NUVISAN

Title Page

Protocol Title:

An open-label, randomized, 3-arm, parallel-group, positive- and negative-arm controlled study to evaluate the mineralocorticoid receptor antagonism effect of vamorolone in healthy subjects

Sponsor Protocol Number:	SNT-I-VAM-026
Nuvisan Protocol Number:	N-A-PH1-23-067
Investigational Medicinal Product:	Vamorolone
Study Phase:	Phase 1
Brief Title:	Evaluation of vamorolone mineralocorticoid receptor antagonism in healthy subjects
Sponsor:	Santhera Pharmaceuticals (Switzerland) AG Hohenrainstrasse 24 4133 Pratteln Switzerland
Sponsor's Legal Representative EU:	PPD Nuvisan GmbH Wegenerstr. 13 89231 Neu-Ulm Germany
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Regulatory Agency Identifier Number(s):	EU CT Number: 2024-512101-60-00
Protocol Version:	26-FEB-2024/Version Final 1.0

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List of Abbreviations

Abbreviations of PK parameters are provided in Section 10.4.

ADaM	Analysis data model
ADR	Adverse drug reaction
AE	Adverse event
ALT	Alanine aminotransferase
anti-HIV	Anti-human immunodeficiency virus antibodies
anti-HCV	Hepatitis C antibody
AST	Aspartate aminotransferase
AxMP	Auxiliary medicinal product
BA	Bioavailability
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 kinase
CKD-EPI	Chronic kidney disease epidemiology collaboration
CRF	Case report form
CRO	Contract research organization
CTS	Clinical trial supplies department
CV	Coefficient of variation
CYP	Cytochrome P450

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
EOS	End of study
EU	European Union
FU	Follow-up
GCP	Good clinical practice
GGT	Gamma-glutamyl transferase
GI	Gastrointestinal
GLDH	Glutamate dehydrogenase
GLP	Good laboratory practice
GR	Glucocorticoid receptor
HBsAG	Hepatitis B virus surface antigen
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
IB	Investigator's brochure
ICF	Informed consent form
ICH	International conference for harmonization
IEC	Independent ethics committee
IMP	Investigational medicinal product

INR	International normalized ratio
IUD	Intrauterine device
K	Potassium
LDH	Lactate dehydrogenase
LLOQ	Lower limit of quantification
MAD	Multiple ascending dose
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
MR	Mineralocorticoid receptor
MRA	Mineralocorticoid receptor antagonist
Na	Sodium
PD	Pharmacodynamic(s)
PK	Pharmacokinetic(s)
PR	Pulse rate
QP	Qualified person
QRS	Part of electrocardiographic wave representing ventricular depolarization
RSI	Reference safety information
SAD	Single ascending dose
SAE	Serious adverse event
SAP	Statistical analysis plan
SDTM	Study data tabulation model
SmPC	Summary of product characteristics

SoA	Schedule of activities
SOC	System organ class
SOP	Standard operating procedure
SUSAR	Suspected unexpected serious adverse reaction
TEAE	Treatment emergent adverse event
TSH	Thyroid-stimulating hormone
ULN	Upper limit of normal
US	United States
WHO	World Health Organization

1. Protocol Summary

1.1. Synopsis

Protocol Title:

An open-label, randomized, 3-arm, parallel-group, positive- and negative-arm controlled study to evaluate the mineralocorticoid receptor antagonism effect of vamorolone in healthy subjects

Brief Title:

Effect of vamorolone on mineralocorticoid receptor in healthy subjects

Regulatory Agency Identifier Number(s):

EU CT number: 2024-512101-60-00

Rationale:

The purpose of this study is to evaluate the PD antagonistic effect of a single dose of vamorolone at the MR. This will be assessed by determining the anti-aldosterone activity after repeated administration of fludrocortisone in healthy subjects.

The potential anti-mineralocorticoid properties of vamorolone suggested by preclinical studies are promising given the known involvement of the renin-angiotensin-aldosterone axis in the pathogenesis of skeletal muscle fibrosis and cardiomyopathy in DMD (Rodriguez-Gonzalez et al., 2020; Howard et al., 2022).

Urinary Na and K levels after fludrocortisone challenge are used to determine the anti-aldosterone activity of vamorolone, as it should reverse the urinary electrolyte effect induced by fludrocortisone, which is known to have a strong sodium retention capacity.

To increase the reliability and validity of the study results by minimizing bias and controlling for confounding variables, the study design will have 3 different arms: vamorolone experimental arm, eplerenone active control arm, and no treatment negative control arm. All study arms will receive a fludrocortisone challenge. The urine levels of Na and K will be compared before and after fludrocortisone administration.

This information is critical to translating the findings to the intended DMD patient population with a better understanding of the potential risks and benefits.

The proposed doses, which have been previously shown to be safe, have undergone extensive in vitro and in vivo evaluation with no genotoxicity observed.

Only male subjects will be enrolled to eliminate potential effects of circulating hormones on endpoints.

Objectives and Endpoints:

Objectives	Endpoints
Primary	
• To investigate the MRA effect of vamorolone by measuring the anti-aldosterone activity using mineralocorticoid challenge with fludrocortisone in healthy subjects	• Determination of the ratio of sodium to potassium (Na/K) in urine
Secondary	
• To investigate the safety and tolerability of vamorolone combined with fludrocortisone challenge	• TEAEs, laboratory assessments, vital signs and ECG evaluation
• To evaluate the PK of a single dose of vamorolone (20 mg/kg) combined with fludrocortisone challenge (Day 2)	• Plasma PK parameters $AUC_{0\text{-tlast}}$, $AUC_{0\text{-inf}}$ and C_{max}
• To evaluate the PK of a single dose of eplerenone (200 mg) combined with fludrocortisone challenge (Day 2)	• Plasma PK parameters $AUC_{0\text{-tlast}}$, $AUC_{0\text{-inf}}$ and C_{max}
Tertiary/Exploratory	
Not applicable.	

ECG=electrocardiogram, K=potassium, MRA=mineralocorticoid receptor antagonist, Na=sodium, PK=pharmacokinetics, TEAE=treatment emergent adverse event.

Overall Design:

This study will be conducted in a single-center, randomized, open-label, 3-arm, parallel-group, positive- and negative-controlled design.

Brief Summary:

The purpose of this study is to investigate the antagonistic effect of vamorolone on the MR following mineralocorticoid challenge by fludrocortisone, compared to eplerenone as a positive control, and to a negative control with no study treatment. Furthermore, the safety and tolerability of vamorolone combined with fludrocortisone challenge will be assessed, and the PK of a single dose of vamorolone and a single dose of eplerenone, combined with fludrocortisone challenge, will be evaluated.

Number of Subjects:

30 healthy male subjects (18 to 55 years, inclusive) will be randomized to one of the 3 study arms:

- Study arm 1 (experimental arm): vamorolone
- Study arm 2 (positive control arm): eplerenone
- Study arm 3 (negative control arm): no treatment

Dropouts may be replaced if the number of evaluable subjects completing the study becomes or is expected to become less than 24 subjects in total, i.e., less than 8 subjects in each study arm.

Study Treatment Groups and Duration:

The maximum total duration for a subject in the study will be approximately 32 days, from screening until the follow-up safety phone call.

Subjects will be screened for eligibility within 19 days of admission. Subjects will be admitted to the study site on Day -2 and will remain inpatient at the study site under medical supervision until discharge on Day 4.

The treatment duration will be 3 days and subjects will receive the study treatments as follows:

- Fludrocortisone challenge on Days 1 to 3 (for all subjects):
    - Day 1:
        - Fludrocortisone 1 mg at 9 h predose vamorolone /eplerenone administration/corresponding timepoint for negative control arm
    - Day 2:
        - Fludrocortisone 0.5 mg at the same time of vamorolone/eplerenone administration in the morning (0 h) /corresponding timepoint for negative control arm.
        - Fludrocortisone 0.1 mg at 2 h, 4 h, 6 h, 8 h, 10 h, 12 h, and 14 h vamorolone/eplerenone postdose administration/corresponding timepoint for negative control arm.
        - Fludrocortisone 0.5 mg at 16 h vamorolone/eplerenone postdose administration/corresponding timepoint for negative control arm.
    - Day 3:
        - Fludrocortisone 0.1 mg at 24 h vamorolone/eplerenone postdose administration on Day 2/corresponding timepoint for negative control arm.

