# QSPainRelief

**Effective combinational treatment of chronic pain in individual patients, by an innovative quantitative systems pharmacology pain relief approach.**

**H2020 – 848068**

# D2.5 – Protocol for future prospective clinical trial (for the best combination)

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## Abbreviations

| | |
|---|---|
| AAC | Area Above the Curve |
| AE | Adverse Event |
| ALT | alanine aminotransferase/serum glutamic pyruvic transaminase (SGPT) |
| ANCOVA | Analysis of Covariance |
| ANOVA | Analysis of Variance |
| AST | aspartate aminotransferase/serum glutamic oxaloacetic transaminase (SGOT) |
| AUC | Area under the concentration – time curve |
| $AUC_{inf}$ | Area under the concentration – time curve from time zero to infinity |
| $AUC_{last}$ | Area under the concentration – time curve from time zero to time of last measurable concentration |
| BLQ | Below the Limit of Quantification |
| BMI | Body Mass Index |
| BP | Blood Pressure |
| bpm | beats per minute |
| BSI | Brief Symptom Inventory |
| CA | Competent authority (also CCMO) |
| CCMO | Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek |
| CHDR | Centre for Human Drug Research |
| CI | Confidence Interval |
| CK | creatine kinase |
| CL/F | Apparent total clearance following extravascular administration |
| $C_{max}$ | Maximum concentration |
| CPM | Conditioned Pain Modulation |
| CV | Coefficient of variation |
| EC | Ethics Committee (also Medical Research Ethics Committee (MREC); in Dutch: Medisch Ethische Toetsing Commissie (METC). |
| ECG | Electrocardiogram |
| EDTA | Ethylene diamine tetra-acetic acid |
| EEG | Electroencephalogram |
| EMA | European Medicines Agency |
| EU | European Union |
| GCP | Good Clinical Practice |
| ICH | International Conference on Harmonization |
| INR | International Normalized Ratio |
| ISB | In Silico Biosciences |
| i.v. | Intravenous(ly) |
| LDH | Lactate dehydrogenase |
| Max | Maximum |
| MED | Minimal erythema dose |

| | |
|---|---|
| MedDRA | Medical Dictionary for Regulatory Activities |
| min | Minutes |
| Min | Minimum |
| M3G | Morphine-3-Glucuronide |
| M6G | Morphine-6-Glucuronide |
| NONMEM | NONlinear Mixed Effects Modelling |
| SAE | Serious Adverse Event |
| SAP | Statistical Analysis Plan |
| SD | Standard Deviation |
| SEM | Standard Error of the Mean |
| SFMPQ | Short Form McGill Pain Questionnaire |
| SOC | System Organ Class |
| SOP | Standard Operating Procedure |
| SPC | Summary of Product Characteristics |
| SRT | Simple Reaction Time |
| SST | Serum Separator Tube |
| STAI | State-Trait Anxiety Inventory |
| SUSAR | Suspected Unexpected Serious Adverse Reaction |
| pEEG | Pharmaco-Electroencephalogram |
| PDT | Pain Detection Threshold |
| PTT | Pain Tolerance Threshold |
| $t_{½}$ | Terminal Elimination Half-life |
| $t_{lag}$ | Absorption lag time |
| $t_{max}$ | Time to attain $C_{max}$ |
| ULN | Upper Limit of Normal |
| VAS | Visual Analog Scale |
| VVLT | Visual Verbal Learning Test |
| WHO | World Health Organization |
| WMO | Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen. |

## Table of Contents

# 1. Executive Summary

The body of this report consists of a clinical trial protocol for the combination of morphine and nortriptyline in patients with chronic neuropathic pain.

# 2. Deliverable Report

# CLINICAL STUDY PROTOCOL

A randomized, double-blind, double dummy, placebo-controlled, two-way cross-over study to investigate the analgesic effects and CNS effects of morphine and nortriptyline in patients with chronic neuropathic pains**.**

## 2.1 SYNOPSIS

This study is a randomized, double-blind, double-dummy, placebo-controlled, two-way crossover trial designed to evaluate the analgesic efficacy and central nervous system effects of morphine and nortriptyline in patients suffering from chronic neuropathic pain.

**Rationale**

Chronic pain is one of the most prevalent and complex medical conditions in the Western world.[1] In general, around 20% of the population in Europe experiences chronic pain[2], resulting in a large socio-economic burden, including the effect on the patient's environment and the health care system. Current pain drug treatment options include opioids, anti-depressants, antiepileptics, benzodiazepines and nonsteroidal anti-inflammatory drugs (NSAIDs)**.**[3]

60% of patients treated for chronic pain responds poorly to the above-mentioned therapies.[2] Prescription of opioids, currently the most effective class of analgesics for moderate to severe chronic pain, has furthermore led to complications related to their adverse effects, including sedation, cognitive side-effects, and drug abuse liability.[4] A solution for this may be by adding a non-opioid analgesic to treatment with an opioid, which may lead to an opioid-sparing effect. For example, therapies with combinations of morphine and anti-epileptics (e.g., pregabalin), selective serotonin and norepinephrine inhibitors (e.g., duloxetine) or anti-depressants (e.g., nortriptyline), may lead to an improved balance of therapeutic benefit and adverse effects, tailored to the needs of individuals and stratified patient groups.[5]

To obtain a better understanding of combination pain therapies a consortium was established through a Horizon 2020 European Union grant: QSPainRelief (H2020-SC1-BHC-2018-2020). The consortium will investigate alternative novel drug combinations with improved analgesic- and reduced adverse effects in the context of a full translational program: from in-silico modelling based on in vitro and in vivo developed results to two healthy volunteer studies and eventually in studies with pain patients. All studies are performed by consortium members (www.qspainrelief.eu).

So far, in silico, in vitro, and preclinical research has been performed by the QSPainRelief consortium to investigate which combination therapy may provide the optimal analgesic-adverse effect profile [Data not yet published]. Data have been gathered in the QSPainRelief model. Initial discussion and results indicate that for this patient with chronic neuropathic pain study morphine (a potent μ-opioid receptor) in combination with nortriptyline (a tricyclic antidepressant) may yield improved clinical utility over either drug used as monotherapy.

Nortriptyline is a tricyclic antidepressant (TCA) widely used in the treatment of neuropathic pain conditions, including diabetic neuropathy and postherpetic neuralgia.[6a] It exerts its analgesic effects primarily through inhibition of norepinephrine reuptake, with modest serotonergic reuptake inhibition, enhancing descending inhibitory pain pathways in the central nervous system.[6b] Nortriptyline also interacts with muscarinic, histaminergic, and α1-adrenergic receptors, contributing to both therapeutic and adverse effects.[7] Clinical studies have shown nortriptyline to be effective in improving pain scores in chronic neuropathic pain and it is included in multiple international guidelines as a first-line treatment.[8a,8b] Common side effects include dry mouth, dizziness, constipation, and sedation, which may limit dose escalation in some patients.[6c]

PainCart® and NeuroCart® are two comprehensive and validated test batteries commonly used in early-phase drug studies to profile pharmacodynamic effects of (novel) drugs. PainCart® consists of an evoked pain test battery that was used previously to amongst others profile morphine, pregabalin and the opioid fentanyl.[10] NeuroCart® is a CNS test battery that is used to assess drug-induced changes in CNS functioning. The batteries have previously been used together to evaluate possible synergistic effects of two analgesics, and to determine the CNS and analgesic profile of a novel investigational drug.[11]

The aim of this first human experimental pain study, the QSPainRelief-novel-B-trial, is to investigate the analgesic effects and effects on CNS functioning of morphine and nortriptyline as an analgesic combination in patients with chronic neuropathic pains, compared to each of the two analgesics alone and to placebo.

## Objectives

The primary objectives are:

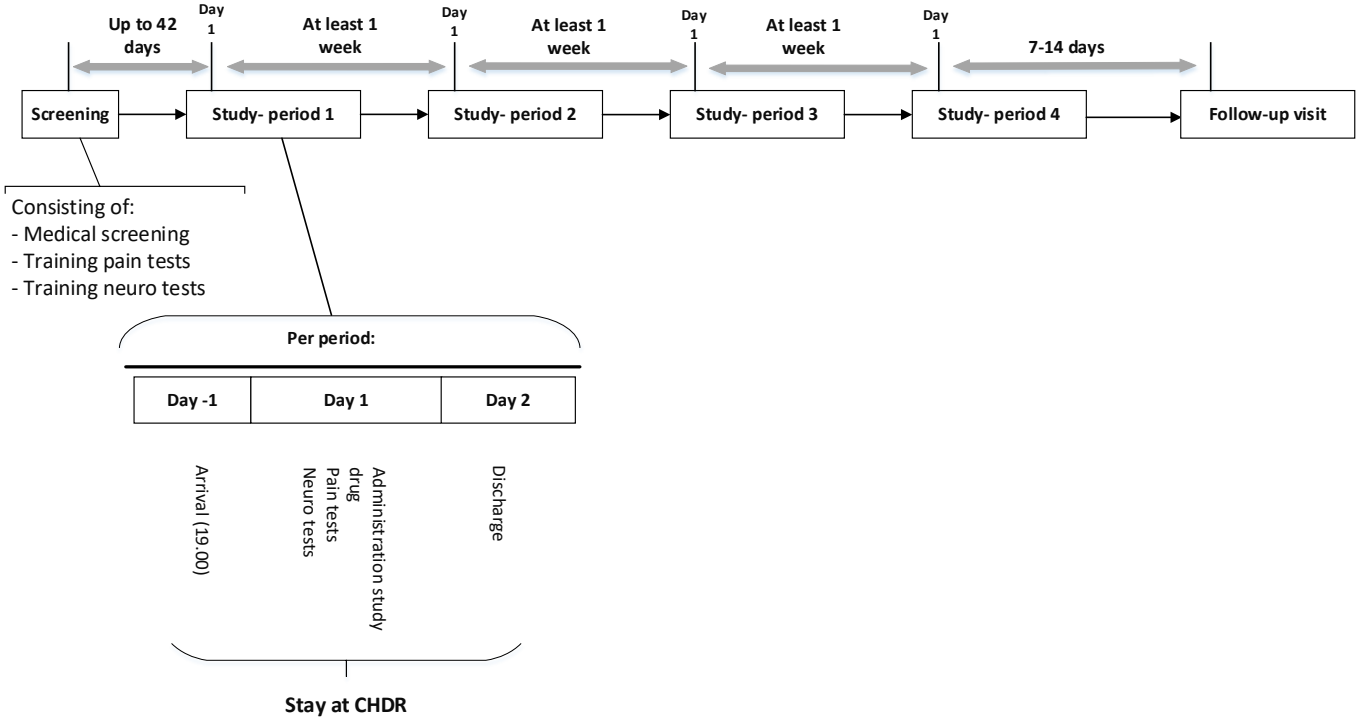
- To evaluate the analgesic effects of morphine, nortriptyline and the two drugs as combination using PainCart®

The secondary objectives are:

- To evaluate the effects on central nervous system (CNS) functioning of morphine, nortriptyline and the two drugs as combination as measured using NeuroCart®
- To compare the tolerability of morphine alone, nortriptyline alone and the two drugs when given together
- To evaluate the blood pharmacokinetics of morphine, nortriptyline and the two drugs as combination

## Trial Design

This is a randomized, double-blind, double dummy, placebo-controlled, two-way cross-over study to investigate the pharmacodynamic effects and adverse effects of morphine and nortriptyline in patients with chronic neuropathic pains.




Each study participant will receive the following study treatments:

- Morphine 3 mg IV + 7 mg IV + placebo
- Nortriptyline 75 mg orally + Morphine 3 mg IV + Morphine 7 mg IV

| Protocol time<br>Treatments | 0h00m | 2h00m | 5h00m |
|---|---|---|---|
| 1 | Placebo | Morphine 3 mg IV | Morphine 7 mg IV |
| 2 | Nortriptyline 75mg | Morphine 3 mg IV | Morphine 7 mg IV |

In total, each subject will be exposed to two treatment options. Each treatment period will consist of one study visit to the clinical research unit. During this visit, arrival to the clinical research unit will be in afternoon of day before dosing (Day-1). In the morning of the next day (Day 1), subjects will receive the study treatment prior which and after which pharmacokinetic (PK) assessments and the pharmacodynamics (PD) will be performed. Subjects will be discharged in the afternoon of Day 2. Each study period will be followed by a wash-out period of at least 7 days and a final follow-up visit 7-14 days after the last dosing day.

### Trial Population

For this study, adult male and female patients, aged between 18 and 65 years (inclusive), with a diagnosis of chronic neuropathic pain (e.g., diabetic neuropathy, postherpetic neuralgia) and an average daily pain score of ≥4 on an 11-point numerical rating scale (NRS) will be enrolled.

### Interventions

Nortriptyline 75 mg will be administered orally.
Morphine hydrochloride (HCl) 3 mg and 7mg will be administered intravenously.

## Ethical Considerations relating to the Clinical Trial including the expected Benefit to the individual Subject or Group of Patients represented by the Trial Subjects as well as the Nature and Extent of Burden and Risks

This study will provide more insight in a combination treatment for patients suffering from chronic pain and determine if the addition of nortriptyline is better than morphine alone. Morphine and nortriptyline are registered drugs which are widely used in the clinic, amongst other analgesics. The safety profiles of these compounds are well known. Both analgesic and adverse effects are likely to occur. Symptoms such as sedation, somnolence, dizziness, fatigue, reduced vigilance, are to be expected following administration for both compounds, but will be closely monitored by medically trained staff as part of the study objectives.[4]

This study will produce novel information on the clinical utility of morphine and nortriptyline when co-administered, i.e., potentiation of analgesic effects (profiled using PainCart®) and characterization of side effect profile (using CNS biomarkers and NeuroCart®).

Study subjects will undergo a medical screening and eligibility will be checked multiple times before administering the study drug. During the clinical study, administration will be done in the clinic under medical supervision. Subjects will be closely monitored and will be discharged by a physician from the unit only if their medical condition allows. In case of nausea and/or vomiting anti emetic, treatments will be administered with metoclopramide 10 mg intravenously as a single dose, which can be repeated if nausea or vomiting persists.

At doses above 3 mg, morphine may decrease respiration in dose-dependent manner.[12] While the dose of 7mg be administered are expected to be manageable. The total maximum dose of morphine IV on a single day is expected to provide adequate analgesic properties and measurable effects on neurocognitive tests, while having manageable adverse effects. As an additional safety measure, oxygen saturation will be measured after the first morphine administration at fixed timepoints as indicated in the Schedule of Assessments, and additionally when deemed necessary.

The measurement techniques performed in this study (PainCart® and CNS tests) are considered to be safe and with minimal risk. Capsaicin application on the volar forearm may cause some transient skin irritation on the application site. The skin may become red and/or more sensitive to e.g., warm water, and to touch (a slight burning sensation). After one or two days the effects will generally resolve.

