Study Title: Clinical study to investigate the pharmacokinetics of multiple repeated doses of intranasal naloxone

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Statistical Analysis Plan

SCR-011: Clinical Study to Investigate the Pharmacokinetics of Multiple Repeated Doses of Intranasal Naloxone

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CONFIDENTIAL

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Contents

Abbreviations and definitions

AE	Adverse event
AUC0-inf	Area under the plasma concentration time curve from time 0 extrapolated to infinity
AUC_{0-t}	Area under the concentration-time curve from 0 to last quantifiable concentration
C_{last}	Last quantifiable concentration
Cmax	Maximum observed plasma concentration
CV	Coefficient of variation
ECG	Electrocardiogram
eCFRs	Electronic case report forms
FDA	Food and Drug Administration
IN	Intranasal
inf	Infinity
K_{el}	Elimination rate constant
pAUC	partial area under the curve
PD	Pharmacodynamic
PK	Pharmacokinetic
SAP	Statistical analysis plan
SD	Standard deviation
$t_{1/2}$	Terminal half-life
TEAE	Treatment-emergent adverse event
Tmax	Time of maximum concentration (C_{max})

Change Log

Version / Date	Section	Summary of Changes
1.0 / 10 Mar 2021	General	Developed initial statistical analysis plan

1. Introduction

This document outlines the proposed statistical methods for data analysis of data collected from Protocol 'SCR-011: Clinical study to investigate the pharmacokinetics of multiple repeated doses of intranasal naloxone'.

The following statistical analysis plan (SAP) provides the framework for the summarization of the data from this study. The SAP may change due to unforeseen circumstances. Any changes made to the SAP will be documented in a change log within the SAP along with a rationale for the changes.

2. Study Objectives

The study objectives are:

1. To determine and compare the pharmacokinetics of intranasal (IN) naloxone between the 4-dose naloxone arms and the 2-dose naloxone arm.
2. To use the pharmacokinetic data from each of the 3 naloxone dosing schedules/doses to predict the time to reverse opioid-induced respiratory depression following different overdose scenarios based on pharmacokinetic/pharmacodynamic (PK/PD) models

3. Study Overview

3.1 Study Design

This study will be a randomized, unblinded, three-way crossover study to determine naloxone plasma concentration after administration of multiple doses of intranasal (IN) naloxone.

Table 3-1: Study Schedule

Day -1	Day 1	Days 2-3	Day 4	Days 5-6	Day 7	Day 8
Check-in	Treatment Period 1	Washout	Treatment Period 2	Washout	Treatment Period 3	Check-out

The following 3 treatments will be evaluated in a randomized order over the 3 treatment periods.

Table 3-2: Study Treatments

Treatment	Description
A	Four 4 mg IN naloxone doses (1 dose every 2.5 min; L at 0 min, R at 2.5 min, L at 5 min, R at 7.5 min)
B	Four 4 mg IN naloxone doses (2 doses every 2.5 min; L and R at 0* min, L and R at 2.5* min)
C	Two 4 mg IN naloxone doses (L at 0 min, R at 2.5 min)

*L = Left Nostril, R = Right Nostril, * doses will be ~5 seconds apart (one after the other)*

Healthy subjects will be randomized to one of six treatment sequences (i.e., ABC, ACB, BAC, BCA, CAB, CBA). FDA will prepare the randomization schedule. Subjects will report to the study site for screening from Days -28 to -2 and then will return to the site on Day -1 for baseline assessments and check-in. After check-in (Day -1), subjects will receive dosing for the 3 respective treatment periods on Days 1, 4 and 7. There will be two days of washout between each treatment period. Participants will be confined in the study clinic from Day -1 until the morning of Day 8. On dosing days, dosing will occur as per the treatment description and PK assessments will occur at the following time points:

- PK assessment: 0 (pre-dose), 2, 4.5, 7, 10, 12.5, 15, 20, 30, 45, 60, 120, 180, 240, 360, and 720 minutes

The subject should remain fully supine for approximately one-hour post-dose. Subjects should be instructed not to breathe through the nose during administration of the nasal spray into the nose. Subjects will be instructed to leave their masks off for twenty minutes after drug administration.

A summary of all assessments prior to and following study drug administration on Day 1, is described in the Protocol, Schedule of Events.

Subjects will be discharged from the study after completion of all study procedures. If a subject discontinues from the study prematurely, all procedures scheduled for the end of the study will be performed.

