Statistical Analysis Plan

An open-label, single-arm study to evaluate the CYP3A4 induction potential of vamorolone on the pharmacokinetics of midazolam (a sensitive CYP3A4 probe) in healthy subjects.

Sponsor: Santhera Pharmaceuticals (Switzerland) Ltd

Version: Final 1.0

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Version History

SAP Final Version	Date	Change	Rationale
Final 1.0	20-SEP-2024	Not Applicable	Not Applicable

1. ABBREVIATIONS

Abbreviations and definitions of PK parameters are provided in Section 12.1.

AE	Adverse event
ANOVA	Analysis of variance
ATC	Anatomical Therapeutic Chemical Classification
BMI	Body mass index
BP	Blood Pressure
CI	Confidence interval
CRF	Case report form
CSR	Clinical study report
CV	Coefficient of variation
CYP	Cytochrome P450
CYP3A4	Cytochrome P450 3A4
DDI	Drug-drug interaction
DEC	Dose Escalation Committee
DRM	Data Review Meeting
ECG	Electrocardiogram
GCP	Good clinical practice
ICF	Informed consent form
ICH	International Council for Harmonisation
IMP	Investigational medicinal product
LLOQ	Lower limit of quantification
LS	Least square
MCAR	Missing completely at random
MedDRA	Medical dictionary for regulatory activities
MW	Molecular weight
N, n	Number
NCA	Non-compartmental analysis
PD	Pharmacodynamics
PK	Pharmacokinetics

QRS	Part of electrocardiographic wave representing ventricular depolarization
QTcF	QT interval frequency-corrected according to Fridericia's formula
Rel.	Relative
SAE	Serious adverse event
SAP	Statistical analysis plan
SAS	Statistical Analysis System
SD	Standard Deviation
SOP	Standard operating procedure
TEAE	Treatment-emergent adverse event
TLF	Tables, listings, figures
TOC	Table of Contents
WHO	World Health Organization

2. INTRODUCTION

The objective of this statistical analysis plan (SAP) is to specify the statistical analysis in more detail than stated in the protocol for the trial. The statistical analysis plan does not change the analysis described in the protocol, but it should be precise enough to serve as a guideline for statistical programming and creation of tables.

This SAP was developed with reference to the valid protocol (final version 1.0, dated 05-JUN-2024). Supplementary text to the protocol gives a full specification of analyses and presentation. If applicable, deviations from the planned methods stated in the protocol are summarized in Section 16. If additional analyses are required to supplement the planned analyses described in this SAP, they may be performed and will be identified in an amendment to this SAP or in the clinical study report (CSR). Any substantial deviations from this SAP will be agreed upon between the Sponsor and Nuvisan and discussed in the CSR.

3. STUDY OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary	
• To investigate the CYP3A4 induction potential of vamorolone by assessing the Pharmacokinetics (PK) of midazolam and of its metabolite 1'-hydroxymidazolam in healthy subjects	• PK parameters ($AUC_{0\text{-tlast}}$, $AUC_{0\text{-inf}}$ and C_{max}) of midazolam and its hydroxyl-metabolite (1'-hydroxymidazolam)
Secondary	
• To investigate the safety and tolerability of concomitant administration of midazolam and vamorolone	• The assessment of safety and tolerability will be based mainly on treatment emergent adverse events (TEAEs), laboratory assessments, vital signs, and electrocardiogram (ECG) evaluation
• To investigate CYP3A4 induction by endogenous biomarkers of the CYP3A4 activity	• Plasma 4β-hydroxycholesterol and urinary 6β-hydroxycortisol to cortisol ratio

For definitions of PK endpoints refer to Section 12.1.

