet and Informed Consent Form (ICF), and who had the opportunity to discuss the clinical trial with the Investigator or designee
3. Males or females aged ≥ 18
4. Patients on maintenance haemodialysis
5. Patients with at least a minimum level of pain on VAS scale or on pain-killers stronger than NSAIDs
6. Females of child-bearing potential should use a highly effective contraceptive measure throughout the study AND have a negative serum pregnancy test at entry. Male patients having sexual relationship in which pregnancy can occur should take adequate contraceptive precautions (wear a condom).

6.3.2 Exclusion Criteria

Patients who meet one or more of the following criteria will not be considered eligible to participate in the clinical trial:

1. Body weight above 150 kg
2. BMI > 35 and central (abdominal) ulcers
3. History of bisphosphonate treatment within 12 months before entering into the study
4. Severely ill patients without reasonable expectation of survival for > 6 months according to the treating physician
5. Patients with scheduled parathyroidectomy during the run-in or study period
6. Female patients who are either intending to get pregnant or are undergoing treatment to get pregnant, as well as breast-feeding females
7. Participation in another clinical trial with an experimental drug within 90 days prior the inclusion
8. Any psychological, emotional problems, any disorders or resultant therapy that is likely to invalidate informed consent, or limit the ability of the patient to comply with the Clinical Trial Protocol requirements
9. Patients who, in the opinion of the Investigator, are considered unsuitable for any other reason

6.3.3 Criteria for subject withdrawal

A subject is defined as having entered the clinical trial when he/she has provided written informed consent. A withdrawal is a subject who receives a study subject code and for whom treatment is prematurely terminated for any reason.

Possible reasons for withdrawal of a subject from the clinical trial:

- Adverse Event(s)
- Subject lost to follow-up
- Withdrawal of consent
- Pregnancy
- Death
- Other (specify)

6.4 Sample size

Given the exploratory purpose of the study, the sample size will not be recalculated.

The original sample size justification was:

Based on recent historical data from a U.S. large dialysis provider organization, approximately 25% of calciphylaxis skin lesions are considered to be improved over a 3-6-month period (data unpublished; personal communication). A total of 15 subjects was determined necessary to show with 80% power that the percentage of subjects for which the primary ulcer is totally or partially healed after 12 weeks of treatment is greater than 25%. For these calculations, it was assumed that the percentage of totally or partially healed subjects for SNF472 will be 60%.

6.5 Randomization and blinding

This was an open-label, single-arm study with no randomization. Efforts to minimize assessment bias were implemented by including an independent review of the images. A protocol for the review and scoring of the images was produced.

6.6 Study methods

6.6.1 Bates-Jensen Wound Assessment Tool (BWAT)

The Bates-Jensen Wound Assessment Tool (BWAT) score is collected through a questionnaire of 13 items, which are evaluated individually with a score of 1 to 5. BWAT is summarized by a total score which is calculated by adding up the scores of all individual items. Therefore, the total score ranges from 13 to 65 and can be only calculated if all items are answered. However, for the purpose of this analysis missing items were imputed as described in Section 8.4. Note that the BWAT contains two extra variables to indicate the location and shape of the lesion that are not used for the calculation of the total score.

6.6.2 Independent Image Review

Standardized lesion images were evaluated by two independent reviewers. The independent reviewers evaluated all images and assessed the evolution of the lesions compared to Week 1 by indicating if the lesion improved, worsened or did not change.

The assessment was performed in two separate steps in the following order:

1. Blinded Review: reviewers compared Week 1 to Week 12 on a blinded manner without knowing the order of the visits.
2. Unblinded Review: reviewers assessed the evolution of the lesions (primary, secondary, and tertiary) for all visits comparing them to Week 1.

For any discordant scores, the evaluators had to discuss and try to find agreement. In situations where concordance could not be reached, the worst result will be used for the analysis.

6.6.3 Pain Visual Analog Scale (VAS)

A VAS is a measurement instrument that aims to measure a characteristic or attitude that is believed to range across a continuum of values and cannot easily be directly measured.

6.6.4 Wound Quality of Life (Wound-QoL)

The Wound-QoL measures the disease-specific, health-related quality of life of subjects with chronic wounds. It consists of 17 items on impairments which are always assessed in retrospect to the preceding seven days. Answers to each item are coded with numbers (0='not at all' to 4='very much').

A Wound-QoL global score on overall disease-specific quality of life is computed by averaging all items. A global score can only be computed if at least 75% of the items have been answered (i.e., at least 13 in 17 items are valid). Otherwise, the global score will be considered missing.

In addition, subscales of the Wound-QoL can be calculated representing different dimensions of disease specific quality of life by averaging the respective items. A subscale can only be computed if no more than 1 item of the subscale is missing.

