[redacted] pharmacokinetic modelling of intravenous and intranasal formulations of naloxone in healthy volunteers

Internal reference: IMIMFCTL/NLX_1

Development Phase: Phase I

NCT number: NCT06306391

Statistical Analysis Plan

(version 1.0, 15th May 2024)

TABLE OF CONTENTS

1. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	Adverse event
BP	Blood Pressure
CNS	Central nervous system
CRS	Clinical Study Report
ECG	Electrocardiogram
EOS	End of Study
HR	Heart Rate
HVD	Half value duration
IM	Intramuscular
IN	Intranasal
IV	Intravenous
LLOQ	Lower Limit of Quantification
MedDRA	Medical Dictionary for Regulatory Activities
N	Number
PSCA	Potentially Clinically Significant Abnormalities
PBPK	Physiologically Based Pharmacokinetic
SAP	Statistical Analysis Plan
TEAE	Treatment-Emergent Adverse Event
URC	Clinical Research Unit

2. VERSIONS AND INVESTIGATOR

2.1. Version

Version	Date	Comments
1.0	15th May 2024	-

2.2. Investigators

Principal Investigators	[redacted]
Sponsor	[redacted]

1. INTRODUCTION

This Statistical Analysis Plan (SAP) describes in detail the statistical methodology applied for the analysis of pharmacokinetic (PK), pharmacodynamic (PD), and safety data in the Phase I crossover study:

"[redacted] **pharmacokinetic modelling of intravenous and intranasal formulations of naloxone in healthy volunteers."**

This SAP is aligned with the approved protocol and reflects the analyses conducted in the Clinical Study Report (CSR).

2. BACKGROUNG INFORMATION

Naloxone is a semisynthetic morphine derivative and is a specific opioid antagonist that acts competitively at opioid receptors. It reveals very high affinity for the opioid receptor sites. Naloxone does not possess the "agonistic" or morphine-like properties characteristic of other opioid antagonists. In the absence of opioids or agonistic effects of other opioid antagonists, it exhibits essentially no pharmacologic activity. Naloxone has not been shown to produce tolerance or cause physical or mental dependence.

Intranasal (IN) administered naloxone has been demonstrated to be rapidly absorbed, as evidenced by very early appearance (as early as 1 minute after administration) of the active substance in systemic circulation.

A study investigating IN naloxone (Nyxoid™) at doses of 1, 2, 4 mg shows that the median (range) t_{max} associated with IN administration of naloxone was 15 (10, 60) minutes for 1 mg, 30 (8, 60) minutes for 2 mg and 15 (10, 60) minutes for 4 mg intranasal doses. Onset of action following IN administration can reasonably be expected to occur in each individual before the t_{max} is reached.

The half value duration (HVD) for IN administration was longer than for intramuscular (IM) administration (IN, 2 mg, 1.27h, IM, 0.4 mg, 1.09h) from which we can infer a longer duration of action of naloxone given by the IN rather than the intramuscular route.

The overall aim is Physiologically Based Pharmacokinetic (PBPK) model for drug release, disposition and dissolution following IN drug delivery. Following delivery, drug release from the formulations and disposition in the nasal cavity will be described. The model will also account for drug transport through epithelial tissue and mucociliary clearance. Predicted local brain and systemic PK will therefore be linked to drug release, dissolution, and disposition in the brain and the rest of the body. In vitro permeability, transporter kinetic and proteomics data from the olfactory region, as well as published clinical data following IV and IN delivery will be used to support this. Integration of all these processes into a dynamic model will help to disentangle different kinetic processes which contribute to this complex system, which will help us to explore the interplay between various processes.

- Intranasal: 1.8 mg nasal spray, solution in single-dose container.
- Intravenous (IV): 1 mg in total (from 0.4 mg/mL injectable solution)

Doses selected, in the low range of those tested previously in clinical opioid overdose situations, were already tested experimentally in a bioavailability study in healthy subjects comparing the two routes of administration of naloxone that will be evaluated in this protocol.

3. STUDY DESIGN

This study follows an open-label, randomized, two-period, two-sequence crossover design in approximately eight healthy adult volunteers. Subjects are randomized in a 1:1 ratio to one of two treatment sequences. In Sequence 1, subjects receive IN naloxone in Period 1 followed by IV naloxone in Period 2. In Sequence 2, the order of administration is reversed. A washout period of at least 72 hours separates the two treatment periods, ensuring adequate elimination of naloxone prior to crossover.

PK blood samples are collected intensively from pre-dose up to 6 hours post-dose in each period. PD parameters, including vital signs, are measured repeatedly over the same timeframe. Safety assessments are conducted throughout both periods and include adverse event (AE) monitoring, laboratory evaluations, vital signs, and electrocardiograms.

3.1. Study drugs

IV naloxone (Naloxona Kern Pharma® 0.4 mg/mL solution for injection and infusion) and IN naloxone (Nyxoid® 1.8 mg nasal spray solution in single-dose, Mundipharma Pharmaceuticals, S.L.).

3.2. Number of subjects

8 healthy male and female subjects will be randomly assigned to one of two sequences in the crossover study. All subjects will receive the same dose of IN or IV naloxone and the sequence will be determined following randomization.

4. OBJECTIVES

4.1. General objective

To characterize the PK and PD of two formulations of naloxone (IN and IV) in healthy subjects, which will be used to verify/validate nasal- central nervous system (CNS)-PBPK model predictions following IN dosing.