***Table 1.*** *Visit and Assessment Schedule*

| | SCR | Treatment period 1 - 4[1] | | | | | | | | | | | | | | FU[2] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SCR | Day -1 | Day 1 | | | | | | | | | | | | Day 2 | |
| Time point<br>Assessment | Up to<br>-42 d to -5 days | Day -1 | Pre-dose | 0 h | 1h | 2 h | 3 h | 3.5 h | 4 h | 5 h | 6 h | 6.5 h | 7.5 h | 8 h | 9 h | 24 h | +14 d<br>± 2 d |
| Informed consent | X | | | | | | | | | | | | | | | | |
| Demography | X | | | | | | | | | | | | | | | | |
| Inclusion and exclusion criteria | X | | X | | | | | | | | | | | | | | |
| Medical history | X | | | | | | | | | | | | | | | | |
| Height, Weight[3], BMI | X | | | | | | | | | | | | | | | | X |
| Admission to clinic | | X | | | | | | | | | | | | | | | |
| Physical examination[4] | X | | X[5] | | | | | | | | | | | | | | X |
| Virology | X | | | | | | | | | | | | | | | | |
| BsHaem, BsChem, BsGluc, Urinalysis | X | X | | | | | | | | | | | | | | | X |
| UrDrug, BrAlc, UrPreg[6] | X | X | | | | | | | | | | | | | | | X |
| Temperature | X | X | X | | | | | | | | | | | | | X | X |
| ECG | X | | X | | | | | | | | | | | X | | X | X |
| General symptoms | X | | X | | X | X | | | | X | | | | X | | X | |
| Vital Signs (Pulse Rate, BP, RR, $O_2$ saturation)[7] | | | X | | | | | | | X | | X | | X | | X | X |
| Meals / snack[8] | | | X[8] | | X | | | | X | | | | | X | X | X | |
| Drug (-placebo) administration | | | | X | | X | | | | X | | | | | | | |
| PK sample | | | X | | X | X[9] | | | X | X[9] | X | | | X | | X | |
| Capsaicin application | X[10] | | X[11] | | | | | | | | | | | | | | |
| Capsaicin task – Von Frey test[11] | | | X | | X | | X | | X | | X | | X | | X | | |
| PainCart®[12,13] | X[14] | | X[15] | | X | | X | | X | | X | | X | | X | | |
| NeuroCart®[12] | X[14] | | X[15] | | X | X | | X | | X | | X | | | X | | |
| pEEG | | | X[16] | | | X | | | | X | | X | | | X | | |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| VVLT[17] | X | | | | | X | | | | X | | | | | | | |
| STAI | X | | X | | X | X | | X | | X | | X | | | X | | |
| BSI | | X | | | | | | | | | | | | | X | | |
| Discharge | X | | | | | | | | | | | | | | | X[18] | |
| (S)AE | | continuous | | | | | | | | | | | | | | | X |
| Concomitant medication | X | continuous | | | | | | | | | | | | | | | X |

BP = Blood Pressure, HR = Heart Rate, RR = Respiratory Rate, SCR = Screening, EEG = Electroencephalogram, PK = Pharmacokinetics, AE = Adverse Event, (S)AE = Serious Adverse Event, UrDrug = Urine Drug Screen, UrPreg = Urine Pregnancy test, BsHaem = Blood Sample Haematology, BsChem = Blood Sample Chemistry, BsGluc = Blood Sample Glucose, BsBiomarker = Blood Sample Biomarker, BrAlc = Breath Alcohol Test., BMI = Body Mass Index

1. At least 7 days between treatment periods.
2. Follow up (FU) visit after last treatment.
3. Only weight will be measured at FU or upon early termination.
4. Physical examination at screening includes assessment of the Fitzpatrick skin type and evaluation of acne, freckles, tattoos or scarring on the back.
5. A symptoms based physical examination will be performed.
6. For women of childbearing potential: Urine beta HCG will be performed either during screening, on the first day of each treatment period or the day before the first day of each Treatment Period at discretion of Investigator. In either case, the results must be negative prior to dosing study participants.
7. Vital signs will be measured in supine position. Oxygen saturation will be monitored after dosing at 2h until the end of study day 1 of each treatment period.
8. Lunch at 0.5h and dinner 8h post dose, snack at 4.5h, time points are indicative. Light breakfast on Day 1 is shortly after wake activity around -3.5hours, after breakfast the subject starts fasting until after dosing. No meal restrictions on day -1 and day 2.
9. PK sample taken 3 minutes after dosing.
10. Capsaicin application at screening must be performed on or before Day -5 to ensure sufficient washout between the capsaicin that is applied at screening and applied on Day 1.
11. Capsaicin application will only be performed after enrolment in subjects not allergic or intolerable to the capsaicin solution, as determined at screening.
12. Sequence of assessments is NeuroCart® before PainCart®, for specific order of assessments see section 6.4.
13. Capsaicin heat pain test (and heat pain test on control skin on volar forearm) will only be performed on subjects not allergic or intolerable to the capsaicin solution. Capsaicin heat pain task only applicable for Day 1.
14. PainCart® training up to 42 days before dosing and NeuroCart® training up to 21 days before dosing.
15. PainCart® and NeuroCart® will be done twice for baseline measurements.
16. NeuroCart® round with pEEG measurement only in second baseline measurement.
17. VVLT at 2u post dose will be measured is the immediate recall. VVLT at 5u post dose will be the delayed recall and recognition.
18. Discharge time is approximate and only when deemed safe.

## 2.2 INTRODUCTION AND RATIONALE

### 2.2.1 Context

Chronic pain is one of the most prevalent and complex medical conditions in the Western world.[1] In general, around 20% of the population in Europe experiences chronic pain[2], resulting in a large socio-economic burden, including the effect on the patient's environment and the health care system. Current pain drug treatment options include opioids, anti-depressants, antiepileptics, benzodiazepines and nonsteroidal anti-inflammatory drugs (NSAIDs)**.**[3]

60% of patients treated for chronic pain responds poorly to the above-mentioned therapies.[2] Prescription of opioids, currently the most effective class of analgesics for moderate to severe chronic pain, has furthermore led to complications related to their adverse effects, including sedation, cognitive side-effects, and drug abuse liability.[4] A solution for this may be by adding a non-opioid analgesic to treatment with an opioid, which may lead to an opioid-sparing effect. For example, therapies with combinations of morphine and anti-epileptics (e.g., pregabalin), selective serotonin reuptake inhibitor (e.g., fluvoxamine) or anti-depressants (e.g., amitriptyline), may lead to an improved balance of therapeutic benefit and adverse effects, tailored to the needs of individuals and stratified patient groups.[5]

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So far, in-silico, in-vitro, and preclinical research has been performed by the QSPainRelief consortium to investigate which combination therapy may provide the optimal analgesic-adverse effect profile. [Data not yet published] Data have been gathered in the QSPainRelief model. Results indicate that for this patient with chronic neuropathic pain study morphine (a potent μ-opioid receptor) in combination with nortriptyline (a tricyclic antidepressant) may yield improved clinical utility over either drug used as monotherapy.

Nortriptyline is a tricyclic antidepressant (TCA) widely used for neuropathic pain conditions such as diabetic neuropathy and postherpetic neuralgia. Its analgesic effects are primarily attributed to potent inhibition of norepinephrine reuptake and moderate inhibition of serotonin reuptake.[6a,6b] This enhances descending pain inhibitory pathways within the CNS.

Combination analgesic strategies involving opioids and tricyclic antidepressants may offer superior pain relief with a reduced opioid burden. Nortriptyline is a well-established treatment for neuropathic pain and, when used in combination with opioids like morphine, may produce additive or synergistic effects on analgesia.

PainCart® and NeuroCart® are two comprehensive and validated test batteries commonly used in early-phase drug studies to profile pharmacodynamic effects of (novel) drugs. PainCart® consists of an evoked pain test battery that was used previously to amongst others profile morphine, pregabalin and the opioid fentanyl.[10] NeuroCart® is a CNS test battery that is used to assess drug-induced changes in CNS functioning. The batteries have previously been used together to evaluate possible synergistic effects of two analgesics, and to determine the CNS and analgesic profile of a novel investigational drug.[11]

The aim of this experimental pain study is to investigate the analgesic effects and effects on CNS functioning of morphine and nortriptyline as an analgesic combination in patients with chronic neuropathic pains, compared to each of the two analgesics alone and to placebo. This trial aims to characterize these effects in a patient population rather than in healthy volunteers.

### 2.2.2 Clinical information

## Clinical pharmacology

*Nortriptyline*

Nortriptyline is a tricyclic antidepressant (TCA) that is widely used in the treatment of neuropathic pain. Its analgesic effects are primarily mediated through potent inhibition of norepinephrine reuptake and modest inhibition of serotonin reuptake, which enhances descending inhibitory pain pathways in the spinal cord and brain. Nortriptyline also acts as an antagonist at muscarinic cholinergic, histamine H1, and alpha-1 adrenergic receptors, contributing to both its therapeutic effects and side effects profile. Although nortriptyline does not bind directly to opioid receptors, preclinical and clinical studies support its use in combination with opioids like morphine, potentially offering additive or synergistic analgesia. Its pharmacokinetics involve extensive hepatic metabolism, primarily by CYP2D6, with a typical elimination half-life of 18–44 hours, supporting once-daily dosing.

In addition to its reuptake inhibition, nortriptyline antagonizes muscarinic cholinergic, histamine H1, and alpha-1 adrenergic receptors, which contributes to both its analgesic efficacy and side effect profile, including sedation and anticholinergic effects.[7] It does not act on opioid receptors and does not have known activity at the sigma-1 receptor.

Nortriptyline is rapidly absorbed after oral administration and undergoes extensive first-pass hepatic metabolism, primarily via cytochrome P450 2D6 (CYP2D6).[25b] The absolute bioavailability is approximately 45-60%, with peak plasma concentrations occurring within 2 to 8 hours.

The exact mechanisms by which nortriptyline exerts its analgesic effects are not yet fully understood. However, its ability to modulate serotonin and other neurotransmitters involved in pain processing suggest a multifaceted approach to pain relief.

*Morphine*

The active substance, morphine is an agonist for the μ opioid receptor, a weak agonist for the κ opioid receptor, and can be administered orally, intravenously, or subcutaneously.[13] Morphine is obtained from opium, which acts mainly on the central nervous system and smooth muscle. Morphine is a potent analgesic with competitive agonist actions for the μ-receptor, which is thought to mediate many of its other actions of respiratory depression, euphoria, inhibition of gut motility and physical dependence. It is possible that analgesia, euphoria and dependence may be due to the effects of morphine on a μ-1 receptor subtype, while respiratory depression and inhibition of gut motility may be due to actions on a μ-2 receptor subtype. Morphine is also a competitive agonist at the κ-receptor that mediates spinal analgesia, miosis and sedation. Morphine has no significant actions at the other two major opioid receptors, the δ- and the σ-receptors.

Morphine directly suppresses cough by an effect on the cough centre in the medulla. Morphine also produces nausea and vomiting by directly stimulating the chemoreceptor trigger zone in the area postrema of the medulla. Morphine is also able to provoke the release of histamine (SPC morphine, section 5.1).

Morphine has a few active metabolites, with variable analgesic effects, of which Morphine-6-Gluceronide (M6G) is one. M6G is a strong μ-receptor agonist with higher affinity than morphine itself. It has been suggested that M6G contributes to the analgesic effect after administration of morphine.[14]

**Clinical pharmacokinetics and metabolism**

*Nortriptyline*

Nortriptyline is approximately 92% bound to plasma proteins and has a large volume of distribution (20–40 L/kg), indicating wide tissue distribution. It exhibits a half-life of 18 to 44 hours depending on metabolic phenotype (extensive vs. poor metabolizers).[25a,25b]

Metabolism occurs primarily through demethylation to E-10-hydroxynortriptyline and Z-10-hydroxynortriptyline. These hydroxylated metabolites are less active but may contribute modestly to the therapeutic effect. The drug and its metabolites are primarily excreted renally.[25b]

Nortriptyline exhibits linear pharmacokinetics within the therapeutic range. Steady-state concentrations are typically achieved within 4 to 5 days, and dosing is often titrated slowly to mitigate side effects, especially in older adults.[25b]

Nortriptyline does not act on opioid receptors. Instead, it is a potent norepinephrine reuptake inhibitor with secondary serotonin reuptake inhibition.[6a,7] Its analgesic effects stem from enhancement of descending inhibitory pain pathways and receptor antagonism at cholinergic, histamine, and adrenergic receptors.[7]

*Morphine*

Morphine, which is a substrate for membrane efflux- transporter-P glycoprotein is almost 100% absorbed from the gastrointestinal tract after oral administration. Morphine distribution can be described by either one -, two-, and three – compartment models. The mean bioavailability is 20-30% after oral administration and is considered to be low due to hepatic first pass- metabolism. Several studies have been examining the pharmacokinetic profile of (intravenous) morphine. In one of the studies, Hasselstrom et al 10,13 studied the pharmacokinetics properties in 7 patient with chronic neuropathic pains after single intravenous 5 mg and oral 20mg doses over 72h period. Systemic plasma clearance of morphine was on average 21.1 ± 3.4 ml/min/kg (1.27 ± 0.20 L/h/kg), M6G clearance was 3.4 ± 1.5 ml/min/kg, volume of distribution for morphine was 2.9 ± 0.8 L/kg and oral bioavailability was 29.2 ± 7.2%. A slowly declining terminal phase of morphine was observed in plasma and urinary excretion. The half-life of morphine is 15.1h. The half-life of M6G was 12.9h. Comparison of oral with intravenous excretion curves of morphine indicated that a greater part of morphine and metabolites were excreted during the slowly declining phase after the oral dose than was observed with the intravenous dose, which is highly suggestive of enterohepatic cycling. 15

Only few covariates have been identified for morphine PK, but in small studies with homogenous populations it can be difficult to find significant covariate relationships, and it can therefore not be excluded that other covariate relationships exist for morphine. Concerning the difference in pharmacodynamic effects between male and female a previous study suggests no significant difference in analgesic effect between male and female. [16]

Morphine is rapidly metabolized in the liver into the (presumably) inactive morphine-3-glucuronide (M3G) and the active M6G (see section 1.3.1) Martini et al17. Martini et al constructed a PK-PD model of morphine and M6G using data obtained from patient with chronic neuropathic pains that were injected with either intravenous morphine or intravenous M6G, with which they could determine M6G's contribution to morphine analgesia 18. Fraction M6G metabolized from morphine was 6.0 ± 0.2% (median value ± standard error); M6G formation was sex independent. 17

## Clinical toxicology and safety pharmacology

*Nortriptyline*

Nortriptyline, an antidepressant medication belonging to the tricyclic antidepressant (TCA) class, exhibits its pharmacological effects by inhibiting the reuptake of norepinephrine and serotonin in the brain. Clinical studies have extensively studied the toxicology profile of nortriptyline. Similarly, to other TCAs, the most commonly include general malaise, excessive sweating, nausea, vomiting, constipation, diarrhoea, reduced appetite, dizziness, headache, somnolence and tremor. Additionally, acting as a psychiatric medication, patients may also feel increased agitation, fear, insomnia and anxiety. Monitoring regarding these potential side effects are therefore crucial to ensure participant safety.

*Morphine*

Morphine appears to mimic the body's endorphins that are responsible for analgesia causing sleepiness, and feelings of pleasure. Morphine is a phenanthrene opioid receptor agonist which binds to and activates opioid receptors in the central nervous system and is studied extensively in clinical studies over the years. The most serious and very common side effect of morphine therapy is respiratory depression. Other very common side-effects are nausea, vomiting, constipation, drowsiness and dizziness. Tolerance generally develops with long term use; however this is not the case for constipation. (SPC morphine, section 4.8). Similar effects are observed in studies conducted in healthy volunteers, where the most frequently reported adverse effects were respiratory depression, constipation, sedation, dizziness, and nausea.[19,20]

*Drug dependence and withdrawal (abstinence) syndrome*

Use of opioid analgesics may be associated with the development of physical and/or psychological dependence or tolerance. An abstinence syndrome may be precipitated when opioid administration is suddenly discontinued or opioid antagonists administered or can sometimes be experienced between doses.

Physiological withdrawal symptoms include: body aches, tremors, restless legs syndrome, diarrhoea, abdominal colic, nausea, flu-like symptoms, tachycardia and mydriasis. Psychological symptoms include dysphoric mood, anxiety, and irritability. In drug dependence, “drug craving” is often involved.

The euphoric activity of morphine has led to its abuse (see SPC morphine, section 4.8)

## 2.3 STRUCTURED RISK ANALYSIS

### 2.3.1 Potential issues of concern

Please refer to subsection 2.2.2

***Level of knowledge about mechanism of action***

Please refer to Subsections 2.2.2

***Previous exposure of human beings***

Please refer to Subsections 2.2.2

***Selectivity of the mechanism***

Please refer to Subsections 2.2.2

***Analysis of potential effect***

Please refer to Subsections 2.2.2

***Pharmacokinetic considerations***

Please refer to Subsections 2.2.2

***Predictability of effect***

Please refer to section 2.4

***Interaction with other products***

Please refer to Subsections 2.2.2

***Managing of effects***

Cardiovascular and respiratory safety will be managed by frequently measuring the HR, BP, RR, and oxygen saturation (only on clinical indication) of the subjects. Adequate medical support will be provided in case of emergencies. All subjects must be using appropriate methods of birth control to avoid pregnancy in female partners of female participants, as applicable – please refer to Subsection 8.2.1 for details. Naloxone will be available in the emergency equipment at the CRU, and emergency care (of the Leiden University Medical Centre [LUMC], Leiden) is available within 5 min – in case this is needed. For other potential risks as described in this section, adequate measures to avoid or minimize the risks have been implemented (refer to Visit and Schedule of Assessment for measures that are performed during the study).