The items are assigned to subscales as follows:

- Subscale 'Body': Items #1 to #5
- Subscale 'Psyche': Items #6 to #10
- Subscale 'Everyday life': Items #11 to #16

Item #17 does not belong to any of the subscales.

7. SEQUENCE OF PLANNED ANALYSES

7.1 Interim analysis

An interim analysis is planned when approximately 50% subjects have completed 12 weeks of treatment. The objective of this interim analysis is to determine whether the study should continue to enroll subjects, given the difficulty in finding qualifying subjects. The interim analysis will also be used to help inform the design of future clinical trials in this population.

7.2 Final analysis

The final analysis will be performed when all subjects have finished the study and the database has been successfully locked.

8. STATISTICAL METHODS

8.1 General considerations

██ Statistical analyses will be reported with tables, figures, and subject data listings and presented in rich text format and pdf. Output specifications for all tables, listings and figures will be in conformance with guidelines specified by the International Council on Harmonisation (ICH E3). In general, continuous variables in demographic and baseline characteristics will be summarized by displaying: n (non-missing sample size), mean, standard deviation (SD), standard error (SE), median, quartiles, maximum and minimum. The frequency and percentages (based on the non-missing sample size) of observed levels will be reported for all categorical measures. Percentages will be calculated out of the total number of subjects in the analysis set or the nonmissing sample size, as appropriate.

Numerical efficacy variables will be summarized displaying n, mean, 95% confidence interval, median, SD, SE, quartiles, minimum, maximum.

Time-to-event variables will be calculated and analyzed in days if not specified otherwise.

All summary tables will be structured in a column and will be annotated with the total population size relevant to that table/treatment, including any missing observations.

Differences within groups will be assessed using paired t-test or Wilcoxon test as considered appropriate.

No inferential statistics will be applied to baseline characteristics and safety variables unless specified otherwise.

In general, all data recoded in this study will be listed, sorted by site and subject, and when appropriate by visit number (or assessment date). A description of the content and format of all planned listings is provided in the Appendix, Section 12.2.

Report summaries will be generated using validated SAS® software, version 9.3 or higher, on a PC platform. Additional validated software may be used to generate analyses, as needed.

All SAS programs that create outputs or supporting analysis datasets will be validated by a second statistical programmer or biostatistician. At a minimum, validation of programs will consist of a

review of the program log, review of output or dataset format and structure, and independent review of all outputs.

8.2 Analysis populations

8.2.1 All Subjects

Subjects included in the study who have signed informed consent and received at least one dose of SNF472.

8.2.2 Safety Analysis Set

Subjects included in the study who have signed informed consent and received at least one dose of SNF472.

8.2.3 Efficacy Analysis Set

The definitions given in the protocol about the efficacy analysis sets were considered very restrictive, therefore two new analysis sets for efficacy have been defined:

8.2.3.1 *Intention to Treat Set*

Subjects who have received at least one dose of SNF472 and for whom at least one post-baseline efficacy measurement is obtained.

8.2.3.2 *Per Protocol Set*

The Per Protocol Set includes all subjects who receive at least 75% of SNF472 doses and who did not violate the protocol in a way that might affect the evaluation of the effect of the study drug on the primary endpoint.

Major and minor protocol violations will be identified and documented by the Sponsor.

8.2.4 PK Analysis Set

All subjects who receive at least 1 dose of SNF472 and for whom either of the primary PK parameters (C_{max}) can be calculated. Further analysis sets might be defined if considered appropriate to assess the sensitivity of the PK results, e.g., subjects without any major protocol deviation thought to interfere with the absorption, distribution, metabolism, and excretion of the compound to be measured.

The following table indicates the correspondence between the above defined analysis sets and the planned statistical analysis:

Planned analysis / Analysis sets	All Subjects*	Safety*	Intention to treat	Per Protocol	PK
----------------------------------	---------------	---------	--------------------	--------------	----

Disposition, Protocol Deviations and Analysis Sets	X				
Baseline and disease characteristics		X			
Previous and Concomitant medications		X			
Treatment compliance and exposure		X			
Primary endpoint			X	X	
Secondary endpoints			X		
Safety variables		X			
PK analysis					X
PD/Biomarker analysis					X
Subgroup analysis			X		
All subject listings	X				

* Includes all patients who received at least 1 dose.

8.3 Covariates and subgroups

The primary efficacy endpoint will be summarized by various subgroups of interest based on baseline and demographic categories including age (e.g., < 65 years of age and \geq 65 years of age), gender, race, and country, and based on certain medications (e.g. STS: sodium thiosulfate). Summaries by subgroup will only be produced if there are at least 2 subjects in the category of interest. Otherwise, categories might be grouped as appropriate.