- Day 2: drug administration period (vamorolone or eplerenone or no treatment)
    - 10 subjects randomized to study arm 1 will receive a single oral dose of 20 mg/kg vamorolone
    - 10 subjects randomized to study arm 2 will receive a single oral dose of 200 mg eplerenone
    - 10 subjects randomized to study arm 3 will receive no treatment

Subjects will be served diet-controlled meals from Day -2 to Day 3, i.e., containing similar quantities of sodium and potassium.

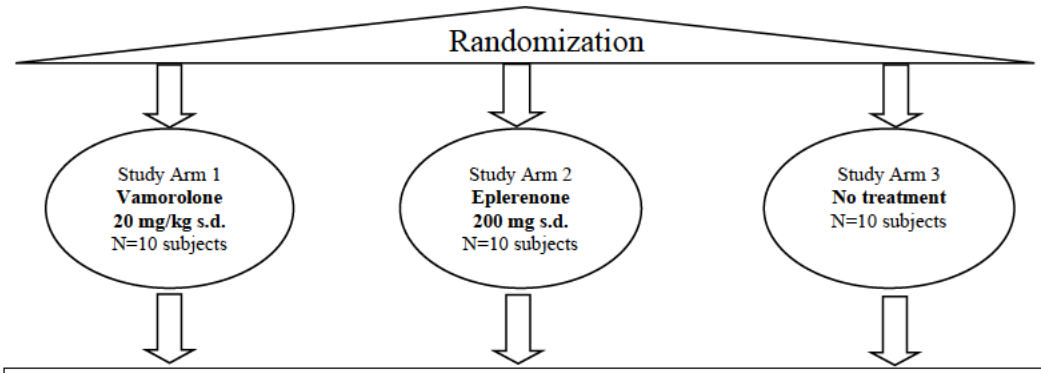
In-house follow-up assessments are planned on Day 4, 1 day after the last dose of study treatment. A follow-up safety phone call is planned on Day 11.

Early discontinuation assessments will be conducted at an early discontinuation visit for subjects who have withdrawn prematurely.

Data Monitoring/Other Committee: No

1.2. Flow Chart

Figure 1: Overall Study Design – Schematic Overview



- **Day -2**: Admission to the study center approximately 63 h prior to fludrocortisone evening dosing on Day 1.
- **Days -2 to -1**: Baseline visit/diet calibration period.
- **Days -2 to 3**: Diet-controlled meals.
- **Days 1 to 3**: Fludrocortisone challenge.
- **Day 2**: Vamorolone or eplerenone administration.
- **Day 2**: PK vamorolone and PK eplerenone (each at predose; 1, 2, 4, 8, 12, 24 h postdose)
- **Days 1 to 4**: Timed urine collection for sodium and potassium measurements.
  Day 1: 24-9 h and 9-0 h predose of vamorolone/eplerenone[a];
  Day 2: 0-2 h, 2-4 h, 4-6 h, 6-8 h, 8-10 h, 10-12 h, 12-14 h, 14-16 h, 16-24 h postdose vamorolone/eplerenone[a]
  Day 3: 24-32 h, 32-40 h, 40-48 h postdose vamorolone/eplerenone[a]
  [a] or corresponding timepoint for negative control arm (no treatment)

End of study visit

- **Day 4**: End of hospitalization with final assessments.
- Discharge from the study center after the last urine collection interval (40-48 h postdose vamorolone/eplerenone[a]) is completed.
- **Day 11**: Safety phone call.

[a] or corresponding timepoint for negative control arm (no treatment)

ECG=electrocardiogram, PK=pharmacokinetics, s.d=single dose

1.3. Schedule of Activities

A 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 -2 evaluations):
    1. 12-lead ECG / vital signs
    2. Urine sampling as most important for the primary objective in this study will end immediately before blood sampling each.
    3. Blood sampling (PK and safety) as close as possible to the nominal time
    4. All other assessments and procedures
- Any assessments / actions at the timepoint of dosing will be performed predose and administration of the study treatments is the last action for the respective timepoint, unless indicated otherwise.
- Any meals or fluid intake will be provided once 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	Scree-ning	Treatment Period					FU/ED		Notes
Day	**-21 to -3**	**-2[1]**	**-1[1]**	**1**	**2**	**3**	**4**	**11**	
Informed consent	X								To be obtained prior to any screening activity. Refer to Section 10.1.
Hospitalization/In-house stay[2]		X	X	X	X	X	X		Day -2: Admission. Day 4: Discharge after completion of Day 4 assessments.
Safety phone call								X	Day 11: Safety phone call for review of AEs and concomitant medication.
Inclusion/exclusion criteria	X								Refer to Sections 5.1.1 and 5.2.1.
Confirmation of eligibility		X							Re-check of clinical status on Day -2 and subject eligibility. Refer to 5.2.2.
Demographic data	X								Refer to Section 8.1.1.
Relevant medical history	X	X							Including history of illegal drugs, alcohol, tobacco, and caffeine use. Refer to Section 8.1.3.
Prior medication	X	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. Refer to Section 6.9.
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.

Procedure	Screening	Treatment Period					FU/ED		Notes
Day	**-21 to -3**	**-2[1]**	**-1[1]**	**1**	**2**	**3**	**4**	**11**	
TSH	X								Refer to Section 10.2.
Serology	X								HIV, Hepatitis B and C screening. Refer to Section 10.2.
Urine drug screen, cotinine screen, alcohol breath test	X	X							Refer to Section 8.3.5 and Section 10.2.
Safety laboratory assessments	X	X			X	X	X		Hematology, coagulation, clinical chemistry, urinalysis. Refer to Section 10.2.
12-lead ECG	X	X			X	X	X		Day 2: At predose and 8 h, 12 h postdose vamorolone/eplerenonea Day 2: For study arm 1 only in addition at 2h postdose vamorolone Day 3: At 24 h postdose vamorolone/eplerenone[a] [a] or corresponding timepoint for negative control arm For details refer to Section 8.3.3.
Vital signs (blood pressure, pulse rate and body temperature)	X	X		X	X	X	X		Day 2: At predose and 8 h, 12 h postdose vamorolone/eplerenone[a] Day 3: At 24 h postdose vamorolone/eplerenone[a] . [a] or corresponding timepoint for negative control arm For details refer to Section 8.3.2.
Diet-controlled meal		X	X	X	X	X			Refer to Sections 5.3.1 and 6.1.
Randomization				X					Prior to administration fludrocortisone. Refer to Section 6.3.

Procedure	Scree-ning	Treatment Period					FU/ED		Notes
Day	**-21 to -3**	**-2[1]**	**-1[1]**	**1**	**2**	**3**	**4**	**11**	
Vamorolone dosing (Study Arm 1)					X				Refer to Section 6.1.
Eplerenone dosing (Study Arm 2)					X				Refer to Section 6.1.
Fludrocortisone dosing				X	X	X			Day 1: 1 mg single dose at 9 h predose vamorolone/eplerenone[a] dosing. Day 2: 0.1 mg and 0.5 mg multiple doses. For detailed timepoints, see Section 4.1. Day 3: 0.1 mg single dose at 24 h postdose vamorolone/eplerenone[a] dosing. [a] or corresponding timepoint for negative control arm Refer to Section 6.1.
PD urine collection for sodium (Na) and potassium (K) measurements[3]				X	X	X	X		Day 1: 24-9 h predose of vamorolone/eplerenone[a], 9-0 h predose of vamorolone/ eplerenone[a]. Day 2: 0-2 h, 2-4 h, 4-6 h, 6-8 h, 8-10 h, 10-12 h, 12-14 h, 14-16 h, 16-24 h, postdose vamorolone/eplerenone[a]. Day 3: 24-32 h, 32-40 h, 40-48 h postdose vamorolone/eplerenone[a]. [a] or corresponding timepoint for negative control arm Refer to Section 8.6.
PK blood sampling for vamorolone (Study Arm 1)					X	X			At predose; 1, 2, 4, 8, 12, 24 h postdose. Refer to Section 8.5.

Procedure	Scree-ning	Treatment Period					FU/ED		Notes
Day	**-21 to -3**	**-2[1]**	**-1[1]**	**1**	**2**	**3**	**4**	**11**	
PK blood sampling for eplerenone (Study Arm 2)					X	X			At predose; 1, 2, 4, 8, 12, 24 h postdose. Refer to Section 8.5.
AE/SAE review	X	X	X	X	X	X	X	X	Any event with start after ICF signature. Refer to Sections 8.4 and 10.3.
Concomitant medication review				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; EOS=end of study; FU=follow-up visit; HIV=human immunodeficiency virus; PD=pharmacodynamics; PK=pharmacokinetics; SAE=serious adverse event; TSH=thyroid-stimulating hormone.