***Study population***

For this study, adult male and female patients, aged between 18 and 65 years (inclusive), with a diagnosis of chronic neuropathic pain (e.g., diabetic neuropathy, postherpetic neuralgia) and an average daily pain score of ≥4 on an 11-point numerical rating scale (NRS) will be enrolled. This population is representative of individuals for whom novel combination analgesics may be appropriate.

### 2.3.2 Overall synthesis of the direct risks for the research subjects

This study will provide more insight in a combination treatment for patients suffering from chronic pain. Morphine and nortriptyline are registered drugs which are widely used in the clinic, amongst other analgesics. The safety profiles of these compounds are well known. Both analgesic and adverse effects are likely to occur. Symptoms such as sedation, somnolence, dizziness, fatigue, reduced vigilance, are to be expected following administration for both compounds, but will be closely monitored by medically trained staff as part of the study objectives.[4]

Combination therapy of nortriptyline and morphine has, to the authors knowledge, not been examined in patients with chronic neuropathic pains before. However, the combination of morphine and amitriptyline, a close analogue of nortriptyline, has been studied in cancer neuropathic pain patient populations and has shown additive or synergistic analgesic effects.[21] 16 patients on "systemic morphine therapy, no longer receiving oncologic treatment, presenting moderate pain (about 4 or more, but less than 7, on a numerical scale of 0-10) in the last week, and given a stable morphine dose in the last 2 days were admitted to the study. During the first week of study, patients were administered 25 mg of amitriptyline or equivalent drops of placebo at night for 3 days and 50 mg for the following 4 days." The combination of morphine and amitriptyline showed a significant improvement for the worst pain. As a result, this combination could contribute to a reduction in adverse effects and an overall improvement in the patients' quality of life.

The benefit of performing this study while the above has been completed, is that the data are utilized in context of a full translational program to develop opioid-sparing therapies (i.e., QSPainRelief model), which is a different objective than the above study. This study will produce novel information on the clinical utility of morphine and nortriptyline when co-administered, i.e. potentiation of analgesic effects (profiled using PainCart®) and characterization of side effect profile (using CNS biomarkers and NeuroCart®).

Study subjects will undergo a medical screening and eligibility will be checked multiple times before administering the study drug. During the clinical study, administration will be done in the clinic under medical supervision. Subjects will be closely monitored and will be discharged by a physician from the unit only if their medical condition allows. In case of nausea and/or vomiting anti emetic, treatments will be administered with metoclopramide 10 mg intravenously in a single dose, which can be repeated if nausea or vomiting persists.

After administration of morphine and nortriptyline, it is not advised to drive a car or operate machinery for 48 hours after drug administration. Therefore, subjects participating in the study will be instructed not to drive a car and not to engage in activities that require operating vehicles or dangerous machinery for up to 48 hours following administration of the study drug. Thus, the subjects will remain in the clinic under supervision and will be discharged by a physician only if their medical condition allows.

At doses above 3 mg, morphine may decrease respiration in a dose-dependent manner.[12] While the doses be administered are expected to be manageable. The total maximum dose of morphine IV on a single day is expected to provide adequate analgesic properties and measurable effects on neurocognitive tests, while having manageable adverse effects. As an additional safety measure, oxygen saturation will be measured after the first morphine administration at fixed timepoints as indicated in the Schedule of Assessments, and additionally when deemed necessary. The measurement techniques performed in this study (PainCart® and CNS tests) are considered to be safe and with minimal risk. Capsaicin application on the volar forearm may cause some transient skin irritation on the application site (only for eligible subjects). The skin may become red and/or more sensitive to e.g., warm water, and to touch (a slight burning sensation). After one or two days the effects will generally resolve.

## 2.4 OBJECTIVES AND ENDPOINTS

***Table 2.*** *Objectives and Endpoints*

| **Objectives** | **Endpoints** |
|---|---|
| Primary | |
| • To evaluate the analgesic effects of morphine, fluvoxamine and the two drugs as combination using PainCart | 1. Pressure Pain: Pain Detection Threshold (PDT), Pain Tolerance Threshold (PTT), Area Under the Curve (AUC), post-test Visual Analogue Scale (VAS)<br>2. Heat pain (pre-cold pressor: unexposed/normal and capsaicin-exposed skin) (only for eligible subjects): PDT, and post-test VAS<br>3. Cold Pressor: PDT, PTT, Area Above the Curve (AAC), post-test VAS<br>4. Electrical Stair test: PDT, PTT, AUC, post-test VAS<br>5. Electrical burst test: PDT, PTT, AUC, post-test VAS<br>6. Conditioned Pain Modulation (CPM) Response (change from heat pain pre- and post-cold pressor): PDT<br>7. Short Form McGill Pain Questionnaire (SFMPQ) for pressure pain, heat pain, cold pressor, electrical stair test and electrical burst test |
| Secondary | |
| • To evaluate the drug-sensitive central nervous system (CNS) functioning of morphine, fluvoxamine and the two drugs as combination by biomarker profiling and the NeuroCart | 1. Body sway:<br>o antero-posterior sway (mm)<br>2. Visual Analog Scales (VAS) according to Bond and Lader to assess:<br>o mood (mm),<br>o alertness (mm), and<br>o calmness (mm)<br>3. Visual Analog Scales (VAS) according to Bowdle to assess:<br>o Feeling high (mm)<br>o Internal perception (mm)<br>o External perception (mm)<br>4. N-back<br>o Average reaction time (ms) (zero-, one-, two-back)<br>o Number of correct targets (zero-, one-, two-back)<br>o Number of incorrect targets (zero-, one-, two-back)<br>o Number of faulty non-target responses (zero-, one-, two-back)<br>5. Saccadic Eye Movement |

| | |
|---|---|
| | ○ Peak velocity (degrees/sec)<br>○ Reaction time (sec)<br>○ Inaccuracy (%)<br>6. Smooth pursuit<br>○ Smooth pursuit (%)<br>7. Pupillometry<br>○ Left and right, pupil/iris ratio<br>8. Adaptive Tracking<br>○ Average performance (%)<br>9. Visual Verbal Learning Test (VVLT) memory testing<br>○ Immediate recall trial 3 (number correct)<br>○ Delayed recall (number correct)<br>○ Delayed recognition (number correct)<br>○ Delayed recognition (reaction time correct) (msec)<br>10. Electroencephalography<br>○ Frequency ranges for spectral analysis, Delta, Theta, Alpha, Beta, Gamma<br>11. Simple Reaction Time Task (SRT)<br>○ Reaction time (ms)<br>**Questionnaires:**<br>12. State-Trait Anxiety Inventory (STAI)<br>○ State anxiety score<br>13. Brief Symptom Inventory (BSI)<br>○ General somatic symptoms<br>○ Cognitive symptoms<br>○ Interpersonal sensitivity<br>○ Depressed mood<br>○ Anxiety<br>○ Hostility<br>○ Phobic anxiety<br>○ Paranoid thoughts<br>○ Psychoticism<br>○ Global severity index |
| • To evaluate the blood pharmacokinetic parameters of morphine, fluvoxamine and the two drugs as combination | PK parameters of morphine (and metabolite morphine-6-glucuronide), fluvoxamine by noncompartmental analysis of the plasma concentration-time data: AUCinf, AUClast, CL(/F), Cmax, t1/2, tlag, tmax, Vz(/F) |
| • To evaluate the safety and tolerability of morphine, fluvoxamine and the two drugs as combination | 1. Treatment-emergent (serious) adverse events ((S)AEs) throughout the study at every study visit<br>2. Concomitant medication throughout the study at every study visit |

| | |
|---|---|
| | 3. Vital signs (Pulse Rate (bpm), Systolic blood pressure (mmHg), Oxygen saturation, Diastolic blood pressure (mmHg)) as per assessment schedule<br>4. Clinical laboratory tests (Hematology, blood chemistry, glucose, and urinalysis) as per assessment schedule<br>5. ECG parameters (Heart Rate (HR) (bpm), PR, QRS, QT, QTcF) as per assessment schedule |

Baseline for PD measurements is defined as the **average** value prior to dosing in the morning of Day 1 for each treatment period.

## 2.5 STUDY PLAN AND DESIGN

### 2.5.1 Trial design

This is a single centre, double blind, double dummy, placebo-controlled, study to investigate the effect of morphine and nortriptyline on pain thresholds and cognitive functioning. The overview of all assessments including time schedule is provided in Table 1.

*Screening*

The Screening Visit may be conducted anytime up to 42 days prior to Baseline/admission to CHDR. The screening phase will only be started after full written, verbal and signed informed consent has been obtained, according to CHDR standard operating procedures. A full medical screening will be performed to assess a subject's eligibility for this study. The overview of the assessments of this visit is provided in the study flow chart (Table 1).

*Re-screening*

It is permitted to re-screen subjects, if the reason for non-eligibility is considered transient to the investigator's opinion. During a re-screening, only assessments that are susceptible to change within the timeframe between screenings are repeated (e.g. medical history, demographics, virology are not repeated).

In addition, retests are permitted in case false-positives are expected and adequately documented by the investigator (e.g. positive drug screen due to accidental poppy seed consumption).

*Treatment and observation period*

The overview of all assessments including time schedule is provided in the study flow chart (Table 1). Subjects will present to the CRU in the afternoon of Day -1 (i.e., the day before the study treatment administration) for baseline procedures conducted prior to dosing. Baseline procedures will be performed prior to dosing and study procedures after dosing. Subjects will be discharged in the afternoon of Day 2, approximately 24 hours post-dose.

*Follow-up*

Follow-up will occur between 7 and 14 days after the last study drug administration. The overview of the assessments of this visit is provided in the study flow chart (Table 1). End of Study (EOS) is defined as the last visit of the last subject. Upon early termination, the follow-up visit will also be performed between 7 and 14 days after the last study drug administration.

### 2.5.2 Number of patients

A total of 24 subjects is planned to be enrolled. All subjects will participate in four study occasions.

### 2.5.3 Overall study duration and follow-up

The total duration of the study for each subject will be approximately 82 days divided as follows:

1. Screening: Up to 42 days before dosing;
2. Four identical In Clinic periods; each: Days -1 to 2; washout of at least 7 days between each dosing;
3. Follow-up visit: 7 to 10 days after last dose.

Subjects will be admitted to the study unit on Day -1 and will be discharged in the afternoon of day 2.

## 2.6 STUDY POPULATION

### 2.6.1 Population

For this study, a total of 24 patients with chronic neuropathic pains, male and female subjects, aged between 18 and 65 years of age (inclusive) will be enrolled.
Subject recruitment to be determined.

### 2.6.2 Inclusion criteria

1. Patient with chronic neuropathic pains, 18 to 65 years of age, inclusive. The patient's ability to be included will be determined following a detailed medical and surgical history, a complete physical examination including vital signs, 12-lead ECG, haematology, blood chemistry, and urinalysis.
2. Current treatment of their chronic pain with an opioidergic-acting drug (along with possible other drugs).
3. Body mass index (BMI) between 18 and 30 kg/m2, inclusive, and with a minimum weight of 50 kg and a maximum weight of 100 kg.
4. Able to participate and willing to give written informed consent and to comply with the study restrictions.
5. Normal laboratory assessment of renal and hepatic function (assessed using available clinical biological tests).

### 2.6.3 Exclusion criteria

1. Evidence of any active or chronic disease or condition that could interfere with, or for which the treatment of might interfere with, the conduct of the study, or that would pose an unacceptable risk to the subject in the opinion of the investigator (following a detailed medical history, physical examination, vital signs (systolic and diastolic blood pressure, pulse rate, body temperature) and 12-lead electrocardiogram (ECG)). Minor deviations from the normal range may be accepted, if judged by the Investigator to have no clinical relevance.
2. Clinically significant abnormalities, as judged by the investigator, in laboratory test results (including hepatic and renal panels, complete blood count, chemistry panel and urinalysis). In the case of uncertain or questionable results, tests performed during screening may be repeated before randomization to confirm eligibility or judged to be clinically irrelevant for patient with chronic neuropathic pains.
3. Positive Hepatitis B surface antigen (HBsAg), Hepatitis C antibody (HCV Ab), or human immunodeficiency virus antibody (HIV Ab) at screening.
4. Systolic blood pressure (SBP) greater than 140 or less than 90 mm Hg, and diastolic blood pressure (DBP) greater than 90 or less than 50 mm Hg at screening.
5. Abnormal findings in the resting ECG at screening defined as:
    a. QTcF> 450 or < 300 msec for men and QTcF> 470 or < 300 msec for women;
    b. Notable resting bradycardia (HR < 45 bpm) or tachycardia (HR > 100 bpm);
    c. Personal or family history of congenital long QT syndrome or sudden death;
    d. ECG with QRS and/or T wave judged to be unfavourable for a consistently accurate QT measurement (e.g., neuromuscular artefact that cannot be readily eliminated, arrhythmias, indistinct QRS onset, low amplitude T wave, merged T- and U-waves, prominent U waves);

e. Evidence of atrial fibrillation, atrial flutter, complete branch block, Wolf-Parkinson-White Syndrome, or cardiac pacemaker.

6. Use of any medications (prescription or over-the-counter [OTC]), within 14 days of study drug administration, or less than 5 half-lives (whichever is longer). Exceptions are paracetamol (up to 4 g/day) and ibuprofen (up to 1g/day), which are allowed up to 2 days before study drug administration and screening period. Other exceptions will only be made if the rationale is clearly documented by the investigator.
7. Planned chemotherapy, hormonotherapy or radiotherapy within 1-week of the testing.
8. Diabetes mellitus, liver disease, kidney disease, hypo- or hyperthyroidism
9. Use of any vitamin, mineral, herbal, and dietary supplements within 7 days of study drug administration, or less than 5 half-lives (whichever is longer). Exceptions will only be made if the rationale is clearly documented by the investigator.
10. Participation in an investigational drug or device study (last dosing of previous study was within 90 days prior to first dosing of this study).
11. History of abuse of addictive substances (alcohol, illegal substances) or current use of more than 21 units (for males) or 14 units (for females) of alcohol per week, drug abuse, or regular user of sedatives, hypnotics, tranquilizers, or any other addictive agent
12. Positive test for drugs of abuse at screening or pre-dose.
13. Alcohol will not be allowed from at least 48 hours before screening or each admission.
14. Current use of tobacco or nicotine products and unable to abstain from use of these products within the previous 3 months before the first dose administration.
15. Is demonstrating excess in caffeine consumption (more than eight cups of coffee or equivalent per day).
16. Any confirmed significant allergic reactions (urticaria or anaphylaxis) against any drug, or multiple drug allergies (non-active hay fever is acceptable).
17. Loss or donation of blood over 500 mL within three months (males) or four months (females) prior to screening or intention to donate blood or blood products during the study.
18. If a woman, pregnant, or breast-feeding, or planning to become pregnant during the study.
19. Not willing to practice effective contraception during the study and not willing and able to continue contraception for at least 90 days after their last dose of study treatment.
20. Any known factor, condition, or disease that might interfere with treatment compliance, study conduct ECG or interpretation of the results such as drug or alcohol dependence or psychiatric disease.
21. Known hypersensitivity to the investigational drug or comparative drug or drugs of the same class, or any of their excipients.
22. Fitzpatrick skin type IV, V and VI, wide-spread acne, tattoos or scarring interfering with the area of interest (i.e., volar forearm).
23. Any current, clinically significant, known medical condition in particular any existing conditions that could have affected sensitivity to cold (such as atherosclerosis, Raynaud's disease, urticaria, hypothyroidism) or pain (paraesthesia, etc.).
24. Subjects who indicated nociceptive tests intolerable at screening or who achieved tolerance at >80% of maximum input intensity for the cold pressor or electrical pain tasks.