4. DESIGN OF THE STUDY

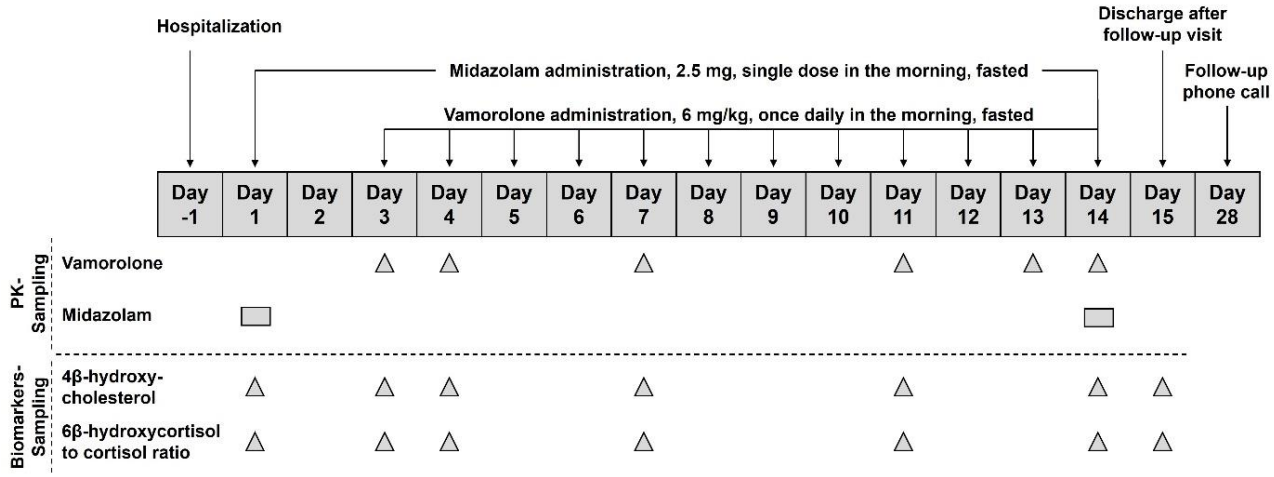
This drug-drug interaction (DDI) study will be conducted in a non-randomized, single-center, open-label, single-arm, fixed-sequence phase 1 design, in 18 healthy male and female subjects.

Subjects will be screened for eligibility between Day -28 and Day -2. They will be admitted to the study site on Day -1 and will remain inpatient until discharged on Day 15, after a follow-up visit 24 hours after last midazolam dosing was performed. A safety follow-up phone call is planned 14 days after the last vamorolone dose, on Day 28.

To investigate the combined interaction potential, midazolam will be administered alone on Day 1, and again after 12 days of daily vamorolone administration, which is considered adequate to achieve the CYP3A4 induction potential of vamorolone, on Day 14 directly after vamorolone dosing.

All subjects are to receive the same study treatments in the same sequence on the same study days. They will receive a single oral dose of midazolam (solution with 2.5 mg) in the morning of Days 1 and 14, fasted for 10 hours, and multiple oral doses of vamorolone (suspension with 6 mg/kg) once daily in the morning, within 30 minutes after start of a standard breakfast on Days 3 to 13 and fasted on Day 14. Day 2 serves as wash-out day.

For an extensive PK profile of midazolam and its metabolite 1'-hydroxymidazolam, plasma samples will be collected on the midazolam dosing days (Day 1 midazolam alone and Day 14 midazolam co-administered with vamorolone) from predose through 10 hours following the midazolam dose. For vamorolone, single predose plasma samples (trough values) will be collected on Days 3, 4, 7, 11, and 14 during the vamorolone induction phase. Plasma and urine samples, to measure biomarkers for CYP3A4 induction, will be taken on Days 1, 3, 4, 7, 11, 14, and 15, each predose (on Day 15 24 hours postdose for plasma) and in the morning from approximately 8:00 to 12:00 a.m., respectively. Safety and tolerability parameters will be collected during the entire study phase from screening to follow-up.



5. STATISTICAL HYPOTHESES

No formal statistical hypotheses have been defined for this exploratory study (protocol section 9.1).

6. SAMPLE SIZE AND POWER ESTIMATION

Sample size is based on feasibility and to ensure adequate precision in estimation of treatment differences. Assuming the intra-subject CV of 22% for midazolam ([1]) with a total of 18 subjects the precision or half-width of 90% CI for treatment difference on the log-scale will extend

approximately 0.179 from the observed difference in means. The above half-width translates into the following 90% CIs when the observed mean ratio for comparison is 1; 90% CI = (0.84 ,1.20).