1 Day -2 to Day -1: Baseline visit/diet calibration period.

2 Subjects must check in on Day -2 (baseline), approximately 63 hours prior to fludrocortisone evening dosing on Day 1 and will be confined to the clinic until Day 4.

3 At the end of each collection interval, all subjects will be instructed to void their bladder.

2. Introduction

Vamorolone 40 mg/mL oral suspension has received Marketing Authorization in the European Union, UK, and the US 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 this study is to evaluate the PD antagonistic effect of a single dose of vamorolone at the MR. This will be assessed by determining the anti-aldosterone activity after repeated administration of fludrocortisone in healthy subjects.

The potential anti-mineralocorticoid properties of vamorolone suggested by preclinical studies are promising given the known involvement of the renin-angiotensin-aldosterone axis in the pathogenesis of skeletal muscle fibrosis and cardiomyopathy in DMD (Rodriguez-Gonzalez et al., 2020; Howard et al., 2022).

Urinary Na and K levels after fludrocortisone challenge will be used to determine the anti-aldosterone activity of vamorolone, as it should reverse the urinary electrolyte effect induced by fludrocortisone, which is known to have a strong sodium retention capacity. To increase the reliability and validity of the study results by minimizing bias and controlling for confounding variables, the study design will have 3 different arms: vamorolone experimental arm, eplerenone active control arm, and no treatment negative control arm. All study arms will receive a fludrocortisone challenge. The urine levels of Na and K will be compared before and after fludrocortisone administration.

This information is critical to translating the findings to the intended DMD patient population with a better understanding of the potential risks and benefits.

The proposed doses, which have been previously shown to be safe, have undergone extensive in vitro and in vivo evaluation with no genotoxicity observed.

Only male subjects will be enrolled to eliminate potential effects of circulating hormones on endpoints.

2.2. Background

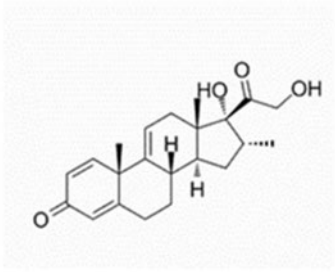
2.2.1. Vamorolone

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.

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 belongs to the structural class of synthetic steroidal drugs, which includes the glucocorticoids prednisone, methylprednisolone, and dexamethasone (Reeves, 2013). It is a structural analogue of prednisolone in which sub-activities have been dissociated, i.e., transrepression retained, transactivation reduced, membrane stabilization increased, cross-reactivity with mineralocorticoid receptor changed from agonist to antagonist.

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 United States in October 2023, in the European Union in December 2023, and in the UK in January 2024 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.

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

2.2.2. Eplerenone

Eplerenone is classified as an aldosterone antagonist and is used in the management and treatment of heart failure with reduced ejection fraction and hypertension.

Eplerenone binds with high selectivity to MRs. It prevents the binding of aldosterone, a key hormone in the renin-angiotensin-aldosterone-system, which is involved in the regulation of blood pressure and the pathophysiology of cardiovascular disease. This results in increased excretion of water and sodium with simultaneous retention of potassium. Due to the narrow therapeutic limits of serum potassium, it must be monitored regularly during therapy with eplerenone to prevent hyperkalemia.

C_{max} is reached after approximately 1.5 to 2 hours and steady state is reached within 2 days, absorption is independent from food intake. The metabolism of eplerenone is mainly mediated via CYP3A4. Approximately 32% is excreted in the feces and approximately 67% in the urine. The elimination plasma half-life $t_{1/2}$ of eplerenone is approximately 3 to 6 hours, the apparent plasma clearance is approximately 10 L/h. Preclinical studies of safety pharmacology, genotoxicity, carcinogenic potential and reproductive toxicity revealed no special hazard for humans.

Eplerenone is administered orally with a daily dose between 25 mg and 50 mg.

More details on eplerenone can be found in the SmPC (eplerenone SmPC).

2.2.3. Fludrocortisone

Fludrocortisone is a synthetic aldosterone analogue that belongs to the group of mineralocorticoids and is used for the partial replacement therapy for primary adrenal insufficiency in Addison's disease and for the treatment of salt-losing adrenogenital syndrome. Fludrocortisone binds to MR and thus increases the reabsorption of sodium and chloride ions in the kidneys. In addition, the renal excretion of potassium, hydrogen and ammonium ions is also increased. The resulting increase in blood volume raises blood pressure. Fludrocortisone has an approximately 400-fold higher potency and 1,000-fold higher MR affinity than hydrocortisone, as it is not inactivated by 11β-hydroxysteroid dehydrogenase.

Fludrocortisone is rapidly and completely absorbed after oral administration. C_{max} is reached after 4 to 8 hours. It is hydrolyzed in the liver and 80% are excreted via the kidneys and 20% in the feces. The plasma half-life $t_{1/2}$ is about 3.5 hours. The effect lasts for 12 to 36 hours.

Clinically, fludrocortisone is administered orally with a daily dosage range of 0.05 to 0.3 mg fludrocortisone acetate tablets orally. The dosing regimen chosen for this study is based on the design used to evaluate the activity of the MRA (Nakamura and Kawaguchi, 2021).

More details on fludrocortisone can be found in the SmPC (fludrocortisone SmPC).

2.2.4. 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 GI 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 low, but the pharmacologically mediated adverse effects were generally reversible and monitorable in the clinic.
- As DMD is a predominantly male disease, a focused microscopic analysis of the male reproductive organs as well as sperm analysis were included in chronic toxicology studies of both, the mouse and dog.
- There were no signals for either mutagenicity or genotoxicity 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).

2.2.5. Summary of Clinical Studies

6 clinical studies have been completed with healthy volunteers 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 short 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 the 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%), diarrhea (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%), diarrhea (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.

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

2.3. Benefit/Risk Assessment

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.

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

Based on the available nonclinical and clinical data to date, the conduct of the study, as specified in this protocol, is considered justifiable.

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 (single dose), eplerenone (single dose) and fludrocortisone (multiple dose), as outlined in the tabular summary below.

To demonstrate MRA activity, vamorolone is administered as a single dose of 20 mg/kg in this clinical study, which is 4-6 times higher than the daily dose indicated in the SmPC. As most events depend on the duration of the therapy in conjunction with the doses administered, it is not expected that the risks identified to date (e.g., adrenal insufficiency, weight gain mainly in the first months of treatment, increased risk of infections, increased risk of diabetes mellitus, altered responses to vaccines, ophthalmic effects) will have an impact on subjects participating in the study. The selected dose of 20 mg/kg vamorolone was determined by considering the maximum dose administered in both SAD and MAD studies in healthy adult subjects, which was 20 mg/kg and has been shown to be well tolerated and safe in this population (see the current IB).

More detailed information about the warnings and precautions for administration of vamorolone, eplerenone, and fludrocortisone can be found in the current IB (IB) and the SmPCs of eplerenone and fludrocortisone (SmPC eplerenone, SmPC fludrocortisone).

Identified and Potential Risks of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Study Treatment Vamorolone		
Hypersensitivity to active substance or excipients	Potential risk of allergic reaction to vamorolone or excipients.	Exclusion of subjects who were exposed to vamorolone previously or are allergic (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 Eplerenone		
Hyperkalemia	Potential risk of high levels of potassium in the blood.	Health monitoring during the study. Safety laboratory controls, as outlined in Sections 1.3 and 10.2.

Identified and Potential Risks of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Dermatologic disorders	Potential risk of rash and pruritus.	Health monitoring during the study.
Study Treatment Fludrocortisone		
Hypertension	Potential risk of hypertension.	Health monitoring during the study.
Fluid retention and edema	Potential risk of fluid retention and edema, which may cause swelling of lower limbs.	Health monitoring during the study.
Congestive heart failure.	Potential risk of congestive heart failure.	Health monitoring during the study.
Dermatologic disorders	Potential risk of increased sweating, poor wound healing, hirsutism, thinning of the skin, severe allergic reaction.	Health monitoring during the study.
Metabolic disorders	Potential risk of elevated blood and urine glucose, weight gain.	Health monitoring during the study. Safety laboratory controls, as outlined in Sections 1.3 and 10.2.
Electrolytes disturbances	Potential risk of severe hypokalemia, metabolic alkalosis.	Health monitoring during the study. Safety laboratory controls, as outlined in Sections 1.3 and 10.2.
Study Procedures		
Blood drawn	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.
Study Procedures (continued)		
Complications from indwelling catheters	Local reactions, infections, nerve, or tissue damage may occur.	Standard medical care to be applied when catheters are used.