# 2.7 STUDY TREATMENTS

## 2.7.1 Investigational Medicinal Product(s) (IMP(s))

### Name and description of the IMP

*Nortriptyline*

Nortriptyline 75 mg will be administered orally.

*Morphine*

Morphine hydrochloride (HCl) 3 mg and 7mg will be administered intravenously.

### Status of development of the IMP

Both compounds are marketed approved drugs.

### Description and justification of dosage and route of administration

Study drug or placebo will be administered to the subjects as detailed in Table 1. Subjects will participate in four identical study periods. In each study period subject will receive a different drug combination, as described below. Two strengths of active (IV) morphine (see SmPC of morphine), 3 mg and 7 mg of morphine and 75 mg oral nortriptyline (see SmPC of nortriptyline), and matching placebo formulations will be manufactured. Each study participant will receive the following study treatments:

- Morphine 3 mg IV + 7 mg IV + placebo nortriptyline
- Nortriptyline 75 mg + morphine 3 mg IV + morphine 7 mg IV

| Protocol time<br>Treatments | 0h00m | 2h00m | 5h00m |
|---|---|---|---|
| 1 | Placebo | Morphine 3 mg IV | Morphine 7 mg IV |
| 2 | Nortriptyline 75mg | Morphine 3 mg IV | Morphine 7 mg IV |

*Nortriptyline*

Given its long half-life (18–44 hours), a single morning dose is expected to maintain pharmacologically active plasma levels throughout the assessment period. This supports the choice of 75 mg nortriptyline as an appropriate comparator in this crossover trial.

A previous study in healthy volunteers found that nortriptyline exhibits dose-proportional pharmacokinetics[25b]. Reported plasma exposure metrics (Cmax and AUC) at single doses are shown below:

| Dose (mg) | Cmax (ng/mL) | AUC (ng·h/mL) |
|---|---|---|
| 25 | 15 | 450 |
| 50 | 30 | 900 |
| 75 | 45 | 1350 |

A 75 mg oral dose is predicted to yield a peak concentration of approximately 45 ng/mL and an AUC of about 1350 ng·h/mL. This exposure lies below the therapeutic plasma range for *antidepressant* efficacy (50–150 ng/mL), but is expected to provide sustained CNS effects relevant for this trial's pharmacodynamic endpoints.[25c] Insomnia and dry mouth are the most common dose-limiting side effects at higher doses.

*Morphine*

Morphine and M6G notably contribute to the analgesic effect after administration of morphine, which is rapidly metabolized in the liver.26 As the study aims to have a better understanding of the analgesic effects of morphine and M6G in patients with chronic neuropathic pains and generate data that may be used for PK-PD modelling, an IV dose is deemed preferable over an oral dose.

Both morphine HCl 3 mg and 7 mg will be administered as intravenous bolus injection in 1 minute. In both the treatment arm with nortriptyline + morphine, and treatment arm with placebo + morphine, the bolus injections will be administered at t = 2 h (3 mg) and t = 5 h (7mg).

The 3 mg and 7 mg doses of morphine are equianalgesic to minimally therapeutic and clearly therapeutic doses of buprenorphine as observed in a previous study where potentiation of buprenorphine and milnacipran was assessed. [11] Buprenorphine is 30-40 times more potent than IV morphine.[27] Testing two morphine doses, one minimally therapeutic and one clearly therapeutic, on a single study day will allow for detailed exploration of possible synergistic/potentiating effects of the combination treatment during the PKPD analysis. Furthermore, the 3 mg and 7 mg morphine doses are based on a calculation using pre-existing and published morphine[28] and pregabalin [29] PK/PD models. Refer to Figure 1 for the predictions of effect of these doses on PTT for the cold pressor test. As illustrated in Figure 1, the proposed doses are predicted to yield an increased analgesic effect, while expected to have manageable side effects.

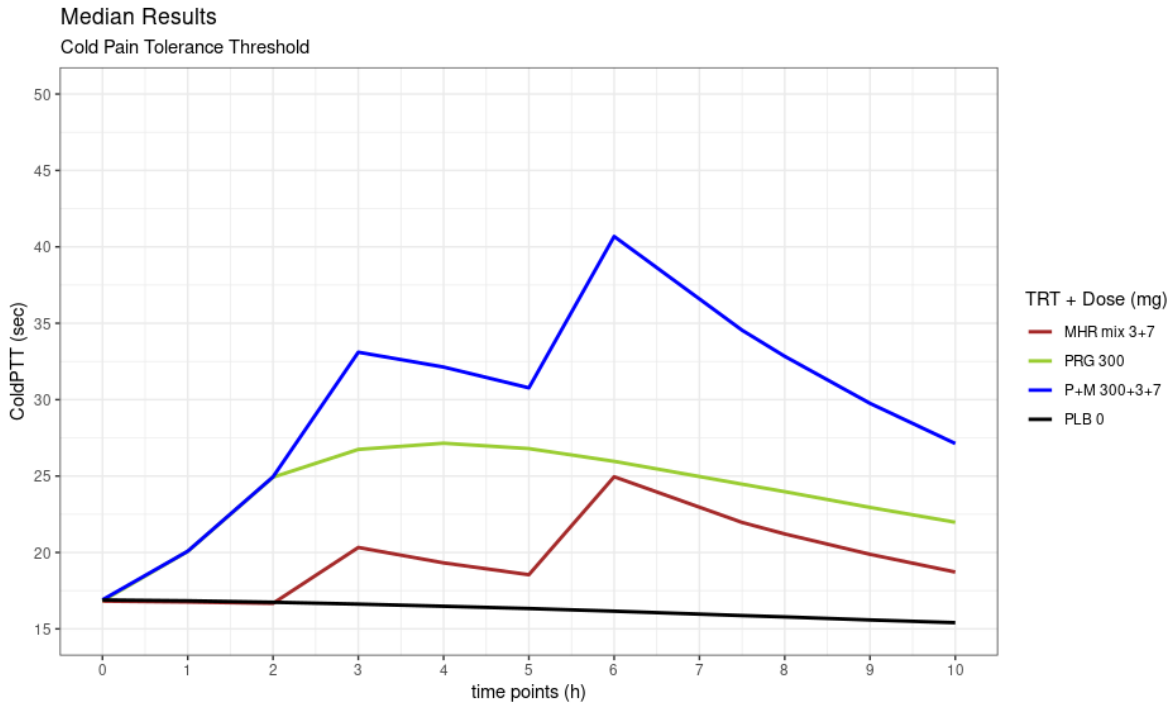


***Figure 1.*** *Predicted effect on cold pressor PTT based on the integrated PKPD model of morphine and pregabalin. Black line is the predicted placebo response, red line the proposed morphine dose of 3mg IV at t = 2 h and 7mg IV at t = 5 h, the green line the single oral dose of pregabalin 300mg at t=0 and blue line the combination treatment of above-mentioned doses and drugs.*

### 2.7.2 Comparator IMP(s)

NA

### 2.7.3 Placebo

A matching dosage form, indistinguishable from active treatment will be used as placebo treatment.

### 2.7.4 Auxiliary Medicinal Product(s) (AxMP(s))

**Name and description of the AxMP**

Capsaicin 1% ethanolic solution will be used as a challenge agent in this trial and therefore is considered an AxMP.

**Statement on authorisation and justification unauthorised AxMP (if applicable)**

The capsaicin model is commonly used to simulate neuropathic pain. This substance selectively activates the primary nociceptive afferents of C-fibres and multimodal Aδ-fibres via the Transient Receptor Potential cation channel subfamily V member 1 (TRPV1) receptor.[1,30] Previous studies have shown that topical application of capsaicin can induce characteristics of neuropathic pain, i.e. peripheral and (possibly) central sensitization.[2,3] These sensitization effects may be expressed by primary and secondary hyperalgesia, respectively. To further test these sensitization effects, the following pain tests may be used in combination with this model: Von Frey – secondary hyperalgesia, Heat pain test on capsaicin exposed skin.

The capsaicin 1% model is included as a model for heat and mechanical (secondary) allodynia. This induced allodynia sensation of the skin on the volar forearm cannot be induced with capsaicin compound that are market approved. Therefore, the AxMP capsaicin 1% solution will be used as part of the pharmacodynamic tests.

**Description and justification of dosage and route of administration**

Previous studies have shown that topical application of capsaicin can induce characteristics of neuropathic pain, i.e. peripheral and (possibly) central sensitization.[2,3]

### 2.7.5 Preparation and labelling of the study treatment(s)

Pre-printed, waterproof labels will be used to identify the tubes used during sample collection and for storage of separated plasma. Each label will contain the following information:

- Protocol number
- Subject number
- Occasion number (date)
- Protocol (delta) time
- Activity: Sample type (blood) & purpose (PK)

## 2.8 OTHER TREATMENTS AND RESTRICTIONS

### 2.8.1 Concomitant therapy

No prescription medications and OTC medications other than contraceptive medication will be permitted within 14 days prior to study drug administrations, or less than 5 half-lives (whichever is longer), and during the study. Exceptions are paracetamol (up to 4 g/day) and ibuprofen (up to 1g/day), which are allowed up to 2 days before all screening visits and 2 days before each study drug administration.

No vitamin, mineral, herbal, and dietary supplements will be permitted within 7 days prior to study drug administrations, or less than 5 half-lives (whichever is longer), and during the study.

*Permitted medication(s)*

Contraceptive medication.

*Prohibited medication(s)*

Paracetamol (up to 4 g/day) and ibuprofen (1 g/day) are not allowed within 2 days before screening and each study drug administration. Other exceptions will only be made if the rationale is clearly documented by the investigator.

### 2.8.2 Lifestyle restrictions

*Contraception measures*

Female subjects are required to have an intrauterine device, a contraceptive implant or are willing to continuously use oral contraceptives (i.e., skip their menstruation) during the study period. Other exceptions will only be made if the rationale is discussed and clearly documented.

All women of childbearing potential and all males must practice effective contraception during the study and be willing and able to continue contraception for at least 90 days after their last dose of study treatment.

Women of childbearing potential are defined as all women physiologically capable of becoming pregnant, unless they meet one of the following conditions:

- Postmenopausal: 12 months of natural (spontaneous) amenorrhea or 6 weeks after surgical bilateral oophorectomy with or without hysterectomy.
- Post-hysterectomy.

For the purposes of the study, effective contraception is defined as follows:

- Women of childbearing potential: Using 1 or more of the following acceptable methods of contraception: surgical sterilization (e.g., bilateral tubal ligation), intrauterine contraception/device, hormonal contraception, or any 2 barrier methods (a combination of male or female condom with diaphragm, sponge, or cervical cap).
- Males: Effective male contraception includes a vasectomy with negative semen analysis at follow up, or the use of condoms.

Abstinence can be considered an acceptable method of contraception at the discretion of the investigator. Periodic abstinence (e.g., calendar, ovulation, sympto-thermal, post ovulation methods) and withdrawal are not considered acceptable methods of contraception.

*Other requirements*

- Any nutrients known to modulate CYP enzymes activity (e.g., grapefruit or Seville orange containing products or quinine containing drinks (tonic water or bitter lemon)) will not be permitted from 3 days before dosing until EOS.
- The use of (illicit) drugs including cannabis can influence the measurements. Therefore, using 'drugs' is not permitted from 14 days before dosing and until EOS. Since poppy seeds can cause a positive 'drugs' result; these should be avoided. If a positive result occurs without an explanation, the subject cannot participate in the study.
- Alcohol will not be allowed from at least 24 hours before screening, dosing, at each scheduled visit, and whilst in the study unit until EOS. At other times throughout the study, subjects should not consume more than 2 units of alcohol daily on average (one unit is 10 grams of alcohol). Subjects may undergo an alcohol breath test at the discretion of the investigator.
- Subjects will not be allowed to have excessive caffeine consumption, defined as >800 mg per day from 7 days prior to the first dose of the study drug until 24 hours prior to dosing. Subjects will abstain from caffeine-containing products for 24 hours prior to the start of dosing until EOS. Caffeine quantities defined as: one cup of coffee contains 100 mg of caffeine; one cup of tea, or one glass of cola, or potion of chocolate (dark:100 g, milk 200 g) contains approximately 40 mg of caffeine; one bottle of Red Bull contains approximately 80 mg of caffeine.
- Subjects will abstain from the use of tobacco-or nicotine-containing products (including e-cigarettes and patches) within the previous 3 months before the first dose administration until EOS.
- Strenuous physical activity (e.g., heavy lifting, weight, or fitness training) is not allowed from 48 hours prior to each study day (including screening visits) and follow-up visit, till the end of each visit. Light ambulatory activities (e.g., walking at normal pace) will be permitted, with the level of activities kept as similar as possible on all days in the study unit.
- Morphine and nortriptyline affect your ability to drive. Driving a vehicle under the influence of morphine and nortriptyline can be dangerous for yourself and others. There is a maximum permitted concentration of morphine and nortriptyline that makes it legal to drive a vehicle in the Netherlands. Violating this may result in you being fined or having your driving licence revoked. Therefore, you are not allowed to drive vehicles until 2 days after taking the study drugs on day 1 of each period. However, you may use a bicycle, public transport, or taxi. 2 days after the dosing days, there are no more restrictions.

## 2.9 TRACEABLILITY, STORAGE, ACCOUNTABLILITY AND COMPLIANCE

### 2.9.1 Traceability and storage of the study treatment(s)?

Specific procedures related to the traceability and storage of the study treatments are described in CHDR SOP CGEDRADM.

### 2.9.2 Accountability of the study treatment(s) and compliance

Drug accountability will be maintained by the Leiden University Medical Centre Pharmacy and assessed by maintaining adequate study drug dispensing records.

The investigator is responsible for ensuring that dosing is administered in compliance with the protocol. Delegation of this task must be clearly documented and approved by the investigator. All study drug administration will occur under medical supervision according to CHDR SOP CGEDRAM.

## 2.10 STUDY ASSESSMENTS AND PROCEDURES

### 2.10.1 Screening procedure

The Screening Visit may be conducted anytime up to 42 days prior to Baseline/admission to CHDR. The screening phase will only be started after full written, verbal and signed informed consent has been obtained, according to CHDR standard operating procedures. A full medical screening will be performed to assess a subject's eligibility for this study. The overview of the assessments of this visit is provided in Table 1.

The screening will include a session in which the PD tasks are performed in order to familiarize the subject with the procedures and to evaluate whether the subject is capable of executing the tests. The training is valid for 42 days.

### 2.10.2 Randomisation, blinding and treatment allocation

*Randomization and treatment assignment*

***Table 3.*** *Randomization and Treatment Assignment*

| | |
|---|---|
| Study type | Cross-over |
| Number of treatments * | 4 |
| Total number of subjects | 24 |
| Number of cohorts | 1 (subjects enrolled per 2 subjects to accommodate logistical planning) |
| Ratio of active : placebo per cohort | NA |
| Sentinel | NA |
| Stratification | No |
| Randomise in blocks | Yes |
| Block size | 4 |
| Subject numbering | Subjects must be randomized in a consecutive order starting with the lowest number. 1 to 24, replacements 101 to 124 |
| Extra relevant information | For each cohort of subjects (n=3) that will start, 1 reserve subject is required, in total 8, with number 920 to 928. |

*Placebo is counted as a separate treatment

The randomization code will be generated using specify the name of the software and its version by a study independent, CHDR statistician. The randomization code will be unblinded/broken and made available for data analysis only after study closure, i.e., when the study has been completed, the protocol deviations determined, and the clinical database declared complete, accurate and locked. The randomization code will be kept strictly confidential. Sealed individual randomization codes, per subject and per treatment, will be placed in a sealed envelope with the label 'emergency decoding envelopes' in a safe cabinet at CHDR.

*Blinding*

This study will be performed in a double-blind fashion. The investigator, study staff, subjects and monitor will remain blinded to the treatment until study closure. The investigational drugs and matching placebo are indistinguishable and will be packaged in the same way. The investigator will receive a set of sealed emergency codes to be broken in case of emergency situations. If the identity of the study drug administered needs to be known in order to manage the subject's condition i.e., in case of a medical emergency or in the case a SAE occurs, the treatment emergency code for that subject may be broken and the study drug identified. All such occurrences should be documented in the study file. Treatment emergency codes should not be broken except in emergency situations and, if possible, the investigator should be contacted before the emergency code is opened. Just prior to database lock the unused emergency code labels will be checked and a statement to the effect that all are intact (or not as the case may be) will be made on the database lock form.

