CLINICAL STUDY PROTOCOL

Title Page

Protocol Title:

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 Protocol Number:	SNT-I-VAM-025
Nuvisan Protocol Number:	N-A-PH1-23-054
Investigational Medicinal Product:	Vamorolone
Study Phase:	Phase 1
Brief Title:	Evaluation of vamorolone CYP3A4 induction on midazolam (a sensitive CYP3A4 substrate) pharmacokinetics
Sponsor:	Santhera Pharmaceuticals (Switzerland) Ltd Hohenrainstrasse 24 4133 Pratteln, Switzerland
Sponsor's Legal Representative EU:	PPD Nuvisan GmbH Wegenerstr. 13 89231 Neu-Ulm, Germany
Principal Investigator:	Michael Lissy Nuvisan GmbH Wegenerstr. 13 89231 Neu-Ulm, Germany
Regulatory Agency Identifier Number(s):	EU CT number: 2024-513845-36-00
Protocol Version:	05-JUN-2024/Version Final 1.0

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List of Abbreviations

Abbreviations of PK parameters are provided in Section 10.6.

ADaM	Analysis data model
ADR	Adverse drug reaction
AE	Adverse event
ALT	Alanine aminotransferase
anti-HIV	Anti-human immunodeficiency virus antibody
anti-HCV	Anti-hepatitis C virus antibody
AST	Aspartate aminotransferase
AxMP	Auxiliary medicinal product
BfArM	Bundesinstitut für Arzneimittel und Medizinprodukte (German Federal Institute for Drugs and Medical Devices)
BMD	Becker Muscular Dystrophy
BMI	Body mass index
CA	Competent authority
CDISC	Clinical data interchange standards consortium
COVID-19	Coronavirus disease 2019
CK	Creatine phosphokinase
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CRF	Case report form
CTIS	Clinical Trials Information System
CTS	Clinical trial supplies
CV	Coefficient of variation
CYP	Cytochrome P450
CYP3A4	Cytochrome P450 3A4
DDI	Drug-drug interaction
DMD	Duchenne Muscular Dystrophy
DRM	Data Review Meeting
ECG	Electrocardiogram

eCRF	Electronic case report form
ED	Early discontinuation
eGFR	Estimated glomerular filtration rate
ENT	Ear, nose, throat
EU	European Union
FSH	Follicle stimulating hormone
FU	Follow-up
GCP	Good clinical practice
GGT	Gamma-glutamyl transferase
GLDH	Glutamate dehydrogenase
GR	Glucocorticoid receptor
HBsAg	Hepatitis B virus surface antigen
hCG	Human chorionic gonadotropin
HIV	Human immunodeficiency virus
HRT	Hormonal replacement therapy
IB	Investigator's Brochure
ICF	Informed consent form
ICH	International conference for harmonization
IEC	Independent ethics committee
IgM	Immunoglobulin M
IMP	Investigational medicinal product
INR	International normalized ratio
IUD	Intrauterine device
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LDH	Lactate dehydrogenase
MedDRA	Medical Dictionary for Regulatory Activities
MR	Mineralocorticoid receptor
NIMP	Non-investigational medicinal product
PK	Pharmacokinetic(s)

PR(Q)	ECG parameter: PR(Q) interval
QRS	ECG parameter: QRS duration; part of electrocardiographic wave representing ventricular depolarization
QT	ECG parameter: QT interval uncorrected
QTc	ECG parameter: QT interval corrected
QTcF	ECG parameter: QT interval corrected for ventricular rate calculated according to the formula of Fridericia
RNA	Ribonucleic acid
SAE	Serious adverse event
SAP	Statistical analysis plan
SDTM	Study data tabulation model
SmPC	Summary of Product Characteristics
SoA	Schedule of activities
SOP	Standard operating procedure
SUSAR	Suspected unexpected serious adverse reaction
TEAE	Treatment emergent adverse event
TSH	Thyroid-stimulating hormone
ULN	Upper limit of normal
USA	United States of America
USPI	United States prescribing information
WHO	World Health Organization
WOCBP	Woman of child-bearing potential
WONCBP	Woman of non-childbearing potential

1. Protocol Summary

1.1. Synopsis

Protocol Title:

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.

Brief Title:

Evaluation of vamorolone CYP3A4 induction on midazolam (a sensitive CYP 3A4 substrate) pharmacokinetics

Regulatory Agency Identifier Number(s):

EU CT number: 2024-513845-36-00

Rationale:

The purpose of this study is to investigate the CYP3A4 induction properties of vamorolone in humans. *In vitro* experiments showed a concentration-dependent CYP3A4 induction, with less than a 2-fold increase in enzyme activity and mRNA levels at concentrations of 5 μM and below. The therapeutic dose of vamorolone (6.0 mg/kg) in patients with DMD results in unbound C_{max} values at least 5-fold lower than concentrations that cause *in vitro* CYP3A4 induction. However, the potential for reduced plasma concentrations due to co-administration of vamorolone with other CYP3A4 substrates cannot be excluded. Using midazolam as a CYP3A probe drug, the study will measure pharmacokinetics on Days 1 and 14 to assess potential DDIs. Conducted in healthy subjects, the study is designed to predict outcomes in the intended patient population for vamorolone. The results will guide labeling for CYP3A4-related DDIs with vamorolone.

Objectives and Endpoints:

Objectives	Endpoints
Primary	
• To investigate the CYP3A4 induction potential of vamorolone by assessing the PK of midazolam and 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 TEAEs, laboratory assessments, vital signs, and ECG evaluation
• To investigate CYP3A4 induction by endogenous biomarkers of the CYP3A4 activity	• Plasma 4β-hydroxycholesterol and urinary 6β-hydroxycortisol to cortisol ratio

ECG=electrocardiogram, PK=pharmacokinetics, TEAE=treatment emergent adverse event.

Overall Design:

This study will be conducted in a single-center, open-label, single-arm, fixed-sequence, design. Healthy male and female subjects (18 to 55 years, inclusive) are eligible.

Brief Summary:

The purpose of this study is to measure the potential of vamorolone to induce CYP3A4 activity using midazolam as a sensitive CYP3A4 probe. The plasma 4β-hydroxycholesterol and urinary 6β-hydroxycortisol to cortisol ratio, endogenous CYP3A4 biomarkers, will be also determined as clinical markers of the CYP3A4 activity. Also, the safety and tolerability of vamorolone will be assessed.

Number of Subjects:

It is planned to assign approximately 18 subjects to study treatments to have at least 16 evaluable subjects completing the study. Dropouts might be replaced if the number of evaluable subjects completing the study becomes or is expected to become less than 16 subjects in total.

Study Treatment Groups and Duration:

The maximum duration for each participating subject is expected to be approximately 8 weeks, from screening until the safety follow-up call.

Subjects will be screened for eligibility within 28 days prior to first administration of study treatment. Subjects will be admitted to the study site on Day -1 and will remain inpatient at the study site under medical supervision for 15 overnight stays until discharge on Day 15.

The treatment duration will be up to 2 weeks and each subject will receive the following treatments:

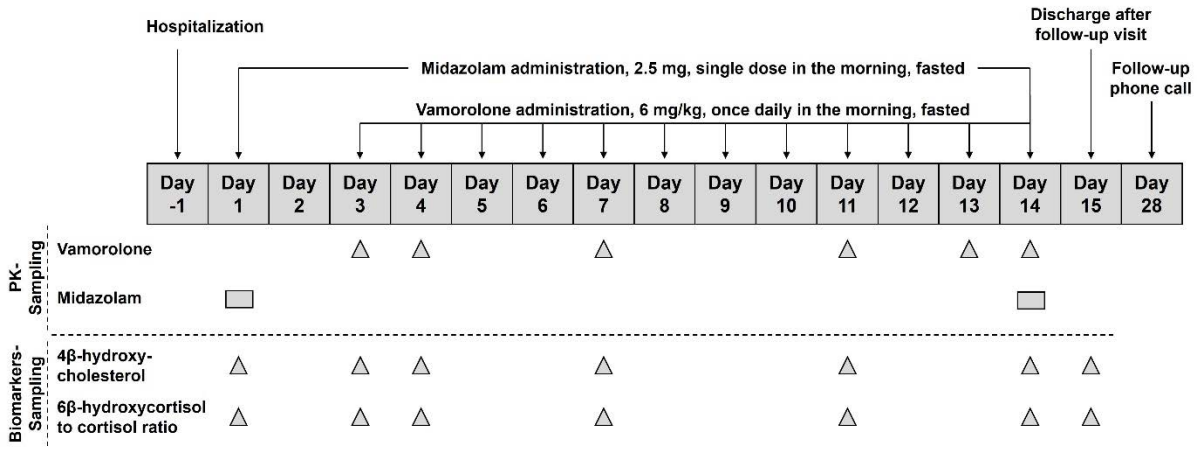
- Day 1: Single oral dose of 2.5 mg midazolam.
- Day 2: Wash-out day.
- Days 3 to 13: Daily oral doses of 6 mg/kg vamorolone.
- Day 14: Single oral doses of 2.5 mg midazolam and 6 mg/kg vamorolone.

The follow-up visit is planned 24 hours after last midazolam dose, on Day 15. A safety follow-up call is planned 14 days after the last vamorolone dose, on Day 28. Early discontinuation assessments will be conducted at an early discontinuation visit for subjects who withdraw prematurely.

Data Monitoring/Other Committee: No

1.2. Schema

Figure 1: Overall Study Design



Screening procedures will be performed within 28 to 2 days prior to the first administration of study treatment.

1.3. Schedule of Activities

The SoA presenting the timepoints for the study-related measures / actions is provided in Table 1.

- Whenever different assessments and procedures are to be performed at the same nominal time, the following sequence will be followed (except for screening and Day -1 evaluations):
    1. 12-lead ECG, vital signs;
    2. Blood sampling (PK, CYP3A4 activity biomarkers, and safety) as close as possible to the nominal time;
    3. All other assessments and procedures.

Any meals or water intake will be served after the assessments / actions for a given timepoint are completed.

Assessment time windows will be defined according to Nuvisan SOP in a separate document. Any time deviations falling into the allowed time windows will not be considered a protocol deviation.

Table 1: Schedule of Activities

Procedure	SCR	Study Day															FU/ED		Notes
	-28 to -2	–1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15*	28	*Not earlier than 24 hours after the last midazolam dose / after early discontinuation.
Informed consent																			To be obtained prior to any screening activity. Refer to Section 10.1.
Ambulatory visit	X																		
Hospitalization		X																	In the morning in fasted state.
In-house stay		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Discharge																	X		After completion of FU/ED assessments.
Safety Phone Call																		X	
Inclusion/exclusion criteria	X																		Refer to Sections 5.1.1 and 5.2.1.
Confirmation of eligibility		X																	Recheck of clinical status on Day -1, and subject eligibility.
Demographic data	X																		Refer to Section 8.1.1.
Relevant medical history	X																		Including history of illegal drugs, alcohol, tobacco, and caffeine use. Refer to Section 8.1.3.
Prior medication	X	X																	Any medication (including prescription, non-prescription drugs, dietary and herbal supplements) taken which had been stopped prior to the first administration of study treatment.
Physical examination	X	X															X		Refer to Section 8.3.1.
Height, weight	X	X															X		Height will be measured at screening only. BMI will be calculated. Refer to Section 8.1.2.
Serum pregnancy test	X	X																	In all female subjects. Refer to Section 8.3.5.
TSH, FSH	X																		Refer to Section 10.2. FSH only for suspected postmenopausal females.
Serology	X																		HIV, Hepatitis B and C screening. Refer to Section 10.2.
Urine drug and cotinine screen and alcohol breath test	X	X																	Refer to Section 8.3.6 and Section 10.2.
Safety laboratory assessments	X	X			X						X						X		Hematology, coagulation, clinical chemistry, urinalysis. Refer to Section 10.2. Day 3 and 9: predose; no urinalysis.

Procedure	SCR	Study Day															FU/ED		Notes
	-28 to -2	–1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15*	28	*Not earlier than 24 hours after the last midazolam dose / after early discontinuation.
12-lead ECG	X	X			X											X			Days 3 and 14: approximately 2 hours postdose. For details refer to Section 8.3.3.
Vital signs including body temperature	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		Day 1 and Days 3 to 14: Predose. Days 2 and 15: 24 hours postdose. For details refer to Section 8.3.2.
Study treatment administration midazolam			X													X			Refer to Section 6.1.
PK blood sampling for midazolam and 1'-hydroxymidazolam			X													X			At predose and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 10 hours after each midazolam dose. Refer to Section 8.5.
Study treatment administration vamorolone					X	X	X	X	X	X	X	X	X	X	X	X			Refer to Section 6.1. For dose calculation, weight of Day –1 will be taken.
PK blood sampling vamorolone					X	X			X				X		X	X			Predose. Refer to Section 8.5.
Blood sampling for 4β-hydroxycholesterol			X		X	X			X				X			X	X		On Day 15: 24 hours postdose. On other days: predose. Refer to Section 8.8.
Urine collection for 6β-hydroxycortisol and cortisol			X		X	X			X				X			X	X		From approximately 8:00 to 12:00 a.m.. Refer to Section 8.8.
(S)AE review	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Any event with start after ICF signature. Refer to Sections 8.4 and 10.5.
Concomitant medication review			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Any medication taken at/after time of first study treatment, regardless of whether it had started prior to the study or not. Refer to Section 6.9.

AE=adverse event; BMI=body mass index; ECG=electrocardiogram; ED=early discontinuation visit; FSH=follicle-stimulating hormone; FU=follow-up visit; HIV=human immunodeficiency virus; ICF=informed consent form; PK=pharmacokinetics; SAE=serious adverse event; SCR: Screening; TSH=thyroid-stimulating hormone.

2. Introduction

Vamorolone belongs to the structural class of synthetic steroidal drugs but shows unique pharmacodynamic properties combining selective glucocorticoid receptor agonism with mineralocorticoid receptor antagonism (Reeves et al, 2013).

Vamorolone 40 mg/mL oral suspension (commercial name AGAMREE®) received Marketing Authorisation in the EU and in the USA for use in patients with DMD. Vamorolone is also being developed for use in BMD; this indication is under clinical investigation.

2.1. Study Rationale

The purpose of the present study is to address the CYP3A4 induction properties of vamorolone in humans.

Based on *in vitro* experiments, CYP3A4 induction by vamorolone was found to be concentration-dependent, resulting in less than a 2-fold increase in enzyme activity in hepatocytes at concentrations of 5 µM and below. Similarly, less than a 2-fold induction based on mRNA was observed in hepatocytes at concentrations of 1.5 µM and below. The unbound C_{max} values associated with the therapeutic dose of 6.0 mg/kg administered daily to patients with DMD (0.327 µM) are at least 5-fold lower than the concentrations causing CYP3A4 induction *in vitro*. However, it is not ruled out that co-administration of vamorolone with other compounds acting as substrates of CYP3A4 may potentially lead to reduced plasma concentrations.

Midazolam is primarily metabolized in the liver and gut by human CYP3A4 to its pharmacologic active metabolite, 1'-hydroxymidazolam and has been used widely as a sensitive CYP3A probe drug for evaluating the effect of an inducer on CYP3A activity *in vivo*.

Pharmacokinetics of midazolam will be measured on Day 1, and then on Day 14 to investigate the potential DDI between the two compounds resulting from potential induction by vamorolone for the CYP3A4 enzyme.