4. Study Endpoints

4.1 Primary Endpoint

First time point when there is a higher naloxone plasma concentration in the 4-dose naloxone arms compared to the 2-dose naloxone arm

4.2 Secondary Endpoints

1. First time point when there is a higher naloxone plasma concentration in the 4-dose naloxone arm B (2 doses every 2.5 minutes) compared to the 4-dose naloxone arm A (1 dose every 2.5 minutes)

2. Dose-proportionality of the 4-dose naloxone arms in reference to the 2 -dose naloxone arm based on Cmax, $AUC_{0\text{-}inf}$, and $AUC_{0\text{-}t}$

3. Predicted time to rescue a patient from simulated opioid-induced respiratory depression from fentanyl and carfentanil following medium and high overdose scenarios

4.3 Exploratory Endpoint

Naloxone C_{max}, $AUC_{0\text{-}inf}$, $AUC_{0\text{-}t}$, t_{max}, and partial AUC [pAUC] within the first 30 minutes of dosing

4.4 Sample Size

Twenty healthy participants are planned for enrollment with up to 4 replacements. Naloxone plasma concentration profiles and variabilities were calculated based on summary pharmacokinetic data from the IN naloxone product label. Predicted naloxone concentrations were determined for each of the proposed dosing treatments based on principles of superposition (i.e., for linear pharmacokinetics the total concentration of a drug in circulation is the sum of remaining concentration from each administered dose prior to the current measurement time) with a focus on comparing plasma concentrations at early timepoints. Power calculations were based on the ratio between concentrations for each of the two primary comparisons (Treatment A vs Treatment C and Treatment B vs Treatment C). Of the comparisons, the power for the 4-dose naloxone arm A (test) versus the 2-dose naloxone arm C (reference) was expected to be lower than the 4-dose naloxone arm B (test) versus the 2-dose naloxone arm C (reference). For the different proposed treatments, the ratio in concentrations and the coefficient of variation (CV) at each timepoint based on principles of superposition are shown in Table 4-1.

Table 4-1: Predicted concentration ratio and CV between treatments at different timepoints and power calculation at a 0.0221 significance level

Comparison	Time (min)	Concentration Ratio	CV	Power (n=18) at $p = 0.0221$
Primary Endpoint (comparisons of 4-dose treatment arms vs. 2-dose treatment arm reference)				
Treatment A vs Treatment C	**10**	1.36	68	49
	12.5	1.57	58	91
	15	1.78	48	100
Treatment B vs Treatment C	**4.5**	2	111	86
	7	2	90	94
	10	2	68	99
Secondary Endpoint (comparison between the 4-dose treatment arms)				
Treatment B vs Treatment A	**4.5**	2	111	86
	7	1.85	90	88
	10	1.47	68	68

As the intent of administering naloxone is rapid reversal of an overdose, the planned primary analysis is a comparison of naloxone concentrations between treatment arms across pre-specified consecutive time points. Each of the paired comparisons was assumed to include three pre-specified timepoints (timepoints correspond to those listed in Table 4-1). The time points will be evaluated sequentially with adjustments for multiplicity using Pocock boundaries (Pocock, 1977). For three pre-specified assessments, this would correspond to assessments at a 0.0221 significance level for comparisons at individual time points to maintain an overall 0.05 significance level.

Based on these considerations and the concentration ratios and CVs in Table 4-1, a sample size was determined so that at least one of the planned time points would have greater than 90% power at a 0.0221 significance level for a one sided, log-transformed paired comparison (for the primary endpoint). For concluding an increase in naloxone concentration for Treatment A compared to Treatment C at 15 minutes, at least 12 subjects would be necessary assuming a 1.78-fold increase in exposure and 48% CV for the treatments. For concluding an increase in naloxone concentration for Treatment B compared to Treatment C at 10 minutes, at least 12 subjects would have the desired power assuming a 2-fold increase in exposure and 68% CV for the treatments. The sample size was further increased to 20 subjects to account for discontinuations and situations where the CV values in Table 4-1 may be underestimated for repeat naloxone dosing.

5. Analysis Populations

The PK population will include all subjects who receive study drug and have at least one estimable PK parameter after dosing. The safety population will include all subjects who receive

at least 1 dose of the study drug. The analysis population will include all subjects in the PK population with sufficient data to calculate PK parameters. Only subjects with results from two or more periods will be included in comparisons. Treatment in all analysis populations will be assigned based upon the treatment which the subjects actually received.