### 2.10.3 Study procedures and assessments

*Efficacy assessments*

N.A.

*Safety assessments*

Safety parameters include AE and concomitant medication monitoring, VSs, ECG, physical and neurological examination, C-SSRS, and laboratory assessments. The definitions, reporting and follow-up of AEs, SAEs and potential pregnancies are described in section 2.12.

*Vital signs*

Evaluations of systolic and diastolic blood pressure, pulse rate, respiratory rate, oxygen saturation and temperature will be performed throughout the study. Pulse and blood pressure will be taken after 5 minutes in the supine position. Automated oscillometric blood pressures and pulse rate will be measured using a Dash 3000, Dash 4000, Dynamap 400, Dynamap ProCare 400, Nonin Xpod or Philips Intellivue MP70.

*Weight and height*

Weight (kg) will be recorded at screening and the follow-up visit or upon early termination. Height (cm) will be recorded, and body mass index (BMI) calculated at screening.

*Physical examination*

Physical examination (i.e., inspection, percussion, palpation, and auscultation) is performed during the course of the study. Clinically relevant findings that are present prior to study drug initiation must be recorded with the subject's Medical History. Clinically relevant findings found after study drug initiation and meeting the definition of an AE (new AE or worsening of previously existing condition) must be recorded.

*MDRO/MRSA screening*

At screening, the risk of colonization with MDRO/MRSA will be assessed according to CHDR SOP CGEBRMO. Subjects with an increased risk for colonization with MDRO and/or MRSA can only participate in a clinical trial at CHDR with negative screening cultures. MDRO and /or MRSA swabs are only performed in subjects with an increased risk for MDRO/MRSA colonization and if the benefit of participating in the subsequent trial outweighs the burden of the swabs/cultures, as jointly assessed by the subject and screening physician, if needed in consultation with the study physician or his/her designee.

*Electrocardiography*

ECGs will be obtained during the course of the study using Marquette 2000/5500 and stored using the MUSE Cardiology Information System. ECGs will be taken after at least 5 minutes in the supine position. When timings coincide, ECGs should be performed before blood sampling. The investigator will assess the ECG recording as 'normal', 'abnormal - not clinically significant', or 'abnormal - clinically significant' and include a description of the abnormality as required. The ECG parameters assessed will include heart rate, PR, QRS, QT, and QTcF (calculated using Fredericia's method).

*Laboratory assessments*

Blood and other biological samples will be collected for the following clinical laboratory tests:

***Table 4.*** *Laboratory assessments*

| Lab | Tests | Collection & Analysis |
|---|---|---|
| Haematology | Haemoglobin [including Mean Corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC)], haematocrit, red cell count (RBC), total white cell count (WBC)and Platelet count. Differential blood count, including: basophils, eosinophils, neutrophils, lymphocytes, and monocytes. | 2 mL of venous blood in a BD Vacutainer® K2EDTA tube. Samples will be analysed by the Clinical Chemistry Laboratory (AKCL) of Leiden University Medical Center (LUMC). |
| Chemistry and electrolytes | Sodium, potassium, calcium, inorganic phosphate, total protein, albumin, triglycerides, blood urea nitrogen (BUN), creatinine, uric acid, total bilirubin[2], alkaline phosphatase, AST, ALT gamma-GT and LDH. | 3.5 mL of venous blood in a BD Vacutainer® SST Gel and Clot Activator tube. Samples will be analysed by the AKCL of LUMC |
| Virology | HIV1 and HIV2 antigen and antibodies, Hepatitis B surface antigen, Hepatitis B antibodies and Hepatitis C antibodies | 8.5 mL of venous blood in a BD Vacutainer® SSTGel and Clot Activator tube. Samples will be analysed by the Microbiology Laboratory (CKML) of the LUMC |
| Glucose | Glucose[1] | 2 mL of venous blood in a BD Vacutainer® Sodium Fluoride tube. Samples will be analysed by the AKCL of LUMC |
| Urinalysis | Leucocytes, blood, nitrite, protein, urobilinogen, bilirubin, pH, specific gravity, ketones, glucose. If there is a clinically significant positive result, urine will be sent to the AKCL for microscopy and/or culture. | A midstream, clean-catch urine specimen will be analysed by dipstick (Multistix® 10 SG, Siemens Healthcare Diagnostics, Frimley, UK). |
| Pregnancy[3] | hCG. If there is a clinically significant, positive result, urine will be sent to the AKCL for confirmation. | A urine specimen will be analysed at CHDR by test kit (InstAlert, Innovacon, San Diego, USA). |

| | | |
|---|---|---|
| Alcohol | Alcohol Breath Test | The hand-held Alco-Sensor IV meter (Honac, Apeldoorn, the Netherlands) will be used to measure the breath ethanol concentrations. |
| Urine drug screen | Cocaine, amphetamines, opiates (morphine), benzodiazepines and cannabinoids. | A urine specimen will be analysed at CHDR by test kit (InstAlert, Innovacon, San Diego, USA). |
| [1]After 4-hours fasting. [2]Conjugated bilirubin will be reported only when total bilirubin is outside the reference range. [3]Pregnancy test for women of childbearing potential will be performed at screening and every study visit and if pregnancy is suspected during the study. | | |

*Pharmacokinetic and (blood) pharmacodynamic assessments*

Details of sampling and sample processing will be included in a separate laboratory manual that will be finalized prior to study start.

*Labelling*

Pre-printed, waterproof labels will be used to identify the tubes used during sample collection and for storage of separated plasma. Each label will contain the following information:

- CHDR Protocol number
- Subject number
- Occasion number (date)
- Protocol (delta) time
- Activity: Sample type (blood) & purpose (PK)

*Shipping Procedures*

CHDR will arrange shipment of the samples. The samples must be packed securely together with completed shipment forms in polystyrene insulated shipping containers together with enough dry ice to last for 48 hours. Samples must be shipped to Ardena Bioanalysis BV at Assen (the Netherlands) at the end of the clinical trial.

*Pharmacodynamic assessments and questionnaires*

*PainCart® assessments (see CHDR SOP CCNPAIN)*

Nociceptive (pain) detection and tolerance thresholds will be measured using the PainCart®. All measurements will be performed in a quiet room with ambient illumination and temperature. A training session is part of the screening procedures to reduce any possible learning effects, as well as to exclude any subjects indicating intolerable to pain tests, or achieving tolerance at more than 80% of the maximum input intensity for the cold pressor- or electrical-, pain test. Each subject is assigned to a separate room to minimize any distraction. During nociceptive tests, subjects will be sitting comfortably in a chair (with knees supported). Except for the heat pain assessment, pain intensity will be measured continuously for each of the following nociceptive tests with subjects rating their pain intensity using a 100% electronic visual analogue scale (eVAS)-slider, with 0 and 100 defined as 'no pain' and 'worst pain tolerable', respectively.

The equipment is programmed to cease giving stimuli if pain intensity reaches the maximum possible score. Data from the eVAS will inform the pain detection threshold (PDT: the start of pain, when a subject moves the eVAS slider away from 0) and the pain tolerance threshold (PTT, when a subject

moves the eVAS slider to the end point, triggering a stop for any particular pain test – eVAS at 100). In addition, the area under the VAS pain curve, and/or post-test VAS measuring peak pain intensity of the test just performed (no pain – worst pain imaginable) is determined for the cold pressor-, electrical-, and pressure pain test. The following tests will be performed:

*Capsaicin 1% application*

Capsaicin application and related tests (heat pain test on capsaicin-treated skin and control skin on contralateral arm, and Von Frey test) will only be performed for subjects found eligible at screening (i.e., not to be allergic and/or tolerable to the capsaicin solution). Subjects allergic and/or intolerable to the capsaicin solution may participate in the trial; no capsaicin-related measurements will be performed for them.

The capsaicin 1% model is included as a model for heat and mechanical allodynia. Capsaicin is applied during screening procedures, to evaluate whether subjects are allergic to the solution, and applied 60 minutes prior to study drug administration. A 3x3 cm surface on the dominant volar forearm is used as application site; the non-dominant volar forearm serves as a non-stimulated control. At 30 minutes post-application, abundant solution is wiped off towards the centre of the application site, if necessary. Immediately after, and subsequently at given time points, Von Frey hair filament assessments and heat pain tests are performed on the 3x3 cm area.

Capsaicin application and related tests (heat pain test on capsaicin-treated skin and control skin on contralateral arm, and Von Frey test) will only be performed for subjects found eligible at screening (i.e., not to be allergic and/or tolerable to the capsaicin solution). Subjects allergic and/or intolerable to the capsaicin solution may participate in the trial; no capsaicin-related measurements will be performed for them.

*Von Frey hair filament assessment*

Von Frey hair filament assessment on capsaicin treated skin will only be performed on subjects who are not allergic or intolerable to the capsaicin solution as confirmed at screening. At screening and prior to dosing on each treatment day, it will be evaluated which Von Frey filament is felt as 'nearly painful' using a set of 128 mN, 256 mN and 512 mN filaments, just outside the area to be stimulated. This filament will be used during the treatment day to determine the secondary mechanical allodynic area. From the centre of the primary area, eight spokes (each 45°) dividing an octagon in equal parts will be drawn. On the two lateral-medial spokes 8 points and on the six remaining spokes 9 points, each 5mm apart, will be the designated Von Frey hair stimulation location. Stimulation method will be starting from the stimulation point furthest away towards the middle. Once a change in sensation is felt, from nearly painful to painful, that point is determined to be the border of the secondary allodynic area. The area is determined by connecting the allodynic points to span an octagon. To minimize the risk of measuring false positive central sensitization, the primary area will be surrounded by a border of 5 mm, in which no secondary mechanical allodynia measurements will be performed.

Endpoint:

- Area of secondary mechanical allodynia ($mm^2$) using Von Frey hairs (volar forearm only for capsaicin eligible subjects).

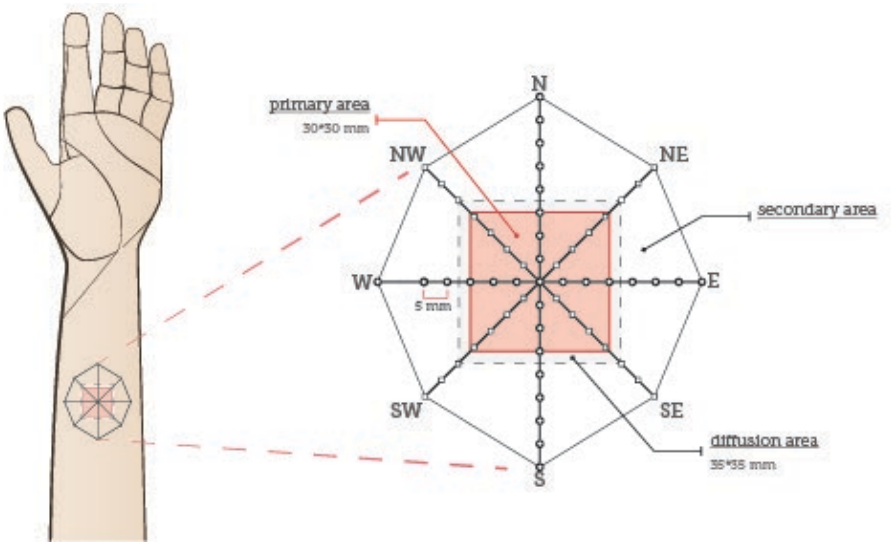


***Figure 2.*** *Visualization Von Frey hair filament assessment*

*Heat pain assessment (normal skin, and capsaicin exposed skin)*

Thermal PDTs are determined using a thermode (Medoc Q-Sense, Israel, contact area: 30mm x 30mm), that is placed on the subject's back. The initial temperature of the thermode is 32 °C and gradually increases in temperature with 0.5 °C/s, until the subject perceives the stimulus as painful (pain detection threshold, PDT), or if a temperature of 50 °C is reached. The subject indicates his PDT by pushing the button on a hand-held feedback control. The average of a triplicate measurement is used for further analysis.

Endpoint

- Heat pain (pre-cold pressor test): capsaicin treated area - PDT (°C), and post-test VAS (mm) (volar forearm only for capsaicin eligible subjects).
- Heat pain (pre-cold pressor test): control area - PDT (°C), and post-test VAS (mm).

*Pressure pain assessment*

This method of pressure pain induction has been shown to primarily assess nociception generated from the muscle with minimal contribution by cutaneous nociceptors [31,32]. Briefly, an 11 cm wide tourniquet cuff (VBM Medizintechnik GmbH, Sulz, Germany) will be placed over the gastrocnemius muscle with a constant pressure rate increase of 0.5 kPa/s controlled by an electro-pneumatic regulator (ITV1030-31F2N3-Q, SMC Corporation, Tokyo, Japan), Power1401mkII analogue–to-digital converter and Spike2 software (CED, Cambridge, UK). The subject will sit comfortably with their foot flat on the floor and rate their pain intensity using the eVAS, with 0 and 100 defined as "no pain" and "worst pain tolerable", respectively. eVAS > 0 is used as PDT. The pneumatic pressure will be increased until the subject indicates their PTT, or a maximum pressure of 100 kPa is achieved, at which point the device releases pressure to the tourniquet.

Endpoints:

- Pressure pain: PDT (kPa), PTT (kPa), Area Under the VAS pain Curve (AUC) (kPa*mm), and post-test VAS (mm).

*Cold pressor pain assessment*

The method of cold pressor pain is based on the methods described by Eckhardt *et al.* and Jones *et al.*[33,34] Subjects place their non-dominant hand into a water bath (minimal depth 200 mm) at 35 ± 0.5 °C for 2 minutes. At 1 minute 45 seconds, a blood pressure cuff on the upper-arm will be inflated to 20 mmHg below resting diastolic pressure. At 2 minutes the subject will then move their hand from the

warm water bath, directly placing their hand into a similar sized bath at 1.0 ± 0.5 °C. The subjects will be instructed to indicate when PDT is reached (first change in sensation from cold non painful to painful) as well as the increase in pain intensity, by moving the eVAS slider. When PTT is reached (sensation is no longer tolerable; eVAS slider at 100mm), or when a time limit (120s) is reached, subjects are instructed to remove their hand from the water, at which point the blood pressure cuff will deflate.

Endpoints:

- Cold pressor: PDT (s), PTT (s), AAC (s*mm), and post-test VAS (mm).

*Electrical Stimulation assessment (Electrical Burst and Stair)*

For cutaneous electrical pain, two electrodes (Ag-AgCl) are placed on clean (scrubbed) and if required, shaved skin overlying the left tibial bone 100 mm distal from the caudal end of the patella; middle of the first electrode is placed 100 mm distal the caudal end of the patella and middle of the second electrode directly (±135 mm) underneath the first. For single stimulus (electrical stair pain test), each sole stimulus (10 Hz tetanic pulse with a duration of 0.2 ms) is controlled by a constant current stimulator. Current intensity increases from 0 mA in steps of 0.5 mA·s-1 (cutoff 50 mA).

For repeated stimulus (electrical burst pain test), each single stimulus (train of five, 1 ms square wave pulses repeated at 200 Hz) is repeated 5 times with a frequency of 2 Hz at the same current intensity with a random interval of 3 to 8 seconds between the repetitions. Current intensity increases from 0 mA in steps of 0.5 mA (cutoff 50 mA). PDT is taken as the value (mA) whereby a subject indicates either: all 5 stimuli are painful, or the train of 5 stimuli started feeling non-painful but ends feeling painful (VAS > 0)[35]. The pain intensity will be measured using the eVAS, until PTT (eVAS = 100) is reached, or a maximum of 50 mA is reached.

Endpoints:

- Electrical Burst: PDT (mA), PTT (mA), AUC (mA*mm), and post-test VAS (mm).
- Electrical Stair: PDT (mA), PTT (mA), AUC (mA*mm), and post-test VAS (mm).