The study will be conducted in healthy subjects as the findings in this population should predict findings in the patient population for which vamorolone is intended. The planned administrated doses have been shown to be safe and no genotoxicity was observed *in vitro* and *in vivo* assessments.

The study results will provide guidance for labelling with respect to CYP3A4-related DDIs with vamorolone.

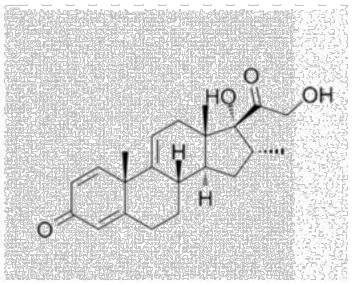
2.2. Background

2.2.1. Vamorolone

Vamorolone is a structural analogue of prednisolone in which the sub-activities have been dissociated, i.e., transrepression retained, transactivation reduced, membrane stabilization increased, and cross-reactivity with the mineralocorticoid receptor changed from agonist to antagonist.

Vamorolone (17α,21-dihydroxy-16α-methyl-pregna-1,4,9(11)-triene-3,20-dione) is a synthetic steroidal drug and differs from traditional glucocorticoids by having a double bond between carbons 9 and 11 of the steroid C ring instead of the 11β-hydroxy or carbonyl moiety on C11 found in most members of the corticosteroid class. The chemical structure of vamorolone is shown in Figure 2.

Figure 2: Chemical Structure of Vamorolone



Molecular formula: $C_{22}H_{28}O_4$

Molecular weight: 356.46

Vamorolone as a dissociative corticosteroid selectively binds to the GR, triggering anti-inflammatory effects, and binds to the MR, inhibiting MR activation by aldosterone. Vamorolone changes the subsequent activity of the receptors, which may possibly dissociate its effectiveness from the known side effects of corticosteroids. A detailed description of the chemistry, pharmacology, efficacy, and safety of vamorolone is provided in the current IB (IB).

Vamorolone oral suspension has been approved in the USA in October 2023 and in the EU in December 2023 for use in patients with DMD and is being developed for BMD. Both are allelic disorders, where DMD is marked by the absence of dystrophin in skeletal muscle, while BMD exhibits the presence of abnormal dystrophin in skeletal muscle.

More detailed information on DMD and BMD can be found in the current IB (IB).

2.2.1.1. Summary of Non-Clinical Studies

A comprehensive package of non-clinical pharmacology, PK, and toxicology studies was completed for vamorolone. A summary of the non-clinical experience is presented below.

- *In vitro* and *in vivo* mechanistic studies show that the pharmacological mode of action of vamorolone is through agonist activity towards the GR and antagonist activity towards the MR. Unlike all members of the corticosteroid class, vamorolone is likely not a substrate for 11β-hydroxysteroid dehydrogenase enzymes (HSD11B1, HSD11B2), either systemically or locally in cells and tissues, as it lacks the 11β moiety on the steroidal C ring. Thus, it is not subject to systemic or local pro-drug/drug conversion.
- A tissue distribution study using radioactively labelled vamorolone showed widespread distribution among body organs with a peak concentration between 2 to 6 hours for most tissues/organs. The highest exposure was noted in the gastrointestinal organs (cecum, large intestine, small intestine) and the liver. Vamorolone is not excreted unchanged into bile or urine. In the rat mass balance study 87% of drug related material was recovered in feces and 10% in urine. The PK of vamorolone is generally dose proportional and shows no systematic sex differences. The elimination half-life ($t_{1/2}$) after oral single administration is 0.68 hours in mice, 2.29 hours in rats, and 2.25 hours in dogs. Plasma protein binding is ~77% to 88% across species.
- Vamorolone can be metabolized via multiple Phase 1 and Phase 2 pathways, such as glucuronidation, hydroxylation, and reduction. Based on *in vitro* studies, it is unlikely that vamorolone inhibits CYP isoenzymes, uridine 5′diphosphoglucuronosyltransferase isoenzymes, solute-linked carrier transporters or efflux transporters at clinically relevant concentrations. Induction of CYP3A4 was observed in-vitro at high doses ≥ 1.5 μM.
- The nonclinical toxicology program showed a consistent safety profile across both the mouse and dog studies. There was a dose-dependent decrease in body weight gain and the adrenal glands, liver, spleen, and thymus were identified as target organs. Animal safety margins relative to the human exposure at the highest therapeutic dose are overall low, however the pharmacologically mediated adverse effects were generally reversible and are monitorable in the clinic.
- As DMD is a predominantly male disease, a focused microscopic analysis of the male reproductive organs and sperm analysis were included in chronic toxicology studies both the mouse and dog.
- There were no signals for either mutagenicity or genotoxicity in any of the studies and there were no preneoplastic lesions in the chronic toxicology studies; carcinogenicity studies will be conducted as a post-approval commitment.

Please refer to the current IB for additional information (IB).

2.2.1.2. Summary of Clinical Studies

6 clinical studies have been completed with healthy subjects as part of the clinical pharmacology program for vamorolone. 4 clinical studies have been completed in subjects first enrolled at ages

4 to < 7 years with DMD. An additional study to expand the age range (ages 2 to < 4 years, 7 to < 18 years) was initiated in April 2022 and is currently ongoing.

A summary of the human experience is presented below.

- The completed clinical trials included 153 adult subjects treated with 0.1 to 20 mg/kg vamorolone (including 8 subjects with hepatic impairment) in clinical pharmacology trials and 164 subjects from 4 years of age and older with DMD who received vamorolone at daily doses ranging from 0.25 to 6 mg/kg. The exposure duration in completed clinical trials ranged from 1.4 to 32 months.
- The PK of vamorolone is dose proportional following single and multiple ascending doses. After oral administration with food, vamorolone is rapidly absorbed with median t_{max} about 2 hours. Consistent with a half-life $t_{1/2}$ of 2 hours, no accumulation is observed after repeated daily administration. Vamorolone undergoes direct glucuronidation and hydrogenation with subsequent glucuronidation. 2 plasma metabolites are observed at greater than 10% of the parent drug in human plasma, but both are pharmacologically inactive O-glucuronides. Approximately, 30% of the dose is excreted in feces (15.4% unchanged) and 57% of dose is excreted in urine.
- *In vitro*, vamorolone is metabolized by CYP3A4/5, CYP2C8, and CYP2C9. A rise of 33.7%. for $AUC_{0\text{-}inf}$ and a t_{max} delay by 2 hours are observed when combined with itraconazole, a potent CYP3A4 inhibitor. The recommended dose of vamorolone when administered with strong CYP3A4 inhibitors (e.g., telithromycin, clarithromycin, voriconazole, grapefruit juice) is 4 mg/kg/day. Acid reducing agents are not expected to impact the absorption of vamorolone.
- Moderate hepatic impairment elevates vamorolone exposure by a 1.7- and 2.6-fold increase in C_{max} and $AUC_{0\text{-}inf}$, respectively, compared to matched healthy adults. Exposure parameters of subjects with mild hepatic impairment are expected to be less affected and should represent an increase in vamorolone concentration of between 10 to 15% (1.1- to 1.5-fold increase in $AUC_{0\text{-}inf}$) compared to those individuals with normal liver function, which is not considered clinically relevant.
- In the pivotal double-blind controlled (placebo and prednisone) study, significant and clinically meaningful improvements were seen with vamorolone 2 mg/kg and 6 mg/kg compared with placebo for multiple measures of lower limb function. When evaluated globally, the improvements seen with vamorolone 6 mg/kg were similar to those seen with prednisone while the improvements seen with vamorolone 2 mg/kg were slightly smaller.
    - TEAEs reported ≥ 5% of subjects in the vamorolone 6 mg/kg group and at a higher frequency (at least 1 subject difference) than placebo: cushingoid (28.6%), abdominal pain upper (7.1%), diarrhoea (7.1%), vomiting (14.3%), rhinitis (7.1%), arthropod

bite (7.1%), fall (10.7%), weight increased (10.7%), Vitamin D deficiency (10.7%), headache (7.1%), irritability (10.7%), and cough (7.1%).

- In the vamorolone 2-6 mg/kg integrated safety group (i.e., 163 subjects with DMD treated with vamorolone 2 mg/kg or 6 mg/kg in any study), 86.5% of subjects experienced a TEAE, regardless of drug relatedness, and 48.5% experienced a TEAE assessed by the investigator as drug related. The TEAEs, regardless of drug relatedness, reported for ≥ 10% of subjects in the vamorolone 2-6 mg/kg group across all DMD studies were: pyrexia (20.2%), nasopharyngitis (18.4%), cough (18.4%), upper respiratory tract infection (17.2%), vomiting (16.6%), pain in extremity (13.5%), headache (12.9%), constipation (11.7%), cushingoid (11.7%), diarrhoea (11.0%), and weight increased (10.4%). 9 SAEs have been reported in subjects receiving 2-6 mg/kg vamorolone in the clinical studies for the DMD program. Additionally, SAEs of pneumonia were reported for 2 subjects receiving 0.75 mg/kg vamorolone. All these SAEs were considered unrelated to study treatment.
- As of 30 Sep 2023, there have been a total of 24 SAEs in the expanded access/compassionate use setting. Except for 1 case (adrenal insufficiency), each of these SAEs was considered unrelated to study treatment by both the Investigator and the Sponsor, and none of them resulted in discontinuation from the study or program. There was no fatal event.

Additional detailed information about the clinical studies of vamorolone can be found in the current IB (IB).

2.2.2. Midazolam

Midazolam is a benzodiazepine available as midazolam solution for oral administration. Oral midazolam provides safe and effective sedation and anxiolysis prior to surgical procedures requiring anesthesia and prior to other procedures requiring sedation without anesthesia. Midazolam is also used for short-term treatment of sleep disorders. Time to onset of effect is most frequently reported as 10 to 20 minutes (Nordt et al, 1997).

Pharmacokinetics and metabolism of midazolam:

Following a single oral dose, peak midazolam concentration is usually achieved at 0.5 to 1 hour. The C_{max} values of midazolam are reported to average 10 ng/mL and 24 ng/mL, following a single oral dose of 2 mg and 5 mg, respectively. The elimination half-life of midazolam is approximately 3.6 hours. The extent of plasma protein binding of midazolam is moderately high and concentration independent. In adults and pediatric patients older than 1 year, midazolam is approximately 97% bound to plasma protein, principally albumin. Midazolam is metabolized via CYP3A4 to 1'-hydroxymidazolam and is a sensitive substrate of CYP3A4. In healthy subjects, 1'-hydroxymidazolam is bound to the extent of 89%.

Safety profile of midazolam:

Midazolam has been associated with reports of respiratory depression, airway obstruction, oxygen desaturation, hypoxia, and apnea, most often when used concomitantly with other central nervous system depressants (e.g. opioids).

Midazolam must only be administered to subjects if they will be monitored by direct visual observation by a health care professional.

More detailed information on midazolam can be found in the SmPC (SmPC midazolam).

2.2.3. Biomarkers for CYP3A4 Activity

Endogenous 4β-hydroxycholesterol is mediated by CYP3A4 and CYP3A5 and had been proposed as a reliable marker of CYP3A4/5 activity. Patients treated with drugs known to be strong inducers of CYP3A4/5 (e.g. rifampicin) have shown dose-related increases in circulating 4β-hydroxycholesterol levels (Diczfalusy et al, 2011). 4β-Hydroxycholesterol is a metabolite of cholesterol formed by CYP3A4-catalyzed metabolism, for which plasma concentrations show no diurnal variation and low intra-individual variability (Kanebratt et al, 2008). In addition, the 6β-hydroxycortisol-to-cortisol ratio will be also measured in urine samples as a conservative approach (Tran et al, 1999).

2.3. Benefit/Risk Assessment

As of November 2023, subjects in 10 completed and 2 ongoing studies have been exposed to vamorolone including healthy subjects, patients with DMD and patients with BMD.

In the clinical pharmacology program, 125 healthy volunteers and 8 subjects with moderate hepatic impairment, males and females, were exposed to vamorolone. 164 subjects have received vamorolone across all of the completed DMD clinical studies. As of 30 September 2023, 44 DMD subjects had been treated with vamorolone in the VBP15-006 study and 8 subjects enrolled in the BMD clinical study VBP15-BMD-001 had been treated with vamorolone 500 mg (250 mg for body weight < 50 kg) daily or placebo.

Vamorolone was generally safe and well tolerated in all subjects. The TEAEs have been primarily mild to moderate in severity.

The vamorolone treatment duration in this study will be 12 days in total. The maximum daily dose of 6 mg/kg corresponds to the maximum clinical dose in DMD pediatric patients. This regimen does not exceed the maximum exposure of 20 mg/kg given daily for up to 14 days which was shown to be well tolerated in previous studies with healthy subjects.

Considering the nonclinical and clinical data available to date, the study is considered reasonable and adequate according to the specifications outlined in this protocol.

2.3.1. Risk Assessment

Potential risks that subjects in the study might be exposed to may arise from study-related procedures as well as from the administration of vamorolone or midazolam, as outlined in the tabular summary below.

More detailed information about the known and expected benefits and risks and reasonably AEs of vamorolone and midazolam may be found in the IB of vamorolone (IB) and in the SmPC of midazolam (SmPC midazolam).

Identified and Potential Risks of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Study Treatment: Vamorolone		
Hypersensitivity to active drug or excipients	Potential risk of allergic reaction to vamorolone or excipients.	Exclusion of subjects who were exposed to vamorolone previously or are allergic to excipients (see Section 5.2).
Increased susceptibility to infections and their severity	While no increased incidence or severity of infections was observed in the clinical studies, limited long-term experience does not allow to exclude an increased risk for infections.	Subjects will stay in-house and will be closely monitored for signs and symptoms of infections.
Study Treatment: Midazolam		
Somnolence or fatigue	Midazolam has a known sedative effect.	Midazolam dose used in this study is markedly lower than recommended therapeutic dose (see Section 4.3). Subjects will be in-house on midazolam administration days.

Identified and Potential Risks of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Hypersensitivity including angioedema, and anaphylactic shock, rash, urticaria, pruritus (frequency unknown)	Contraindication and a known side effect of midazolam according to the SmPC.	Subjects with known hypersensitivity to midazolam will not be included in the study (see Section 5.2). Subjects will stay in-house during the midazolam dosing periods and will be closely monitored for signs and symptoms of hypersensitivity.
Respiratory depression, apnea, respiratory/cardiac arrest, dyspnea, bradycardia, laryngospasm	Contraindication and known side effects.	Midazolam dose used in this study is markedly lower than recommended therapeutic dose (see Section 4.3). Subjects will be in-house on midazolam administration days. Subjects with known respiratory/cardiac impairment and sleep apnea will not be included in the study (see Section 5.2).
Use in subjects with hepatic and renal impairment.	In patients with renal impairment (creatinine clearance < 30 mL/min) midazolam may be accompanied by more pronounced and prolonged sedation possibly including clinically relevant respiratory and cardiovascular depression. Hepatic impairment reduces the clearance of intravenous midazolam with a subsequent increase in terminal half-life.	Subjects with known hepatic and renal impairment will not be included in the study (see Section 5.2).

Identified and Potential Risks of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Study Procedures		
Blood draw	Blood draws have the potential to cause AEs such as fainting or hematoma.	The amount of blood drawn will be strictly controlled. Subjects will be in a hospital setting with support from highly trained professionals.
Complications from indwelling catheters	Local reactions, infections, nerve or tissue damage may occur.	Standard medical care to be applied when catheters are used.
Allergic reactions to ECG electrodes or dressing adhesive	Local intolerance may occur.	Subjects with known contact allergies will not be included in the study.
Other		
Not applicable		

2.3.2. Warnings and Precautions

No special warnings and precautions are considered necessary for the substances investigated in the present study.