8.4 Missing data

This section provides a general description about methods used to impute missing data. Specific details for imputed variables will be given in Section 9.2.

Missing imputation will be only applied for the primary and key secondary endpoints, i.e. BWAT, VAS and Wound QoL. The main analysis will be based on Multiple Imputation (MI). Sensitivity analyses will be conducted to assess the robustness of the results with regards to handling of missing data. The following methods will be applied: Last Observation Carried Forward (LOCF), and Observed Cases (OC) (Molenberghs & Kenward, 2007).

Multiple Imputation

Multiple imputation was selected as the method for imputing missing data as the natural progression of calciphylaxis skin lesions under standard of care treatment varies considerably [Nigwekar et al, 2013]. Rather than making assumptions under the LOCF method that may either be too optimistic or conservative relative to the natural progression of skin lesions under standard

of care treatment, multiple imputation uses all available data for a given variable to impute for missing data. Missing data will be imputed 100 times to generate 100 complete data sets, using the MI SAS procedure (using *Markov Chain Monte Carlo (MCMC)* for continuous variables or the *Fully Conditional Specification (FCS) Discriminant* analysis for categorical variables). The absolute change from baseline at Week 12 will be then derived from observed and imputed data at this time point. The 100 complete data sets will be then analyzed using a paired t-test, and the MIANALYZE procedure will be used to generate valid statistical inferences by combining results from the 100 analyses using Rubin's formulae.

The number of imputations (100) will be informally verified by replicating sets of 100 imputations and checking whether the combined results are stable. If not stable, the number of imputations will be increased and informally checked as above, and thus until stable estimates are obtained.

The imputation model will include:

- The endpoint values from all non-missing scheduled weeks.
- The endpoint values from other items within the same scale, if applicable.

LOCF

This analysis imputes the last measured value of the endpoint to all subsequent evaluations. In case of missing data at baseline, the missing value will be imputed using the first available visit, i.e., First Observation Carried Backward, FOCB.

Observed Cases

The analysis under this scenario will be conducted without any missing data imputation.

8.5 Censored Subjects

Subject 16001 had a surgical closure of the primary lesion at Week 11. This subject's efficacy data was censored for the timepoints after the surgical procedure, and will be treated as missing.

8.6 Multi-centre studies

This is a multicenter study, with sites enrolling subjects in the USA, UK and Spain with each site enrolling 1 -2 subjects. Efficacy data collected from all study centers will be pooled for data analysis.

8.7 Multiple testing

There will be no adjustments for multiple comparisons in this proof of concept study.

8.8 Baseline values

Baseline is considered the value collected at the Day 1 Week 1 visit. If Day 1 Week 1 value is missing, the Screening value will be used as baseline. If neither a Day 1 Week 1 or Screening value

is available, then the baseline value will be imputed according to the MI and LOCF methods described in Section 8.4.

Some analyses will be also presented comparing pre-dose and post-dose effects. Unless otherwise specified, post-dose and pre-dose comparisons will be done using data from the same study day.

8.9 Reporting conventions

8.9.1 General reporting conventions

All tables, figures and data listings will be presented in landscape orientation. Legends will be used for all figures with more than one variable or item displayed. Figure lines should be wide enough to see the line after being copied.

All tables, figures, and listings, (TFLs) will have the name of the relevant SAS program (output name), the author, and a run time stamp on the bottom of each output.

Titles should contain the following information:

- Output number.
- Description of the data that is being summarized.
- Type of analysis performed, covariates used (if applicable).
- Analysis set.

8.9.2 Statistical summary conventions

For tables, sample sizes for each population will be presented as totals in the column header (N=xx), where appropriate. Sample sizes shown with summary statistics are the number (n) of subjects with non-missing values at a visit.

Summaries for categorical variables will include all categories (even if some categories are empty). All summaries for continuous variables will include: N, mean, and SD. Other summaries (e.g. SE, median, quartiles, range, 95% intervals, CV or %CV) will be used as appropriate. All percentages should be rounded and reported to a single decimal place (xx.x%). If percentages are reported as integers, percentages greater than 0% but <1% will be reported as <1%, whereas percentages greater than 99% but <100% will be reported as >99%. A percentage of 100% will be reported as 100%. No value of 0% should be reported. Any computation of percent that results in 0% is to be reported as a blank. P-values will be reported with up to three decimal places with a leading zero (0.001). P-values <0.001 will be reported as <0.001. Summaries for continuous variables will be presented with the same number of decimal places as originally provided in the CRF.

9. STATISTICAL ANALYSIS

9.1 Baseline and other relevant data

9.1.1 Subject disposition

The number of subjects who were enrolled, completed each visit, and discontinued, and reasons for discontinuation will be summarized (Shell T - 1).