6. Data Screening and Acceptance

6.1 Handling of Missing and Incomplete Data

The following approaches will be used for the handling of missing and incomplete data:

- Missing PK data (e.g., skipped plasma sample collected) will not be imputed.
- Missed dose, dose administered outside the window, or other issues with dose administration (e.g., product delivery fails) will result in PK data from that period for that subject being excluded from all treatment arm comparisons and summary statistics from that time point forward. Other situations, such as a subject sneezing in close proximity to dosing will be carefully documented by study staff and handling of these cases will be determined and documented before any concentration data is available.
- PK samples collected outside the protocol-defined sampling window (i.e., more than ± 0.5 minute from the scheduled time for the first 15 minutes of collections) will be excluded from analyses comparing naloxone concentrations at the specific time point that is outside the window.
- PK measurements below the lower limit of quantification will be set to zero for all analyses.

7. General Statistical Considerations

All data will be presented in data listings. Data from subjects excluded from an analysis population will be presented in the data listings, but not included in the calculation of summary statistics. Demographic and baseline characteristics will be summarized overall and by treatment for all subjects.

7.1 Subject Disposition

The number of subjects who enroll in the study and the number and percentage of subjects who complete each assessment will be presented. The frequency and percentage of subjects who withdraw or discontinue from the study and the reason for withdrawal or discontinuation will be summarized.

7.2 Demographic and Baseline Characteristics

Continuous demographic and baseline characteristic variables (age, height, weight, and body mass index) will be summarized overall and by treatment using descriptive statistics (number of subjects, mean, standard deviation [SD], median, minimum, and maximum). The number and percentage of subjects in each class of categorical demographic and baseline characteristic variables will also be summarized.

7.3 Pharmacokinetic Analyses

7.3.1 Naloxone Concentration Comparisons

The primary endpoint is the first timepoint when there is a higher naloxone plasma concentration in the 4-dose naloxone arms compared to the 2-dose naloxone arm. Separate comparisons will be performed with the 4-dose naloxone arm A (test) versus the 2-dose naloxone arm C (reference) and the 4-dose naloxone arm B (test) versus the 2-dose naloxone arm C (reference). No adjustment for multiplicity is planned for these separate comparisons.

Secondary endpoints will include determination of the first time point when there is a higher naloxone plasma concentration in the 4-dose naloxone arm B (2 doses every 2.5 minutes) as test compared to the 4-dose naloxone arm A (1 dose every 2.5 minutes) as reference. The same approach outlined for the primary endpoint analysis above will be used for this secondary endpoint comparison. The secondary endpoint analysis will be conducted regardless of the primary endpoint results.

Table 7-1: Pre-specified Timepoints and *p*-value Threshold for Comparing Naloxone Concentrations Across Treatments (Maintain Overall *p*-value Per Comparison at 0.05) (Pocock, 1977)

Comparison	Time (min)	Interim Analysis	*p*-value Threshold
Treatment A vs Treatment C (primary)	**10**	1	0.0221
	12.5	2	0.0221
	15	3 (final)	0.0221
Treatment B vs Treatment C (primary)	**4.5**	1	0.0221
	7	2	0.0221
	10	3 (final)	0.0221
Treatment B vs Treatment A (secondary)	**4.5**	1	0.0221
	7	2	0.0221
	10	3 (final)	0.0221

For both the primary and secondary analyses, up to three different time points have been pre-specified for each comparison. Time points will be evaluated from earliest to latest time (Table 7-1). Testing will be conducted sequentially along planned interim analyses (i.e., start at the initial time and progress forward, stopping if success is achieved) at a 0.0221 significance level. Testing will proceed through each time point until either one passes (reported as the earliest time where a difference in concentration is observed) or all pre-specified time points fail (no difference in naloxone concentrations between treatments for the selected time points).

For comparing treatment A to treatment C, the analysis will use naloxone concentration data collected at 10, 12.5, and 15 min. For both the treatment B to treatment C comparison and the treatment B to treatment A comparison, the pre-specified time points are 4.5, 7, and 10 min. All

subjects contributing naloxone concentration measures from both treatments at the pre-specified time points will be included in the analysis. At each of these timepoints, subject-level naloxone concentration will be log-transformed. Naloxone concentration values that are below the lower limit of quantification (i.e., zero) will result in that time point from that subject being removed for comparisons involving that treatment arm.