2.3.3. Benefit Assessment

Healthy subjects may expect no direct benefits from participating in this clinical study.

2.3.4. Overall Benefit Risk Conclusion

Considering the currently available nonclinical and clinical safety data, the measures taken to minimize risk to subjects participating in this study, in conjunction with the predicted exposures of 6 mg/kg of vamorolone daily dose and implementing close medical monitoring of safety and tolerability the potential risks identified in association with vamorolone are justified by the anticipated benefits that may be afforded to patients with DMD and BMD.

Risk minimization measures routinely implemented in early phase clinical studies are considered adequate, including exclusion criteria for laboratory parameters (Section 5.2), close biochemical and hematology laboratory monitoring (Section 8.3.4), and observation of vital signs and ECGs (Sections 8.3.2 and 8.3.3). Administration will be discontinued in case of events that unacceptably endanger the safety of the subjects (Section 7).

This clinical study will start after the favorable opinion of the IEC and once permission of the CA has been obtained. All investigations will be conducted in compliance with the clinical study protocol, ICH GCP, and any additional applicable regulatory requirements.

3. Objectives and Endpoints

Table 2: Objectives and Endpoints

Objectives	Endpoints
Primary	
• To investigate the CYP3A4 induction potential of vamorolone by assessing the PK of midazolam and 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 TEAEs, laboratory assessments, vital signs, and ECG evaluation
• To investigate CYP3A4 induction by endogenous biomarkers of the CYP3A4 activity	• Plasma 4β-hydroxycholesterol and urinary 6β-hydroxycortisol to cortisol ratio

ECG=electrocardiogram, PK=pharmacokinetics, TEAE=treatment emergent adverse event.

For definitions of PK endpoints refer to Section 8.5.2. Refer to Section 9 for the statistical aspects of the endpoints.

4. Study Design

4.1. Overall Design

This will be a non-randomized, single-center, open-label, single-sequence, single-arm Phase I study in 18 healthy male and female subjects.

- The total duration in the study for a subject is approximately 8 weeks, including the screening period (maximum 4 weeks), 2 weeks of treatment period, and a safety follow-up period of 2 weeks.
- A screening period will occur from Day -28 to Day -2. Subjects will be admitted to the study site on Day -1 and will remain resident throughout the study conduct period until follow-up visit on Day 15. A follow-up safety phone call is planned on Day 28.
- Day 1: Subjects will receive a single dose of 2.5 mg midazolam in fasted state in the morning.
- Day 2: Wash-out period.
- Days 3 to 13: Subjects will receive daily doses of 6 mg/kg vamorolone in the morning within 30 minutes after start of a standard breakfast.
- Day 14: Subjects will receive single doses of 2.5 mg midazolam and 6 mg/kg vamorolone in fasted state in the morning.
- Safety and tolerability parameters will be collected during the entire study phase from screening to follow-up.
- Blood samples for PK assessment of midazolam and 1'-hydroxymidazolam will be collected from predose through 10 hours following the midazolam doses on Days 1 and 14.
- Blood samples for PK assessment of vamorolone will be collected throughout the vamorolone dosing period.
- Blood and urine biomarkers samples for CYP3A4 induction assessment will be collected throughout the treatment period.

A study scheme and a detailed SoA with more information on the specific time points indicated for the scheduled study visits and required procedures are provided in Sections 1.2 and 1.3.

4.2. Scientific Rationale for Study Design

The trial design and endpoints are typical for a drug interaction trial of this type.

The CYP3A4 induction effect of vamorolone would be examined on Day 14, i.e. 12 days after vamorolone first dosing, which is considered adequate to achieve the induction potential of vamorolone.

Midazolam will be administered as a single dose once alone (Day 1) and once after 12 days of daily vamorolone administration (Day 14) to investigate the combined interaction potential.

Based on the apparent elimination half-life of up to 2.5 hours for midazolam and up to 1 hour for 1'-hydroxymidazolam in healthy adult subjects (SmPC midazolam), a one-day washout period on Day 2 should be sufficient between Day 1 midazolam and Day 3 vamorolone administration to avoid carryover of midazolam concentrations.

PK blood samples for the determination of plasma midazolam and 1'-hydroxymidazolam will be collected on Day 1 (PK midazolam alone) and Day 14 (PK midazolam when co-administrated with vamorolone) from predose to 10 hours postdose.

PK blood samples for the determination of plasma vamorolone will be collected on Days 3, 4, 7, 11, 13 and 14 at predose to determine the vamorolone levels during the induction phase.

The follow-up visit is planned 24 hours after the last midazolam and vamorolone dose. That exceeds 5 times the elimination half-lives of both vamorolone and midazolam.

The follow-up phone call on Day 28 is foreseen to monitor potential AEs after vamorolone withdrawal that are mainly but not exclusively related to adrenal suppression. Vamorolone was shown to cause adrenal suppression as seen in low morning cortisol levels (see IB). Cortisol levels were shown to be back to baseline 2 weeks after vamorolone withdrawal.

The single arm, sequential design allows for intra-individual comparison of the effects of CYP3A4 induction by vamorolone and reduces the impact of inter-individual variability.

4.3. Justification for Dose

Vamorolone

The recommended clinical dose for the paediatric population, as defined in DMD, is 6 mg/kg, to be administered orally once daily, with a maximum daily dosage of 300 mg for patients weighing more than 50 kg (USPI vamorolone). The objective of this study is to assess the potential for vamorolone to induce the CYP3A4 enzyme in healthy adults. It is plausible that the maximum daily flat dose of 300 mg, when administered to paediatric patients with a body weight of over 50 kg, may result in reduced exposure in adults, particularly those weighing over 70 kg. Therefore, the selected daily single dose of vamorolone for this study is 6 mg/kg. For dose calculation, weight of Day –1 will be taken.

The selected dosing regimen for this study is considered safe as in previous studies single and multiple doses of vamorolone up to 20 mg/kg given daily for 14 days were well tolerated in healthy adult subjects (see IB).

Midazolam

The clinically recommended dose for midazolam is from 0.25 mg/kg to 1.0 mg/kg in adults. An oral dose of 2.5 mg midazolam is considered appropriate to achieve the study objectives for drug interaction studies and is considered safe (Wiebe et al, 2020).

4.4. Start / End of Study Definition

The study start is defined as the date of the first informed consent signature of the first subject.

Subjects are considered to have completed the study, if they have completed all phases of the study including the last scheduled procedure shown in the SoA (refer to Section 1.3).

The end of the study is defined as the date of the last scheduled procedure shown in the SoA for the last subject.

Information on study termination or discontinuation criteria is provided in Section 7.

5. Study Population

The study will be conducted in a total of 18 healthy adult male and female subjects.

No subject may be assigned to study treatment unless adherence to all eligibility criteria as given in Sections 5.1 and 5.2 is established.

Prospective approval of protocol deviations to recruitment and enrolment criteria, also known as protocol waivers or exemptions, is not permitted.

Individuals who do not meet the eligibility criteria due to medical findings will be advised to consult a doctor as applicable.

5.1. Inclusion Criteria

A subject is eligible to be included in the clinical study only if all the following criteria apply:

5.1.1. Inclusion Criteria to be Checked at Screening

Age

1. Age of 18 to 55 years inclusive, at the time of signing the informed consent.

Type of Subject and Disease Characteristics

2. Subject is overtly healthy as determined by medical evaluation including medical history, physical examination, vital signs, laboratory tests, and ECG.

Weight

3. Body weight ≥ 50 kg and a BMI ≥ 18 kg/m^2 and ≤ 29.9 kg/m^2 at screening.

Sex and Contraceptive/Barrier Requirements

4. Male and female.

Contraceptive use by men and women should be consistent with local regulations regarding the methods of contraception for those participating in clinical studies.

Male subjects:

- If the subject is a sexually active man and not surgically sterilized, he must be willing to:
  - Abstain from sexual intercourse or
  - Use a condom plus another form of contraception, (e.g., spermicide, IUD, birth control pills taken by female partner) if engaging in sexual intercourse with a woman who could become pregnant.
  - Use a condom during sexual intercourse with pregnant or lactating women.
  - Must not father a child and must refrain from donating sperm from administration of the first dose and up to 3 months after the last dose of study treatment.

Female subjects:

All women (regardless of their status, i.e. WOCBP and WONCBP; for definitions see Section 10.4.1) must have a negative serum β-hCG pregnancy test prior to the initiation of the study treatments. FSH levels of suspected postmenopausal females must be > 30 mIU/mL.

Vamorolone has the potential to induce CYP3A4, which may result in a reduction in the effectiveness of contraceptives that are metabolized by CYP3A4 such as hormonal contraceptives when co-administered with vamorolone. Therefore, hormonal contraceptives by any route of administration are contraindicated.

Women participating in the study must be either:

- WONCBP or
- WOCBP using, during the length of the study and for at least 3 months after the stop of the study treatments, 1 of the following contraceptive methods plus a condom:
  - IUD during the study and up to 3 months after the last administration of the study treatments
  - Any IUD with published data showing that the lowest expected failure rate is less than 1% per year (not all IUDs meet this criteria)
  - Any other methods with published data showing that the lowest expected failure rate for birth control is less than 1% per year, or
  - Abstinent from intercourse for 2 weeks before exposure to the study treatments, throughout the clinical trial until 3 months following discontinuation of the study treatments, or
  - Have a male partner who is sterile prior to the woman's entry into the study and is the sole sexual partner for that woman during the study period.

The Investigator is responsible for review of medical history, menstrual history, and recent sexual activity to decrease the risk for inclusion of a woman with an early undetected pregnancy.

Drugs and Stimulants

5. Subject is a non-smoker for at least 3 months prior exposure to the study treatments. Subject must also have abstained from use of other nicotine containing products (e.g., nicotine patch, chewing gum or e-cigarettes) for at least 3 months before exposure to the study treatments.

Informed Consent

6. Capable of giving signed informed consent as described in Section 10.1, which includes compliance with the requirements and restrictions listed in the ICF and in this protocol prior to any clinical study specific procedure.

Vital Signs

7. Supine systolic blood pressure ≥ 90 mmHg and ≤ 140 mmHg; diastolic blood pressure ≥ 50 mmHg and ≤ 90 mmHg and pulse rate ≥ 45 bpm and ≤ 90 bpm, and tympanic body temperature ≥ 35.0 °C and ≤ 37.5 °C at screening.

Other Inclusion Criteria

8. Subjects must be able to communicate well with the Investigator and comply with the protocol requirements, instructions, and protocol related restrictions (e.g. dietary, fluid and lifestyle restrictions from screening to study completion; Section 5.3).
9. Subjects must be able to swallow the study treatments as per protocol.

5.2. Exclusion Criteria

Subjects are excluded from the study if any of the following criteria apply:

5.2.1. Exclusion Criteria to be Checked at Screening

Medical Conditions

10. A past medical history of clinically significant abnormalities or a history/family history of long QT interval syndrome, structural cardiac abnormalities (including but not limited to hypertrophic cardiomyopathy, valvulopathy, and congenital defects), or cardiogenic syncope.
11. An abnormal ECG, defined as:
    - PR > 215 msec and < 100 msec, QRS complex > 120 msec; QTcF > 450 msec by automated reading
    - Any clinically significant ST/T wave abnormalities
    - Any atrial or ventricular arrhythmias

12. A past medical history of myocardial infarction, angina pectoris, atherosclerosis or other clinically significant heart disease (e.g., congestive heart failure, uncontrolled hypertension, history of labile hypertension).
13. A past medical history of peptic ulcers, diverticulitis, and non-specific ulcerative colitis.
14. History of complaints of frequent dizziness and /or vomiting spells or lightheadedness ("frequent" defined as incidence occurs more than once every week) or history of/or present sleep apnea.
15. Any history or evidence of any clinically relevant, gastrointestinal, respiratory, hepatic, renal, endocrinologic, hematologic, immunologic, metabolic, genitourinary, pulmonary, neurologic, dermatologic, musculoskeletal, and/or other major disease or malignancy as determined by medical evaluation (including physical examination) capable of significantly altering the absorption, metabolism, or elimination of drugs; constituting a risk when taking the study treatments; or interfering with the interpretation of data.
16. Known Gilbert's syndrome.
17. Any clinically relevant history of allergic conditions requiring hospitalization or prolonged systemic treatment (including drug allergies, allergic asthma, eczema, allergies requiring therapy with corticosteroids or anaphylactic reactions); but excluding untreated, asymptomatic, seasonal allergies at time of dosing or allergic contact sensitizations (e.g., nickel allergy).
18. Known or suspected hypersensitivity or contraindications to vamorolone and/or midazolam or any components of the formulation used.
19. Relevant current acute or chronic/recurrent viral, bacterial, fungal, or parasitic infections (e.g., pulmonary/upper respiratory, gastrointestinal, urinary, skin, or ENT infections) at screening or within 28 days prior to administration of the study treatments.

Prior/Concomitant Therapy

20. Use of any concomitant medication or any drugs / medicines (including dietary supplements, natural and herbal remedies, and hormone replacement therapy) within 2 weeks or 5 times the half-life of the respective drug, whichever is longer, prior to the first administration of the study treatments.

    Occasional use of paracetamol up to 2 g/day or ibuprofen up to 1.2 g/day (medicinal products in their original packaging, approved and marketed in Germany) is permitted.

    Oral, injectable, and implantable contraceptives as outlined in Section 5.1 are permitted.
21. Previous exposure to vamorolone.
22. Any use of corticoids within 6 months prior to the first administration of the study treatments.

23. Administration of live, attenuated, replication-competent vaccines within 6 weeks prior to the first administration of the study treatments until 2 weeks after the follow-up visit. Administration of vector-based or mRNA COVID-19 vaccines within 2 weeks prior to the first administration of the study treatments until 2 weeks after the follow-up visit.
24. Treatment with biologic agents (such as monoclonal antibodies including marketed drugs) within 3 months or 5 half-lives (whichever is longer) prior to the first administration of the study treatments.

Prior/Concurrent Clinical Study Experience

25. Use of any investigational drug or participation in any clinical study within 30 days or 5 half-lives (whichever is longer) prior to the expected date of first administration of study treatments or planning to take other investigational drugs during the study.

Diagnostic assessments

26. Positive results for HBsAg, anti-HCV, anti-HIV 1 and 2, and HIV 1-p24 antigen at screening.
27. Positive screen for alcohol, drugs of abuse and cotinine at screening.
28. Elevations in ALT > 1.1 x ULN, AST > 1.2 x ULN, serum bilirubin > 1.2 x ULN, creatinine > 1.1 x ULN and and HbA1c > ULN at screening. A case-by-case decision for any abnormality must be discussed with the Sponsor before inclusion.
29. TSH outside of normal ranges.
30. eGFR based on the CKD-EPI (details for calculation see Section 10.2) of < 90 mL/min at screening.
31. Potassium or magnesium blood concentration below the lower limit of normal at screening.