Approximately 18 subjects will be assigned to study treatment such that 16 evaluable subjects complete the study (protocol section 9.5).

Dropouts might be replaced on a case-by-case basis, in agreement between the Sponsor and the Principal Investigator if the number of evaluable subjects completing the study becomes or is expected to become less than 16 subjects in total. The data obtained from dropouts will be used in the evaluation to the largest possible extent.

7. ANALSYIS SETS AND PROTOCOL DEVIATIONS

7.1. Analysis Sets

For purposes of analysis, the following analysis sets are defined:

Set	Description
Full	All subjects enrolled in the study.
Safety	All subjects assigned to study treatment and who receive at least 1 dose of study treatment. Subjects will be analysed according to the treatment they actually received. This population will be used for safety and biomarker analyses, if not stated otherwise.
PK Set - midazolam	This analysis set is a subset of the Safety Set and includes all subjects who complete the scheduled vamorolone and midazolam dose and provide evaluable midazolam PK profile on at least one of the following days: Day 1 and Day 14, without any findings/events or with findings/events unlikely affecting PK. No vomiting should occur within 4 hours following midazolam and/or vamorolone administration. This PK Set will be used for PK concentration summary and PK parameter summary including results from the statistical model for midazolam and 1'-hydroxymidazolam.
PK Set - vamorolone	This analysis set is a subset of the Safety Set and includes all subjects who completed at least one scheduled vamorolone dose without vomiting within 4 hours immediately following that vamorolone administration and provided at least one valid PK concentration data for vamorolone. This PK Set will be used for PK analyses for vamorolone.

7.2. Protocol Deviations

- A protocol deviation is any change, divergence, or departure from the study design, or procedures defined in the protocol. Protocol deviations will be identified prior to database lock.

- Important protocol deviations are a subset of protocol deviations that may significantly affect the completeness, accuracy and/or reliability of the study data or that may significantly affect a subject's rights, safety, or well-being.

- Important protocol deviations will be listed.

- A protocol deviation may also be declared as finding/event that led to an exclusion of data or complete subjects from an analysis set.

- Protocol deviations as well as findings/events leading to the exclusion from analysis sets will be pre-specified in a deviation manual and collected in a quality issue log on an ongoing basis. This quality issue log will finally be discussed and agreed on in a Data Review Meeting (DRM) prior to database lock and prior to availability of bioanalytical data to those who decide on the allocation of subjects to analysis sets.
- The listings that are compiled by the biostatistics department for the DRM are marked with an asterisk ('*') in Section 19.2.

8. GENERAL CONSIDERATIONS

8.1. General Rules

Generally, all data entered to the database will be listed by subject and summarized descriptively by the following study treatments, where applicable: "Midazolam Alone", "Vamorolone Alone" and "Vamorolone + Midazolam".

If not stated otherwise, descriptive statistics for qualitative data include number of cases including percentages. The percentages will be calculated based on the number of subjects in the population for overall summaries and on the number of subjects in the population receiving the respective treatment(s) for summaries by treatment (denoted as "N"). Descriptive statistics for quantitative data include, if not stated otherwise: number of evaluable cases/measurements (denoted as "n"), arithmetic mean, standard deviation (SD), coefficient of variation (CV, in %), minimum, median, maximum, interquartile range (Q1; Q3). For PK concentrations and PK parameters related to concentrations, geometric mean, geometric SD, and geometric CV (in %) and for PK concentrations geometric "n" will also be derived.

In general, for repeated measurements after the first administration of study treatment, the first one will be used in the statistical calculations for presentation in the tables of descriptive statistics (unless the scheduled measurement was considered unreliable, e.g., due to technical reasons, and needed to be replaced by an unscheduled repeat measurement). For repeated measurements before first administration of study treatment, the last one will be used in the statistical analysis. Although only one value per visit is selected for analysis, all data are presented in the data listings.

Data listings will include all subjects, i.e., evaluable and not evaluable, and will be sorted by subject and study time and treatment. Subjects will be identified by the assignment number, where applicable. Only demographic and population listing will show the screening and assignment number.