Identified and Potential Risks of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
ECG	Contact allergies can develop during ECG procedures.	Subjects with known contact allergies will not be included in the study.

AE=adverse event, ECG=electrocardiogram.

2.3.2. Warnings and Precautions

Detailed information about the warnings and precautions for administration of vamorolone can be found in the current IB (IB).

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 20 mg/kg of vamorolone single 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. Similarly, a single dose of eplerenone is unlikely to be associated with risk to healthy volunteers. The short-term fludrocortisone challenge used in this study has been shown to be safe and well tolerated in healthy subjects (Nakamura and Kawaguchi, 2021).

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 10.2), and observation of vital signs (Section 8.3.2) and ECGs (Section 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 MRA effect of vamorolone by measuring the anti-aldosterone activity using mineralocorticoid challenge with fludrocortisone in healthy subjects	• Determination of the ratio of sodium to potassium (Na/K) in urine
Secondary	
• To investigate the safety and tolerability of vamorolone combined with fludrocortisone challenge.	• TEAEs, laboratory assessments, vital signs and ECG evaluation
• To evaluate the PK of a single dose of vamorolone (20 mg/kg) combined with fludrocortisone challenge (Day 2)	• Plasma PK parameters $AUC_{0\text{-tlast}}$, $AUC_{0\text{-inf}}$ and C_{max}
• To evaluate the PK of a single dose of eplerenone (200 mg) combined with fludrocortisone challenge (Day 2)	• Plasma PK parameters $AUC_{0\text{-tlast}}$, $AUC_{0\text{-inf}}$ and C_{max}
Tertiary/Exploratory	
Not applicable	

ECG=electrocardiogram, MRA=mineralocorticoid receptor antagonist, PK=pharmacokinetics, TEAE=treatment emergent adverse event.

For definitions of PK endpoints refer to Section 8.5.2. Refer to Section 9.3 for the statistical aspects of the endpoints.

4. Study Design

4.1. Overall Design

This will be a single-center, randomized, open-label, 3-arm, parallel-group, positive- and negative-controlled, Phase 1 study in 30 healthy male subjects (Sections 1.2 and 1.3). Only male subjects will be enrolled to eliminate potential effects of circulating hormones on the endpoints. Subjects aged 18 to 55 years will be randomized to 3 study arms (see below).

The purpose of this study is to investigate the antagonistic effect of vamorolone on the MR by measuring the anti-aldosterone activity using mineralocorticoid challenge with fludrocortisone, compared to eplerenone as a positive control and to a negative control without treatment.

Furthermore, the safety and tolerability of concomitant administration of fludrocortisone and vamorolone will be assessed and the PK of single dose vamorolone and single dose eplerenone will be evaluated.

- The total time in the study for a subject is approximately 32 days, including the screening period (maximum 19 days), a baseline/diet calibration visit (2 days), a treatment period (3 days), and a safety follow-up period of 8 days (in-house follow-up assessments on Day 4 and a safety follow-up phone call on Day 11).
- The screening period will occur from Day -21 to Day -3. Eligible subjects will be admitted to the study site on Day -2 and will remain resident throughout the treatment period until the safety follow-up on Day 4. Subjects will be discharged from the study center after the last urine collection interval (40-48 h postdose vamorolone/eplerenone/corresponding timepoint for negative control arm) and Day 4 assessments are completed. A follow-up safety phone call is planned on Day 11.
- 10 subjects each will receive vamorolone, eplerenone, or no treatment, randomly allocated to 1 of 3 study arms:
    - Study arm 1 (experimental arm): vamorolone oral suspension
    - Study arm 2 (positive control arm): eplerenone tablets
    - Study arm 3 (negative control arm): no treatment
- Fludrocortisone challenge on Days 1 to 3 (for all subjects):
    - Day 1:
        - Fludrocortisone 1 mg at 9 h predose vamorolone/eplerenone administration/corresponding timepoint for negative control arm.

  - Day 2:
    - Fludrocortisone 0.5 mg at the same time of vamorolone/eplerenone administration in the morning (0 h) /corresponding timepoint for negative control arm.
    - Fludrocortisone 0.1 mg at 2 h, 4 h, 6 h, 8 h, 10 h, 12 h, and 14 h vamorolone/eplerenone postdose administration/corresponding timepoint for negative control arm.
    - Fludrocortisone 0.5 mg at 16 h vamorolone/eplerenone postdose administration/corresponding timepoint for negative control arm.
  - Day 3:
    - Fludrocortisone 0.1 mg at 24 h vamorolone/eplerenone postdose administration on Day 2/corresponding timepoint for negative control arm.

- Day 2: Drug administration period (vamorolone or eplerenone or no treatment; in fasted state in the morning):
  - Subjects randomized to study arm 1 will receive a single dose of 20 mg/kg vamorolone.
  - Subjects randomized to study arm 2 will receive a single dose of 200 mg eplerenone.
  - Subjects randomized to study arm 3 will receive no treatment.
- Subjects will be served diet-controlled meals from Day -2 to Day 3, i.e., containing similar quantities of sodium and potassium (Section 5.3.1).
- Blood samples for safety laboratory assessments (hematology, coagulation, and clinical chemistry) will be collected at screening (Day -21 to Day -3), at baseline (Day -2), on Days 2 and 3, and at EOS.
- Urine samples for routine urinalysis will be collected at screening (Day -21 to Day -3), at baseline (Day -2), on Days 2 and 3, and at EOS.
- Blood samples for PK assessments of plasma vamorolone (study arm 1) or plasma eplerenone (study arm 2) will be collected from predose through 24 hours following vamorolone / eplerenone dosing on Day 2.
- Urine samples for sodium and potassium measurement will be collected during 14 collection intervals (see Table 8):
  - Day 1: 24-9 h predose and 9-0 h predose vamorolone/eplerenone[a]
  - Day 2: 0-2 h, 2-4 h, 4-6 h, 6-8 h, 8-10 h, 10-12 h, 12-14 h, 14-16 h, 16-24 h postdose[a] vamorolone/eplerenone
  - Day 3: 24-32 h, 32-40 h, 40-48 h postdose[a] vamorolone/eplerenone[a]

[a] or at corresponding timepoint for negative control arm

A flow chart and a detailed SoA with more information on the specific timepoints for the scheduled study visits and required procedures are provided in Sections 1.2 and 1.3.

4.2. Scientific Rationale for Study Design

In this study, the MRA effect of vamorolone will be evaluated by determining the anti-aldosterone activity after repeated administration of fludrocortisone in healthy subjects.

The primary endpoint for evaluating the MRA effect of vamorolone is to assess urinary sodium (Na) and potassium (K) levels following a fludrocortisone challenge. The goal is to demonstrate the anti-aldosterone activity of vamorolone, specifically its ability to reverse the urinary electrolyte effects induced by fludrocortisone, a substance known for its strong sodium retention capacity.

The pharmacological effect of the MRA is measured against a background of mineralocorticoid excess induced by the administration of an exogenous mineralocorticoid, such as fludrocortisone. Fludrocortisone is a renal mineralocorticoid agonist that binds to aldosterone receptors, resulting in increased sodium reabsorption and potassium excretion.

The methodology is based on the observation that oral doses of mineralocorticoid fludrocortisone affect the sodium/potassium (Na/K) ratio in overnight urine. This effect follows a logarithmic dose-response pattern similar to aldosterone. The ratio tends to return to normal levels in the presence of aldosterone antagonists, providing a reliable quantitative assay for these substances in humans. Antagonist activity is identified by increased sodium excretion, decreased potassium excretion, and an increased Na/K ratio observed in overnight urine 2-10 hours after treatment.

Eplerenone is selected as positive control because of its enhanced MR selectivity and superior safety profile compared to spironolactone, particularly in terms of a reduced incidence of sex hormone-related AEs (Nakamura and Kawaguchi, 2021).

This trial will be conducted in 30 healthy subjects to standardize the trial population and to minimize exposure variability.

The trial will be conducted in 30 healthy subjects in 3 arms of 10 each, and this sample size is determined by the need for adequate representation rather than statistical power. The rationale for the determination of the sample size is described in Section 9.5.

Safety and tolerability of vamorolone co-administrated with fludrocortisone will be reported and the blood samples will be collected to follow the plasma levels of vamorolone and eplerenone.