Other Exclusions

32. Subjects who are unwilling to adhere to contraceptive requirements.
33. Higher than low-risk alcohol consumption i.e., consumption of an average weekly alcohol intake of > 21 units/week for men and > 14 units/week for women. 1 unit (12 g) corresponds to 0.3 L of beer/day or 0.12 L of wine/day or 1 glass (at 2 cL) of spirits/day.
34. Excessive consumption of caffeine- or xanthine-containing food or beverages (> 5 cups of coffee a day or equivalent) or inability to stop consuming from 48 hours prior to first planned administration of study treatments.
35. Consumption of alcohol from 48 hours prior to admission.

36. Consumption of high dose resveratrol-containing products or products with enzyme-inducing or enzyme-inhibiting properties (for details refer to Section 5.3.1) 14 days prior to first administration of study treatment.
37. Regular consumption of poppy seed containing food prior to first administration of study treatment.
38. Any use of drugs-of-abuse or alcohol abuse within 1 month prior to dosing.
39. Subject with vegetarian, vegan, or restricted diet (e.g., gluten-free) or not willing or able to eat the complete standard meals.
40. Female subject who has been pregnant within 6 months prior to screening or breastfeeding or lactating within 3 months prior to screening or plans to become pregnant during the clinical study period and for 3 months after final study treatment administration.
41. Donation or loss of more than 400 mL of blood or received a transfusion of any blood or blood products within 30 days, or donated plasma within 30 days prior to first administration of study treatment.
42. Strenuous physical activity within 72 h to admission.
43. Employee of the Sponsor, the Nuvisan Group, or other Contract Research Organization involved in the clinical study.
44. Legal incapacity or limited legal capacity, or incarceration and vulnerable subjects.
45. Inability to understand or communicate reliably with the Investigator or considered by the Investigator to be unable to or unlikely to co-operate with the protocol requirements, instructions, and study-related restrictions.
46. History of non-compliance to medical regimens and subjects who are considered potentially unreliable (e.g., refuse to comply with study regulations).
47. Any other conditions or factors which in the opinion of the Investigator may interfere with study conduct.

5.2.2. Exclusion Criteria to be re-checked upon Admission

Medical Conditions

48. Changes in medical conditions compared to screening, as judged by the Investigator.
49. Body weight < 50 kg.

Prior/Concomitant Therapy

50. Changes in prior/concomitant therapy compared to screening, as judged by the Investigator.

Diagnostic assessments

51. Positive screen for alcohol, drugs of abuse and cotinine test upon admission.

Other Exclusions

52. Changes in other exclusion criteria compared to screening, as judged by the Investigator.

5.3. Lifestyle Considerations

5.3.1. Meals and Dietary Restrictions

- Subjects will be required to refrain from consumption of red wine, Seville oranges, grapefruit, or grapefruit juice, pomelos, exotic citrus fruits, grapefruit hybrids, or all fruit juices from 14 days before the start of study treatment until after collection of the final blood PK sample.
- Subjects will be required to refrain from consumption of poppy seeds from 72 hours before the start of study treatment until the end of confinement.
- During the in-house stay, the subjects may only consume food and beverages provided by the study site. The diet provided (maximum of 4 meals per day) will consider the imposed restrictions. Meals will be served at customary times. For further restrictions on dosing days refer to Section 6.1.
- On Days 1 and 14, subjects will fast for at least 10 hours before and 4 hours after administration of study treatments. On Days 3 to 13 administration of vamorolone will be done within 30 minutes after start of a standard breakfast.
- On Days 1 and 14, water consumption will be allowed ad libitum (as desired) except for 1 hour before until 1 hour after study treatment intake (apart from the water taken with the dose). Subjects should drink at least 1.5 L/day.

5.3.2. Caffeine, Alcohol, and Tobacco

- Subjects will abstain from ingesting caffeine- or xanthine-containing food and beverages (e.g., coffee, tea, cola, and chocolate) from 48 hours before admission to the study until after the end of confinement.
- Subjects will abstain from alcohol for 48 hours before admission to the study site until the end of confinement.
- The use of tobacco / nicotine containing products is not permitted during the study.

5.3.3. Activity

- Subjects will abstain from strenuous exercise and sauna from 72 hours prior to admission until the end of confinement.
- Subjects may participate in light recreational activities during hospitalization phase (e.g., watching television, reading).
- Subjects will remain in semi-supine position until 4 hours after dosing of midazolam but may get up to go to the toilet.
- Subjects will remain in semi-supine position until 1 hour after dosing on Days 3 to 13 but may get up to go to the toilet.

5.3.4. Other Restrictions

Not applicable.

5.4. Screen Failures

Screen failures are defined as subjects who consented to participate in the clinical study but are not subsequently assigned to study treatment. A minimal set of screen failure information is required to ensure transparent reporting of screen failure subjects to meet the Consolidated Standards of Reporting Trials publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any SAE.

Subjects who do not meet the criteria for participation in this study (screen failure) may be rescreened, however, only under the following conditions:

- The subject had successfully passed the screening procedures but could not start subsequent treatment on schedule.
- Initial screening occurred too early to complete the required wash-out period after prior therapy.
- The in- / exclusion criteria preventing the subject's initial attempt to take part in the study have been changed (via protocol amendment).
- The violation of the respective in-/exclusion criteria is considered minor and temporary by the Investigator.

Up to 2 rescreenings will be allowed. Rescreened subjects will be assigned a new screening number for every rescreening event. The previous screening number of the subject will be documented in the eCRF in order to effectively track the screening processes. Subjects who are rescreened are required to sign a new ICF, even if it was not changed after the subject's previous screening (Section 10.1).

In case of abnormal results caused by intercurrent diseases, short-term treatable conditions, or other temporary health disorders (e.g., acute infection, iron deficiency, blood pressure outside defined range), the Investigator may decide to repeat the respective screening parameter(s) within the predetermined screening period. As a rule, up to 2 repetitions are acceptable. Outside of the predetermined screening period, the subject shall be rescreened as described above.

In any case, the Investigator has to ensure that the repeated procedures, whether the recheck during initial screening or a rescreening, do not expose the subject to an unjustifiable health risk.

5.5. Criteria for Temporarily Delaying Enrollment, Study Treatment

Not applicable.

6. Study Treatments and Concomitant Therapy

Study treatments are all prespecified investigational and non-investigational medicinal products, medicinal devices, and other treatments (e.g., surgical, and behavioral) including marketed product(s), or placebo, or a combination of the same as per study plan, intended to be administered / applied to a study subject during the study conduct.

6.1. Study Treatments Administered

The treatments to be administered in this study are displayed in Table 3.

Table 3: Study Treatments

Treatment Label	**Test Drug**	**Reference Drug**
Treatment Name	Vamorolone	Midazolam
Treatment Description	Oral suspension	Oral solution
Type	Drug	Drug
Dose Formulation	Suspension	Solution
Unit Dose Strength(s)	4.0% w/v (40 mg/mL)	To be described in the IMP manual.
Dosage Level(s)	6 mg/kg once daily dosing over 12 days. For dose calculation, weight of Day –1 will be taken.	2.5 mg once daily dosing on 2 days
Route of Administration	Oral	Oral
State for Administration	Days 3 to 13: after a standard breakfast Day 14: fasted	Fasted
Use	Experimental	Experimental
Dosing Instructions	7.5 mL to be taken in the morning.	To be taken in the morning.
Manufacturer/Marketing Authorization Holder	To be described in the IMP manual.	To be described in the IMP manual.
IMP and NIMP/AxMP	IMP	IMP
Sourcing	The Sponsor will provide Nuvisan GmbH with the medication released by the Sponsor Qualified Person (QP) according to Good Manufacturing Practice Annex 13.	Nuvisan GmbH will source medication available on the local market.
Packaging and Labelling	Study medication will be primary packaged in amber glass bottles. Each bottle will be labelled according to country requirement.	Study medication will be used as unchanged product with market authorization.

Subjects will receive single oral dose of midazolam on Days 1 and 14 and multiple oral doses of vamorolone on Days 3 to 14. On Day 14, when both study treatments are administered together, the subjects will first take vamorolone and directly afterwards midazolam.

The cumulative doses per subject will be 5 mg midazolam and 3.6 g vamorolone.

Both study treatments will be administered in an upright position with a total of 240 mL of non-carbonated water. Prior to each midazolam administration on Days 1 and 14 subjects will be fasting for at least 10 hours and abstain from consumption of additional fluid from 1 hour before until 1 hour after dosing (apart from the water taken with the dose). After each midazolam administration subjects will continue fasting for 4 hours postdose. On Days 3 to 13 vamorolone will be administered after a standard breakfast.

Standardized meals will be served 4 hours (lunch), 7 hours (snack), and 10 hours (dinner) after midazolam dosing on Days 1 and 14.

Other meals will be served at customary times during inpatient period.

6.2. Preparation, Handling, Storage, and Accountability

The Sponsor will supply sufficient amounts of vamorolone to Nuvisan CTS Department. The Sponsor will provide administration devices (8 mL oral syringes) in an appropriate quantity.

IMPs will be stored and handled by Nuvisan according to product specific storage conditions (for storage conditions of Vamorolone 4% suspension, refer to the current Quality Agreement between Nuvisan and the Sponsor). IMPs will be stored in a secure, environmentally controlled and monitored area with access limited to authorized staff.

Nuvisan will source commercially available midazolam. Details will be described in the IMP manual.

Nuvisan CTS Department will document the receipt of vamorolone and must confirm appropriate conditions (e.g., temperature) have been maintained during transit for all study treatment received and any discrepancies are reported and resolved before use of the study treatment.

Vamorolone will be packed and labelled study specifically by the Sponsor before the clinical part of the study starts including final QP release as per the German clinical study labelling requirements.

Nuvisan CTS Department will be responsible for transfer of vamorolone and dosing devices to site. Details will be described in an IMP manual.

Only subjects enrolled in the study may receive study treatment and only authorized site staff may supply, prepare, or administer study treatment. All study treatment must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labelled storage conditions with access limited to the Investigator and authorized site staff.

All study treatment administrations will be performed in accordance with the specifications in the IMP manual, by site staff authorized by the Principal Investigator.

The Investigator is responsible for study treatment accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records).

The number of unused study treatment will be documented by Nuvisan CTS Department. According to the provisions of the Sponsor the unused study treatment will be destroyed or sent back to the Sponsor.

Further guidance and information for the final disposition of unused study treatments are provided in the IMP manual.

6.3. Assignment to Study Treatment

Randomization is not applicable in this study. All subjects receive the same study treatments in the same sequence on the same study days.

Each subject is identified by the study site's unique subject identification number. After informed consent procedure, every subject is given a screening number. Only subjects who comply with all eligibility criteria can be included into the study. On Day 1 subjects will be assigned a unique 3-digit assignment number (e.g. 101, 102, 103, etc.) in ascending numerical order (in accordance with Nuvisan SOP).

If subjects who already have been assigned to study treatment need to be replaced, the assignment number will be the number of the original subject plus 100. For example, the replacement for assignment number 102 will receive the assignment number 202. For details on replacement of subjects see Section 7.2.2.

The Investigator will keep a record relating the subject identifiers (screening and assignment number) and the names of all subjects who have given their informed consent, to allow easy checking of data in subject files, when required. This record will also include the date of subject's enrolment and completion, as well as subjects who could not be assigned to study treatment for whatever reason.

6.4. Blinding

Not applicable. This is an open-label study.

6.5. Study Treatment Compliance

The administration of the study treatment will be performed in the clinical unit by qualified clinical professionals of the Investigator's team. Deviation(s) from the planned dosage regimen should be recorded in the eCRF.

If the individual dose for a subject is distributed from a bulk supply, the preparation of the dose will be confirmed and documented by a second member of the study site staff.

The dose of study treatment and subject identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the study treatment.

Study site staff will examine each subject's mouth to ensure that the study treatment was ingested.

6.6. Dose Modification

No dose modifications are envisaged during this study.

6.7. Continued Access to Study Treatment after the End of the Study

In patients, dose tapering is foreseen if vamorolone has been administered for more than one week. Due to the short treatment duration, i.e. less than 2 weeks, and the previous experience with vamorolone in two studies treating patients and healthy subjects for up to 2 weeks and then stopping without tapering, a tapering for this study is not foreseen (IB).

Furthermore, as this is a study in healthy subjects who are not under concomitant therapy, no treatment is planned after the end of the study. If there are findings that are unclear in the final examination, a detailed assessment, a follow-up and, if necessary, specific medical treatment may be required.

6.8. Treatment of Overdose

For this study, any dose of vamorolone greater than the protocol-defined dose will be considered an overdose. Treatment of acute overdosage is by immediate supportive and symptomatic therapy. Gastric lavage or emesis can be considered (IB).

For this study, any dose of midazolam greater than the highest recommended clinical dose in the SmPC will be considered an overdose (SmPC midazolam). In the event of an overdose with midazolam, subjects should be closely monitored e.g. for signs and symptoms of sedation, confusion, lethargy, muscle relaxation and paradoxical excitation. Further absorption of midazolam may be prevented using an appropriate method e.g. treatment within 1-2 hours with activated charcoal. If activated charcoal is used, airway protection is imperative for drowsy patients. Midazolam can be antagonized by flumazenil, a benzodiazepine antagonist. However, it should only be used under closely monitored conditions and in case of severe central nervous system depression.

In the event of an overdose, the Investigator should:

1. Evaluate the subject to determine, in consultation with the medical expert of the Sponsor, if possible, whether study treatment should be interrupted or whether the dose should be reduced.
2. Closely monitor the subject for any AE/SAE and laboratory abnormalities as medically appropriate and at least until scheduled follow-up.
3. If requested by the medical expert of the Sponsor (determined on a case-by-case basis), obtain one or more plasma samples for PK analysis as soon as possible and on further timepoints thereafter.
4. Document the quantity of the excess dose as well as the duration of the overdose in the eCRF.

The Investigator will use appropriate clinical judgment when treating an overdose of an investigational drug.

Decisions regarding dose interruptions or modifications will be made by the Investigator in consultation with the Sponsor based on the clinical evaluation of the subject.

6.9. Prior and Concomitant Therapy

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 study treatment.

Subjects must abstain from taking prescription or non-prescription drugs (including vitamins and dietary or herbal supplements) within 2 weeks or 5 half-lives (whichever is longer) before the start of study treatment until completion of the safety follow-up phone call, unless, in the opinion of the Investigator and Sponsor, the medication will not interfere with the study. Information on non-permitted prior or concomitant therapy including drugs and herbal remedies is provided in the list of exclusion criteria (Section 5.2).

Except when necessary to treat an AE, subjects are not allowed to use those medications, starting from the specified timepoints until completion of the study.

Paracetamol up to 2 g per day and ibuprofen up to 1.2 g per day may be allowed for the symptomatic treatment of AEs (e.g., headache) after consulting the Investigator when medically indicated. Contraceptive methods as outlined in Section 5.1.1 are also permitted. Other concomitant medication may be considered on a case-by-case basis to avoid immediate hazard to the subjects by the Investigator or in consultation with the Sponsor if required.

For any concomitant therapy used, the name of the drug, the reason for use, dates of administration including start and end dates and dosage information including dose and frequency will be recorded in the eCRF. In consultation with the Sponsor, a decision may be taken by the Investigator to withdraw a subject from the study when other concomitant medication is required or has been taken without consulting the Investigator.

6.9.1. Rescue Medicine

No specific antidote is available for vamorolone.

Flumazenil, a specific benzodiazepine-receptor antagonist, is an antidote for midazolam (see Section 6.8).