8.2. Imputation of Missing Data

All data will be used to its largest possible extent. The number of missing observations is expected to be very low in this Phase I healthy volunteer study. Any missing observation will be assumed to be missing completely at random (MCAR). Missing data will not be replaced or imputed in any way, if not stated otherwise in the SAP.

8.3. Definition of Relative Days and Duration

For the calculation of relative days, the following definitions will be applied:

- Day 1 is the day of first administration of study treatment Midazolam Alone. Each study day before and thereafter is defined relative to Day 1.
  Day 3 is the day of first administration of study treatment Vamorolone Alone.
  Day 14 is the first day of the Vamorolone + Midazolam study treatment combination with dosing of both drugs in the morning of Day 14.
- Relative (Rel.) Day of events on or after Day 1 as defined above = start date of the event – date of first administration of study treatment (midazolam) + 1
- Rel. Day of events before Day 1 = start date of the event – date of first administration of midazolam

The following definitions and calculations of duration, will be applied, as applicable:

- Duration of event (in days hh:mm) = end date and time – start date and time of the event
- Days hh:mm from dosing (onset post administration) = start date and time of the event – date and time of last administration of study treatment
- Treatment duration (in days) = date of last administration of study treatment – date of first administration of study treatment + 1
- If start time of an event is missing, then it will be imputed by “00:00”, if end time of an event is missing then it will be imputed by “23:59”

9. SOFTWARE

Software to perform final statistical analyses will be SAS® version 9.4 or higher, if not stated otherwise. The non-compartmental PK analysis of the data will be performed by using Phoenix WinNonlin® version 8.3 or higher, if not stated otherwise. In case a PK parameter is not automatically calculated within WinNonlin®, then the concentration data itself or WinNonlin® derived parameters will be used to determine these subsequent PK parameters and will be calculated within SAS®.

10. STUDY SUBJECTS AND DEMOGRAPHIC CHARACTERISTICS

The number and percentage of subjects who are screened, assigned (N), treated with Midazolam Alone, treated with Vamorolone Alone, treated with Vamorolone + Midazolam, completed or discontinued study as well as the reason for study termination will be summarized overall in a disposition frequency table for all assigned subjects.

A frequency table of number and percentage of subjects in the Safety Set, PK Set - midazolam, and PK Set - vamorolone will be provided overall for all assigned subjects (N).

Demographic variables and baseline physical characteristics will be listed by subject and summarized in total for the Safety Set (and all other analysis sets, if different) using descriptive statistics for continuous variables (age, height, weight, body mass index (BMI)) and using frequencies for categorical variables (sex, ethnicity, race).

Violation of inclusion and exclusion criteria will be listed.

Prior medication, concomitant medication and procedures will be coded with World Health Organization Drug Global Dictionary (WHO Drug Global) and will be listed by subject. Any medication taken at or after the time of first study treatment, regardless of whether it had started prior to the study or not, is to be recorded as concomitant medication. Prior medications are defined as any medication taken which had been stopped prior to the first administration of study treatment.

Medical history will be listed by subject.

11. EFFICACY ANALYSES

Not applicable.

12. PHARMACOKINETIC ANALYSES

12.1. Pharmacokinetic Parameters

Midazolam and 1'-hydroxymidazolam:

The following PK parameters will be calculated for subjects in the PK Set - midazolam. The PK parameters will be calculated for the two treatments Midazolam Alone and Vamorolone + Midazolam.

The PK parameters that will be calculated for each subject by non-compartmental analysis (NCA) for levels of midazolam and 1'hydroxymidazolam in plasma are listed below. Parameters will be calculated relative to the midazolam treatment administration on Day 1 and Day 14.

$AUC_{0\text{-}tlast}$ = The area under the concentration-time curve (AUC) from time zero (= dosing time) to the time of the last quantifiable concentration (t_{last}), calculated using linear trapezoidal rule.

$AUC_{0\text{-}inf}$ = The AUC from time zero (dosing time) extrapolated to infinity estimated using the log-linear regression for λ_z determination (see below):
$AUC_{0\text{-}inf} = AUC_{0\text{-}tlast} + C_{last}/\lambda_z$.