*Conditioned Pain Modulation (CPM)*

Effects on the centrally acting descending inhibitory control pathway are measured using the conditioned pain modulation (CPM) response, which is quantified by calculating the difference of pain detection and pain tolerance threshold of the heat pain test on unexposed/normal skin, directly after the cold pressor pain test, and the heat pain detection and tolerance thresholds prior to the cold pressor pain test.

Endpoints:

- Conditioned Pain Modulation Response (change from heat pain on unexposed/normal skin pre- and post-cold pressor): PDT (°C).

*Subjective (pain) perception*

The subject is asked to rate their pain after the heat-, cold pressor-, electrical-, and pressure pain tests using a digital, amended, Dutch language version of the short form of the McGill pain questionnaire (SF-MPQ)[36–38]. The questionnaire consists of:

- 16 questions regarding type of pain experienced measured with a 4-point Likert -type scale). From these measurements, two main factors are the calculated as described by Melzack: sensory (from 11 scores) affective (from 4 scores).
- A choice of one of 5 descriptors measuring peak pain intensity of the test just performed.

Endpoints:

- McGill Pain Questionnaire (score)

*NeuroCart® (see CHDR SOP CCNNCART)*

The NeuroCart® will include pEEG (at applicable timepoints), Saccadic eye movement, Smooth pursuit eye movement, Pupillometry, Adaptive tracking, Bond & Lader, Bowdle, N-Back, VVLT, Body Sway, STAI. These measurements will be conducted according to CHDR standard operating procedures. One NeuroCart® round will take ±40 minutes.

*Saccadic eye movement & Smooth pursuit eye movement*

Analysis of smooth pursuit and saccadic eye movements are frequently used for the assessment of (side) effects of drugs involving the central nervous system. The use of a computer for measurement of saccadic eye movements was originally described by Baloh *et al.*[39]. and for smooth pursuit by Bittencourt *et al.*[40] and has been extensively validated at the CHDR by Van Steveninck *et al.*[41]

*Saccadic eye movement*

Saccadic peak velocity is one of the most sensitive parameters for sedation[4]. The use of a computer for measurement of saccadic eye movements was originally described by Baloh et al.[39], and has been validated at CHDR by Van Steveninck et al.[41] While the sedative effects of 20 mg oral temazepam were detectable by subject self-report, visual analogue scales and SEM testing, at a dose of 5 mg were only detectable with measures of SEM[2]. The effects of 1 night of sleep deprivation (suggested as a threshold level of clinically significant sedation) were consistently detectable by SEM testing with a sustained decrease in saccadic peak velocity of 9 to 10% observed[3].

Saccadic eye movements will be recorded at a training session, pre-dose and at times specified in the protocol. Recording of eye movements will be performed in a quiet room with dimmed lightning. There will be only one subject per session in the same room.

Disposable silver-silver chloride electrodes (Ambu Blue Sensor N) will be applied on the forehead and beside the lateral canthi of both eyes of the subject for registration of the electro-oculographic signals. Skin resistance is reduced to less than 5 kOhm before measurements. Head movements are restrained using a fixed head support. The target consists of a moving dot that is displayed on a computer screen. This screen is fixed at 58 cm in front of the head support.

Saccadic eye movements are recorded for stimulus amplitudes of approximately 15 degrees to either side. Fifteen saccades are recorded with interstimulus intervals varying randomly between 3 and 6 seconds. Average values of latency (reaction time), saccadic peak velocity of all correct saccades and inaccuracy of all saccades will be used as parameters. Saccadic inaccuracy is calculated as the absolute value of the difference between the stimulus angle and the corresponding saccade, expressed as a percentage of the stimulus angle.

The measurements of saccadic eye movements will take approximately 2 min.

*Smooth pursuit eye movement*

The same system as used for saccadic eye movements is also used for measurement of smooth pursuit. For smooth pursuit eye movements, the target moves at a frequency ranging from 0.3 to 1.1 Hz, by steps of 0.1 Hz. The amplitude of target displacement corresponds to 22.5 degrees eyeball rotation to both sides. Four cycles are recorded for each stimulus frequency. The method has been validated at CHDR by Van Steveninck et al.[41] based on the work of Bittencourt et al.[40]and the original description of Baloh et al.[39]

The time in which the eyes are in smooth pursuit of the target will be calculated for each frequency and expressed as a percentage of stimulus duration. The average percentage of smooth pursuit for all stimulus frequencies will be used as parameter.

This measurement takes approximately 1 minute.

*Adaptive tracking*

The adaptive tracking test will be performed as originally described by Borland and Nicholson[42] (Borland and Nicholson 1975) using customized equipment and software developed by Hobbs & Strutt.

The assessment includes a run-in time of 0.5 min, during which data are not recorded. Adaptive tracking is a pursuit-tracking task. A circle moves pseudo-randomly on a screen. The subject must try to keep a dot inside the moving circle by operating a joystick. If this effort is successful, the speed of the moving circle increases. Conversely, the velocity is reduced if the test subject cannot maintain the dot inside the circle. The average tracking performance (%) will be used as variable and recorded into the subject's eCRF.

The adaptive tracking test is more sensitive to impairment of eye-hand coordination by drugs than compensatory pursuit tasks or other pursuit tracking tasks, such as the pursuit rotor. The adaptive tracking test has proved to be useful for measurement of central nervous system effects of alcohol, various other psychoactive drugs, and sleep deprivation. The tracking performance test will take in total approximately 4 min.

*Body sway*

The body sway meter allows measurement of body movements in a single plane, providing a measure of postural stability. Body sway is measured with a pot string meter (celesco) based on the Wright ataxiameter[43]. At CHDR, the method has been used to demonstrate effects of sleep deprivation[44], alcohol[45], benzodiazepines[46] and other psychoactive agents (data on file). With a string attached to the waist, all body movements over a period of time are integrated and expressed as mm sway. Subjects will be instructed to wear a pair of comfortable, low-heeled shoes on each session. Before starting a measurement, subjects will be asked to stand still and comfortable, with their feet approximately 10 cm part and their hands in a relaxed position alongside the body and eyes closed. Subjects may not talk during the measurement. The total period of body-sway measurement will be two minutes.

*N-Back*

The N-back test measures working memory. Following Rombouts et al.[47], the N-Back test consists of three conditions, with increased working memory load: (Condition 0) "X" condition, in which participants are required to indicate whether the presented letter is a "X" (=target) or another letter; In Condition 1 and 2, letters will be presented sequentially (1.5 seconds for a letter [consonant, except for the letter "z"] presentation, followed by a black screen for 0.5 seconds). "z" is pressed on the keyboard for a target and "/" is pressed for a non-target. Condition 1, "1-back" condition, in which participants are required to indicate whether the letter presented is a repetition without any other letter intervening (e.g., B … B); Condition 2, "2-back" condition, in which participants are required to indicate whether a letter is repeated with one other letter in between (e.g., B … C … B).The 3 conditions are presented in 3 blocks with increasing working memory load. Each condition starts with a training (7 consonants; ratio target : non-target 3:4), followed by the test (24 consonants; ratio target : non-target 1:3). This test takes approximately 10 minutes.

*Pupillometry*

The pupil size will be measured using the digital single-lens reflex camera Canon EOS 1100D[48]. This procedure is performed on the NeuroCart®, which is equipped with Windows 7. To perform this measurement, a picture is taken from both eyes simultaneously. Consequently, the ratio between the pupil- and iris diameter are measured. In order to make sure that the measurement of the pupil size is independent of the distance between the camera and the subject, the ratios of the diameters are used.

During the measurement the subject will be seated in a chair while the subjects head is resting in a head support system. The measurements are taken in a room with lighting set at a marker. The marker is set for a certain amount of LUX, depending on the study protocol. Each measurement should be made under the same lighting conditions and with the subject's head in the same position.

The pupil measurements will be done with the help off the program Qpupil that has been designed by Jasper Janssen (radiology department, LUMC, the Netherlands). This program has been designed to automatically calculate the ratio between the diameter of the iris and the pupil of both eyes.

*Visual analogue scales (Bond & Lader)*

Visual analogue scales as originally described by Norris have often been used previously to quantify subjective effects of a variety of sedative agents[49,50].Dutch versions of the scales have been frequently employed at CHDR, for a variety of sedative agents and circumstances[51]. A set of VAS assessing alertness, mood, and calmness (Bond and Lader)[52] will be used for subjective assessment. The VAS allows the subjects to evaluate their current subjective states. The VAS Bond and Lader consists of 16 visual analogue scales, which allow the subjects to rate their subjective feelings and will be used to determine effects on alertness, mood, and calmness. Nine VAS items are combined to assess alertness, five VAS items are combined to assess mood, and two VAS items are combined to assess calmness. The algorithm to derive the variables is described in the SOP CGEVAS. Each VAS scale consists of a 100 mm horizontal line. Two words representing opposite feelings are placed to the left and right of the horizontal line. The subject is asked to mark his/her current feelings. The VAS will be scored from 0 to 100, with 0 reflecting the word placed to the left, e.g., alert and 100 reflecting the word placed to the right of the line, e.g., drowsy. It is important that the investigator checks for completeness to avoid omitted scales.

*Visual analogue scales (Bowdle)*

Potential subjective psychotomimetic (psychedelic) effects of CNS-active agents can be evaluated using specific VAS[53,54]. Bowdle Psychotomimetic Effects Scores have been used to quantify the psychotomimimetic effects of ketamine[55]. No validated VAS is available for the Dutch language and population, but a translated version of the scales originally developed by Bowdle et al. has been used at CHDR to study CNS-active drug effects in multiple studies (data on file). Bowdle Psychotomimetic Effects Scores consist of thirteen 10 cm visual analogue lines ranging from 0 ('not at all) to 100 mm ('extremely')[41], addressing various abnormal states of mind.

*Visual Verbal Learning Test (VVLT)*

The Visual Verbal Learning Test (VVLT) contains three different subtests that cover merely the whole scope of learning behaviour[56,57] (Schmitt et al 2001, De Haas *et al.* 2009). Subjects that perform the VVLT will be presented 30 words in three consecutive word trials, i.e. word learning test (VVLT30), according to CHDR's SOP CCNVVLT. Each trial ends with a free recall of the presented words (Immediate Recall- a test to determine acquisition and consolidation of information). Approximately thirty minutes after start of the first trial, the volunteers are asked to recall as many words as possible (Delayed Recall- this test measures active retrieval from long term memory). Immediately thereafter, the subjects will undergo memory recognition test, which consists of 15 presented words and 15 'distractors' (Delayed Recognition- testing memory storage). Importantly, subjects are not allowed to write words down at any time during the whole test procedure.

The VVLT scheduled at the 1-hour timepoint will test the “immediate recall, the “delayed recall and the VVLT at the 8-hour timepoint will assess the “delayed recognition”.

*Pharmaco-electroencephalography*
Pharmaco-Electroencephalography (pEEG) will be used to monitor any drug effects, which can be interpreted as evidence of penetration and activity in the brain. EEG provides non-specific measures of CNS functions. EEG recordings will be performed according to the guidelines of the International Pharmaco-EEG Society (IPEG).

### 2.10.4 Sequence of assessments and time windows

When the following assessments are scheduled to be performed at the same time-point, the order (of priority) will be as follows: ECG, vital signs, blood sampling for safety, NeuroCart® (pEEG (at applicable timepoints), Saccadic eye movement, Smooth pursuit eye movement, Pupillometry, Adaptive tracking, Bond & Lader, Bowdle, N-Back, VVLT, Body Sway, STAI), PainCart® (Heat pain (pre-cold pressor: unexposed/normal and Capsaicin-exposed skin) Pressure Pain test, Electrical Burst test, Electrical Stair test, heat pain test on unexposed/normal skin Cold pressor pain test, heat pain on unexposed/normal skin test), BSI and physical examination. When a PK assessment is scheduled for the same nominal time as another scheduled assessment, the blood sampling will take precedence.

The deviations of actual time points from the expected time points will be within ten percent, calculated from the zero point, time of drug administration. The expected timepoints are defined as the timepoints in Promasys. Deviations of more than 10% will be explained in a note. These time deviations only apply for ECG, vital signs, blood sampling for PK , first test of a NeuroCart® round, and first test of a PainCart® round.

Pre-dose assessments are given in indicative expected times.

## 2.11 STUDY DISCONTINUATION AND COMPLETION

### 2.11.1 Definition End of Trial

End of Study (EOS) is defined as the last visit of the last subject, i.e. subject 24.

### 2.11.2 Criteria for temporary halt and early termination of the clinical trial

In accordance with CTR: Annex I D17p, dose escalation will be stopped (temporarily) when one of the following conditions applies:

- in case of an unacceptable tolerability profile based on the nature, frequency, and intensity of observed AEs, judged jointly by the investigator and the sponsor.
- if two or more subjects on active drug experience a serious adverse event (SAE) or severe AEs, which in the opinion of the PI or the Sponsor's medical representative is likely to be causally related to IMP.

If dose escalations are suspended because one or more of the above defined stopping criteria are met, the study team will review all available PK, safety, and tolerability data and may decide to unblind. Based on this review, it may be decided to repeat a dose level or, if observed changes are deemed to have occurred by chance (i.e., similar changes are observed in placebo-treated subjects), to proceed with further dose escalations.

If the reason(s) for temporary halt did not impact the risk-benefit ratio for subjects (Article 37 CTR), the EC shall be informed, and the study can be resumed. If the reason(s) for temporary halt impacted the risk-benefit ratio for subjects (Article 38 CTR), a substantial amendment shall be submitted to CCMO and EC. In such case, the trial will not be resumed until approval has been received.

### 2.11.3 Discontinuation/withdrawal of individual subjects

*Study drug interruption or discontinuation*

Before trial medication is administered, changes in the subject health status including laboratory results if applicable, since the previous visit or previous dose in case of a multiple dose regimen, must be checked. The investigator must temporally interrupt or permanently discontinue the study drug if continued administration of the study drug is believed to be contrary to the best interests of the subject. The interruption or premature discontinuation of study drug might be triggered by an AE, a diagnostic or therapeutic procedure, an abnormal assessment (e.g., ECG or laboratory abnormalities), or for administrative reasons in particular withdrawal of the subject's consent. The reason for study drug interruption or premature discontinuation must be documented.

*Subject withdrawal*

Subjects have the right to withdraw from the study at any time for any reason. Should a subject decide to withdraw from the study, all efforts should be made to complete and report the observations, particularly the FU examinations, as thoroughly as possible.

*Replacement policy*

Subjects withdrawing, or being withdrawn, for reasons other than (possibly or probably related) AEs or any other tolerability issues with the treatment can be replaced at the discretion of the investigator. The rationale for replacement must be clearly documented in the source (e.g., note in Promasys, Filenotes, etc.)

### 2.11.4 Arrangements for subjects after their participation in the clinical trial ended

Not applicable.

## 2.12 SAFETY REPORTING

### 2.12.1 Definitions

**Adverse events (AEs)**

Adverse events are defined as any untoward medical occurrence in a subject to whom a medicinal product is administered, and which does not necessarily have a causal relationship with this treatment.

The intensity/severity of clinical AEs is graded on a 3-point scale as defined below:

- Mild: discomfort noticed but no disruption of normal daily activity,
- Moderate: discomfort sufficient to reduce or affect normal daily activity,
- Severe: inability to work or perform daily activity.

For each AE the causal relationship to the drug will be judged by the investigator as defined below:

- Probably related,
- Possibly related,
- Not related.

The chronicity of the AE will be classified by the investigator on a 3-item scale as defined below:

- Single occasion: single event with limited duration,
- Intermittent: several episodes of an event, each of limited duration,
- Persistent: event which remained indefinitely.

**Serious adverse events (SAEs)**

Serious adverse event is any untoward medical occurrence in a patient or trial subject that at any dose:

- results in death,
- is life-threatening,
- requires inpatient hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity,
- is a congenital anomaly/birth defect, or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate medical judgement by the investigator.

An elective hospital admission will not be considered as a SAE.

**Suspected unexpected serious adverse reactions (SUSARs)**

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. The event must be serious;
2. There must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. The adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in the reference safety information (RSI, i.e. the IB).

### 2.12.2 Recording of AEs/SAEs/SUSARS

Recording of AEs will be done according to CHDR SOP CGEAE.