7. Discontinuation of Study Treatment and Subject Discontinuation/Withdrawal

7.1. Discontinuation of Study Treatment

In rare instances, it may be necessary for a subject to permanently discontinue study treatment. If study treatment is permanently discontinued, the subject will remain in the study. Safety, and tolerability data as well as PK and biomarker data will be collected to the furthest possible extent and the subject will be asked to participate in the early discontinuation visit.

See the SoA (Section 1.3) for data to be collected at the time of discontinuation of study treatment and safety follow-up / early discontinuation visit and for any further evaluations that need to be completed.

7.1.1. Stopping Rules for Individual Subjects

The Investigator will withdraw a subject from receiving further study treatment in the following cases:

- Occurrence of an AE of severe intensity in case of causal relationship to study treatment or an SAE.
- Relevant signs or symptoms affecting subject safety. Any events that unacceptably endanger the safety of the subject.
- If, in the Investigator's opinion, continuation of the study would be harmful to the subject's well-being.
- Hypersensitivity reactions classified as severe.
- Vomiting within 4 hours after vamorolone and within 4 hours after midazolam administration (corresponding to approximately 2 times expected median t_{max} for each study treatment).
- Impossibility to obtain blood samples in general.
- Abnormal blood pressure including hypotension defined as systolic < 70 mmHg and/or diastolic < 40 mmHg, or hypertension defined as systolic > 160 mmHg and/or diastolic > 110 mmHg (evaluated with the subject in supine position for a minimum of 5 minutes and confirmed by 2 repeat measurements). In case of repeat abnormal findings, see above, this should lead to direct study withdrawal.
- Creatinine > 1.3 x ULN.
- Use of nonpermitted concomitant medications. However, any medications considered necessary for the subject's wellbeing may be given at the discretion of the Investigator.
- Occurrence of pregnancy. See Section 8.4.5.

7.1.1.1. Liver Chemistry Stopping Criteria

Discontinuation of study treatment for abnormal liver tests is required by the Investigator when a subject meets one of the following conditions or if the Investigator believes that it is in best interest of the subject:

- Increase in ALT or AST ≥ 3 x ULN and/or total bilirubin ≥ 2 x ULN considered by the Investigator to be at least possibly related to the study treatment.
- ALT ≥ 3 x ULN and bilirubin ≥ 2 x ULN (> 35% direct bilirubin) has to be reported as SAE (confirmed by analysis of 2 blood samples taken on different days; additional laboratory examinations may be necessary) (refer to Section 10.5).

7.1.1.2. QTc Stopping Criteria

The Investigator will withdraw a subject from receiving further study treatment in the following cases:

- QTcF ≥ 500 ms and/or an increase of QTcF from baseline of ≥ 60 ms, confirmed by repeated ECGs.

7.2. Subject Discontinuation / Withdrawal from the Study

7.2.1. Discontinuation / Withdrawal Criteria for Individual Subjects

- A subject may withdraw from the study at any time at their own request without giving reasons. The subject will not suffer any disadvantage as a result. A subject may be withdrawn at any time at the discretion of the Investigator for safety, behavioral, compliance, or administrative reasons. This is expected to be uncommon.
- At the time of discontinuing from the study, if possible, an early discontinuation visit should be conducted. See SoA in Section 1.3 for data to be collected at the time of study discontinuation and follow-up and for any further evaluations that need to be completed.
- The subject will be permanently discontinued from both the study treatment and the study at that time.
- If the subject withdraws consent for disclosure of future information, the Sponsor may retain and continue to use any data collected before such a withdrawal of consent.
- If a subject withdraws from the study, they may request destruction of any samples taken and not tested, and the Investigator must document this in the site study records.
- Subjects who discontinue may not re-enter the study.
- Depending on the timepoint of discontinuation, a withdrawn subject is referred to as either “screening failure” or “dropout” as specified in Sections 5.4 and 7.2.2.

The Investigator will withdraw a subject from the study in the following cases:

- Request by the subject to discontinue (withdrawal of consent).
- At the specific request of the Sponsor and in liaison with the Investigator (e.g., obvious non-compliance).
- Any clinically relevant symptom or sign which in the opinion of the Investigator and/or Sponsor warrants subject withdrawal.
- Impossibility to obtain samples.
- Positive results from alcohol, cotinine, pregnancy and drug testing.
- Protocol deviation judged as significant by the Investigator, including non-compliance to the required study considerations (e.g., food/diet requirements).
- Depending on the timepoint of withdrawal, a withdrawn subject will be referred to as either "screening failure" or "dropout" as specified in Sections 5.4 and 7.2.2.

7.2.2. Replacement of Subjects

A subject who discontinues the study prematurely for any reason is defined as a "dropout" if the subject has already been assigned to study treatment. Dropouts might be replaced if the number of evaluable subjects completing the study becomes or is expected to become less than 16 subjects in total. The decision to replace a subject will be taken on a case-by-case basis, in agreement between the Sponsor and the Principal Investigator.

The data obtained from dropouts will be used in the evaluation to the largest possible extent.

7.3. Further Stopping Rules

7.3.1. Premature Discontinuation of the Complete Study

The Sponsor may discontinue the complete study at any time, for ethical or scientific reasons. The Principal Investigator is entitled to stop the study at any time due to medical reasons. In such a case, the Principal Investigator should consult the Sponsor at the earliest opportunity.

The study may be terminated prematurely or temporarily halted if any unacceptable findings are identified. The occurrence of 1 of the following stopping criteria shall result in an immediate stop of dosing and a temporary halt of the study:

- A serious adverse reaction (i.e., an SAE considered at least possibly related to the study treatment administration) in 1 subject.
- AEs of at least moderate severity in ≥ 50% of the subjects for which a causal relationship to the study treatment or study related procedures cannot be excluded.

- Severe non-serious adverse reaction (i.e., severe non-serious AEs considered as, at least, possibly related to the study treatment administration) in 2 subjects, independent of within or not within the same system organ class.
- Unacceptable risks, any relevant toxicity, or a negative change in the risk/benefit assessment is identified. This might include the occurrence of AEs which character, severity or frequency is new in comparison to the existing risk profile.
- Any data derived from other clinical studies or toxicological studies become available which negatively influence the risk/benefit assessment.

If the study is prematurely terminated or suspended, the Sponsor shall promptly inform the Investigators, the IECs, the regulatory authorities, and any contract research organization(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The Investigator shall promptly inform the subject and should ensure appropriate subject therapy and/or follow-up.

Further information on study and site closure is provided in Section 10.1.12.

7.4. Lost to Follow-up

Subjects will be considered lost to follow-up if they are unable to be contacted by the study site for the follow-up safety call on Day 28 or an ad-hoc safety follow-up visit.

Before a subject is deemed lost to follow-up, the Investigator or designee must make every effort to regain contact with the subject (where possible, contact via email along with 3 telephone calls and, if necessary, a certified letter to the subject's last known mailing address or local equivalent methods). These contact attempts should be documented in the subject's medical record.

Should the subject continue to be unreachable, they will be considered to have withdrawn from the study.

8. Study Assessments and Procedures

- Prior to performing any study assessments, the Investigator will obtain written informed consent as specified in Section 10.1.4.
- Study procedures and their timing are summarized in the SoA (Section 1.3). Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.
- Protocol waivers or exemptions are not allowed.
- Immediate safety concerns should be discussed with the Sponsor immediately upon occurrence or awareness to determine if the subject should continue or discontinue study treatment.
- All screening evaluations must be completed and reviewed to confirm that potential subjects meet all eligibility criteria. The Investigator will maintain a screening log to record details of all subjects screened and to confirm eligibility or record reasons for screening failure, as applicable.
- In the event of a significant study-continuity issue (e.g., caused by a pandemic), alternate strategies for subject visits, assessments, medication distribution and monitoring may be implemented by the Sponsor or the Investigator, as per local health authority/ethics requirements. All procedures adapted to the situation must be submitted, if required as per local regulations, through a protocol amendment to the regulatory authority and IEC for approval prior to implementation of mitigation procedures.
- The maximum amount of blood collected from each subject over the duration of the study, including any extra assessments that may be required, will be approximately 150 mL (approximately 66 mL for safety laboratory samples and 84 mL for PK and biomarkers samples; exact volumes will be defined in the laboratory manual). Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.
- If subjects agree, samples collected during this clinical study may be used for future research outside the clinical protocol when additional consent for this purpose is given (see Section 10.1.4). Any such testing will not be reported in the CSR.

8.1. Administrative and General Procedures

8.1.1. Demographic

For demographic assessment the following parameters will be recorded: age (incl. year of birth), sex, race / ethnicity.

8.1.2. Body Weight and Height, BMI

Body weight will be measured by a member of the Investigator's team under the following conditions:

- Subject in underwear and without shoes after having emptied their bladder
- Electronic physician (column) scale with digital display, measurement units 0.1 kg

The subject's height (without shoes) will be measured to calculate the BMI. The BMI will be calculated by data management directly in the eCRF. The study site might calculate in addition for eligibility check.

8.1.3. Medical History

Medical history findings (i.e., previous diagnoses, diseases, or surgeries) considered relevant to the study will be collected:

Any relevant findings from the past that occurred prior to signing the ICF or started prior to signing the ICF, are still ongoing but resolve before start of first study treatment administration will be recorded in the Medical History section of the eCRF.

Any relevant findings after obtaining informed consent or presently occurring and worsening after signing the informed consent form will be recorded in the AE section of the eCRF.

8.1.4. Other Baseline Characteristics

Information on smoking and alcohol consumption will be collected.

8.2. Efficacy and Immunogenicity Assessments

This section is not applicable as efficacy and immunogenicity are not assessed in this study.

8.3. Safety Assessments

Planned timepoints for all safety assessments are provided in the SoA (Section 1.3). Additional assessments during the study will be conducted, if required, at the discretion of the Investigator.

8.3.1. Physical Examinations

- A comprehensive physical examination will be performed by a physician and will include at a minimum, assessment of the eyes, ears, nose, and throat as well as assessment of the cardiac, peripheral vascular, pulmonary, musculoskeletal, neurologic, abdominal, lymphatic, and dermatologic system.
- Investigators should pay special attention to clinical signs related to previous serious illnesses.

- All preexisting and relevant medical events must be recorded and described in the source documents. All efforts to attach support documentation (i.e., reports, results, etc.) must be done.

8.3.2. Vital Signs

- Vital signs will be measured after at least 5 minutes rest in a supine position and will include body temperature, pulse rate, and systolic and diastolic blood pressure at the timepoints specified in the SoA (Section 1.3).
- Blood pressure and pulse should not be measured on the same arm where blood samples are taken from.
- Blood pressure and pulse measurements will be assessed with a completely automated device. Manual techniques will be used only in case of doubt or if an automated device is not available.
- Body temperature will be measured auricularly with a calibrated device.
- Vital sign measurements will be repeated as appropriate.

8.3.3. Electrocardiograms

- Single 12-lead ECGs will be obtained as outlined in the SoA (Section 1.3) using an ECG machine that automatically calculates the heart rate and measures PR(Q), QRS, QT, and QTcF. Refer to Section 7.1.1.2 for QTc withdrawal criteria.
- Subjects should rest for at least 5 minutes in supine position before ECG collection is performed and should be obtained prior to blood sampling.
- Per timepoint, the ECG results will be stored electronically, timely reviewed, and approved electronically by the Investigator.
- Each ECG should be interpreted (normal/abnormal) by the Investigator. For abnormal ECGs, the clinical significance (yes/no) should be judged by the Investigator and the abnormality is to be specified. ECG recording will be repeated as appropriate.

8.3.4. Clinical Safety Laboratory Tests

- Clinical laboratory assessments will be performed by Nuvisan GmbH.
- All protocol-required laboratory assessments, as defined in Section 10.2, must be conducted in accordance with the laboratory manual and the SoA (Section 1.3).
- Any abnormalities in any of the laboratory parameters will be judged by an Investigator individually in relation to the reference ranges.

- The Investigator must review the laboratory results, document this review, and record any clinically significant changes occurring during the study after first dosing as an AE. The laboratory results must be retained with source documents.
- All laboratory tests with values considered clinically significantly abnormal, with major deviation and/or possible pathological relevance during participation in the study should be repeated until the values return to normal or baseline or the absence of clinical relevance can be confirmed. If clinically significant values do not return to normal/baseline within a period judged reasonable by the Investigator, the etiology should be identified, and the Sponsor notified.
- Additional tests may be performed at any time during the study as determined necessary by the Investigator or required by local regulations.

8.3.5. Pregnancy Testing

- Female subjects should only be included after a negative highly sensitive serum pregnancy test at screening and admission day.
- Pregnancy testing will be performed whenever a menstrual cycle is missed or when pregnancy is otherwise suspected during the time from ICF signature until follow-up visit/early discontinuation visit.

8.3.6. Other Assessments

Alcohol breath tests will be performed as outlined in the SoA (Section 1.3).

8.4. Adverse Events and Serious Adverse Events

- The definitions of AEs and SAEs can be found in Section 10.3.
- **SAEs and pregnancy cases will have to be reported to the Sponsor within 24 hours of awareness, filling in the adequate safety form provided. Contact details are provided in Section 10.3.4.**
- An AE will be reported by the subject or observed by members of the study team elicited by general questioning or by the Investigator/designee. The Investigator will review this data and determine the seriousness, the severity/intensity, the causality, and the action taken with the IMP.
- AEs will be documented, and the following information will be given for each AE: description of the AE, onset date and time, end date and time, maximum severity, action taken, outcome, seriousness, and relationship to an IMP.

- The Investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up unresolved AEs.
- The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Section 10.3.

8.4.1. Time Period and Frequency for Collecting AE and SAE Information

- All AEs and SAEs will be collected from the signing of the informed consent until safety follow-up call / early discontinuation visit at the timepoints specified in the SoA (Section 1.3).
- All SAEs will be recorded and reported to the Sponsor or designee within the time frames indicated in Section 10.3.
- Investigators are not obligated to actively seek AEs or SAEs after conclusion of the study participation. However, if the Investigator learns of any SADR (SAE considered related to the IMP), at any time it has to be reported to the Sponsor.

8.4.2. Method of Detecting AEs and SAEs

During the reporting period unfavorable changes in the subject's condition will be recorded as AEs, regardless, if reported by the subjects or observed by the investigative team. Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the subject is the preferred method to inquire about AE occurrences (e.g., "How do you feel?" or "How have you been feeling since the last questioning?").

8.4.3. Follow-up of AEs and SAEs

After the initial AE/SAE report, the Investigator is required to proactively follow each subject at subsequent visits/contacts. All AEs and SAEs will be followed until resolution, stabilization, the event is otherwise explained, or the subject is lost to follow-up (as defined in Section 7.4). Further information on follow-up procedures is given in Section 10.3.

8.4.4. Regulatory Reporting Requirements for SAEs

- Prompt notification by the Investigator to the Sponsor of an SAE is essential so that legal obligations and ethical responsibilities towards the safety of subjects and the safety of a study treatment under clinical investigation are met.
- The Sponsor has a legal responsibility to notify both the competent authority and other regulatory agencies about the safety of a study treatment under clinical investigation. The Sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authorities, IEC, and Investigators.

- The Sponsor is responsible for assessing whether an SAE is expected or not (see also Section 10.3). The IB of vamorolone and the SmPC of the selected midazolam product to be administered will be used as reference safety information for this clinical study.
- SUSARs must be reported by the Sponsor as detailed in Article 42 of European Regulation 536/2014.
- The Sponsor must report all unexpected events which affect the benefit-risk balance of the clinical study but are not SUSARs as detailed in Article 53 of European Regulation 536/2014.
- An Investigator who receives an Investigator safety report describing an SAE or other specific safety information (e.g., summary or listing of SAEs) from the Sponsor will review and then file it along with the IB and will notify the IEC, if appropriate according to local requirements.
- Investigator safety reports must be prepared for SUSARs according to local regulatory requirements and Sponsor policy and forwarded to Investigators as necessary.