(C_{last} = observed concentration at the last sampling time (t_{last}) at which the concentration is at or above the lower limit of quantification)

$\%AUC_{extrap}$ = Ratio of the extrapolated part within $AUC_{0\text{-}inf}$ in %.

C_{max} = Maximum observed concentration.

$t_{½}$ = Terminal half-life; $t_{½} = \ln(2)/\lambda_z$

t_{max} = The time to reach the maximum observed concentration collected during a dosing interval (unless otherwise defined, take the 1st occurrence in case of multiple/ identical C_{max} values).

λ_z = Terminal first order (elimination) rate constant. Determined from the terminal slope of the concentration curve using log-linear regression on terminal data points of the curve.

V_z/f = The apparent volume of distribution during the terminal phase following extravascular administration. $V_z/f = CL/f/\lambda_z$ (for midazolam only)

CL/f = The apparent total body clearance of study treatment following extravascular administration. $CL/f=Dose/AUC_{0\text{-}inf}$ (for midazolam only)

Phoenix WinNonlin® method linear trapezoidal linear/log interpolation will be used for calculation of AUC.

The pre-dose plasma sample will always be considered as if it had been taken simultaneously with the drug administration. If there should have been any deviations in post-dose sampling, the actual sampling times relative to drug administration will be used unless stated otherwise. Missing data will not be replaced or imputed in any way. Scheduled time will be used for the calculation of PK parameters in case of missing sampling times. Plasma concentrations below the lower limit of quantification (LLOQ) up to the sampling time, for which the last measured concentration was equal to or larger than LLOQ, will be treated as zero.

AUC will be regarded as unreliable if more than two consecutive results are missing or if the concentrations were quantifiable and/or non-missing for fewer than 2/3 of the time points. In this case all derived parameters will be calculated but also considered as unreliable.

C_{max} and t_{max} will be regarded as unreliable if the maximum was observed preceding or following a sample with missing data. In case of multiple peaks, C_{max} and t_{max} refer to the highest measured concentration even if there should be earlier peaks. In case of two or more samples with the same concentration (as supplied by the analyst), t_{max} refers to the earlier of these.

Log-linear regressions for determination of λ_z should exclude t_{max} and will be performed using at least 3 data points of the terminal elimination phase. Concentrations < LLOQ after the last quantifiable data point > LLOQ should not be used in the regression analysis.

λ_z will not be calculated, if the terminal elimination phase is not apparent.

λ_z will be calculated but considered as unreliable, if the R^2 value is less than 0.8. In this case, all derived parameters (e.g. $AUC_{0\text{-}inf}$, $\%AUC_{extrap}$, $t_{½}$, CL/f, and V_z/f) will still be calculated but also considered as unreliable.

The value of $AUC_{0\text{-}inf}$ will also be considered unreliable if the terminal area beyond the last quantified sample is greater than 20% of the total $AUC_{0\text{-}inf}$ ($\%AUC_{extrap}$). In this case all derived parameters (e.g. CL/f, V_z/f) will be calculated but also considered as unreliable.

Unreliable parameters will be listed and flagged accordingly and set to missing for calculation of descriptive statistics and statistical analysis. If a PK parameter is unreliable for more than 20% of the subjects, this parameter will additionally be evaluated as sensitivity including the unreliable cases.

Vamorolone:

The following PK parameter will be determined for each subject in the PK Set – vamorolone for levels of vamorolone in plasma. The PK parameter will be derived for the treatment Vamorolone Alone.

The predose concentrations collected during the vamorolone induction phase on Days 3, 4, 7, 11, 13 and 14 correspond to the C_{trough} definition.

C_{trough} = The concentration observed at the end of a dosing interval immediately before next dosing.

12.2. Presentation of Data

Samples with concentrations below LLOQ will be identified in listings (i.e. <LLOQ). Individual concentration values below LLOQ will be set to zero for the calculation of summary statistics and for plotting.