The open-label design is considered acceptable and justified because the main assessment criterion is a PD parameter that is unlikely to be subject to Investigator or subject induced bias.

The treatment allocation is conducted in a randomized manner to support the scientific integrity of the trial by creating comparable treatment groups, reducing the risk of bias, and maintaining the statistical and ethical fundamentals. Details about randomization are provided in Section 6.3.

4.3. Justification for Dose

Vamorolone

The selected dose was determined by considering the maximum dose administered in both SAD and MAD studies in healthy adult subjects, which was 20 mg/kg. This has been shown to be well tolerated and safe in this population (see the current IB).

Eplerenone

A single dose of 200 mg eplerenone was selected since this dose has been shown to be safe and well tolerated in healthy adults while also confirming the mineralocorticoid antagonist effect (Nakamura and Kawaguchi, 2021).

Fludrocortisone

All subjects in the 3 arms will receive fludrocortisone at the doses specified in Section 4.1. This choice of dose is based on its established efficacy in influencing the overnight Na/K ratio. In addition, fludrocortisone has proven to be safe and well tolerated when administered over a 2-day period, even in subjects not treated with a MRA (Nakamura and Kawaguchi, 2021).

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 (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

A total of 30 healthy adult males will be randomized to the 3 study arms with 10 subjects assigned to each arm.

No subject may be randomly assigned to study treatments unless adherence to all eligibility criteria as given in Sections 5.1and 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 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. 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, diaphragm with spermicide) 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.

Drugs and Stimulants

5. Subject is a non-smoker for at least 3 months prior to exposure to the study treatments. Subjects 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 treatment.

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 of ≥ 90 mmHg and ≤ 140 mmHg, diastolic blood pressure of ≥ 50 mmHg and ≤ 90 mmHg, pulse rate of ≥ 45 bpm and ≤ 90 bpm, and tympanic body temperature of ≥ 35.0 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

1. A past medical history of clinically significant abnormalities or a history/family history of long QT interval syndrome.
2. An abnormal ECG, defined as:
    - PR > 215 msec and < 120 msec, QRS complex > 120 msec; QTcF > 440 msec by automated reading
    - Any clinically significant cardiac conduction abnormalities
    - Any atrial or ventricular arrhythmias
3. 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).

4. A past medical history of peptic ulcers, diverticulitis, and non-specific ulcerative colitis.
5. History of complaints of frequent dizziness and /or vomiting spells or lightheadedness ("frequent" defined as incidence occurs more than once every week).
6. 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.
7. Known Gilbert's syndrome.
8. 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).
9. Known or suspected hypersensitivity or contraindications to the study treatments or any components of the formulation used, e.g., vamorolone, eplerenone and fludrocortisone (see vamorolone IB, eplerenone SmPC, fludrocortisone SmPC).
10. 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

11. 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 (medicinal product in its original packaging, approved and marketed in Germany) is permitted.

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

14. Administration of live, attenuated, replication-competent vaccines within 6 weeks prior to the first administration of study treatment until 2 weeks after the follow-up visit. Administration of inactivated vaccines or 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.
15. 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

16. 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

17. Positive results for HBsAg, anti-HCV, anti-HIV 1 and 2, and HIV 1-p24 antigen at screening.
18. Positive screen for alcohol, drugs of abuse and cotinine at screening.
19. Elevations in ALT ≥ 1.1 x ULN, AST ≥ 1.1 x ULN, serum bilirubin ≥ 1.0 x ULN, creatinine ≥1.0 x ULN and HbA1c > ULN at screening. A case-by-case decision for any abnormality must be discussed with the Sponsor before inclusion.
20. Sodium, potassium, magnesium, chloride blood concentration below the lower limit of normal at screening.
21. TSH and coagulation outside of normal ranges.
22. eGFR based on the CKD-EPI (details for calculation see Section 10.2) of < 90 mL/min at screening.

Other Exclusions

23. Subjects who are unwilling to adhere to contraceptive requirements.
24. Higher than low-risk alcohol consumption i.e., consumption of an average weekly alcohol intake of > 21 units/week for men. 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.
25. 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.
26. Consumption of alcohol from 48 hours prior to admission.

27. 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 treatments.
28. Regular consumption of poppy seed containing food prior to first administration of study treatments.
29. No sweets/teas containing liquorice are allowed within 2 weeks prior to first administration of study treatments up to the follow-up examination.
30. Any use of drugs-of-abuse or alcohol abuse within 1 month prior to dosing.
31. Subject with vegetarian, vegan, or restricted diet (e.g., gluten-free) or not willing or able to eat the complete standard meals.
32. 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 treatments.
33. Strenuous physical activity within 72 h to admission.
34. Employee of the Sponsor, the Nuvisan Group, or other Contract Research Organization involved in the clinical study.
35. Legal incapacity or limited legal capacity, or incarceration and vulnerable subjects.
36. 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.
37. History of non-compliance to medical regimens and subjects who are considered potentially unreliable (e.g., refuse to comply with study regulations).
38. 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

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

Prior/Concomitant Therapy

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

Diagnostic Assessments

42. Positive for an acute common cold/respiratory or other infection upon admission.

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

Other Exclusions

44. 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 treatments until after collection of the final blood PK sample. Abstinence from liquorice containing sweets and teas 14 days before admission until follow-up examination.
- Subjects will be required to refrain from consumption of poppy seeds from 72 hours before the start of study treatments until the end of confinement.
- During the in-house stay (Day -2 to Day 4), the subjects may only consume food and beverages provided by the study site.
- From Day -2 to Day 3, subjects will be served diet-controlled meals, i.e., containing similar quantities of sodium and potassium. The diet provided (maximum of 4 meals per day) will consider the imposed restrictions. To standardize the meals as much as possible, for breakfast, lunch and dinner, the same meals will be served on each of the 5 days, based on a diet plan. For lunch and dinner, the meals will be prepared once for this period (1 for lunch, 1 for dinner), split in 5 portions for each day, vacuum-packed, refrigerated and reprepared when needed. A diet-controlled breakfast with similar quantities of sodium and potassium will be provided based on a diet plan.
- On Day 2, subjects will fast for at least 10 hours before first administration of vamorolone/eplerenone/corresponding timepoint for negative control arm and will continue fasting for 2 hours.
- Fluid consumption will be controlled as outlined in Table 3.
- From Day -2 to Day 3, subjects must drink at least 2.5 L/day, on Days 1 to 3 exactly as defined in Table 3 (non-carbonated water of defined electrolyte composition). This is to ensure urine flow during the urinary Na/K assessment period.
- All study treatments will be administered in an upright position with 240 mL of non-carbonated water of defined electrolyte composition.

- Diet-controlled meals will be served 2 hours (breakfast), 5 hours (lunch), 7 hours (snack), and 10 hours (dinner) after dosing, referring to the timepoint of administration of vamorolone/eplerenone/negative control on Day 2 (for Days -2 to Day 2), or referring to the timepoint of administration of fludrocortisone 0.1 mg (for Day 3 only).
- Apart from food and drink specified in Table 3, no additional or in-between food or drinks are allowed.
- Further details on standardized food and drink are presented in Table 3.
- For further restrictions on dosing days, refer to Section 6.1.

Table 3: Food and drink before and after dosing of study treatments

Time	Food and Drink
Day -2	
Corresponding timepoint for breakfast on Day 2	Diet-controlled breakfast
Corresponding timepoint for lunch on Day 2	Diet-controlled lunch
Corresponding timepoint for snack on Day 2	Diet-controlled snack
Corresponding timepoint for dinner on Day 2	Diet-controlled dinner
During the whole day	At least 2.5 L non-carbonated water[b] over 24 h
Day -1	
Corresponding timepoint for breakfast on Day 2	Diet-controlled breakfast
Corresponding timepoint for lunch on Day 2	Diet-controlled lunch
Corresponding timepoint for snack on Day 2	Diet-controlled snack
Corresponding timepoint for dinner on Day 2	Diet-controlled dinner
During the whole day	At least 2.5 L non-carbonated water[b] over 24 h
Day 1	
Corresponding timepoint for breakfast on Day 2	Diet-controlled breakfast
Corresponding timepoint for lunch on Day 2	Diet-controlled lunch
Corresponding timepoint for snack on Day 2	Diet-controlled snack
Corresponding timepoint for dinner on Day 2	Diet-controlled dinner
Within 24 h to 12 h prior to administration of vamorolone/eplerenone[a] on Day 2	2.0 L non-carbonated water[b] over 12 h
Within 12 h to 8 h prior to administration of vamorolone/eplerenone[a] on Day 2	500 mL non-carbonated water[b] over 4 h
Fludrocortisone 1 mg dosing 9 h predose administration of vamorolone/eplerenone[a] on Day 2	**240 mL non-carbonated water[b,c]**
Day 2	
Previous evening / overnight	Fasting for at least 10 h before administration of vamorolone/eplerenone[a]. Drinking of non-carbonated water[b] allowed until 1 h before dosing.
From 2 h to 1 h before dosing	No food. 500 mL non-carbonated water[b] over 1 h.
During 1 h before dosing	No food or drink.
0 h: Vamorolone/eplerenone[a] dosing (together with **fludrocortisone 0.5 mg**)	**240 mL non-carbonated water[b,c]**
During the first 2 h postdose vamorolone/eplerenone[a]	No food or drink.
2 h: Fludrocortisone 0.1 mg dosing	**240 mL non-carbonated water[b,c]**
4 h: Fludrocortisone 0.1 mg dosing	**240 mL non-carbonated water[b,c]**
6 h: Fludrocortisone 0.1 mg dosing	**240 mL non-carbonated water[b,c]**