8.4.5. Pregnancy

Adequate birth control measures are defined for the study subjects to prevent pregnancies (Section 5.1). However, in case, a pregnancy occurs in the period from start of study treatment until 3 months after last administration of study treatment, the following applies:

- The Investigator will attempt to collect pregnancy information on the pregnant female (whether it is a female subject or a male subject's female partner).
- After obtaining the necessary signed informed consent from the pregnant female, she will be followed to determine the outcome of the pregnancy.
- The Investigator will record pregnancy information on the appropriate form and submit it to the Sponsor within 24 hours of learning of the pregnancy (after obtaining the necessary signed informed consent from the female partner).
- Whenever possible, a pregnancy should be followed to term, any premature terminations should be reported, and the status of the mother and child should be reported to the Sponsor after delivery. Any termination of pregnancy will be reported, regardless of fetal status (presence or absence of anomalies) or indication for the procedure.
- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be reported as an AE or SAE. Abnormal pregnancy outcomes (e.g., spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs and will be reported as such.
- Any poststudy pregnancy-related SAE considered reasonably related to the study treatment by the Investigator will be reported to the Sponsor as described in Section 8.4.4. While the

Investigator is not obligated to actively seek this information in former pregnant females, he or she may learn of an SAE through spontaneous reporting.

- The Investigator will collect follow-up information on the pregnant female and the neonate, and the information will be forwarded to the Sponsor. Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the estimated delivery date for a healthy newborn. In case of a congenital anomaly or other illness of the newborn, follow-up will continue until the illness has resolved or there is a definite outcome of the event.
- Any female subject who becomes pregnant while participating in the study will discontinue study treatment or be withdrawn from the study.

8.4.6. Adverse Events of Special Interest

No AEs of special interest are defined for this study.

8.5. Pharmacokinetics

Blood samples will be taken by either direct venipuncture or an indwelling cannula inserted in a forearm vein in accordance with the SoA (Section 1.3). Detailed information on sample collection, handling, storage, and shipment will be provided in a laboratory manual.

All sample handling procedures, including the date/time of each sample collection, the date/time of placement into frozen storage (at the end of the sample workup), and the date/time of transfer or shipment of the samples to the responsible analyst will be documented in detail.

22 blood samples for determination of midazolam and 1'-hydroxymidazolam concentrations will be collected up to 10 hours after midazolam dosing on Days 1 and 14 as specified in Table 4 and in the SoA (Section 1.3).

Table 4: Pharmacokinetic Blood Collection Schedule for Midazolam and 1'-Hydroxymidazolam

Study Day	Time (hours postdose)	Volume (mL)
Day 1	0 (predose)	2
	0.25	2
	0.5	2
	1	2
	1.5	2
	2	2
	3	2
	4	2
	6	2
	8	2
	10	2
Day 14	0 (predose)	2
	0.25	2
	0.5	2
	1	2
	1.5	2
	2	2
	3	2
	4	2
	6	2
	8	2
	10	2
Total Blood Collected		**44**

6 blood samples for determining trough plasma concentration of vamorolone will be collected as specified in Table 5 and in the SoA (Section 1.3).

Table 5: Pharmacokinetic Blood Collection Schedule for Vamorolone

Study Day	Time (hours)	Volume (mL)
Day 3	0 (predose)	2
Day 4	0 (predose)	2
Day 7	0 (predose)	2
Day 11	0 (predose)	2
Day 13	0 (predose)	2
Day 14	0 (predose)	2
Total Blood Collected		**12**

The actual date and time (24-hour clock time) of each sample will be recorded. In case the actual sampling time deviates from the scheduled sampling time, a comment must be given in the e-source. Samples collected within the time windows defined (Section 1.3), will not be considered a protocol deviation. Actual time will be considered when calculating the final PK parameters.

Samples collected for PK analyses may also be used to evaluate safety or efficacy aspects related to concerns arising during or after the study.

8.5.1. Bioanalytical Methods

- All bioanalytical analyses will be performed at Nuvisan GmbH Bioanalytical Department.
- Bioanalytical analyses will be performed in accordance with the applicable principles of Good Laboratory Practice and GCP.
- The procedure for determination of concentrations of vamorolone, midazolam, and 1'-hydroxymidazolam in plasma samples will be described in bioanalytical protocols.
- Information relating to the responsible persons, specification of communication rules, handling of the informed consent and confidentiality of personal data, specification of the analytical method (matrix, calibration range, samples volume, etc.), sample storage, "Incurred Samples" (ISR), specification of the applicable regulatory guidelines and archiving process will be described in the bioanalytical protocol.
- Samples for midazolam, 1'-hydroxymidazolam, and vamorolone will be analyzed using validated LC-MS/MS methods.

8.5.2. Pharmacokinetic Evaluation

- The following PK parameters will be calculated from the individual plasma concentration-time data of midazolam and 1'-hydroxymidazolam for each treatment by non-compartmental analysis using Phoenix® WinNonlin® Version 8.0 or higher (applying linear trapezoidal linear/log interpolation method for calculation of AUC):

Table 6: Non-compartmental Pharmacokinetic Parameters for Midazolam and 1'-Hydroxymidazolam

Main Parameters	$AUC_{0\text{-tlast}}$ $AUC_{0\text{-inf}}$ C_{max}
Additional Parameters	$t_{1/2}$ t_{max} λ_z V_z/f (for midazolam only) CL/f (for midazolam only)

For definitions of PK parameters refer to Section 10.6.

- Trough values (C_{trough}) will be calculated from the individual plasma concentration time data of vamorolone only.
- Individual PK parameters will be calculated using actual sampling times.
- For calculation of PK parameters, concentrations below the lower limit of quantification will be treated as zero.
- Unreliable parameters will be listed and flagged accordingly and set to missing for calculation of descriptive statistics and statistical analysis.
- More details will be provided in the SAP.

8.6. Pharmacodynamics

Not applicable.

8.7. Genetics

Pharmacogenetics are not evaluated in this study.

8.8. Biomarkers

4β-Hydroxycholesterol

The 4β-hydroxycholesterol plasma concentration will be measured by a validated LC-MS/MS assay and used as an *in vivo* marker for the assessment of CYP3A4 induction as specified in Table 7 and in the SoA (Section 1.3).

Table 7: Blood Collection Schedule for 4β-Hydroxycholesterol

Study Day	Time (hours)	Volume (mL)
1	0 (predose)	4
3	0 (predose)	4
4	0 (predose)	4
7	0 (predose)	4
10	0 (predose)	4
14	0 (predose)	4
15	24 (postdose)	4
Total Blood Collected		**28**

6β-Hydroxycortisol to Cortisol Ratio

The ratio of 6β-hydroxycortisol to cortisol will be measured by a validated LC-MS/MS assay and used as an *in vivo* marker for the assessment of CYP3A4 induction. 7 urine samples will be collected as specified in Table 8 and in the SoA (Section 1.3).

Table 8: Urine Collection Schedule for 6β-Hydroxycortisol to Cortisol Ratio

Study Day	Period	Aliquot Volume (mL)
1	Morning	up to 5
3	Morning	up to 5
4	Morning	up to 5
7	Morning	up to 5
10	Morning	up to 5
14	Morning	up to 5
15	Morning	up to 5
Total Urine Aliquot Volume		**up to 35**

Urine will be collected in the morning from approximately 8:00 to 12:00 a.m.. Each subject should void his/her bladder at the beginning and at the end of each urine sampling period. During each sampling interval the urine portions will be pooled and the total volume of urine will be determined and recorded together with the exact time for start and end in the eCRF.

Detailed information on sample collection, handling, storage, and shipment will be provided in a laboratory manual.

The procedures for determination of 4β-hydroxycholesterol in plasma samples and 6β-hydroxycortisol and cortisol in urine samples will be described in the respective bioanalytical protocols.

8.9. Immunogenicity Assessments

Immunogenicity is not evaluated in this study.

8.10. Medical Resource Utilization and Health Economics

Medical resource utilization and health economics parameters are not evaluated in this study.

9. Statistical Considerations

The statistical analyses will be performed by Nuvisan.

9.1. Statistical Hypotheses

No formal statistical hypotheses have been defined for this exploratory study.

9.2. Analysis Sets

For purposes of analysis, the following populations are defined:

Set	Description
Full set	All subjects enrolled in the study.
Safety set	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 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 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 concentration summary and PK parameter summary for vamorolone.

Finding/events leading to the exclusion from analysis sets will be prespecified in a deviation manual.

The final decision to exclude subjects from any analysis set will be made during a DRM prior to database lock. The respective meeting minutes will be signed together with the Sponsor.

9.3. Statistical Analyses

Statistical analysis will be performed using SAS® and the version used will be specified in the SAP, which will be finalized before database lock. The SAP will contain a more comprehensive explanation than described below of the methodology used in the statistical analyses. The SAP will also contain the rules and data handling conventions to be used to perform the analyses.

Objectives and endpoints are described in Section 3 (Table 2).

9.3.1. Efficacy Analyses

This section is not applicable as efficacy is not assessed in this study.

9.3.2. Safety Analyses

All safety analyses will be performed on the Safety Analysis Set.

- Safety data will be listed by subject.
- Summary tables by treatment will be generated for TEAEs (as defined in Section 10.3.1), sorted by system organ class and preferred term, in which the number and percentages of subjects with treatment-emergent AEs and frequency of the events are reported.
- AE occurring after 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 treatment. An AE that worsens after a later treatment period will be counted towards both study treatments.
- Laboratory values outside the normal ranges will be flagged. A listing of abnormal laboratory values and clinically relevant abnormal laboratory values will also be provided.
- Descriptive statistics of vital signs and ECG data will be presented for each timepoint by treatment.
- The ECG interpretation of the Investigator will be tabulated as the number and percentage of subjects with “normal”, “abnormal, not clinically significant”, or “abnormal, clinically significant” results by treatment and timepoint.

9.3.3. Pharmacokinetic Analyses

- All PK analyses will be performed on the PK Set, if not stated otherwise.
- For midazolam and 1’-hydroxymidazolam, concentration-time courses and PK parameters will be tabulated by treatment.

- The following statistics will be calculated for each of the sampling points: number of cases, geometric "n", geometric mean, geometric standard deviation (re-transformed standard deviation of the logarithms) and coefficient of variation (CV), arithmetic mean, standard deviation and CV, minimum, median, maximum value, and the number of measurements.
- For midazolam and 1'-hydroxymidazolam, individual and arithmetic mean plasma concentration versus time curves (using the actual sampling times for individual plots and the planned sampling times for mean plots) will be plotted by treatment using both linear and log-linear scale.
- A linear mixed model with a fixed effect for TREATMENT and a random effect for SUBJECT will be applied to log-transformed PK parameters $AUC_{0\text{-tlast}}$, $AUC_{0\text{-inf}}$, and C_{max} of midazolam and 1'-hydroxymidazolam based on the PK Set to assess treatment differences on the log scale of midazolam with vamorolone vs midazolam alone (Day 14 vs Day 1). Least-squares (LS) means, LS treatment difference, and 90% confidence intervals (CI) for the treatment differences on the log-scale will be obtained. The results will be back-transformed to the original scale by exponentiation to provide geometric least-square means, point estimate of the geometric mean ratio (Day 14 vs Day 1) and its corresponding 90% CI.
- Missing data will not be replaced or imputed in any way.
- The trough values of vamorolone will also be listed and tabulated by treatment as well as displayed graphically as described above.

9.3.4. Pharmacodynamic Analyses

Not applicable.

9.3.5. Other Analyses

Analyses for CYP3A4 biomarkers will be performed on the safety set.

Data of plasma 4β-hydroxycholesterol as well as urine 6β-hydroxycortisol, cortisol, and 6β-hydroxycortisol to cortisol ratio by visit will be listed and appropriate summary tables will be provided.

Concentration of plasma 4β-hydroxycholesterol and both urine 6β-hydroxycortisol and cortisol versus time curves will be plotted using the actual sampling times. Ratio of 6β-hydroxycortisol to cortisol will also be plotted using the actual sampling times. In addition, for 4β-hydroxycholesterol summary statistics will be also provided for change from baseline and the baseline is defined as the assessment taken predose on Day 1.

9.4. Interim Analysis

Not applicable.

9.5. Sample Size Determination

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 (Prueksaritanont et al, 2000) 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% confidence intervals when the observed mean ratio for comparison is 1; 90% CI = (0.84 ,1.20).

Approximately 18 subjects will be assigned to study intervention such that 16 evaluable subjects complete the study. For information on subject replacement refer to Section 7.2.2.

9.6. 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.
- Protocol deviations will be prespecified in a deviation manual, discussed on an ongoing basis and finally categorized as important/non-important during the DRM. A protocol deviation may also be declared as finding/event that leads to an exclusion of data or complete subjects from an Analysis Set (see Section 9.2).
- Important protocol deviations will also be described in the clinical study report.

9.7. Data Management

Nuvisan will be responsible for clinical data management activities for this study. The full details of procedures for data handling will be described in the study specific Data Management Plan.

Medical coding will be done using the latest version of the coding dictionaries for MedDRA for AEs and medical history to the primary system organ class and WHO Drug Global Dictionary for concomitant medications. Coding will be performed by Data Management and needs to be approved by the Investigator and Sponsor.

9.7.1. Database Lock

Study database must be soft and hard locked to ensure their integrity for the generation of results, analysis, and submissions.

9.7.2. Soft Lock

When validation (including external data reconciliation), SAE reconciliation and all coding activities, as well as medical review activities, quality control activities have been completed and there are no further open issues or open queries the database will be soft locked.

9.7.3. Data Review Meeting

A DRM will be held for the study by the biostatistician to check the data status, discuss any open issues and provide proposals for resolution, as well as finally agree on the categorization of the protocol deviations and assignment of subjects to analysis sets.

9.7.4. Hard Lock

After finalization and approval of the DRM minutes and after all actions resulting from the DRM minutes are completed, e.g. additional solving of queries, the database will be hard locked. Hard lock needs to be authorized by the study team.

9.7.5. Data Standards

The database and the electronic external data will be converted to CDISC / SDTM and ADaM. The procedure will be described in the Data Management Plan and in the SAP.

10. Supporting Documentation and Operational Considerations

10.1. Appendix 1: Regulatory, Ethical, and Study Oversight Considerations

Before the start of the study, Nuvisan on behalf of the Sponsor will apply for approval for the performance of the study at the CA German Federal Institute for Drugs and Medical Devices (BfArM) and the IEC.

10.1.1. Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with the following:
    - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences International Ethical Guidelines
    - Applicable ICH GCP Guidelines
    - Applicable laws and regulations
- The protocol, modifications to the protocol (modifications), ICF, IB, and other relevant documents (e.g., advertisements) must be submitted to an IEC and reviewed and approved by the IEC before the study is initiated.
- Any substantial modification to the protocol (substantial modification) will require competent authority and IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study subjects.
- The Investigator will be responsible for the following:
    - Notifying the IEC of SAEs or other significant safety findings as required by IEC procedures.
    - Providing oversight of the conduct of the study at the site and adherence to ICH guidelines, the IEC, European Regulation 536/2014, and all other applicable local regulations.
    - Performing a benefit-risk assessment on an ongoing basis and informing the IEC about any changes. Moreover, making sure that any substantial modifications to the protocol (substantial modifications) will be submitted and approved prior to implementation.