Midazolam and 1'-hydroxymidazolam:

The results of subject plasma concentrations measured will be listed separately for each analyte and for each of the treatments Midazolam Alone and Vamorolone + Midazolam. Tables will be prepared showing descriptive statistics as described for concentrations in Section 8.1 at each sampling time by analyte and treatment.

Similar listings and tables will also be prepared for the PK parameters each analyte. Tables will show descriptive statistics as described in Section 8.1, except for t_{max}, for which only minimum, median, and maximum will be reported.

%AUCextrap will be listed only. The auxiliary parameters R^2, the starting and time (lower and upper limit) and number of points considered in calculation of λ_z will also be listed only.

For each subject with plasma concentration data and for each analyte separately, individual plasma concentration-time profiles will be plotted on a linear as well as on a log-linear scale, showing both treatments in one figure. Furthermore, synoptic plots will be provided on a linear scale, for each treatment separately.

Similar plots will also be generated for the mean plasma concentration-time profiles showing both treatments in one figure (incl. figures showing the arithmetic mean course without standard deviation on a linear as well as on a log-linear scale and a figure showing the arithmetic mean

course with standard deviation on a linear scale). The actual sampling times for individual plots and the scheduled sampling times for mean plots will be used.

The main individual PK parameters $AUC_{0\text{-tlast}}$, $AUC_{0\text{-inf}}$ and C_{max} of midazolam and

1'-hydroxymidazolam of all subjects will furthermore be displayed in spaghetti plots comparing the treatments.

Vamorolone:

The results of vamorolone plasma concentrations measured will be listed for each subject. Tables will be prepared showing descriptive statistics as described for concentrations in Section 8.1 at each sampling time.

Similar listings and tables will also be prepared for the PK parameter. Tables will show descriptive statistics as described in Section 8.1.

Arithmetic mean (with standard deviation) vamorolone predose values during the induction phase will be graphically displayed on a linear scale. The scheduled sampling times in hours (presented also in days) will be used for this plot.

12.3. Analysis of Pharmacokinetic Parameters

PK parameters will be summarized descriptively by treatment as mentioned above in Section 12.2.

In order to achieve a better approximation to a normal distribution, the PK parameters $AUC_{0\text{-tlast}}$, $AUC_{0\text{-inf}}$ and C_{max} of midazolam and 1'-hydroxymidazolam will be logarithmically transformed. Afterwards, they will be evaluated statistically using an analysis of variance (ANOVA) with TREATMENT as fixed effect and SUBJECT as random effect. Based on the model, least-squares (LS) means, LS mean differences, and 90% confidence intervals (CI) for the differences on the log-scale will be obtained for each of $AUC_{0\text{-tlast}}$, $AUC_{0\text{-inf}}$ and C_{max}.

The results will be back-transformed to the original scale by exponentiation to provide geometric least-square means with 90% CI, point estimate of the geometric mean ratio midazolam with vamorolone vs. midazolam alone (Day 14 / Day 1) in % and its corresponding 90% CI in the table. Intra-subject CV will be calculated from the residual error of the model.

Only subjects that have a valid PK parameter for both treatments will be included.

13. PHARMACODYNAMIC ANALYSES

Not applicable.

14. SAFETY ANALYSES

14.1. Adverse Events

Definition of AE are given in protocol section 8.4. AEs will be coded using the medical dictionary for regulatory activities (MedDRA) dictionary, using the latest version as in the Data Manual Plan.

For each subject within a treatment arm, AEs that continue with changing intensity will be counted as one AE and only the worst severity will be considered in the corresponding frequency tables. Moreover, a recurrent AE (e.g. a headache for a couple of hours each day) will be counted as several AEs.

AEs occurring after the administration of any study treatment will be counted towards the last treatment received before the onset, even if the event is not resolved at the beginning of the following study treatment. An AE that worsens after a later study treatment will be counted towards both study treatments.