Time	Food and Drink
8 h: Fludrocortisone 0.1 mg dosing	**240 mL non-carbonated water[b,c]**
10 h: Fludrocortisone 0.1 mg dosing	**240 mL non-carbonated water[b,c]**
12 h: Fludrocortisone 0.1 mg dosing	**240 mL non-carbonated water[b,c]**
14 h: Fludrocortisone 0.1 mg dosing	**240 mL non-carbonated water[b,c]**
16 h: Fludrocortisone 0.5 mg dosing	**240 mL non-carbonated water[b,c]**
Approx. 2 h postdose vamorolone/eplerenone[a]	Diet controlled breakfast
Approx. 5 h postdose vamorolone/eplerenone[a]	Diet controlled lunch
Approx. 7 h postdose vamorolone/eplerenone[a]	Diet controlled snack
Approx. 10 h postdose vamorolone/eplerenone[a]	Diet controlled dinner
Day 3	
24 h postdose vamorolone/eplerenone[a]: Fludrocortisone 0.1 mg dosing	**240 mL non-carbonated water[b,c]**
24 h to 32 h postdose vamorolone/eplerenone[a]	1.5 L non-carbonated water[b] over 8 h
32 h to 40 h postdose vamorolone/eplerenone[a]	1.0 L non-carbonated water[b] over 8 h
Approx. 2 h postdose fludrocortisone 0.1 mg	Diet controlled breakfast
Approx. 5 h postdose fludrocortisone 0.1 mg	Diet controlled lunch
Approx. 7 h postdose fludrocortisone 0.1 mg	Diet controlled snack
Approx. 10 h postdose fludrocortisone 0.1 mg	Diet controlled dinner
Day 4	
Upon discharge	Provision of packed lunch

[a] or at corresponding timepoint for negative control arm
[b] non-carbonated water of defined electrolyte composition of ambient temperature
[c] together with the study treatment(s)
Approx. = approximately; h = hour(s); min = minute(s)

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 site 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 2 hours after dosing of vamorolone / eplerenone 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 randomly 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.
- 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 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 (see 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, Randomization, Study Treatment

Not applicable.

6. Study Treatments(s) and Concomitant Therapy

Study treatments are all prespecified investigational and non-investigational medicinal products, medicinal devices, and other interventions (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

30 subjects will be randomized to 1 of 3 study arms with 10 subjects assigned to each treatment. All subjects will receive multiple doses of fludrocortisone as a challenge agent (see Table 4):

- Experimental arm: 20 mg/kg vamorolone single dose
- Positive control arm: 200 mg eplerenone single dose
- Negative control arm: no treatment
- All subjects will receive multiple doses of fludrocortisone as a challenge agent.

Table 4: Study Treatments

Arm No. / Arm Title	**1 Vamorolone**	**2 Eplerenone**	**3 No treatment**	**NA**
Arm Type	Experimental	Active comparator	Negative control	NA
Associated Treatment Labels	**Vamorolone**	**Eplerenone**	**No treatment**	**Fludrocortisone**
Treatment Description	Test drug	Positive control, comparator	Negative control	Mineralocorticoid agonist
Type	Drug	Drug	No treatment	Drug
Dose Formulation	Oral suspension	Tablet	NA	Tablet
Unit Dose Strength(s)	4% w/w (40 mg/g) vamorolone	To be described in the IMP manual	NA	To be described in the IMP manual

Arm No. / Arm Title	1 Vamorolone	2 Eplerenone	3 No treatment	NA
Dosage Level(s)	20 mg/kg single dose on Day 2	200 mg single dose on Day 2	NA, on Day 2	Day 1: 1 mg single dose. Day 2: 0.1 mg and 0.5 mg multiple doses; for detailed timepoints and the dosing schedule, see Section 4.1. Day 3: 0.1 mg single dose
Route of Administration	Oral	Oral	NA	Oral
State for Administration	After an overnight fast	After an overnight fast	NA	On Day 2 for morning dosing after an overnight fast. For more details on fludrocortisone administration, see Sections 4.1 and 5.3.1.
Dosing Instructions	20 mg/kg to be taken in the morning	To be taken in the morning	NA	To be taken at the timepoints given in Section 4.1
Manufacturer/ Marketing Authorization Holder	To be described in the IMP manual	To be described in the IMP manual	NA	To be described in the IMP manual
IMP and NIMP/AxMP	IMP	IMP	NA	AxMP
Sourcing	The Sponsor will provide Nuvisan GmbH with the medication, which will be released by the Nuvisan QP according to Good Manufacturing Practice Annex 13 for use in the study.	Nuvisan will source medication available on the local market	NA	Nuvisan will source medication available on the local market

Arm No. / Arm Title	1 Vamorolone	2 Eplerenone	3 No treatment	NA
Packaging and Labelling	Study treatment will be primary packaged in amber glass bottles by Purna Pharmaceuticals, Belgium. Each bottle will be labelled according to country requirement by Nuvisan.	Study treatment will be used as unchanged product with market authorization	NA	Study treatment will be used as unchanged product with market authorization.

AxMP=auxiliary medicinal product, NA=not applicable, IMP=investigational medicinal product.

- Subjects will receive a single oral dose of vamorolone in study arm 1 and a single oral dose of eplerenone in study arm 2 in the morning of Day 2 in fasted state.
- Prior to administration of vamorolone/eplerenone/corresponding timepoint for negative control arm on Day 2, subjects will be fasting for at least 10 hours. After administration subjects will continue fasting for 2 hours postdose. See Section 5.3.1.
- All subjects will be given fludrocortisone on Days 1, 2 and 3 at the timepoints given in Sections 4.1 and 4.1.
- For food and drink restrictions, see Table 3 and Section 5.3.1.

6.2. Preparation, Handling, Storage, and Accountability

1. The Sponsor will supply sufficient amounts of vamorolone to Nuvisan CTS Department. The Sponsor will provide administration devices (8 mL Elm Plastic oral syringes and adapters) in an appropriate quantity. Administration devices (syringes) will be used once only and disposed; details will be described in the IMP manual.
2. IMP and AxMP 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 Santhera [the Sponsor]).
3. Nuvisan will source commercial eplerenone and fludrocortisone. Details will be described in the IMP manual.

4. 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.
5. Vamorolone will be labelled study specifically by the Nuvisan CTS department before the clinical part of the study starts including final QP release as per the German clinical study labelling requirements.
6. Nuvisan CTS Department will be responsible for transfer of vamorolone and dosing devices to the site. Details will be described in an IMP manual.
7. 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.
8. All study treatment administrations will be performed in accordance with the specifications in the IMP manual, by site staff authorized by the Principal Investigator.
9. The Investigator is responsible for study treatment accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records).
10. 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.
11. Further guidance and information for the final disposition of unused study treatments are provided in the IMP manual.

6.3. Assignment to Study Treatment

Subjects will be randomized to 1 of the following 3 study arms, with 10 subjects assigned to each treatment:

- Study arm 1 (experimental arm): vamorolone
- Study arm 2 (positive control arm): eplerenone
- Study arm 3 (negative control arm): no treatment

After informed consent procedure, every subject is given a screening number. Only subjects who comply with all eligibility criteria (Sections 5.1 and 5.2) can be included into the study.