A paired Student's t-test will be used for comparing naloxone concentrations between treatments. To demonstrate an increase in naloxone concentration with the test compared to reference, it is necessary that the lower bound of the one-sided 97.79% interval for the geometric mean ratio excludes 1. The results will be transformed back to the original scale by exponentiation to provide treatment geometric means.

Normality assumption will be verified using the Shapiro-Wilke test for normality and homogeneity of variances will be verified using Levene's test. If either assumption is not valid, a Mann-Whitney-U test will be used for the comparisons rather than a t-test.

7.3.2 Dose Proportionality

Dose proportionality based on C_{max}, AUC_{0-t}, and AUC_{0-inf} will be compared between each of the three naloxone treatments as secondary endpoints. The 4-dose naloxone arms (separate comparisons for 1 dose every 2.5 minutes and 2 doses every 2.5 minutes) will be compared with the 2-dose naloxone arm (1 dose every 2.5 minutes). An additional comparison will be performed between the 4-dose naloxone arm B (2 doses every 2.5 minutes) as test and 4-dose naloxone arm A (1 dose every 2.5 minutes) as reference.

Dose-adjusted values for C_{max}, AUC_{0-t}, and AUC_{0-inf} will be calculated based on noncompartmental PK parameter results, normalized to per mg of naloxone administered. Using linear mixed-effects modeling, comparisons of log-transformed dose-adjusted PK parameters will be performed. Treatment will be a fixed effect. Subject will be included as a random effect on the intercept. Treatment differences on the log-scale will be estimated for the PK parameters. Geometric mean ratio and 90% CIs will be obtained by exponentiation of the treatment effect and 90% CIs based on the log-transformed scale. Dose-proportionality will be concluded if the confidence interval includes 1.

7.3.3 Plasma Pharmacokinetics

The following PK parameters will be determined for naloxone for each subject across all treatments:

- **AUC from time 0 to the sampling time corresponding to the last quantifiable concentration (C_{last}) (AUC_{0-t})**

  AUC_{0-t} will be calculated according to the linear trapezoidal rule from time 0 minutes to the last quantifiable concentration.

- **AUC from time 0 extrapolated to infinity (AUC_{0-inf})**

AUC_{0-t} will be extrapolated to infinity from the AUC from time 0 to the sampling time corresponding to the last quantifiable concentration plus the last quantifiable concentration divided by K_{el}, as follows:

$AUC_{0-inf} = AUC_{0-t} + C_{last}/K_{el}$

- **Partial AUCs (pAUC) over time intervals of 0 to 7 ($pAUC_{0-7}$), 0-10 ($pAUC_{0-10}$), 0-12.5 ($pAUC_{0-12.5}$), 0-15 ($pAUC_{0-15}$), 0-20 ($pAUC_{0-20}$), and 0-30 ($pAUC_{0-30}$) minutes**

  pAUC will be calculated according to the linear trapezoidal rule from time 0 minutes to 7, 10, 12.5, 15, 20, and 30 minutes after time from first dose

- **Maximum concentration (observed peak drug concentration) (C_{max})**

  C_{max} will be read directly from the observed concentrations.

- **Time at which C_{max} occurs (T_{max})**

  T_{max} will be read directly from the observed concentrations as the blood sampling time corresponding to C_{max}. The earlier time will be reported in cases where the same C_{max} value is observed for a subject at multiple time points

- **Terminal half-life ($t_{1/2}$)**

  The terminal half-life will then be calculated using the elimination rate constant as:

  $t_{1/2}=\ln(2)/K_{el}$

- **K_{el}**

  The terminal elimination rate constant, which will be determined from the terminal slope of the log-concentration curve using linear regression

The PK parameters will be analyzed using noncompartmental methods based on actual sampling times. Plasma concentrations below the limits of quantification will be set to zero for the purpose of this analysis (see Section 6). AUC_{0-inf}, $t_{1/2}$, and K_{el} for naloxone by treatment and subject will only be included if the subject has 3 or more concentration values on the terminal portion of the pharmacokinetic curve and with an adjusted coefficient of determination (R^2) greater than 0.80.

These PK parameters will be summarized using descriptive statistics (number of subjects, mean, SD, CV, median, minimum, and maximum) for naloxone.

All parameters will be calculated using R software. Mean and individual concentration-time profiles will be presented in graphs.