4.2. Specific objectives

4.2.1. Pharmacokinetics objectives

To calculate plasma PK parameters listed below using a non-compartmental model:

- Observed maximum concentration: C_{max}.
- Observed last concentration: Cl_{ast}.
- Time to observed maximum concentration: T_{max}.
- Time lag (time to first measurable plasma concentration): t_{lag}.
- Area under the concentration-time curve (AUC0-24h, AUC0-t, AUC0-∞) where t is the latest observed timepoint.
- Terminal elimination half-life: t1/2.
- Apparent clearance: Cl/F.
- Apparent volume of distribution: Vd/F.

- Elimination rate constant (λz).

4.2.2. Pharmacodynamic objectives

To assess the PD effects of naloxone by measuring:

- Primary PD endpoints: Effects of naloxone on Heart Rate (HR) and Blood Pressure (BP).
- Secondary PD endpoints: Effects of naloxone on cortisol in plasma on Visit 1 and Visit 4.

4.2.3. Pharmacodynamic objectives

To evaluate drug safety focusing on:

- Treatment-Emergent Adverse Event (TEAEs) from Day 1 to End of Study (EOS).
- Potentially Clinically Significant Abnormalities (PSCAs) in vital signs, Electrocardiogram (ECG) and safety laboratory parameters from Day 1 to EOS.

5. Analysis Population

5.1. Pharmacokinetic population

The PK population will include all subjects who receive both treatment and have sufficient and valid plasma concentration data to allow reliable estimation of primary PK parameters using non-compartmental analysis.

Subject with major protocol deviation that could meaningfully affect PK interpretation may be excluded from the PK population.

5.2. Pharmacodynamic population

The PD population will include all subjects who receive at least one dose of naloxone and have at least one valid baseline and one post-dose PD measurement within a treatment period.

Subjects with major protocol deviations affecting PD interpretation may exclude from PD analyses, as appropriate.

5.3. Safety population

The safety population will include all subjects who receive at least one dose of naloxone in any study period. Safety analysis will perform according to the actual treatment received in each period.

6. General Statistical Principles

All statistical analyses will be performed using validated statistical software such as R or SAS. Pk parameters will be derived using validated non-compartmental analysis software (Phoenix™ WinNonlin).

Continuous variables will be summarized using descriptive statistics, including the number of observations, arithmetic means, standard deviation, standard error of the mean, median, minimum, and maximum values. Categorical variables will be summarized using frequencies and percentages.

Given the study design and the limited sample size, no formal confirmatory hypothesis testing is planned. Where inferential analyses are conducted for comparative purposes between treatments, these analyses will be considered exploratory and a nominal two-sided significance level of 0.05 will be applied. No adjustments for multiplicity will be performed.

No interim analysis is planned. Missing data will not be imputed, and all analyses will be based on observed data only.

7. DATA HANDLING

Baseline values for each treatment period will be defined as the last valid measurement obtained prior to dosing in that period. For pd variables, change-from-baseline will be calculated for each subject at each post-dose timepoint as the difference between the observed post-dose value and the period-specific baseline value.

For pk analyses, actual recorded sampling times will be used in all calculations. Plasma concentrations below the lower limit of quantification (LLOQ) will be handled as follows: pre-dose concentrations below LLOQ will be set to zero; post-dose concentrations below LLOQ occurring prior to the first quantifiable concentration will also be set to zero; post-dose concentrations below LLOQ occurring after the first quantifiable value will be treated as missing for the purposes of non-compartmental analysis.

No imputation will be applied to missing PK or PD measurements. If insufficient data are available to reliably characterize the terminal elimination phase for a subject, parameters dependent on the terminal phase (such as λz, t½, and AUC extrapolated to infinity) may not be calculated for that subject.

8. PHARMACOKINETIC ANALYSIS

Pharmacokinetic analyses will be performed separately for intranasal and intravenous administration using non-compartmental methods.

The primary PK parameters to be derived for naloxone and its metabolites (including naloxol and naloxone-3-glucuronide) include maximum observed C_{max}, T_{max}, C_{last}, t_{lag}, AUC^{0-t}, $AUC_{0-\infty}$, λz, and $t_{½}$.

For IVs administration, systemic clearance (CL) and volume of distribution (Vd) will be calculated. For IN administration, apparent clearance (CL/F) and apparent volume of distribution (Vd/F) will be estimated.

The area under the concentration–time curve and the terminal elimination rate constant will be derived using standard non-compartmental procedures implemented in validated software.

All pharmacokinetic parameters will be summarized descriptively and plasma concentration–time profiles for naloxone and its metabolites will be summarized descriptively and graphically presented.

9. PHARMACODYNAMIC ANALYSIS

PD parameters include HR, systolic BP and diastolic BP. For each parameter, descriptive statistics will be presented at baseline and at each post-dose timepoint.

Change-from-baseline values will be calculated and summarized for each treatment. Mean change-from-baseline over time will be graphically presented for IV and IN naloxone, with error bars representing standard deviation or standard error as appropriate. These graphical presentations will be analogous in structure to the PK concentration–time curves to allow visual comparison between systemic exposure and cardiovascular response.

Time-course plots will be generated for each PD parameter to illustrate the temporal profile of the response.

Although plasma cortisol sampling was planned, no determinations were performed; therefore, no statistical analysis of cortisol concentrations will be conducted.

All pharmacodynamic analyses are descriptive in nature.

10.SAFETY ANALYSIS

Safety analyses will be conducted using the Safety Population.

AE will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and summarized by system organ class and preferred term. Adverse events will be summarized by system organ class, preferred term, severity, and relationship to study drug. Serious adverse events will be described individually.

Vital signs, ECG parameters, and clinical laboratory parameters will be summarized descriptively at baseline and post-dose time points. Change-from baseline values will be presented. Clinically significant abnormalities will be flagged and listed where applicable.

11.DESVIATION FROM THE SAP

Any deviation from the methods described in this SAP will be documented, justified, and reported in the Clinical Study Report.