On Day 1, prior to administration of fludrocortisone the subjects enrolled will be assigned a unique 3-digits assignment number (randomization number, e.g., 101, 102, 103…/ 201, 202, 203…/ 301, 302, 303…etc.) in ascending numerical order at the study site. The randomization number encodes the subject's assignment to 1 of the 3 study arms, per the randomization schedule generated prior to the study by Nuvisan CTS Department.

In case of replacement of already randomized subjects, the replaced subject will be assigned to the study treatment as the original subject. Randomization number will be the number of the original subject plus 500. For example, the replacement for randomization number 110 will receive the randomization number 610. For details on replacement of subjects, see Section 7.2.2.

The Investigator will keep a record relating the subject identifiers (screening and randomization 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 randomly assigned to study treatment for whatever reason.

6.4. Blinding

Not applicable, as the study will be performed using an open-label design.

6.5. Study Treatment Compliance

The administration of the study treatments will be performed in the clinical unit by qualified clinical professionals of the Investigator's team. The date and time of each dose administered in the clinic will be recorded in the source documents and recorded in the eCRF.

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

A member of the study site staff other than the person administering the study treatment will confirm the study treatment dose and study subject identification at the time of dosing.

Study site personnel 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

After the end of this study, further treatment is not necessary since only healthy subjects will be included in 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 higher 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 eplerenone and fludrocortisone higher than the protocol-defined dose will be considered an overdose.

Overdose following administration of eplerenone should be treated as clinically indicated. No cases of AEs associated with overdose of eplerenone in humans have been reported. The most likely manifestation of human overdose would be anticipated to be hypotension or hyperkalemia. If symptomatic hypotension should occur, supportive treatment should be initiated. If hyperkalemia develops, standard treatment should be initiated. Eplerenone cannot be removed by hemodialysis. It has been shown to bind extensively to charcoal (eplerenone SmPC).

Overdose following administration of fludrocortisone should be treated as clinically indicated. Acute intoxications with fludrocortisone are not known. In the event of an overdose, there may be an increase in side effects, especially on the electrolyte balance. In case of significant overdose, glucocorticoid effects affecting the endocrine system and metabolism may also occur (fludrocortisone SmPC).

In the unlikely event of an overdose, the Investigator should:

1. Evaluate the subject to decide, 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 a plasma sample for PK analysis as soon as possible after the overdose intake and on further timepoints thereafter.
4. Document the quantity of the excess dose as well as the duration of the overdose in the eCRF.
5. In case of symptomatic and clinically significant, it will be reported as an AE.

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

Decisions on 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 as well as liquorice containing sweets and teas) within 2 weeks or 5 half-lives (whichever is longer) before the start of study treatment until completion of the follow-up visit, 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 may be allowed for the symptomatic treatment of AEs (e.g., headache) after consulting the Investigator when medically indicated. 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.

Additional restrictions that subjects should adhere to during the study are detailed in Section 5.3.1.

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, eplerenone and fludrocortisone.

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 PD 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

In the case of stopping rules apply, evaluable parameters can still be used for statistical analysis. 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 (SAE or severe AE) 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, within 4 hours after eplerenone, and within 4 hours after fludrocortisone 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). If there is no other situational explanation for such abnormal findings, see above, this should lead to direct study withdrawal.
- Marked ECG changes, e.g., induced by electrolyte balance disturbances like significant AV blocks, T wave changes, or QT prolongation, significant arrhythmias or extrasystoles.
- Creatinine > 1.5 x ULN, hypokalemia < 3.2 mmol/L, hyperkalemia > 6 mmol/L

- Use of non-permitted concomitant medications. However, any medications considered necessary for the subject's well-being may be given at the discretion of the Investigator.

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 10.3).

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 the 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.
- 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 the destruction 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” (prior to randomization) or “dropout” as specified in Sections 5.4 and Section 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.
- Protocol deviation judged as significant by the Investigator, including non-compliance to the required study considerations (e.g., food/diet requirements).
- Evidence of significantly impacting viral/bacterial infections.
- 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 Section 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 randomly assigned to study treatments.

The decision to replace a subject will be taken on a case-by-case basis, in agreement between the Sponsor and the Principal Investigator.

Drop-outs might be replaced if the number of evaluable subjects completing the study becomes or is expected to become less than 24 subjects in total, i.e., less than 8 subjects in each study arm.

For details regarding treatment assignment of replacement subjects, please refer to Section 6.3.

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., a 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 in one cohort with a reasonable relationship to the study treatment or study-related procedures.
- 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 SOC.
- 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

Not applicable.

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 (within 24 hours) 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 up to approximately 115 mL (approximately 65 mL for safety laboratory samples and up to 50 mL for PK 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 (Appendix 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 and 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 ICF 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/or Immunogenicity Assessments

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

8.3. Safety Assessments

The safety profile of the study treatment will be assessed through the recording, reporting and analysis of baseline medical conditions, AEs, physical examination findings, vital signs, ECGs, and clinical laboratory tests.

Collection of safety assessments will start when the subjects give informed consent and throughout the study. The Investigator will report any AEs, whether observed by the Investigator or reported by the subject; the reporting period is specified in Section 8.3.

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 by a member of the Investigator's team 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.
- Body temperature will be measured auricularly with a calibrated device.
- 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.
- Further vital signs measurements during the clinical study are at the discretion of the Investigator.

8.3.3. Electrocardiograms

- Single 12-lead ECGs will be obtained by a member of the Investigator's team at timepoints provided in the SoA (Section 1.3) using an ECG machine that automatically calculates the heart rate and measures PR(Q) interval, QRS duration, QT interval uncorrected (QT) and corrected for ventricular rate calculated according to the formula of Fridericia (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 recording 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.
- For all study arms, the ECG will be obtained on Day 2 predose, and approximately 8 and 12 hours after administration of vamorolone/eplerenone/corresponding timepoint for negative control arm, as well as on Day 3, 24 hours postdose vamorolone/eplerenone/corresponding timepoint for negative control arm, to monitor ECG alterations, e.g., induced by electrolyte imbalance.
- For study arm 1 only, the ECG will additionally be obtained in the morning of Day 2, approximately 2 hours after vamorolone administration at its expected C_{max}.

8.3.4. Clinical Safety Laboratory Tests

- Clinical laboratory assessments will be performed and analyzed at Nuvisan GmbH.
- All protocol-required laboratory assessments, as defined in Section 10.2 (list of clinical laboratory tests), must be conducted in accordance with the laboratory manual and the SoA (Section 1.3, timing and frequency of clinical laboratory tests).
- 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. Other Assessments

Alcohol breath tests, cotinine, and urine drug screens 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 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 in the eCRF, 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 the 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, or the event is otherwise explained. 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 CA 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 (IB) and the SmPCs of selected eplerenone (eplerenone SmPC) and fludrocortisone products (fludrocortisone SmPC) to be administered will be used as RSI 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. Adverse Events of Special Interest

No AEs of special interest are defined for this study.

8.5. Pharmacokinetics

- All 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 processing), and the date/time of transfer or shipment of the samples to the responsible analyst will be documented in detail.
- Blood samples will be collected for measurement of plasma concentrations of vamorolone (study arm 1; 7 blood samples) or eplerenone (study arm 2; 7 blood samples) up to 24 hours after dosing, as specified in Table 5, Table 6, and in the SoA (Section 1.3).
- 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 (see Section 1.3), will not be considered a protocol deviation. Actual time will be considered when calculating the final PK parameters.
- Samples collected for analyses of vamorolone plasma concentrations may also be used to evaluate safety or efficacy aspects related to concerns arising during or after the study.

Table 5: Pharmacokinetic Blood Collection Schedule for Vamorolone

Study Day	Time (hours postdose[a])	Volume (mL)
Day 2	0 (predose[a])	2
	1	2
	2	2
	4	2
	8	2
	12	2
Day 3	24	2
Total blood collected		14

[a]: Vamorolone administration.

Table 6: Pharmacokinetic Blood Collection Schedule for Eplerenone

Study Day	Time (hours postdose[a])	Volume (mL)
Day 2	0 (predose[a])	up to 5
	1	up to 5
	2	up to 5
	4	up to 5
	8	up to 5
	12	up to 5
Day 3	24	up to 5
Total blood collected		up to 35

[a]: Eplerenone administration.

8.5.1. Bioanalytical Methods

- Bioanalytical analyses will be performed at Nuvisan GmbH Bioanalytical Department.
- Bioanalytical analyses will be performed in accordance to the principles of GLP and GCP. The procedure for determination of concentrations of vamorolone and eplerenone 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”, specification of the applicable regulatory guidelines and archiving process will be described in the bioanalytical protocols.
- Plasma samples will be analyzed for concentrations of vamorolone and eplerenone using validated liquid chromatography-tandem mass spectrometry methods.