10.1.2. Financial Disclosure

- Investigators and sub-Investigators will provide the Sponsor with sufficient, accurate financial information as requested to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities.

- Investigators are responsible for providing information on financial interests during the study and for 1 year after completion of the study.

10.1.3. Clinical Study Insurance and Subject Compensation

- In accordance with local law, insurance coverage will be provided for all subjects participating in this study.
- Subjects will be paid compensation for participation and will be reimbursed for travel-related costs.

10.1.4. Informed Consent Process

- The Investigators or their representative will explain the nature of the study to the subject and answer all questions regarding the study.
- Subjects must be informed that their participation is voluntary. Subjects will be required to sign a statement of informed consent that meets the requirements of local regulations, ICH guidelines, and the IEC.
- The written ICF must be signed and personally dated by the subject and by the Investigator who conducted the informed consent discussion.
- Subjects should be informed of the possibility to withdraw consent without giving any reason and to require that all previously retained identifiable samples will be destroyed to prevent future analyses, according to national provisions. The information should include a statement that the consequence of the subject's withdrawal of consent will be that no new information will be collected from the subject and added to existing data or database.
- All subjects who sign the ICF will be assigned a screening number.
- Subjects must be re-consented to the most current version of the ICF(s) during their participation in the study. Any revised ICF must receive the IEC approval / favorable opinion in advance of use.
- One original of the signed and dated ICF will be provided to the subject. A second original will be retained in the Investigator site file.
- The Investigator should maintain a log of all subjects who signed the ICF.
- Subjects who are rescreened are required to sign a new ICF and receive a new screening number.

A separate ICF will be prepared that addresses the use of remaining mandatory samples for optional exploratory research. The Investigator or authorized designee will explain to each subject the objectives of the exploratory research. Subjects will be told that they are free to refuse to participate and may withdraw their consent at any time and for any reason during the storage period. A signature on this ICF for future use will be required to document a subject's agreement to allow any remaining specimens to be used for exploratory research. Subjects who decline to participate in this optional research will not provide this separate signature.

10.1.5. Protocol Amendment

Modifications of the signed protocol, where substantial, are only possible by approved protocol amendments and with the agreement of all responsible persons. The procedure for approval of a protocol amendment is identical to that for approval of the protocol. The IEC must be informed of all substantial amendments and should be asked for its opinion as to whether a full reevaluation of the ethical aspects of the study is necessary by the committee. This should be fully documented.

The Investigator must not implement any deviation from, or change to the protocol, without discussion with, and agreement by Santhera Pharmaceuticals (Switzerland) Ltd. and prior review and documented approval/favourable opinion of the amendment from the relevant EC, except where it is necessary to eliminate an immediate hazard to study subjects, or where the change(s) involves only logistical or administrative aspects of the study, i.e., non-substantial (e.g., change in monitor(s), change of telephone number(s)).

Protocol amendments will be submitted to the appropriate authority(ies) as required by the applicable regulatory requirement(s).

10.1.6. Recruitment

- The study will be performed in adult subjects fully capable of giving informed consent.
- Healthy subjects for the study will be recruited from suitable candidates in the database of the investigational site. Interested subjects can actively contact the site directly via a public website offering information about studies currently recruiting.
- Detailed description of the recruitment strategy will be provided in country- and site-specific documentation, as applicable.

10.1.7. Data Protection

Personal and sensitive personal data will be treated as confidential. The results of the study will be made available for review by authorized representatives of the Sponsor and/or submitted to one or more Sponsor offices worldwide, the ethics committee and regulatory authorities. Data may be transferred to other countries.

Prior to the subject's enrolment in the study, the subject's consent is required for the data to be used for these purposes and to gain direct access to their medical records for data verification purposes.

Data protection and privacy regulations will be observed in capturing, forwarding, processing, and storing subject data.

Authorized access only is assured by strict rules on Nuvisan firewall. Nuvisan uses strict rules to separate the networks within the company. User groups with various permission sets are maintained within Nuvisan network to ensure confidentiality of records. Connections to the Nuvisan network from the off-site access point have to use a virtual private network. The Nuvisan network is constantly being monitored for potential threats, viruses, and other security related issues by a separate security operation center. To prevent a security breach, all Nuvisan employees are trained on how to proceed in case of receiving suspicious email.

- Subjects will be assigned a unique identifier. Any subject records or datasets that are transferred to the Sponsor will contain the identifier only; subject names or any information which would make the subject identifiable will not be transferred. Only the Investigator and the clinical team will be able to link the subjects' trial data to the subjects via an identification list kept at the site. The subjects' original medical data that are reviewed at the site during source data verification by the monitor, audits and during health authority inspections will be kept strictly confidential.
- The subject must be informed that their personal study-related data will be used by the Sponsor in accordance with the EU General Data Protection Regulation. The level of disclosure must also be explained to the subject.
- The subject must be informed that their medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor and by inspectors from regulatory authorities without violating the confidentiality of the subject to the extent permitted by law and regulations.
- All personal data collected and processed for the purpose of this study will be managed by the Investigator and their staff with adequate precautions to ensure confidentiality of those data, as per national and/or local laws and regulations on personal data.
- Measures are in place to mitigate the possible adverse effects of a data security breach and are in line with the EU General Data Protection Regulation and relevant national legislations. These measures are defined in the CRO's SOPs regarding IT Security and Serious Breaches.

10.1.8. Committees Structure

Not applicable.

10.1.9. Dissemination of Clinical Study Data

This clinical study will be registered in a clinical study database before enrolment of the first subject. All data and results and all intellectual property rights in the data and results derived from the study will be the property of the Sponsor who may utilize them in various ways, such as for submission to government regulatory authorities or disclosure to other Investigators.

10.1.10. Data Quality Assurance

The clinical trial protocol, each step of the data capture procedures, and the handling of the data, including the final clinical trial/study report, will be subject to independent Quality Assurance activities. Audits may be conducted at any time during or after the trial to confirm the validity and integrity of the trial data. The Investigator will give the auditor direct access to all relevant documents and will allocate his/her time and the time of his/her staff to the auditor as may be required to discuss findings and relevant issues. In addition, Regulatory bodies (e.g.: Regulatory Authorities, IECs, at their discretion may conduct inspections and the investigator agrees to cooperate fully during conduct and discuss any relevant issues.

The Investigator has the obligation to immediately inform the Sponsor of an Inspection notification or request by a Regulatory Authority and will ensure direct access to source data and all study related documentation during Regulatory Authority Inspections.

- All subject data relating to the study will be recorded and transmitted to the Sponsor or designee electronically. The Investigator is responsible for verifying that data entries are accurate and correct by signing the eCRF.
- The Investigator must permit study-related monitoring, audits, IEC review, and regulatory agency inspections and provide direct access to source data documents, including electronic medical records.
- Monitoring details describing strategy (e.g., risk-based initiatives in operations and quality such as Risk Management and Mitigation Strategies and Analytical Risk-Based Monitoring), methods, responsibilities, and requirements, including handling of noncompliance issues and monitoring techniques (central, remote, or on-site monitoring) are provided in the Monitoring Plan.
- Nuvisan GmbH is responsible for the data management of this study including quality checking of the data.
- The Sponsor assumes accountability for actions delegated to other individuals (e.g., Contract Research Organizations).

- Study monitors will perform ongoing source data verification to confirm that data entered into the eCRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of subjects are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.
- Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the Investigator for 25 years after study completion unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the Sponsor. No records may be transferred to another location or party without written notification to the Sponsor.

10.1.11. Source Documents

- All source documents must follow the ALCOA principle of data integrity (Attributable, Legible, Contemporaneous, Original and Accurate). Changes to source data should be traceable, should not obscure the original entry, and should be explained, if necessary (e.g.: audit trail). The sponsor ensures that the Investigator has control of and continuous access to the (e)CRF data reported to the sponsor.
- All subject data relating to the study will be recorded on the (e)CRF. In addition, some study related data (e.g., laboratory data, source documentation on adverse events etc.) could be required to be provided to Sponsor or representative and AEs need to be reported to the Sponsor, as further detailed in this protocol. At all times, Subject confidentiality is and will be protected.
- Source documents provide evidence for the existence of the subject and substantiate the integrity of the data collected.
- eCRF data must be consistent with the source documents, or the discrepancies must be explained.
- Definition of what constitutes source data can be found in the source data location form.

10.1.12. Study and Site Closure

- The Sponsor designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the Sponsor.
- Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.
- The Investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

- Reasons for the early closure of a study site by the Sponsor or Investigator may include but are not limited to:
    - Failure of the Investigator to comply with the protocol, the requirements of the IEC or local health authorities, the Sponsor's procedures, or GCP guidelines.
    - Inadequate recruitment of subjects by the Investigator.
    - Discontinuation of further study treatment development.

10.1.13. Publication Policy

- The results of this study may be published or presented at scientific meetings. If this is foreseen, the Investigator agrees to submit all manuscripts or abstracts to the Sponsor before submission. This allows the Sponsor to protect proprietary information and to provide comments.
- The Sponsor will comply with the requirements for publication of study results.
- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

10.1.14. Clinical Study Report

After completion of the study, a clinical study report covering clinical and statistical aspects of the study will be prepared by Nuvisan GmbH in consultation with the Sponsor.

10.2. Appendix 2: Clinical Laboratory Tests

- The tests detailed in Table 9 will be performed by the local laboratory at the timepoints specified in the SoA (Section 1.3).
- Details of all methodology and reference ranges will be provided in the trial master file.
- Investigators must document their review and assess clinical relevance of each laboratory safety report.
- The results of each test will be transferred electronically to the clinical database.
- Protocol-specific requirements for inclusion or exclusion of subjects are detailed in Section 5 of the protocol.
- Additional tests may be performed at any time during the study as determined necessary by the Investigator or required by local regulations.

Table 9: Protocol-Required Safety Laboratory Assessments

Laboratory Assessments	Parameters		
Hematology	Platelet count	Mean corpuscular volume (MCV) Mean corpuscular hemoglobin (MCH) Mean corpuscular hemoglobin concentration (MCHC)	White Blood Cell Count with Differential (absolute and %): Neutrophils Lymphocytes Monocytes Eosinophils Basophils
	Reticulocytes		
	Hemoglobin		
	Hematocrit		
	Red blood cell count		
	White blood cell count		
Clinical Chemistry	Alanine aminotransferase[a]	Calcium	Lactate dehydrogenase
	Albumin	Chloride	Lipase
	Alkaline phosphatase	Cholesterol	Magnesium
	Amylase	Creatinine	Potassium
	Aspartate aminotransferase	Creatine phosphokinase[c]	Sodium
	Bilirubin (total)[a,b]	γ-Glutamyl-transferase	Total protein
	Blood urea nitrogen	Glucose	Triglycerides
	C-reactive protein	Inorganic phosphate	Uric acid
Coagulation	• Activated Partial Thromboplastin Time (aPTT) • Prothrombin time (INR, %)		
Urinalysis	• Specific gravity • pH, hemoglobin, urobilinogen, bilirubin, protein, ketones, nitrite, leukocyte esterase by dipstick • In case of positive results for protein, leukocyte esterase, hemoglobin or nitrite on the dip stick, flow cytometry count and classification will be performed.		
Other Tests	• Urine drug screen: Amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, methamphetamines, methylenedioxymethamphetamine, methadone, opiates, tricyclic antidepressants, phencyclidine • Urine cotinine test • Serum hCG pregnancy test (female subjects only)		

Laboratory Assessments	Parameters

	• At screening only:
	  ○ TSH[d]
	  ○ FSH (to confirm postmenopausal status)
	  ○ HbA1c
	  ○ Fibrinogen
	  ○ Glomerular filtration rate[e]
	• Serology:
	HBsAg, anti-HCV, anti-HIV 1 + 2 and HIV p24 antigen combined

Notes:

[a] Details of liver chemistry stopping criteria and required actions and follow-up assessments after liver stopping or monitoring event are given in Section 7 and Section 10.5. All events of ALT ≥ 3 × upper limit of normal (ULN) and bilirubin ≥ 2 × ULN (> 35% direct bilirubin) which may indicate severe liver injury (possible Hy's Law), must be reported as an SAE.

[b] In case of increased bilirubin (total), the direct bilirubin will be determined.

[c] If increased, creatine kinase (muscle-brain type) and Troponin I (if CK-MB/CK-total x 100 > 6.0% or CK-MB > 25 U/L) will be determined.

[d] If TSH is out of range, additionally free triiodothyronine and free thyroxine will be determined.

[e] eGFR will be calculated as follows based on the CKD-EPI serum creatinine equation (Levey et al, 2009 and 2011) adjusted to the body surface area (BSA=0.20247 x ((height/100)^0.725) x (weight^0.425)) of the subjects.

For male subjects:

If serum creatinine ≤ 80 µmol/L: eGFR [mL/min] = ((141 x (serum creatinine [µmol/L] / 79.6)$^{-0.411}$ x 0.993$^{Age[years]}$)) x (0.20247 x ((height/100)$^{0.725}$) x (weight$^{0.425}$) / 1.73)

If serum creatinine > 80 µmol/L: eGFR [mL/min] = ((141 x (serum creatinine [µmol/L] / 79.6)$^{-1.209}$ x 0.993$^{Age[years]}$)) x (0.20247 x ((height/100)$^{0.725}$) x (weight$^{0.425}$) / 1.73)

For female subjects:

If serum creatinine ≤ 62 µmol/L: eGFR [mL/min] = ((144 x (serum creatinine [µmol/L] / 61.9)$^{-0.329}$ x 0.993$^{Age[years]}$)) x (0.20247 x ((height/100)$^{0.725}$) x (weight$^{0.425}$) / 1.73)

If serum creatinine > 62 µmol/L: eGFR [mL/min] = ((144 x (serum creatinine [µmol/L] / 61.9)$^{-1.209}$ x 0.993$^{Age[years]}$)) x (0.20247 x ((height/100)$^{0.725}$) x (weight$^{0.425}$) / 1.73)

If a subject is Black, the initial result calculated as shown above will have to be multiplied by 1.159 to arrive at the eGFR.

10.3. Appendix 3: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

Investigators are responsible for monitoring the safety of subjects who have entered this study. Each subject will be carefully monitored by the Investigator or a delegate for AEs. All adverse events will be reported and documented as stated below.

The Investigator is responsible for appropriate medical care of subjects during the study.

The Investigator remains responsible for following through an appropriate healthcare option with study subjects who experienced AEs until resolution or until the AE is recognized as stabilized.

10.3.1. Definitions

Adverse Event
An AE is any untoward medical occurrence in a clinical study subject, temporally associated with the use of study treatment, whether or not considered related to the study treatment. NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study treatment.
Treatment-Emergent Adverse Event
All AEs occurring prior to the initiation of study treatment will be referred to as pretreatment-AEs, which include any unintended sign, symptom, or disease that occurs between the screening and the first administration of study treatment. All AEs that emerge during treatment and having been absent pretreatment or worsening relative to the pretreatment state are referred to as TEAEs.
Adverse Drug Reaction
In the preapproval phase of a new medicinal product or its new usages, particularly when the therapeutic dose(s) may still be established, all noxious and unintended responses to a medicinal product related to any dose should be considered an ADR. The phrase “responses to a medicinal product” means that a causal relationship between a medicinal product and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out. An adverse reaction, the nature or severity of which is not consistent with the applicable product Reference Safety information is called an unexpected ADR.