A listing of AEs, according to the ICH Guidelines, will be created. This listing will include, at minimum, a description of AEs as coded and reported, last study treatment administration date and time (including relative day), start/end time and date of occurrence (including relative day), time lag (onset) since last administration of study treatment, duration, seriousness, severity, relationship to study treatment midazolam and/or to vamorolone, reasonable causal relationship to protocol required procedures (yes, no), other causality factors, concomitant medication taken (yes, no), concomitant procedure done (yes, no), other action taken, outcome, and action taken with study treatment midazolam and/or with vamorolone.

All analyses will be restricted to treatment-emergent AEs (TEAEs), defined as all AEs that emerge during study treatment and having been absent pre-treatment or worsening relative to the pre-treatment state. All other AEs will be listed only and defined as pre-treatment AEs.

A frequency table will be compiled showing the number and percentage of subjects per treatment and overall affected by
- any TEAE,
- any Midazolam unrelated and related TEAE,
- any Vamorolone unrelated and related TEAE,
- any mild, moderate and severe TEAE,
- any related severe TEAE,
- any serious TEAE,
- any TEAE leading to death,
- any related TEAE leading to death.

This table will also show the frequencies of the events per treatment.

Further summary tables by study treatment will be generated. These tables will be sorted by system organ class and preferred term. The numbers and percentages of subjects with AEs and the frequency of the events will be reported. Three sets of such summary tables by study treatment will be presented:
- overall,
- by relationship (see definition above),
- by severity.

14.2. Clinical Safety Laboratory Tests

Laboratory data will be listed by subject, study treatment and study time. Variables: at minimum scheduled study time, laboratory parameter, unit, results at each of the assessment times, comments

(if available). Values outside of normal ranges as well as clinically relevant values will be highlighted or annotated.

Another listing will show date and clock times of laboratory sampling.

Separate listings for abnormal laboratory values as well as abnormal laboratory values considered clinically relevant by the investigator will also be provided.

14.3. Vital Signs

Vital Signs will be listed by subject, study treatment, and study time. Variables: at minimum scheduled study time, date, clock time, location of measurement, position, parameter (body temperature, pulse rate, and systolic and diastolic blood pressure), repeated (yes, no), interpretation, and comments (if available).

Descriptive statistics of vital signs will be presented for each scheduled time point by study treatment.

Arithmetic means of vital signs will be graphically displayed with standard deviation over all timepoints. Thus, all study treatments will be shown in one plot. Scheduled sampling times will be used.

14.4. Electrocardiograms

ECG parameters (heart rate, PR(Q), QRS, QT, and QTcF), interpretation (normal/abnormal, clinical significance), comments (if available) will be listed by subject, study treatment, and study time.

Descriptive statistics will be presented for heart rate, PR(Q), QRS, QT, and QTcF for each scheduled study time by study treatment.

The ECG interpretation will be tabulated as the number and percentage of subjects with “normal”, “abnormal, not clinically significant”, or “abnormal, clinically significant” results by study treatment and scheduled study time.

14.5. Physical Examination

Any clinically relevant findings will be reported as adverse event.

14.6. Drug Administration and Pharmacokinetic Plasma, Biomarker Plasma and Biomarker Urine Sampling

Treatment duration (as defined in Section 8.3) as well as the number of administrations of study treatment will be calculated for each study treatment, listed and summarized in an exposure table for each study treatment. For treatment Midazolam + Vamorolone both drugs will be considered separately.

An exposure listing will be provided by subject, scheduled study time, and study treatment. This listing will contain, at minimum, the calculated study treatment duration as well as the calculated number of administration of each drug by study treatment, date and time of each drug administration, calculated dose, actual dose, unit, pharmaceutical strength, unit, route, dose

formulation, water intake, fasting status and reason, where applicable, required position (yes, no), bottle weight before and after use, where applicable, change in dose and reason, dose adjusted and reason, no dose and reason.

A separate listing of start date and time of each drug administration of midazolam and vamorolone and each PK plasma sampling, including time deviations from scheduled sampling times (for midazolam and 1'-hydroxymidazolam samplings only) will be provided sorted by subject and study treatment.

Time deviations (hh:mm) for plasma/urine samplings will be calculated as

time deviation = actual sampling date and time – scheduled sampling date and time.