8.5.2. Pharmacokinetic Evaluation

- The following PK parameters will be calculated from the individual plasma concentration time data of vamorolone and eplerenone for each treatment by noncompartmental analysis using Phoenix® WinNonlin® Version 8.0 or higher.

Table 7: Non-compartmental Pharmacokinetic Parameters for Vamorolone and Eplerenone in Plasma

Main Parameters	$AUC_{0\text{-tlast}}$ $AUC_{0\text{-inf}}$ C_{max}
Additional Parameters	$t_{1/2}$ t_{max} λ_z

For definition of PK parameters, refer to Section 10.4.

- Individual PK parameters will be calculated using actual sampling times.
- For calculation of PK parameters, concentrations below the LLOQ 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 on the PK evaluation will be provided in the SAP.

8.6. Pharmacodynamics

- Urine samples will be collected for measurement of sodium and potassium in the 3 study arms, i.e., vamorolone, eplerenone and the study arm with no study treatment administered, as specified in Table 8 and in the SoA (Section 1.3).

Table 8: Urine Collection Schedule for Sodium and Potassium Measurements

Study Day	Urine Collection Period (hours postdose[a])	Aliquot Volume (mL)
Day 1	24-9 (predose[a])	up to 5
	9-0 (predose[a])	up to 5
Day 2	0-2	up to 5
	2-4	up to 5
	4-6	up to 5
	6-8	up to 5
	8-10	up to 5
	10-12	up to 5
	12-14	up to 5
	14-16	up to 5
	16-24	up to 5
Day 3	24-32	up to 5
	32-40	up to 5
	40-48	up to 5
Total urine volume		up to 70

[a]: Vamorolone/eplerenone administration/corresponding timepoint for negative control arm

- Urine will be collected from Day 1 to Day 3 as specified in Table 8. At the end of each collection interval, all subjects will be instructed to void their bladder. During each collection 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 urine sample collection, handling, storage, and shipment will be provided in a laboratory manual.

- The following data will be provided by Nuvisan Biostatistics for statistical analysis:
  - The ratio of the amount of sodium to potassium (Na/K) as well as the corresponding logarithm of the Na/K ratio in urine will be calculated for each urine collection interval of vamorolone, eplerenone, and for the study arm with no treatment administered (Section 9.3.4). To avoid negative values, ratio will be multiplied by 10 before transformation ($\log_{10}$ 10*Na/K).
  - In cases where the subject is not able to urinate and/or concentration of sodium and/or potassium is missing, the ratio will set to be missing.

8.7. Genetics

Pharmacogenetics are not evaluated in this study.

8.8. Biomarkers

Biomarkers are not evaluated in this study.

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 GmbH.

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.

Table 9: Analysis Sets

Analysis Set	Description
Full set	All subjects enrolled in the study.
Safety	All subjects assigned to study treatment and who receive at least 1 dose of study treatment. Subjects will be analyzed according to the treatment they received. This population will be used for safety analyses, if not stated otherwise
Pharmacokinetics	This set is a subset of the safety set and includes all subjects who complete the study without any or findings/events likely affecting PK. This population will be used for PK analyses, if not stated otherwise.
Pharmacodynamics	This set is a subset of the safety set and includes all subjects who complete the study without any findings/events likely affecting PD. This population will be used for PD analyses, if not stated otherwise.

PD=pharmacodynamics, PK=pharmacokinetics.

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 SOC and PT, in which the number and percentages of subjects with TEAEs and frequency of the events are reported.
- 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 analysis set, if not stated otherwise.
- Plasma concentrations and PK parameters will be listed and 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 CV, arithmetic mean, standard deviation and CV, minimum, median, maximum value, and the number of measurements.
- 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.
- Missing data will not be replaced or imputed in any way.

9.3.4. Pharmacodynamic Analyses

- All PD analyses will be performed on the PD analysis set, if not stated otherwise.
- Concentration and amount of sodium and potassium as well as the ratio of the amount of sodium to potassium (Na/K) and the corresponding logarithm of Na/K ratio will be listed and tabulated by treatment for each collection interval. The amounts of sodium and potassium will be calculated by multiplying the concentrations by the amount of urine.

- In cases where the subject is not able to urinate and/or concentration of sodium and/or potassium is missing, the sodium and potassium concentrations as well as the ratios will be set to be missing.
- The following statistics will be calculated for the concentrations: number of cases, geometric "n", geometric mean, geometric standard deviation (re-transformed standard deviation of the logarithms) and CV, arithmetic mean, standard deviation and CV, minimum, median, maximum value, and the number of measurements. For the amounts as well as the ratios, only arithmetic statistics will be provided.
- Time curves of individual and arithmetic mean $\log_{10}$ 10*Na/K ratio versus time (urinary collection interval) will be plotted by treatment.
- More details on PD analyses will be provided in the SAP.

9.3.5. Other Analyses

Not applicable.

9.4. Interim Analysis

Not applicable.

9.5. Sample Size Determination

30 subjects will be randomly assigned to study treatment such that 24 evaluable subjects complete the study, i.e., 8 subjects in each study arm. For information on subject replacement, refer to Section 7.2.2). The estimated sample size was based on sample sizes from previous studies (Nakamura and Kawaguchi, 2021).

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 GmbH will be responsible for clinical data management activities for this study. The full details of procedures for data handling will be documented in the 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 SOC 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 and 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 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 study specific 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 CA 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/favorable 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 volunteers for the study will be recruited from suitable candidates in the database of the investigational site. Interested volunteers 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-specific documentation as required.

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 EC and regulatory authorities. Data may be transferred to other countries.

Prior to the subject's enrolment in the study, the patient'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.

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.

Regarding public disclosure of study results, the sponsor will fulfil its obligations according to all applicable laws and regulations. The sponsor is interested in the publication of the results of every study it performs.

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 physically or electronically 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, Quality of Life, source documentation on AEs 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.
- All information supplied by the Sponsor in connection with this study will remain the sole property of the Sponsor and is to be considered confidential information. No confidential information will be disclosed to others without obtaining prior written consent from the Sponsor and will not be used except in the performance of this study.

10.2. Appendix 2: Clinical Laboratory Tests

- The tests detailed in Table 10 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 10: 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 (ALT)[a]	Calcium	Lactate dehydrogenase
	Albumin	Chloride	Lipase
	Alkaline phosphatase (ALP)	Cholesterol	Magnesium
	Amylase	Creatinine	Potassium
	Aspartate aminotransferase (AST)	Creatine phosphokinase (CK)[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.		

Laboratory Assessments	Parameters
Other Tests	• Urine drug screen: Amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, methamphetamines, methylenedioxymethamphetamine, methadone, opiates, tricyclic antidepressants, phencyclidine • Urine cotinine test • At screening only: ◦ TSH[d] ◦ Fibrinogen ◦ HbA1c ◦ 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. All events of ALT ≥ 3 × 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 subject.

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)

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 AEs 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 RSI 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.

Examples 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 to the PPD email address in the SAE form provided by the Sponsor. Contact details are provided 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 (maximum intensity):

Mild:

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

Moderate:

A type of AE that is usually alleviated with additional specific therapeutic treatment. 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 AE that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic treatment.

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 (IB) and/or Product Information, for marketed products (eplerenone SmPC, fludrocortisone SmPC).
- 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 AE 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 eCRF.
- 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
- Email transmission of the scanned SAE report forms is the preferred method to transmit this information to the Sponsor. Alternatively, facsimile may be used.
- In rare circumstances, notification by telephone is acceptable with a copy of the SAE data collection tool 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: Pharmacokinetic Parameters

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). $AUC_{0\text{-}inf}$ $AUC_{0\text{-}tlast}$ $+C_{last}$ / λ_z
C_{max}	Maximum observed concentration.
$t_{1/2}$	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.

10.5. Appendix 5: 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) AG
Address:	Santhera Pharmaceuticals (Switzerland) AG Hohenrainstrasse 24 4133 Pratteln Switzerland
Telephone number:	PPD
E-mail address:	PPD

10.6. Appendix 6: 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), ICH guidelines for current 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:	Steffen Haffner, MD
Function/Title:	Principal Investigator
Institution:	Nuvisan GmbH, Wegenerstrasse 13, 89231 Neu-Ulm
Address:	Nuvisan GmbH Wegenerstrasse 13 89231 Neu-Ulm Germany
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