### 2.12.3 Reporting of AEs and SAEs

*Reporting of SAEs by the investigator to the sponsor*

Not applicable, all SAEs will be reported.

*List of SAEs which do not require immediate reporting and procedure for reporting*

Not Applicable.

### 2.12.4 Follow-up of adverse events

All AEs will be followed until they have abated, returned to baseline status or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported till end of study within the Netherlands, as defined in the protocol.

### 2.12.5 Reporting of SUSARs by the sponsor to the EudraVigilance database

The sponsor will keep detailed records of all AEs which are reported to him/her by the investigator or investigators (CTR: Article 41(3)).

The sponsor will report electronically and without delay to EudraVigilance database all relevant information about any SUSAR (CTR: Article 42).

The period for the reporting of SUSARs by the sponsor to the EMA will take account of the seriousness of the reaction and will be as follows:

- In the case of fatal or life-threatening SUSARs, as soon as possible and in any event not later than **7 days** after the sponsor became aware of the reaction (CTR: Article 42(2(a)));
- In the case of non-fatal or non-life-threatening SUSARs, not later than **15 days** after the sponsor became aware of the reaction (CTR: Article 42(2(b)));
- In the case of a SUSARs which was initially considered to be non-fatal or nonlife threatening but which turns out to be fatal or life-threatening, as soon as possible and in any event not later than **7 days** after the sponsor became aware of the reaction being fatal or life-threatening (CTR: Article 42(2(c))).

Where necessary to ensure timely reporting, the sponsor may, in accordance with section 2.4 of Annex III, submit an initial incomplete report followed up by a complete report (CTR: Article 42(2)).

### 2.12.6 Annual safety report

Regarding investigational medicinal products other than placebo, the investigator shall submit annually through CTIS to the Member State concerned a report on the safety of each investigational medicinal product used in a clinical trial (CTR: Article 43).

### 2.12.7 Unblinding procedures for safety reporting

The investigator will only unblind the treatment allocation of a subject in the course of a clinical trial if unblinding is relevant to the safety of the subject (CTR: Annex III 2.5(17)).

When reporting a SUSAR to the EMA, the investigator will only unblind the treatment allocation of the affected subject to whom the SUSAR relates (CTR: Annex III 2.5(18)).

In case of unblinding, describe procedure to maintain blind for persons responsible for the ongoing conduct of the clinical trial such as the management, monitors, investigators) and those persons

responsible for data analysis and interpretation of results at the conclusion of the clinical trial, such as biometrics personnel (CTR: Annex III 2.5(19)).

### 2.12.8 Temporary halt for reasons of subject safety

The sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will submit the notification through CTIS without undue delay of a temporary halt but not later than in 15 days of the date of the temporary halt. It shall include the reasons for such action and specify follow-up measures. The study will be suspended pending a further positive decision by the concerned member state (CTR: Article 38). The investigator will take care that all subjects are kept informed.

### 2.12.9 Urgent safety measures and other relevant safety reporting

Where an unexpected event is likely to seriously affect the benefit-risk balance, the sponsor and the investigator will take appropriate urgent safety measures to protect the subjects. In addition, the sponsor will notify the Member State concerned, through CTIS, of the event and the measures taken. That notification will be made without undue delay but no later than **7 days** from the date the measures have been taken (CTR: Article 54).

### 2.12.10 Data Safety Monitoring Board (DSMB)/Data Monitoring Committee (DMC)

Not applicable.

## 2.13 STATISTICAL ANALYSIS

### 2.13.1 Description of statistical methods

All safety and statistical programming are conducted with SAS 9.4 (maintenance 6) for Windows or newer (SAS Institute Inc., Cary, NC, USA). PK variable programming is conducted with R 4.0.3 for Windows or newer (R Foundation for Statistical Computing/R Development Core Team, Vienna, Austria, 2019).

### 2.13.2 Analysis sets

Data of all subjects participating in the study will be included in the analyses if the data can meaningfully contribute to the objectives of the study.

*Safety set*

The safety population will be defined as all subjects who were validated (randomised) and received at least 1 dose of study treatment.

*Pharmacokinetic analysis set*

The PK analysis population is defined as all subjects who were validated (randomised), received at least one dose of study treatment, and have at least one measurable drug concentration in samples collected.

*Pharmacodynamic analysis set*

The analysis population for pharmacodynamics is defined as all subjects who were validated (randomised), received at least one dose of study treatment, and have at least one post-baseline assessment of the parameter been analysed.

### 2.13.3 Participant demographics and other baseline characteristics

Subject disposition will be listed by subject.

The following subject data will be summarized:

- number and percentage of subjects screened,
- number and percentage of subjects enrolled,
- number and percentage of subjects completed,
- number and percentage of subjects included in safety population.

*Demographics and baseline variables*

Continuous demographic variables (e.g., age, height, weight, BMI) will be summarized by descriptive statistics (n, mean, SD, median, Min, Max). Qualitative demographic characteristics (sex, race/ethnicity) will be summarized by counts and percentages.

*Medical history*

Medical history will only be listed.

*Concomitant Medications*

Previous and concomitant medications will be listed by International Non-proprietary Names, dose, regimen, route and for which indication it was prescribed.

*Treatment compliance/exposure*

Exposure to study treatment is described in terms of duration of treatment and average infusion rate. The average infusion rate (mL/hr) is summarized by mean, SD, median, Q1, Q3, Min, Max.

### 2.13.4 Randomisation and blinding

*Randomization and treatment assignment*

Please refer to Subsection 2.10.2.

*Blinding*

Please refer to Subsection 2.10.2.

### 2.13.5 Sample size, trial power and level of significance used

The power calculation for the pregabalin – placebo contrast was performed with SAS 9.4 (maintenance 6) for Windows (SAS Institute Inc., Cary NC, USA). Variances estimated in four CHDR studies (i.e., CHDR1311 (part 1), CHDR1311 (part 2), CHDR1425 and CHDR143, table 3) were used to calculate the CV estimations (Table 4), for this sample size calculation. Comparability of results from these studies have been discussed by Siebenga et al. previously.[10] To properly estimate the inter-subject CV for a treatment contrast for this study based on five post-dose measurements, the CVs referenced in the article of Siebenga et al. were corrected for the repeated measures. I.e. for the power calculation performed for this study, the partial variance time*subject and residual were divided by five.

Based on data from three CHDR studies (CHDR1311 part 2, CHDR1425 and CHDR1431), mean differences for the pregabalin – placebo contrast, and effect ratio were estimated (Table 5).

***Table 5.*** *Variances observed for the four CHDR studies for cold pressor PTT.*

| Variance component (log scale) | CHDR1311 Part 1 | CHDR1311 Part 2 | CHDR1425 | CHDR1431 |
|---|---|---|---|---|
| Subject | 0.009054 | 0.009930 | 0.007694 | 0.01728 |
| Trt*subject | 0.02615 | 0.02953 | 0.02207 | 0.01357 |
| Time*Subject | 0.000089 | 0.000760 | 0.000769 | 0.000421 |
| Residual | 0.04216 | 0.04115 | 0.02426 | 0.02082 |

***Table 6.*** *CV estimation assuming five post-dose measurements for cold pressor PTT*

| Cold Pressor PTT | Cross-over CV | Parallel CV |
|---|---|---|
| CHDR1311 Part 1 | 26.7% | 21.1% |
| CHDR1311 Part 2 | 28.0% | 22.1% |
| CHDR1425 | 23.5% | 18.8% |
| CHDR1431 | 18.9% | 18.9% |

Example of CV estimation for CHDR1311 part 1:

CV cross-over 5 post dose measurements=(sqrt(exp((2*(0.02615+(0.04216/5))))-1))*100=26.7%

CV parallel 5 post dose measurements = (sqrt(exp(0.009054+(0.02615)+(0.000089/5)+(0.04216/5))-1))*100=21.1%

***Table 7.*** *Effect ratio*

| Cold Pressor PTT | Mean difference (sec) Pregabalin - Placebo | Effect ratio |
|---|---|---|
| CHDR1311 Part 2 | 8.2 | 1.51 |
| CHDR1425 | 5.8 | 1.23 |
| CHDR1431 | 0.3 | 1.07 |

Using that data, a sample size calculation was performed. The sample required to achieve a power of at least 80% to detect an expected difference on the cold pressor task (PTT) of 5 seconds (25% of an average score, based on 4 studies, under placebo of 20 seconds) at a two-sided significance level of 0.05, are given in the following Table (Table 6) for a range of within subject CV values:

***Table 8.*** *Sample size*

| Within subject CV | Actual power | Sample size |
|---|---|---|
| 20% | 0.796 | 20 |
| 25% | 0.845 | 24 |
| 30% | 0.825 | 24 |

Assuming a within subject CV of 30%, a sample size of 24 would yield a power of over 80% (82.5%) and thus suffices, for the contrast pregabalin - placebo.

*Inferential methods*

The study is exploratory, and no formal null hypothesis is set. No adjustments for multiple comparisons will be applied.

### 2.13.6 Planned analysis

*Pharmacokinetic analysis set*

The PK analysis population is defined as all subjects who were validated (randomised), received at least one dose of study treatment, and have at least one measurable drug concentration in samples collected.

*Pharmacodynamic analysis set*

The analysis population for pharmacodynamics is defined as all subjects who were validated (randomised), received at least one dose of study treatment, and have at least one post-baseline assessment of the parameter been analysed.

*Subject disposition*

Subject disposition will be listed by subject.

The following subject data will be summarized:

1. number and percentage of subjects screened,
2. number and percentage of subjects enrolled,
3. number and percentage of subjects completed,
4. number and percentage of subjects included in safety population.

*Analysis of safety and tolerability endpoints*

The safety set is used to perform all safety analyses. Baseline is defined as the last value prior to dosing. Change from baseline will be calculated for all continuous safety parameters.

*Adverse events*

The AE coding dictionary for this study will be Medical Dictionary for Regulatory Activities (MedDRA). It will be used to summarize AEs by primary system organ class (SOC) and preferred term (PT).

All adverse events will be displayed in listings.

A treatment-emergent adverse event (TEAE) is defined as an adverse event observed after starting administration of the specific treatment. If a subject experience an event both prior to and after

starting administration of a treatment, the event will be considered a TEAE (of the treatment) only if it has worsened in severity (i.e., it is reported with a new start date) after starting administration of the specific treatment, and prior to the start of another treatment, if any. All TEAEs collected during the investigational period will be summarized.

The number of treatment emergent AEs will be summarized by:

1. treatment, MedDRA SOC and PT;
2. treatment, MedDRA SOC, PT and severity;
3. treatment, MedDRA SOC, PT and drug relatedness.

*Vital signs*

At each time point, absolute values and change from baseline of supine blood pressure and pulse rate will be summarized with n, mean, SD, SEM, median, Min, and Max values. The number of available observations and out-of-range values (absolute and in percentage) will be presented. Values outside the reference range will be flagged in the listing. 'H' and 'L', denoting values above or below the investigator reference range (when present), will flag out-of-range results.

*ECG*

At each time point, absolute values and change from baseline of ECG numeric variables will be summarized with the number of samples (n), mean, SD, SEM, median, Min, and Max values. The number of available observations and out-of-range values (absolute and in percentage) will be presented. Values outside the investigator's normal range will be flagged in the listing. 'H' and 'L', denoting values above or below the investigator reference range (when present), will flag out-of-range results.

*Clinical laboratory tests*

At each time point, absolute values and change from baseline of clinical laboratory variables will be summarized with n, mean, SD, SEM, median, Min, and Max values. The number of available observations and out-of-range values (absolute and in percentage) will be presented. All laboratory data (including re-check values if present) will be listed chronologically. 'H' and 'L', denoting values above or below the investigator reference range (when present), will flag out-of-range results.

*Analysis of pharmacokinetic endpoints*

The individual plasma nortriptyline, morphine, and M6G concentrations will be listed by treatment, subject, visit and time. Individual plasma drug concentrations versus time will be plotted in panel plots for each treatment using both a linear and log y-axis.

The individual plasma morphine and nortriptyline concentrations will be summarised (number of samples [n], mean, standard deviation [SD], % coefficient of variation [CV], median, minimum [Min] and maximum [Max] values) by treatment and time, and will also be presented graphically as mean over time, with standard deviation as error bars.

When an actual sampling time of a drug concentration sample differs from the protocol time by more than 10% and at least 5 minutes, the concentration will be excluded from calculation of descriptive statistics and a note will be added to the sample in the listing. A sample will in general not be excluded from the non-compartmental analysis due to deviation from the protocol sampling times.

The individual PK parameters (except tmax and tlag) will be summarized (n, mean, SD, %CV, geometric mean, geometric %CV, median, Min and Max) per treatment group and will be presented graphically as boxplots. For tmax and tlag, the n, median, Min and Max statistics will be reported. The procedure for non-compartmental analysis at CHDR including the post and pre-processing of data is described in SOP SDANCA.

*Analysis of pharmacodynamic endpoints*

The final analysis will be preceded by a blind data review which consists of individual graphs per visit by time of all pharmacodynamic measurements by time. The graphs will be used to detect outliers and measurements unsuitable for analysis. The PD parameters will be listed by treatment, subject, visit and time. Individual graphs by time will be generated.

Repeatedly measured PD endpoints (PainCart® and CNS tests) will be summarised (n, mean, SD, SEM, median, Min and Max values) by treatment and time, and will also be presented graphically as mean over time, with standard deviation as error bars.

Single measured PD endpoints will be summarised (mean, SD, SEM, median, Min and Max values) by treatment, and will also be presented graphically as mean in a bar graph, with standard deviation as error bars.

Parameters will initially be analysed without transformation, but if the data suggest otherwise, log-transformation may be applied. Log-transformed parameters will be back-transformed after analysis where the results may be interpreted as percentage change.

To establish whether significant treatment effects can be detected on the repeatedly measured PD parameters, parameters will be analysed with a mixed model analysis of covariance (ANCOVA) with treatment, time, period and treatment by time as fixed factors and subject, subject by treatment and subject by time as random factors and the (average) baseline measurement as covariate.

Single measured PD parameters will be analysed with a mixed model analysis of variance (ANOVA) with treatment and period as fixed factors and subject as random factor.

The Kenward-Roger approximation will be used to estimate denominator degrees of freedom and model parameters will be estimated using the restricted maximum likelihood method.

The general treatment effect and specific contrasts will be reported with the estimated difference and the 95% confidence interval, the least square mean estimates and the p-value. Graphs of the Least Squares Means (LSM) estimates over time by treatment will be presented with 95% confidence intervals as error bars, as well as change from baseline LSM estimates.

The following contrasts will be calculated within the model:

- **morphine + nortriptyline versus morphine**

All the collected PD endpoint will be analysed with descriptive statistics. However, only PD endpoints collected after start of the first IV bolus injection will be used for the mixed model ANCOVA analysis.

Final analysis of specific PD endpoints (PainCart® and CNS tests) will be performed in sequence to limit the number of tests. Refer to Table 3 and 4 below. Initially, only the parameters listed in the 'first round analysis' column will be analysed following methods described further along this paragraph.

Only in case one or more of the endpoints listed in the 'first round analysis' column reaches statistical significance in any of the contrasts, the endpoints mentioned in the 'second round analysis' will be analysed. Whether to proceed with the 'second round analysis' is evaluated separately for each test.

Due to the large amount endpoints obtained from pEEG, those results will be analysed separately to allow for comprehensive evaluation of all power bands, EEG-derivations taken together.

BSI endpoints will be presented in a frequency table.