9.1.2 Protocol deviations

Deviations from the Clinical Study Protocol will be collected by the monitors, including deviations of inclusion/exclusion criteria will be assessed and categorized by the Sponsor. The number of subjects with protocol deviations (any, major, minor and by deviation) will be summarized descriptively (Shell T - 2). All the collected information regarding protocol deviations will be listed.

9.1.3 Analysis sets

The number of subjects included and excluded in each analysis set will be described (Shell T - 3). Subjects excluded from any analysis set will be listed including: analysis set from which it is excluded, and the reasons for exclusion (Shell T - 3).

9.1.4 Demographics and other baseline characteristics

The following variables will be summarized (Shell T - 4):

Demographics: Age, Sex, Race, Ethnicity, Weight, Height, and BMI as recorded at the Screening visit. BMI will be presented as a continuous variable and for the following categories: “<20”, “20-25”, and “>25”.

Disease characteristics: Intense Pain (Yes/No), VAS score, Firm calcified (Yes/No/NA), cutaneous lesions (Yes/No), Skin biopsy (Yes/No/Scheduled/Not done), Results of the biopsy (Yes/No/Not applicable/To be redone).

History of drug and alcohol abuse: Alcohol Consumption (Yes/No), total units of alcohol consumed weekly, and Drug Consumption (Yes/No), recorded in the Medical History CRF section.

9.1.5 Medical History

The following tables will be created (Shell T - 4):

General: contains a Yes/No variable for each domain: Hemodialysis, Heart, Vascular System, Parathyroid, Diabetes, Kidney Diseases, Fractures, Liver Diseases, Other Diseases. For each domain we will consider as “Yes” if the subject has any non-negative (i.e. excluding No, UNK and missings) answer for at least one question related with the domain and “No” in the other cases.

The data related with each domain will be summarized and listed. The information summarized will be presented only for the subjects who has a “Yes” in the General table, but all subjects’ information will be listed.

For each domain, the following variables will be summarized:

Hemodialysis: Ca-Conc, URR and Kt/V.

Heart: NYHA Stage, CHD, Heart failure, LVEF, MI, Bypass Surgery and PTCA.

Vascular System: PAD and Stage Fontaine.

Parathyroid: PTX, and Method.

Diabetes: Diabetes, Type, and Insulin dependent.

Kidney Disease: Renal insufficiency, CKD, Origin, Renal hypert., Metab. Acid., Osteopathy, Anemia, and 2nd HPT.

Additional Diseases, Fractures: Occurrence by each Location.

Additional Diseases, Liver Diseases: Occurrence by each Condition.

Additional Diseases, Other: Primary HPT, Malignant, and Rheumatic.

9.1.6 Prior and Concomitant medications

Prior and concomitant medications will be tabulated and listed for the safety analysis set.

Prior medications are defined as those taken from 6 months prior to Screening until the time of the first treatment dose administration. Concomitant medications are defined as those taken at the time of or after the first treatment dose administration.

Prior and concomitant medication will be coded according to the World Health Organization's Drug Dictionary (WHO-DDE) and the Anatomical Therapeutic Chemical (ATC) classification system.

Prior and concomitant medications will be summarized separately by ATC Name and Preferred Term indicating the number of subjects and percentage (Shell T - 5). When calculating the use of prior/concomitant medications, each subject will only be counted once and any repetitions of medications will be ignored; the denominator will be the total population size.

A separate table will be presented for Pain Medications. Pain medication will be defined from the list of Concomitant Medications, according to coding specified by the study sponsor which should be merged with the Concomitant Medications dataset by Drug Record Number and Sequence 1.

9.1.7 Treatment exposure and compliance

The extent of exposure to SNF472 will be characterised according to the number of subjects exposed, the duration of exposure, and the dose to which they were exposed.

The following data will be summarized descriptively for the total treatment period and by week (Shell T-6):

- **Treatment duration (days)** defined as the total number of days between the first and the last infusion dates (inclusive).

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- **Treatment exposure (infusions)** defined as the number of infusions with the study drug of at least 1 hour that were administered to a subject.
 - **Compliance (%)** defined as the total number of infusions administered to a subject divided by the total number of planned infusions, i.e, 3 administrations x 12 weeks of treatment = 36.
 - **Dose (mg)** presented as a categorical variable indicating the number and percentage of subjects presented on the different planned dose levels (400, 450, 700, and 900 mg).
 - **Total dose (mg) (an estimation of the total dose)**
 - **Dialysis duration (minutes)** defined as the time in minutes registered between the start of the dialysis start and it end.
 - **Infusion duration (minutes)** defined as the time in minutes registered between the start of the infusion and its end.
 - **Number of infusions with any interruptions** counting the number of infusions per subject where the question "Infusion interruptions" is Yes.
 - **Ca in Dialysate Bath** presented as a categorical variable.