The actual date and time (24-hour clock time) of each sample will be recorded. Allowed assessment time windows will be defined in the deviation manual and collected in the quality issue log (see Section 7). Any time deviations falling into the allowed time windows will not be considered a protocol deviation.

Study drug administrations, biomarker plasma collection date/time and urine start and end date/time, will be provided sorted by subject and study treatment in a similar way.

15. ENDOGENE BIOMARKER ANALYSES

Analyses for CYP3A4 biomarkers will be performed on the Safety Set.

The biomarker 4β-hydroxycholesterol will be measured predose in plasma. 6β-hydroxycortisol and cortisol will be measured in pooled urine samples. Additionally, the ratio of 6β-hydroxycortisol to cortisol will be delivered.

In cases where the subject was not able to urinate, the urine volume is set to zero and biomarker concentration is set to missing. Samples with concentrations below LLOQ will be identified in listings (i.e. <LLOQ). Individual concentration values below LLOQ will be set to zero for the calculation of summary statistics and for plotting.

Baseline will be defined as the assessment taken predose on Day 1, whereas absolute changes from baseline are defined as: post-dose value – baseline value.

Urine biomarkers (including change from baseline) and their ratio at each collection day will be listed by subject and visit and study treatment. The urine volume will also be included. Another listing will present the plasma biomarker (including change from baseline).

A table will be prepared showing descriptive statistics for urine biomarkers (including summary statistics for change from baseline) and the ratio for each visit and study treatment, as described in Section 8.1. A similar table will be presented for the plasma biomarker.

For each subject with biomarker data available (including the ratio), individual concentrations will be plotted for each biomarker and the ratio separately on a linear scale over all timepoints . Similar plots will also be generated for the mean plasma/urine concentrations (figure showing the arithmetic

mean with standard deviation on a linear scale). The scheduled sampling times in hours (also presented in days) will be used for all plasma concentration and the study days for urine concentration plots.

16. CHANGES TO PROTOCOL PLANNED ANALYSES

The SAP confirms to the study protocol and its amendment(s).

17. INTERIM ANALYSES

Interim analyses are not anticipated.

18. GENERAL FORMAT OF TABLES, FIGURES AND SUBJECT DATA LISTINGS

There are no Sponsor-specific guidelines or SOPs that must be observed for the analysis or report generation.

The standard report is an integrated study report according to Nuvisan SOPs and ICH-guidelines. The Sponsor does not require any specific formats (e.g. footer, header, margins, fonts) to be observed. The tables, listings, and figures (TLFs) provided for the report will be formatted with font courier new with at least 7 points.

Treatment labels to be used in TLFs will be:

M Alone: 2.5 mg Midazolam on Day 1
V Alone : 6 mg/kg Vamorolone on Days 3 – 13
M+V : 6 mg/kg Vamorolone + 2.5 mg Midazolam on Day 14

The following lists in Section 19 provide an overview of a possible Table of Contents (TOC) of the outputs produced for statistical analysis.

19. APPENDIX

19.1. List of End-of-Text-Tables and Figures

14.1 Demographic Data
14.1.1 Subject Disposition (Full Set)
14.1.2 Exposure (Safety Set)
14.1.3 Analysis Sets (All Assigned Subjects)
14.1.4 Demographics and Other Baseline Characteristics
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Not applicable.

16.2.7 Adverse Event Listing (Safety Set)

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16.2.8.3.6 Individual Concentration Plots of Cortisol (ng/mL) in Urine
16.2.8.3.7 Individual Concentration Plots of 6β-Hydroxycortisol to Cortisol Ratio in Urine

16.2.9 Other Safety Data (Safety Set)

16.2.9.1 Vital Signs
16.2.9.2 12-Lead ECG

* Defines the listings for DRM.

20. References

[1] Prueksaritanont T, Vega JM, Rogers JD, Gagliano K, Greenberg HE, Gillen L, Brucker MJ, McLoughlin D, Wong PH, Waldman SA. Simvastatin does not affect CYP3A activity, quantified by the erythromycin breath test and oral midazolam pharmacokinetics, in healthy male subjects. J Clin Pharmacol. 2000 Nov;40(11):1274-9.