7.4 Additional Secondary Analyses

7.4.1 PK/PD Modeling of Time to Reversal of Opioid Induced Respiratory Depression

PK data from this study will be used to update the naloxone PK model in a previously developed PK/PD model for opioid-induced respiratory depression. The updated model will be used to predict the mean time to rescue a patient from simulated opioid induced respiratory depression from fentanyl and carfentanil following a range of overdose scenarios (e.g. low, medium and high). Full details of the analysis will be described in a separate Modeling Analysis Plan (MAP).

7.5 Safety Analyses

7.5.1 Adverse Events

All adverse events (AEs) will be coded using the latest version of the Medical Dictionary for Regulatory Activities (MedDRA). The incidence of treatment-emergent adverse event (TEAEs), organized by system organ class and frequency, will be summarized by seriousness, severity, relationship to treatment, and by treatment at onset of the TEAE. A detailed listing of serious AEs and TEAEs leading to withdrawal will also be provided.

7.5.2 Clinical Laboratory Tests

Clinical laboratory results (hematology, serum chemistry, and urinalysis) will be summarized using descriptive statistics (number of subjects, mean, SD, minimum, median, and maximum). Clinical laboratory results will be classified as normal or abnormal, according to the reference ranges of the individual parameter. The number and percentage of subjects with abnormal laboratory results will be provided. No statistical testing will be performed on clinical laboratory data.

7.5.3 Vital Sign Measurements

Vital sign measurements and changes from baseline will be summarized using descriptive statistics (number of subjects, mean, SD, minimum, median, and maximum) by treatment and timepoint.

7.5.4 Safety 12-lead Electrocardiograms

Abnormal 12-lead ECG findings will be recorded as AEs (not planned for each treatment period) in a data listing.

7.5.5 Physical Examinations

Abnormal physical examination findings will be recorded as AEs (not planned for each treatment period).

7.5.6 Nasal Irritation Score Analyses

Nasal Irritation Score is an examination of the nasal passage conducted by a trained observer to evaluate evidence of irritation to the nasal mucosa. These results will be summarized using descriptive statistics for all treatment groups.

7.5.7 Other Safety Data

All concomitant medication usage and medications that changed in daily dose, frequency, or both since the subject provided informed consent will be summarized for each subject.

8. Data Quality Assurance

Completed eCRFs are required for each subject randomly assigned to treatment. Electronic data entry will be accomplished through the ClinSpark® remote electronic data capture system, which allows for on-site data entry and data management. This system provides immediate, direct data transfer to the database, as well as immediate detection of discrepancies, enabling site coordinators to resolve and manage discrepancies in a timely manner. Each person involved with the study will have an individual identification code and password that allows for record traceability. Thus, the system, and subsequently any investigative reviews, can identify coordinators, investigators, and individuals who have entered or modified records.

Furthermore, the investigator retains full responsibility for the accuracy and authenticity of all data entered into the electronic data capture system. The completed dataset and their associated files are the sole property of the sponsor and should not be made available in any form to third parties, except for appropriate governmental health or regulatory authorities, without written permission of the sponsor.

9. Appendices

9.1 Randomization schedule

This is a 3-period, unblinded, crossover study where approximately 20 subjects will be enrolled to receive either two or four administrations of 4 mg intranasal naloxone following different administration schedules. Healthy subjects will be randomized to one of six treatment sequences (i.e., ABC, ACB, BAC, BCA, CAB, CBA), where treatment codes are summarized in the table below. The first 18 subjects enrolled will be randomized in blocks of 6. The remaining two subjects will be randomly placed in 2 of the 6 treatment sequences. The study plans for 1:1 enrollment of males and females, but the randomization will not account for sex and treatment sequence interactions.

Table 9-1: Treatment Codes and Treatment Names

Treatment Code	Treatment Name
A	Four 4 mg IN naloxone doses (1 dose every 2.5 min; L at 0 min, R at 2.5 min, L at 5 min, R at 7.5 min)
B	Four 4 mg IN naloxone doses (2 doses every 2.5 min; L and R at 0* min, L and R at 2.5* min)
C	Two 4 mg IN naloxone doses (L at 0 min, R at 2.5 min)

10. References

NARCAN Nasal Spray Label, dated 08/06/2020. Obtained from https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/208411Orig1s004lbl.pdf

Pocock, S. (1977). Group Sequential Methods in the Design and Analysis of Clinical Trials. *Biometrika, 64*(2), 191-199.