9.2 Efficacy Analysis

9.2.1 Primary endpoint

The primary endpoint is the absolute change in BWAT total score between baseline (Week 1) and Week 12 for the primary lesion (the largest one).

Absolute change from baseline will be calculated as differences in the total score from Week 1 to Week 12. Descriptive statistics will be presented. Differences between the two visits will be analyzed using a Paired Student's T-Test. (Shell T-7)

Missing imputation will be done on the item level. For the imputation of baseline missing item scores FOCB will be used for LOCF imputation method if any subsequence item score is available, else the score will be imputed by 3. For the multiple imputation method the baseline values will be imputed by 3. The main analysis will be done using Multiple Imputation, and supported by the different sensitivity analysis as specified in Section 8.4. A forest plot summarizing these results will be produced presenting the estimated change from Week 1 to Week 12 and its 95% confidence interval for the different imputation methods (Shell F - 1).

9.2.2 Secondary endpoints

9.2.2.1 *Change from baseline in BWAT total and component scores by visit*

BWAT Score is collected at Week 1, Week 2, Week 4, Week 6, Week 8, Week 10, Week 12 and Follow-up. Differences in absolute and relative change from baseline will be also presented. Differences from baseline will be tested using a Paired Student's T-Test. (Shell T-7). Additionally, a figure presenting the evolution (mean, and 95% confidence interval) of the total score and

individual items by visit will be presented (Shell F-2). This analysis will be done using Observed Cases.

9.2.2.2 *The previous analysis will also be done for the secondary and tertiary lesions if there is enough data. Change from baseline in imaging score by visit*

Image evaluation will be summarized separately for the blinded and unblinded reviews. Percentages for each category and their exact 95% confidence interval (Clopper-Pearson) will be presented. Imputation methods will not be used for this score.

9.2.2.3 *Change from baseline in the Pain Visual Analog Scale (VAS) by visit*

Absolute change from baseline will be calculated as differences in the total score from Week 1 to Week 12. Descriptive statistics will be presented. Differences between the two visits will be analyzed using a Paired Student's T-Test. (Shell T-7)

The Week 1 to Week 12 analysis will be done using Multiple Imputation, and supported by the different sensitivity analysis as specified in Section 8.4. A forest plot summarizing these results will be produced presenting the estimated change from baseline and 95% confidence interval for the different imputation methods. These analysis will also be done for the PP population.

Additionally, the VAS Score is collected at Screening, Week 1, Week 2, Week 4, Week 6, Week 8, Week 10, Week 12 and Follow-up. VAS scores will be summarized by visit. Differences in absolute and relative change from Week 1 to each visit will be presented descriptively. Finally, differences in absolute change from Week 12 to Follow-up will be summarized. Statistically significant differences from the reference values will be tested using a Paired Student's T-Test (Shell T-7). Additionally, a figure presenting the evolution (mean, and 95% confidence interval) of the VAS score by visit will be presented (Shell F-2). This analysis will be done using Observed Cases.

9.2.2.4 *Change from baseline in total and subscales of wound QoL score by visit*

Missing values (e.g., missing visits, visits with more than 25% of the items missing, or subscales with more than one item missing) will be imputed. Missing imputation will be done on a subscale level. Absolute change from Week 1 to Week 12 will be done using Multiple Imputation, by each subscale and global score and supported by the different sensitivity analysis as specified in Section 8.4. A forest plot summarizing these results will be produced presenting the estimated change from baseline and 95% confidence interval for the different imputation methods (Shell F-1).

Wound Quality of Life was collected at Week 1, Week 6 and Week 12. Differences in percent and absolute change from Week 1 to each visit by each subscale and in the global score will be analyzed using a Paired Student's T-Test (Shell T-7). Means and 95% confidence interval will be presented graphically by visit for the global score and subscales (Shell F-2). This analysis will be done using Observed Cases.

9.2.2.5 Potential Wound Complications

Sponsor will review all AEs to identify potential wound complications (eg., wound infections, systemic infections, wound complications, surgical treatments of the wound).

9.2.3 Hospitalization and Death

Hospitalizations will be identified from the list of Adverse Event for which Seriousness has been reported as “Requires or prolongs hospitalization”.

The number and percentage Hospitalizations will be summarized by System Organ Class and Preferred Term presenting the number of events, the number of subjects and percentage (Shell T-5).

Deaths will be identified from the list of adverse events, for which Seriousness has been reported as “Results in death” or Outcome has been reported as ”Death” or Primary Reason for Premature Termination has been reported as “Death” on the End of Study CRF section.