***Table 9.*** *PainCart® endpoints*

| PainCart® endpoints | | |
|---|---|---|
| **Test** | **First round analysis** | **Second round analysis** |
| **Pain pressure** | **PDT, PTT** | **AUC, VAS, McGill** |
| **Heat Pain** | **PDT** | **VAS, McGill** |
| **Cold pressor** | **PDT, PTT** | **AAC, VAS, McGill** |
| **Electrical stair** | **PDT, PTT** | **AUC, VAS, McGill** |
| **Electrical Burst** | **PDT, PTT** | **AUC, VAS, McGill** |
| **CPM** | **PDT** | **NA** |

***Table 10.*** *CNS test endpoints*

| CNS test endpoints | | |
|---|---|---|
| **Test** | **First round analysis** | **Second round analysis** |
| **Adaptive tracker** | Average performance | NA |
| **Body sway** | Antero-posterior sway | NA |
| **VAS B&L** | Alertness | Mood; calmness |
| **VAS Bowdle** | Feeling High | - Internal perception<br>- External perception |
| **N-back** | - Average reaction time (ms) (two-back)<br>- Number of correct targets (two-back)<br>- Number of incorrect targets (two-back)<br>- Number of faulty non-target responses (two-back) | - Average reaction time (ms) (zero- and one-back)<br>- Number of correct targets (zero- and one-back)<br>- Number of incorrect targets (zero- and one-back)<br>- Number of faulty non-target responses (zero- and one-back) |
| **VVLT** | - Delayed recall (number correct) | - Immediate recall trial 3 (number correct)<br>- Delayed recognition (number correct)<br>- Delayed recognition (reaction time) |
| **Eye Movement** | - Saccadic peak velocity (degrees/sec)<br>- Saccadic reaction time (sec)<br>- Saccadic Inaccuracy (%)<br>- Smooth pursuit (%) | NA |
| **Pupillometry** | - Pupil/iris ration (left and right) | NA |
| **SRT** | Reaction time | NA |
| **STAI** | State Anxiety Score | NA |
| **BSI** | - General somatic symptoms<br>- Cognitive symptoms<br>- Depressed mood<br>- Anxiety paranoid thoughts | NA |

| | | |
|---|---|---|
| | - Global severity index<br>- Interpersonal sensitivity<br>- Hostility<br>- Phobic anxiety<br>- Psychoticism | |
| **pEEG** | Frequency range for spectral analysis (Delta, Theta, Alpha, Beta, Gamma) | NA |

*PK/PD modelling*

Population PK and PK/PD models may be developed to address objectives that require an integrative interpretation of the study results. These include assessment of the dose proportionality, investigation of the nature of the PK/PD relationship, and the use of study results as part of a larger model-based data analysis. If population PK/PD models are developed, a separate Pharmacometrics Analysis Plan will be written.

*Exploratory analyses*

Exploratory data-driven analyses can be performed with the caveat that any statistical inference will not have any confirmatory value.

Deviations from the original statistical plan will be documented in the clinical study report.

### 2.13.7 Interim analysis

No interim analysis is planned.

### 2.13.8 (Statistical) criteria for termination of the trial

Not appliable for this study.

### 2.13.9 Procedure for accounting for missing, unused and spurious data

All missing or incomplete safety and PD data, including dates and times, are treated as such. Missing test results or assessments will not be imputed. Missing PD data, indicated as 'M' in the data listing, will be estimated within the statistical mixed model using SAS PROC MIXED.

For graphical and summary purposes PD and safety values below the limit of quantification will be set to half (½) of the limit of quantification. For analysis no undetermined values will be replaced. For graphical and summary purposes of drug concentrations, values below the limit of quantification (BLQ) will be set to zero.

For calculation of PK parameters, all BLQ plasma concentrations occurring prior to $C_{max}$ will be replaced by 0, except for embedded BLQ values (between two measurable time points) which will be treated as "missing". All BLQ values after $C_{max}$ will be treated as "missing". If data points for plasma concentrations are missing, the AUC parameters will be derived by interpolating with regard to the two neighbouring non-missing concentrations. If the actual sampling time is missing, but a valid concentration value is measured, the scheduled protocol time will be used for the calculation of PK parameters.

The handling of missing, unused and spurious data will be documented in the study report.

### 2.13.10 Procedure for reporting any deviation(s) from the original statistical plan

Deviations from the original statistical plan will be documented in the clinical study report.

## 2.14 ETHICAL CONSIDERATIONS

### 2.14.1 Declaration of Helsinki

The investigator will ensure that this study is conducted in full compliance with the protocol, the principles of the Declaration of Helsinki (www.wma.net), ICH GCP guidelines (http://www.ich.org/products/guidelines.html), and with the laws and regulations of the country in which the clinical research is conducted.

### 2.14.2 Recruitment and informed consent procedures

It is the responsibility of the investigator to obtain written informed consent from each individual participating in this study after adequate information of the study. Potential subjects will reach out to CHDR when they are interested in participation. After a brief explanation as stated in the Subject Information Sheet, a meeting will be planned with the subject for the medical screening. Prior to screening, the potential subjects will receive the informed consent document via mail with detailed information on the study and contact details in case of questions. Before signing the ICF the potential subjects will watch an informational video on the trial. This includes the aims, methods, objectives and potential hazards of the study and an explanation that subjects are completely free to refuse to enter the study or to withdraw from it at any time for any reason. If subjects are interested in the study participation, they can sign the Informed Consent and their study participation can begin. Subjects always have the opportunity to discuss their study participation and questions with a CHDR physician before the information visit by phone or during the information visit, screening or clinical conduct with the CHDR physician. In accordance with regulations, sufficient time is given for subjects to understand what their participation means, but no minimum amount of time is specified or required. The Informed Consent and Subject Information will be provided in the local language/Dutch.

### 2.14.3 Benefits and risks assessment, group relatedness

Please refer to Subsection 3.

### 2.14.4 Compensation for injury

The investigator has an insurance that is in accordance with the legal requirements in the Netherlands (Article 7 WMO, under 1). This insurance provides cover for damage to research subjects through injury or death caused by the study. The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study. The investigator has a liability insurance that is in accordance with article 7, under 9, of the WMO.

### 2.14.5 Compensation for subjects

The subjects will receive a monetary payment as compensation. This compensation is based on a national consensus to avoid financially enticing potential subjects in participating in clinical trials by an agreed standard amount per study characteristics; subject burden, time input, invasive measurements, measurements, restrictions, and duration of the trial. The compensation is a sum of all study characteristics The amount per study characteristic is according to the 'wage payment model'. This is based on the minimum wage, time investment, and burden. The compensation will therefore never be more than necessary as to serve as an enticement for participation.

### 2.14.6 Compensation for investigators

TBD

### 2.14.7 Other ethical considerations

No other ethical considerations are applicable for this study.

## 2.15 ADMINISTRATIVE ASPECTS, MONITORING AND CONFIDENTIALITY

The investigator will ensure that this study is conducted in full compliance with the protocol, ICH Good Clinical Practice (GCP) guidelines (https://www.ich.org/page/ich-guidelines), and with the laws and regulations of the country in which the clinical research is conducted.

This study will be conducted according to applicable CHDR's SOPs. Quality assurance will be performed under the responsibility of CHDR's Quality Assurance manager.

### 2.15.1 Approval initial application and substantial modifications

The trial protocol, informed consent form, subject information leaflet, investigational medicinal product dossier, investigators brochure and any other documents required by the Regulation will be submitted for the regulatory approval before the clinical trial is started via CTIS.

The sponsor will also submit and obtain approval for substantial modifications to the original approved documents via CTIS.

A 'substantial modification' (SM) is defined in the CTR as any change to any aspect of the clinical trial which is made after notification of a decision referred to in Articles 8, 14, 19, 20 or 23 and which is likely to have a substantial impact on the safety or rights of the subjects or on the reliability and robustness of the data generated in the clinical trial.

*Non-substantial modification (NSM)*

Non substantial modifications (i.e., without substantial impact on the safety or rights of the subjects and/or the reliability and robustness of the data and when the information is not necessary for oversight) should not be notified as such. These changes should be implemented during the next substantial modification, whenever the scope of the non-substantial changes matches with the scope of the application under evaluation, meaning:

a. Part I non-substantial changes can be included in an application with a Part I or Part I and II scope;

b. Part II non-substantial changes can be included in an application with Part II or Part I and Part II scope.

c. Both Part I & II changes can be included in an application with Part I (only non-SM Part I will be applicable), Part II (only non-SM Part II will be applicable) or Part I and Part II scope.

Non-substantial modifications will not result in a new version of the protocol. These will be listed in a log which contains the trial title and EU CT number and a table describing the non-substantial changes according to CHDR SOP APRAMEND. In case of more than one non-substantial change simultaneously, the changes are all listed in the same table (first column, second row of the table).

The following changes are by definition non-substantial in this study:

- Significant increase in duration of the overall time of the trial, provided that the following conditions are met (79):
  - the exposure to treatment with the IMP is not extended;
  - the definition of the end of the trial is unchanged; and
  - scheduled subject study visits arrangements are unchanged;
- If there is a change in one or more of these conditions, it would be considered to be a substantial modification.
- In case of low interventional trials, additional diagnostic or medical monitoring procedure which is not requested by a MSC if it does not pose more than minimal additional risk or burden to the participants.

- Minor clarifications to the protocol.
- The addition/deletion of exploratory and/or tertiary endpoints as recorded in the TMF with no significant effect on the conduct of the trial.
- A minor increase in the duration (<10%) of the trial.
- A change in the number of trial participants per Member State if the absolute number of participants in the trial is identical or the decrease/increase is insignificant.
- A change in amount and timing of the samples (maximum of 2 samples without a > 10% increase in the amount of blood taken and not exceed 500 ml of blood in total).
- Changes in assay-type and / or institution where an assay will be performed, provided that validated assays will be used.
- Editorial changes to documents in the submission dossier including the participant information materials and the protocol. An editorial change is defined as a modification in the documents of typographical errors and other modifications that in no way alter the meaning or content of the document.
- Determination of additional parameters in already collected materials, which are in agreement with the study objectives and do not provide prognostic or genetic information;
- Other statistical analyses than described in the protocol.
- A change in clinical staff, when this concerns regular staff members of CHDR who comply with internal regulations for training and authorisation.
- A change in dosing schedule in an ascending dose trial, provided the expected exposure of the subjects does not exceed the preset values indicated in this protocol.

*Urgent safety measures*

Urgent safety measures might become necessary to preserve the safety of the subjects included in the study. The requirements for approval should in no way prevent any immediate action being taken by the investigators in the best interests of the subjects. Therefore, if deemed necessary, an investigator can implement an immediate change to the protocol for safety reasons. This means that, exceptionally, the implementation of urgent safety measures will occur before submission of the changes.

If urgent safety measures are required while any assessment is still ongoing, the sponsor should take the appropriate measure and notify the MSC. A SM can then be submitted once the ongoing SM is finalised.

### 2.15.2 Monitoring

An initiation visit will be performed before the first subject is consented. Monitoring visits and contacts will occur at regular intervals thereafter, according to a frequency defined in the study-specific monitoring plan. A close-out visit will be performed after study closure.

### 2.15.3 Recording, handling and storage of information

The sections below will describe the recording, handling and storage of information.

*Handling of data and data protection*

To substantiate compliance to General Data Protection Regulation (EU) 2016/679 a Privacy Governance Framework has been adopted and implemented. CHDR embedded the governance of the Privacy Framework with the organization by installing a Privacy Team that consist of the CEO and several Directors from relevant departments, as well as by appointing an external Data Protection Officer (DPO) and Privacy Officer (PO) who also takes part in the Privacy Team. The DPO is designated

and registered with the Dutch Data Protection Authority and independently monitors the GDPR compliance of CHDR. The PO provides operational support to all employees who process personal data.

All study data will be handled confidential. The investigator will retain the originals of all source documents generated at CHDR until at least database lock, after which all study-related documents will be archived in an outside storage location. Administrative files are archived when a study is finished, defined as when the protocol status is FIN in the study life cycle of the Promasys. Clinical files are archived after the database lock; this is when the phase is transferred from DAT to ANA in Promasys. Financial files are archived after final completion. This outside storage facility has appropriate environmental controls and adequate protection from fire, flood and unauthorized access to secure long-term archiving. Study data will be archived for 25 years, after which it will be destroyed.

The investigator will permit trial-related monitoring, audits, EC review and regulatory inspections, providing direct access to source data and documents.

*Source documents and case report forms (CRF)*

All data from paper source will be entered into the Promasys database twice, by two different individuals. A quality control check will be done by CHDR staff on all data entered in the Promasys database, using data entry progress checks and database listings (blind data review). Errors with obvious corrections will be corrected before database lock.

Results of computer (NeuroCart®/PainCart®/ECG) tests and electronically captured questionnaires, clinical laboratory and pharmacokinetic analyses will be sent electronically to CHDR and loaded into the database.

After the database has been declared complete and accurate, the database will be locked. Any changes to the database after that time can only be made by joint written agreement between the investigator, and the statistician.

*Clinical trial master file and data archiving*

All study data will be handled confidential. The investigator will retain the originals of all source documents generated at CHDR until at least database lock, after which all study-related documents will be archived in an outside storage location. Administrative files are archived when a study is finished, defined as when the protocol status is FIN in the study life cycle of the Promasys. Clinical files are archived after the database lock; this is when the phase is transferred from DAT to ANA in Promasys. Financial files are archived after final completion. This outside storage facility has appropriate environmental controls and adequate protection from fire, flood and unauthorized access to secure long-term archiving. Study data will be archived for 25 years, after which it will be destroyed.

The investigator will permit trial-related monitoring, audits, EC review and regulatory inspections, providing direct access to source data and documents.

*Collection and storage of biological samples*

Biological samples will be coded and labelled according to section 2.10.3. The procedure for the storage of study samples and the destruction and /or shipment to the sponsor of the spare/ left over plasma, serum and urine samples at the end of a study are according to CHDR SOP LSHARC01.

### 2.15.4 Audits and inspections and direct access to source data/documents

This trial may be subject to internal or external monitoring, auditing or inspections procedure to ensure adherence to GCP. Access to all trial-related documents including direct access to source data will be given at that time.

### 2.15.5 Reporting of serious breaches

The investigator will notify the Member State concerned about a serious breach of the Regulation or of the version of the protocol applicable at the time of the breach through CTIS without undue delay but not later than **seven days** of becoming aware of that breach (CTR: Article 52).

### 2.15.6 Notification of the start and the end of the recruitment

The investigator will notify within 15 days each Member State concerned of the first visit of the first subject in relation to that Member State through CTIS (CTR: Article 36(2)).This is defined as Fist Subject First Screening.

The investigator will notify within 15 days each Member State concerned of the end of the recruitment of subjects for a clinical trial in that Member State through the EU (CTR: Article 36(3)). End of recruitment is being considered equivalent to LSLV for notification purposes.

### 2.15.7 Temporary halt/(early) termination

The investigator will notify within 15 days each Member State concerned of the end of a clinical trial in relation to that Member State through CTIS (CTR: Article 37(1)).

*Temporary halt/early termination for reasons not affecting the benefit-risk balance*

The investigator will notify with 15 days each Member State concerned of a temporary halt of a clinical trial in the Member State concerned for reasons not affecting the benefit-risk balance through CTIS (CTR: Article 37(5)).

When a temporarily halted clinical trial for reasons not affecting the benefit-risk balance is resumed the investigator will notify each Member State concerned through CTIS (CTR: Article 37(6)). The investigator will notify to the EU portal CTIS of early termination of the clinical trial for reasons not affecting the benefit-risk balance through CTIS. The reasons for such action and, when appropriate, follow-up measures for the subjects will be provided as well (CTR: Article 37(7)).

*Temporary halt/early termination for reasons of subject safety*

In accordance to article 38 of the CTR, the investigator will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The temporary halt or early termination of a clinical trial for reasons of a change of the benefit-risk balance will be notified to the Member State concerned through the EU portal CTIS without undue delay but not later than in 15 days of the date of the temporary halt or early termination. It shall include the reasons for such action and specify follow-up measures. The restart of the clinical trial following a temporary halt as referred to in paragraph 1 shall be deemed to be a substantial modification subject to the authorisation procedure laid down in Chapter III of the CTR (CTR: Article 38).

### 2.15.8 Summary of the results

Within one year from the end of a clinical trial in the Member State concerned, the investigator will submit to the EU database CTIS a summary of the results of the clinical trial. The content of the summary of the results is set out in CTR Annex IV. It shall be accompanied by a summary written in a manner that is understandable to laypersons. The content of the summary is set out in CTR Annex V (CTR: Article 37(4)).

### 2.15.9 Public disclosure and publication policy

In accordance with standard editorial and ethical practice, the results of the study will be published. The authorship guidelines of the Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals will be followed regarding co-authorship.

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