Example of Events Meeting the AE Definition
• Any abnormal laboratory test results (e.g., hematology, coagulation, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECG, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the Investigator. • Exacerbation of a chronic or intermittent preexisting condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study treatment administration even though it may have been present before the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment or a concomitant medication. Overdose *per se* will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae.
Example of Events NOT Meeting the AE Definition
• Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is the AE. • Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital). • Anticipated day-to-day fluctuations of preexisting disease(s) or condition(s) present or detected at the start of the study that do not worsen.

10.3.2. Definition of SAE

An SAE is defined as any untoward medical occurrence that, at any dose:
a. Results in death

b. Is life-threatening

The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

c. Requires inpatient hospitalization or prolongation of existing hospitalization

In general, hospitalization signifies that the subject has been admitted (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Procedures done in or visits to a clinic or outpatient facility are not considered SAEs. Admission to a rehabilitation facility, transitional care unit, or nursing home is not considered a hospitalization. A hospitalization for an elective treatment of a preexisting condition that did not worsen from baseline, or a routinely scheduled treatment is not considered an SAE because a "procedure" or "treatment" is not an untoward medical occurrence.

Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfils any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.

d. Results in persistent disability/incapacity

The term disability means a substantial disruption of a person's ability to conduct normal life functions.

This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

e. Is a congenital anomaly/birth defect

Intrauterine development of an organ or structure that is abnormal in form, structure, or position.

f. Other situations

Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.

Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

10.3.3. Recording and Follow-Up of AE and/or SAE

AE and SAE Recording

- When an AE/SAE occurs, it is the responsibility of the Investigator to review all documentation (e.g., hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- The Investigator will then record all relevant AE/SAE information in the eCRF.
- The Investigator shall inform the Sponsor of all SAEs within 24 hours of awareness. Contact details are provided below in Section 10.3.4.
- In case an existing AE changes in intensity the AE will be reported in the eCRF with its maximum intensity.
- It is **not** acceptable for the Investigator to send photocopies of the subject's medical records to the Sponsor in lieu of completion of the AE/SAE eCRF.
- There may be instances when copies of medical records for certain cases are requested by the Sponsor. In this case, all subject identifiers, with the exception of the screening number, will be redacted on the copies of the medical records before submission to the Sponsor.
- The Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

Assessment of Intensity

The Investigator will assess intensity for each AE and SAE reported during the study and assign it to 1 of the following categories:

Mild:

A type of adverse event that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.

Moderate:

A type of adverse event that is usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research subject.

Severe:

A type of adverse event that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.

An AE that is assessed as severe should not be confused with an SAE. Severe is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.

An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality

- The Investigator is obligated to assess the relationship between study treatment and each occurrence of each AE/SAE according to the following:
  - **Not related**: Not reasonably related to the study treatment(s). AE could not medically (pharmacologically/clinically) be attributed to the study treatment(s). A reasonable alternative explanation will be available.
  - **Related**: Reasonably related to the study treatment(s). AE could medically (pharmacologically/clinically) be attributed to the study treatment(s).
- In addition, the Investigator will assess the relationship between protocol required procedure(s) and each occurrence of each AE/SAE according to the following:
  - **Not related**: Not reasonably related to protocol required procedure(s).
  - **Related**: Reasonably related to protocol required procedure(s).

- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- The Investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study treatment administration will be considered and investigated.
- For causality assessment, the Investigator will also consult the IB and/or Product Information, for marketed products.
- The Investigator **must** review and provide an assessment of causality for each AE/SAE and document this in the medical notes.
- There may be situations in which an SAE has occurred, and the Investigator has minimal information to include in the initial report to the Sponsor. However, **it is very important that the Investigator always makes an assessment of causality for every event before the initial transmission of the SAE data to the Sponsor.**
- The Investigator may change their opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Outcome

The outcome of the adverse event should be classified according to the following definitions:

Recovered / resolved: the event has resolved (no further symptoms are present, and no treatment is being received by the subject).

Recovered / resolved with sequelae: the event has resolved but there may be lingering effects present (e.g., a scar following a cut or abrasion).

Fatal: the subject died as a result of the event. This code should only be used for the event that caused the death, not any event that was present at the time of the subject's death. Fatal events require immediately reporting to the Sponsor (or an authorized representative).

Unknown: may only be used in the event that the subject is lost to follow-up and no reliable data can be obtained.

Not resolved: the event is not yet resolved and is ongoing.

All efforts should be made to classify the AE according to the above categories.

Follow-up of AEs and SAEs

- All (S)AEs must be followed by the Investigator until resolved, stabilized, or judged no longer clinically significant. Thus, follow-up visits may be required even after the administration of the study treatment has been discontinued.
- The Investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the Sponsor to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- New or updated information will be recorded in the originally completed CRF.
- The Investigator will submit any updated SAE data to the Sponsor within 24 hours of receipt of the information.

10.3.4. Reporting of SAEs

SAE Reporting via SAE Report Form

- SAEs will be reported from signing of the ICF up to the safety follow-up call / early discontinuation visit. Additionally, any SAEs that occur after this time frame and are considered related to a medicinal (investigational) product by the Investigator must be reported.
- The Investigator will submit any SAE occurring during the study to the Sponsor without undue delay but not later than 24 hours after obtaining knowledge of the event. SAEs considered related to the medicinal (investigational) product occurring after the end of the clinical study must be reported to the Sponsor without undue delay.
- SAE reporting contact:

  PPD
  Phone: PPD
  Fax: PPD
  e-mail: PPD
- E-mail transmission of the scanned SAE report forms is the preferred method to transmit this information to the Sponsor. Alternatively, facsimile transmission may be used.
- In rare circumstances, notification by telephone is acceptable with a copy of the SAE report form sent by overnight mail or courier service.

- Initial notification via telephone does not replace the need for the Investigator to complete and sign the SAE form within the designated reporting time frames.

10.4. Appendix 4: Contraceptive Guidance

10.4.1. Definitions

Woman of Childbearing Potential (WOCBP)

A woman is considered fertile following menarche and until becoming postmenopausal unless permanently sterile (see below).

Women in the following categories are considered WOCBP (fertile):

1. Following menarche
2. From the time of menarche until becoming postmenopausal unless permanently sterile (see below)

- A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
    - A high FSH level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or HRT. However, in the absence of 12 months of amenorrhea, confirmation with more than one FSH measurement (> 30 mIU/mL) is required.
    - Females on HRT and whose menopausal status is in doubt will be required to use one of the non-estrogen hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrolment.
- Permanent sterilization methods (for the purpose of this study) include:
    - Documented hysterectomy
    - Documented bilateral salpingectomy
    - Documented bilateral oophorectomy

For individuals with permanent infertility due to an alternate medical cause other than the above, (e.g., Mullerian agenesis, androgen insensitivity, gonadal dysgenesis), Investigator discretion should be applied to determining study entry.

Note: Documentation can come from the site personnel's review of the subject's medical records, medical examination, or medical history interview.

If fertility is unclear (e.g., amenorrhea in adolescents or athletes) and a menstrual cycle cannot be confirmed before first dose of study treatment, additional evaluation should be considered.

Woman of Nonchildbearing Potential (WONCBP)

Women in the following categories are considered WONCBP:

1. Premenopausal female with permanent infertility due to one of the following (for the purpose of this study):

   a. Documented hysterectomy

   b. Documented bilateral salpingectomy

   c. Documented bilateral oophorectomy

   For individuals with permanent infertility due to an alternate medical cause other than the above, (e.g., Mullerian agenesis, androgen insensitivity, gonadal dysgenesis), Investigator discretion should be applied to determining study entry.

Note: Documentation can come from the site personnel's review of the subject's medical records, medical examination, or medical history interview.

2. Postmenopausal female

- A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
    - A high FSH level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or HRT. However, in the absence of 12 months of amenorrhea, confirmation with more than one FSH measurement (> 30 mIU/mL) is required.
    - Females on HRT and whose menopausal status is in doubt must discontinue HRT to allow confirmation of postmenopausal status before study enrolment.

10.5. Appendix 5: Liver Safety: Suggested Actions and Follow-up Assessments

Phase 1 liver chemistry stopping criteria are designed to assure subject safety and to evaluate liver event etiology.

Phase 1 Liver Chemistry Stopping Criteria and Follow-Up Assessments

<table>
<tr><th colspan="3">Liver Chemistry Stopping Criteria – Liver Stopping Event</th></tr>
<tr><td>ALT/AST-absolute</td><td colspan="2">ALT or AST ≥ 3 x ULN
ALT or AST ≥ 3 x ULN AND total bilirubin ≥ 2 x ULN OR international normalized ratio (INR) > 1.5, report to the Sponsor in expedited manner and as an SAE if SAE criteria are met [a,b].</td></tr>
<tr><th colspan="3">Suggested Actions, Monitoring, and Follow up Assessments</th></tr>
<tr><th>Actions</th><th colspan="2">Follow Up Assessments</th></tr>
<tr><td>• Immediately discontinue study treatment.
• Report the event to the Sponsor within 24 hours.
• Complete the liver event eSource and complete an SAE data collection tool if the event also met the criteria for an SAE[b]
• Perform liver follow-up assessments as described in the Follow Up Assessment column.
• Do not restart/rechallenge subject with study treatment.
• Monitor the subject until liver chemistry test abnormalities resolve, stabilize, or return to baseline (see MONITORING).

MONITORING:
If ALT or AST ≥ 3 x ULN AND total bilirubin ≥ 2 x ULN or INR > 1.5:
• Repeat liver chemistry tests (include ALT, AST, alkaline phosphatase, total bilirubin, and INR) and perform liver event follow-up assessments within 24 hours.
• Monitor subject twice weekly until liver chemistry test abnormalities resolve, stabilize, or return to baseline.</td><td colspan="2">• Viral hepatitis serology[c]
• Obtain blood sample for PK analysis - as soon as possible after the most recent dose[d].
• Obtain CK, LDH, GGT, GLDH and serum albumin.
• Fractionate bilirubin if total bilirubin ≥ 2 x ULN.
• Obtain complete blood count with differential to assess eosinophilia.
• Record the appearance or worsening of clinical symptoms of liver injury, or hypersensitivity, on the AE CRF.
• Record use of concomitant medications (including acetaminophen, recreational drugs, herbal remedies, and other over-the-counter medications) on the concomitant medications CRF.
• Record alcohol use on the liver event alcohol intake CRF.</td></tr>
</table>

• A specialist or hepatology consultation is recommended. **If ALT or AST ≥ 3 x ULN AND total bilirubin < 2 x ULN and INR ≤ 1.5:** • Perform liver chemistry tests (include ALT, AST, alkaline phosphatase, total bilirubin, and INR) and liver chemistry follow-up assessments within **24 to 72 hours.** • Monitor subjects weekly until liver chemistry abnormalities resolve, stabilize, or return to baseline.	**If ALT or AST ≥ 3 x ULN AND total bilirubin ≥ 2 x ULN or INR > 1.5** obtain the following in addition to the assessments listed above**:** • Anti-nuclear antibody, anti-smooth muscle antibody, Type 1 anti-liver kidney microsomal antibodies, and quantitative total immunoglobulin G (IgG) or gamma globulins. • Liver imaging (ultrasound, magnetic resonance, or computed tomography) to evaluate liver disease; complete liver imaging CRF. • Liver biopsy may be considered and discussed with local specialist if available, for instance: o In subjects when serology raises the possibility of autoimmune hepatitis (AIH) o In subjects when suspected DILI progresses or fails to resolve on withdrawal of study treatment o In subjects with acute or chronic atypical presentation • If liver biopsy is conducted, then complete liver biopsy CRF.

Notes:

[a] Serum bilirubin fractionation should be performed if testing is available. If serum bilirubin fractionation is not immediately available, discontinue study treatment if ALT or AST ≥ 3 x ULN **and** total bilirubin ≥ 2 x ULN. Additionally, if serum bilirubin fractionation testing is unavailable, **record the absence/presence of detectable urinary bilirubin on dipstick** which is indicative of direct bilirubin elevations suggesting liver injury.

[b] All events of ALT or AST ≥ 3 x ULN **and** total bilirubin ≥ 2 x ULN (for subjects with known Gilbert's syndrome these criteria only apply if total bilirubin ≥ 2 x ULN, and direct bilirubin > 2 x ULN and at least doubled from baseline value) or ALT or AST ≥ 3 x ULN **and** INR > 1.5 may indicate severe liver injury **(possible 'Hy's Law') and must be reported to Sponsor in an expedited manner and as an SAE if SAE criteria met (excluding studies of hepatic impairment or cirrhosis)**. The INR stated threshold value will not apply to subjects receiving anticoagulants.

[c] Includes: Hepatitis A IgM antibody; HBsAg and Hepatitis B core antibody; hepatitis C RNA; cytomegalovirus IgM antibody; Epstein-Barr viral capsid antigen IgM antibody (or if unavailable, heterophile antibody or monospot testing); and hepatitis E IgM antibody.

[d] Record the date/time of the PK blood sample draw and the date/time of the last dose of study treatment prior to the PK blood sample draw on the CRF. If the date or time of the last dose is unclear, provide the subject's best approximation. Instructions for sample handling and shipping are in the laboratory manual.

10.6. Appendix 6: Pharmacokinetic Parameters Definition

Symbol	Definition
$AUC_{0\text{-}tlast}$	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). $AUC_{0\text{-}inf} = AUC_{0\text{-}tlast} + C_{last} / \lambda_z$
CL/f	The apparent total body clearance of study treatment following extravascular administration. CL/f= Dose /$AUC_{0\text{-}inf}$. This parameter will be estimated for midazolam only.
C_{max}	Maximum observed concentration.
C_{trough}	The concentration observed at the end of a dosing interval immediately before next dosing. This parameter will be estimated for vamorolone only.
$t_{1/2}$	Terminal half-life ($t_{1/2} = \ln(2)/\lambda_z$)
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. $V_z/F = Dose/(AUC_{0\text{-}inf}*\lambda_z)$ following single dose. This parameter will be estimated for midazolam only:

10.7. Appendix 7: Sponsor Signature Page

The people signing hereby declare that they have read this protocol and agree to its contents.

_____ _____

Signature Date of Signature

Name, academic degree:	PPD
Function/Title:	PPD
Institution:	Santhera Pharmaceuticals (Switzerland) Ltd
Address:	Hohenrainstrasse 24 4133 Pratteln, Switzerland
Telephone number:	PPD
E-mail address:	PPD

10.8. Appendix 8: Principal Investigator Signature Page

The people signing hereby declare that they have read this protocol and agree to its contents. They confirm that the study will be conducted and documented in full accordance with the protocol (and modifications), International Conference for Harmonization (ICH) guidelines for current Good Clinical Practice (GCP) specified herein, the national drug law, and applicable regulatory requirements. They will also ensure that sub-Investigator(s) and other relevant members of their staff have access to copies of this clinical study protocol and the ICH GCP guidelines to enable them to work in accordance with the provisions of these documents.

_____ _____

Signature Date of Signature

Name, academic degree:	Michael Lissy, MD
Function/Title:	Principal Investigator
Institution:	Nuvisan GmbH
Address:	Wegenerstrasse 13 89231 Neu-Ulm, Germany
Telephone number:	PPD
Fax number:	PPD
E-mail address:	PPD

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