The number and percentage of deaths will be computed and will be summarized by System Organ Class and Preferred Term (Shell T-5).

9.2.4 Exploratory Analyses

9.2.4.1 Absolute change in BWAT total score between Week 12 and Follow-up for all lesions.

The absolute change in BWAT total score of the primary lesion between Week 12 and Follow-up will be summarized using the missing imputation method specified for the primary endpoint.

The same analysis will also be done for the secondary and tertiary lesions if there is enough data.

9.2.4.2 BWAT vs VAS correlation.

Correlation between VAS and BWAT (absolute and relative) change from baseline to Week 12 will be assessed:

- Absolute VAS change from baseline vs absolute BWAT change from baseline
- Relative VAS change from baseline vs relative BWAT change from baseline

Correlation will be assessed graphically by means of linear regression analysis where VAS and BWAT will be considered as dependent and independent variables, respectively. Linear regression parameters, correlation estimate and their 95% confidence intervals will be presented.

9.3 Safety analyses

9.3.1 Adverse Events

AEs will be summarized presenting the number of events, the number of subjects and percentage. When calculating the incidence of AEs, or by seriousness sub-classification, each subject will only be counted once and any repetitions of AEs will be ignored; the denominator will be the total population size. In case of repetition of an AE the worse seriousness will be considered.

An overview table (Shell T-5) will be prepared showing the number of subjects (percentage of subjects, and events) with:

- Adverse Events
- Adverse Events by relationship to SNF472
- Adverse Events leading to drug discontinuation
- Serious Adverse Events
- Adverse Events leading to death

In addition, frequency tables (Shell T-5) will be prepared for:

- Adverse Events by System Organ Class and Preferred Term
- Adverse Events by Preferred Term in Decreasing Order of Incidence
- Adverse Events by System Organ Class and CTCAE Grade
- Adverse Events by System Organ Class and Relationship to SNF472
- Adverse Events Leading to Discontinuation of SNF472 by System Organ Class
- Serious Adverse Events by System Organ Class and Preferred Term

9.3.2 Clinical Laboratory Evaluations

Summary tables will be presented in groups of related parameters:

- Haematology
- Clinical Chemistry
- Parathyroid Hormone
- Potassium, magnesium, iron (ferritin/transferrin) and zinc
- Ionised Calcium
- Pregnancy test

Analyses are conducted for the laboratory data using SI units.

Haematology and **Clinical chemistry** data will be collected at Screening, Week 1, Week 6, Week 12 and Follow-up. Absolute change from baseline values for each parameter will be summarized descriptively. (Shell T-7).

Parathyroid Hormone data will be collected at Week 1 and Week 12. Absolute change from baseline values for each parameter will be summarized descriptively. (Shell T-7).

Potassium, magnesium, iron (ferritin/transferrin) and zinc data will be collected at Screening, Week 1 (pre and post-dose), Week 12 (pre and post-dose) and Follow-up. Differences in absolute change from the pre-dose measurement of each visit to the post-dose measurement of the same visit, and the differences in absolute change from Baseline to all measures will be summarized (Shell T-7).

Ionised Calcium data will be collected at Screening, Weeks 1, 2, 4, 6, 8, 10 and 12 (pre and post-dose), and Follow-up. Differences in absolute change from the pre-dose measurement of each visit to the post-dose measurement of the same visit, and the differences in absolute change from Baseline to all measures will be summarized (Shell T-7).

Pregnancy test data will be collected at Screening, Week 1, Week 4, Week 8 and Week 12. The following variables will be summarized: Done/ not done, if any, reason for not done. Sample type and results by visit (Shell T-6).

9.3.3 ECG, Physical examination and Body weight

Electrocardiograms are collected at Screening, Week 1 (pre-dose and end of infusion), Week 6 (pre-dose and end of infusion), Week 12 (pre-dose and end of infusion) and Follow-up. The following variables are collected: RR interval, PRT axis, PR interval, QRS complex, QT interval, QTcB, QTcF, Heart rate and ECG Assessment, including clinical evaluation.

Differences in absolute change from the pre-dose to post-dose within the same visit, and the differences in absolute change from Week 1 day 1 pre-dose to all the following pre-dose measurements will be summarized (Shell T-7).

Following the recommendation of the guidance ICH E14, the following additional analyses on QTc for both Bazett's and Fridericia's formulas will be done:

- number of subjects with QTc interval > 450 ms
- number of subjects with QTc interval > 480 ms
- number of subjects with QTc interval > 500 ms
- number of subjects with QTc interval increases from predose > 30 ms
- number of subjects with QTc interval increases from predose > 60 ms

The previous parameters will be measured at any point during the study after the first drug infusion (Shell T-4). In addition, the same parameters will be summarized by visits Week 1 day 1, Week 6 day 1 and Week 12 day 5 for pre-dose and post-dose time-points (Shell T-6). The tables will be also presented for QT.

Shift tables on the ECG clinical assessment will be produced to present:

- Change from baseline on the pre-dose assessments to Week 6 and Week 12 (Shell T-9)
- Change from pre-dose to post-dose within infusion days at weeks 1, 6 and 12 (Shell T-10)

RR, QTcB and QTcF will be re-calculated using the following formulas, if it is possible:

$$RR = \frac{60}{HR}$$

$$\text{Bazzet: } QT_{cB} = \frac{QT}{\sqrt{RR}}$$
$$\text{Fredericia: } QT_{cF} = \frac{QT}{\sqrt[3]{RR}}$$

Physical examination was collected at Screening and Follow up Visit and it will be summarized and listed by subject, time-point and body system including the following information: parameter, assessment and clinical significance (Shell T-6).

Body weight was collected at Screening, Week 6 and Follow up Visit and it will be listed by subject and time-point including the following information: date, weight without shoes, Height without shoes and BMI.

9.4 Pharmacokinetics

The PK analysis will be summarized, listed and printed for the PK analysis set.

Plasma samples collected, at Week 1 and Week 12, pre and end of infusion will be used for PK assessment. The end of the infusion is the approximate time of C_{max}.

All concentration values below the LLQ and samples with no reportable value occurring prior to dosing will be replaced by "0". For tabulation, graphical representation and calculation purposes, all samples LLQ or with no reportable value observed after the drug administration will be set to missing.

Blood samples for the quantitation by LC-MS/MS of SNF472 will be taken on 2 different days at pre-dose and at the end of the infusion time on day 1 (Week 1), and on Week 12. Infusion should be performed during 2.5 to 4 hours at constant rate. The following parameters will be calculated:

- **Actual infusion time (T_{inf})** will be calculated as the difference between the actual clock time of the end of infusion time (EOI) and the actual clock time of the start of the infusion (SOI), i.e., T_{inf} = EOI - SOI. The actual infusion time will be expressed in minutes.
- **Actual sampling time** will be calculated as the difference between the sample collection actual clock time and the clock time of dosing starts. The actual sampling times will be expressed in minutes.

9.4.1 Pharmacokinetic Parameters

The plasma concentrations of SNF472 obtained at predose and at the end of the infusion time (EOI) will be reported. EOI corresponds to the maximum (peak) plasma concentration during the dosing interval (C_{max}), based on data from previous clinical studies. Standard pharmacokinetic parameters will not be calculated due to the lack of experimental points in the plasma concentration-time profile of SNF472.

Descriptive statistics of the pharmacokinetic parameters will be presented by visit and timepoint and will contain the following information:

-
- SNF472 dose: expressed in mg
 - T_{inf} : actual infusion time. Expressed in minutes
 - Actual sampling time: actual sampling time. Expressed in minutes
 - Pre-dose plasma levels: expressed in ng/mL or as below of the lower limit of quantification (BLQ).
 - End of infusion plasma levels (C_{max}): expressed in ng/mL

Individual data will be flagged for exclusion if actual time differs significantly from scheduled time. In those cases when the actual infusion time and actual sampling time at the end of the infusion differs significantly from the protocol time will be excluded from statistics, based on the pharmacokinetic criteria.

PK data will be listed by subject, visit and time-point. In addition, the same information listed using all time-points will be reported in a figure (Shell F-2).

On a case by case basis, it may be necessary to exclude individual infusion time values and concentration values because they are erroneous or abnormal. Any excluded data should be flagged in the individual data listings and the reason for exclusion should also be documented. If the exclusion has a meaningful impact on the overall interpretation of the results, then it should be discussed. Planned infusion and sampling times may be used as a replacement for unknown or missing actual times, based on the pharmacokinetic criteria.

9.4.2 Accumulation

Given the short half-life of SNF472, no accumulation of SNF472 plasma concentration is expected. Accumulation effects from Week 1 to Week 12 will be assessed on the C_{max} by comparing the difference between the two visits. Accumulation will be declared if the 90% CI of the difference does not include the value 0.

9.4.3 End of infusion PK concentration vs BWAT correlation

Correlation between End of Infusion PK Concentration vs Increase in BWAT Total Score from Week 1 to Week 12 will be assessed graphically by means of linear regression analysis where BWAT and EoI PK concentration will be considered as dependent and independent variables, respectively. Linear regression parameters, correlation estimate and their 95% confidence intervals will be presented.

9.4.4 End of infusion PK concentration vs VAS correlation

Correlation between End of Infusion PK Concentration vs Increase in Pain Score (VAS) from Week 1 to Week 12 will be assessed graphically by means of linear regression analysis where VAS and EoI PK concentration will be considered as dependent and independent variables, respectively. Linear regression parameters, correlation estimate and their 95% confidence intervals will be presented.

9.5 Pharmacodynamics

Plasma samples collected, at Week 1 and Week 12 pre and post-dose (around the Tmax for the post-dose measurement), from all subjects, will be used for exploratory pharmacodynamic (PD) assessment by using an in vitro test to assess the propensity for hydroxyapatite (HAP) crystal formation in plasma (see Protocol CT2015-04.2).

9.5.1 % PD Inhibition

The PD assessment will be performed by Laboratoris Sanifit and sent to [REDACTED] for analysis. The readout of the assay is a % inhibition of the propensity for hydroxyapatite crystal formation per each subject and visit.

The percent inhibition of HAP crystallization will be summarized by visit. In addition, the same information will be listed by all subjects and all timepoints together with the PK.

9.5.2 % PD Inhibition vs BWAT correlation

Correlation between % PD Inhibition vs Increase in BWAT Total Score from Week 1 to Week 12 will be assessed graphically by means of linear regression analysis where BWAT and % PD Inhibition will be considered as dependent and independent variables, respectively. Linear regression parameters, correlation estimate and their 95% confidence intervals will be presented.

9.5.3 % PD Inhibition vs VAS correlation

Correlation between % PD Inhibition vs Increase in Pain Score (VAS) from Week 1 to Week 12 will be assessed graphically by means of linear regression analysis where VAS and % PD Inhibition will be considered as dependent and independent variables, respectively. Linear regression parameters, correlation estimate and their 95% confidence intervals will be presented.

9.6 PK/PD modelling

PK/PD modeling links the drug plasma concentration with an efficacy metric, and it is performed to guide in the design for future studies.

The % inhibition of HAP crystallization and the Cmax will be used to calculate a PK/PD correlation between both parameters at Week 1, Week 12 and combining both visits.

9.7 Subgroup analysis

Subgroup analysis will be summarized for the ITT analysis set. Subgroups will be based on baseline and demographic categories including age (e.g., < 65 years of age and \geq 65 years of age), gender, race, and country, and use of STS (sodium thiosulfate).

9.7.1 BWAT

Subgroup analysis will be done for the absolute change in BWAT total score between baseline (Week 1) and Week 12 for the primary lesion as specified in Section 8.3. Missing imputation will be done as specified in Section 9.2.1.

9.7.2 VAS

Subgroup analysis will be done for the absolute change in VAS total score between Week 1 and Week 12 as specified in Section 8.3. Missing imputation will be done as specified in Section 9.2.2.4.

VAS responder analysis for STS subgroup will be done by each imputation method summarizing the percentage of reduction of VAS from Week 1 to Week 12 (Shell T-8). Percentage of reduction will be calculated as follows:

$$\text{Percentage of reduction} = -100 * (\text{Week 12 VAS score} - \text{Week 1 VAS score}) / (\text{Week 1 VAS score})$$

The following categories will be used:

- Worsening (negative percentage of reduction)
- ≥ 0 and < 30
- ≥ 30
- % not available (Week 1 VAS score = 0)
- Missing W12 value

10. SUMMARY OF CHANGES FROM THE PROTOCOL

5.2 The protocol did not provide details about the methods used for the analysis of the study endpoints, nor at which timepoints. The primary endpoint was assessed comparing Week 1 to Week 12. Whereas, secondary endpoints were assessed comparing baseline to all subsequent visits. Differences from baseline were tested using a Paired Student's T-Test. Last Observation Carried Forward (LOCF) and Multiple Imputation (MI) was used to treat missing data.

5.2.2 "Change in dose and type of pain medication assessed as a continuous variable. Name (INN), dose, and number of pills captured" was not done but the use of pain medications will be discussed in the CSR.

6.3.3 Criteria for subject withdrawal - For criteria for withdrawal, data are presented for subjects who received at least one dose of study medication. Subjects who did not meet entry criteria are presented as screen failures.

8.2.2 Efficacy Analysis Set. The efficacy analysis set has been divided in Intention to Treat and Per Protocol sets.

11. REFERENCES

- Nigwekar S et al (2003). Sodium Thiosulfate Therapy for Calcific Uremic Arteriopathy. Clin J Am Soc Nephrol, Vol 8.
- Molenberghs, G. and Kenward, M. G. (2007). Missing Data in Clinical Studies. Wiley.
- ICH E3 (1995) - Structure and content of clinical study reports. Step4.
- ICH E14 (2005) - The clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for nonantiarrhythmic drugs. Step 4.

12. APPENDICES

The appendices contain a list of Tables, Figures, and Listings to be produced, as well as their corresponding shells.