Official Title: A Study to Characterize the Time Course of Reversal of Opioid (Fentanyl)-Induced Respiratory Depression Following Administration of Nalmefene Autoinjector 1.5 mg (0.94% $MgCl_2$) Intramuscular·and Narcan® 4 mg Intranasal in Healthy Subjects

NCT #: NCT06719986

Date of Document: August 7, 2022

STATISTICAL ANALYSIS PLAN

A Study to Characterize the Time Course of Reversal of Opioid (Fentanyl)-Induced Respiratory Depression Following Administration of Nalmefene Autoinjector 1.5 mg (0.94% $MgCl_2$) Intramuscular and Narcan® 4 mg Intranasal in Healthy Subjects

Protocol Number:	NAL1004
Protocol Version and Date:	Amendment 1: 28-JULY-2022
Study Drug Name:	Fentanyl, Nalmefene Hydrochloride, Naloxone Hydrochloride
Phase:	Phase 1
Sponsor:	Purdue Pharma, LP One Stamford Forum Stamford, CT 06901-3431 USA
Analysis Plan Date:	August 7, 2022
Analysis Plan Version:	Version 1.0

SPONSOR SIGNATURE PAGE

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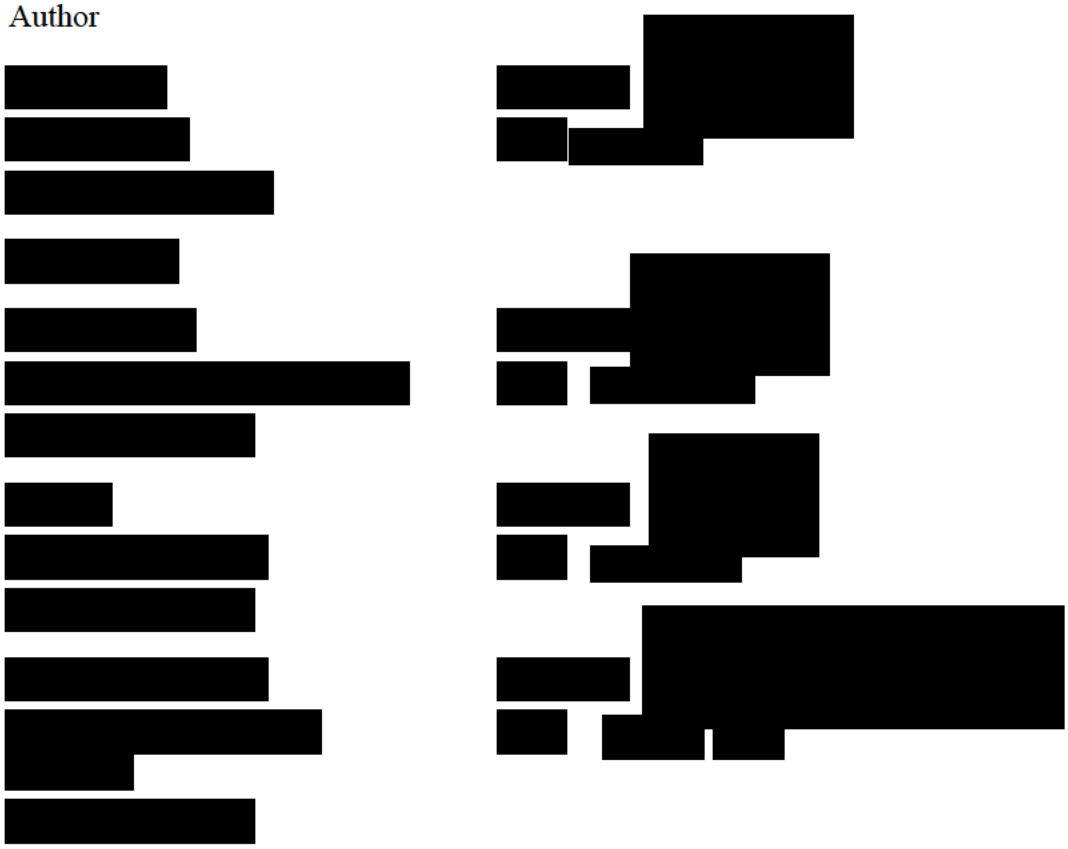
By signing this document, I acknowledge that I have read the document and approve of the planned statistical analyses described herein. I agree that the planned statistical analyses are appropriate for this study, are in accordance with the study objectives, and are consistent with the statistical methodology described in the protocol and all applicable regulatory guidance and guidelines.

Author



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ABBREVIATIONS

®	registered trademark
AE	adverse event
ALT	alanine aminotransferase (alanine transaminase; also SGPT)
AST	aspartate aminotransferase (aspartate transaminase; also SGOT)
bpm	beats per minute
BMI	body mass index
BUN	blood urea nitrogen
CO_2	carbon dioxide
CRF	case report form
DO	Doctor of Osteopathic Medicine
DoR	duration of reversal
dL	deciliter(s)
ECG	electrocardiogram
EOQ	end of qualification
EOS	end of study
ET	end-tidal
fax	facsimile transmission
FDA	Food and Drug Administration
FSH	follicle stimulating hormone
GCP(s)	Good Clinical Practice(s)
gm or g	gram(s)
HBsAg	hepatitis B surface antigen
HCV	hepatitis C virus (antibody)
HIV	human immunodeficiency virus
hr or h	hour
HS	healthy subjects
ICF	informed consent form
ICH	International Conference on Harmonisation
IM	intramuscular
IRB	Institutional Review Board
IV	intravenous
K_2EDTA	dipotassium ethylenediaminetetraacetic acid
kg	kilogram(s)
L	liter(s)
ln	natural logarithm
L.P.	Limited Partnership
LDH	lactate dehydrogenase

LFT	liver function test
LLT	lower level term(s)
mcg	microgram
MD	Medical Doctor
MedDRA	Medical Dictionary for Regulatory Activities
mEq	milliequivalent(s)
mg	milligram(s)
min	minute(s)
mL or ml	milliliter(s)
mm	millimeter(s)
mm Hg	millimeters of mercury
mmol	millimole(s)
MV	minute volume
N/A or NA	not applicable
OIRD	opioid-induced respiratory depression
OOWS	Objective Opioid Withdrawal Scale
$PaCO_2$	arterial partial pressure of carbon dioxide
PCO_2	partial pressure of CO_2 in mm Hg (transcutaneous CO_2)
PD	pharmacodynamics(s)
pH	negative log of hydrogen ion concentration
PI	principal investigator
PK	pharmacokinetic(s)
PO2	Partial pressure of oxygen
PPLP	Purdue Pharma Limited Partnership
PT	preferred term(s)
RBC	red blood cell (count)
ROU	healthy recreational opioid users
RR	respiratory rate
RVM	respiratory volume monitor(ing)
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System
SC	subcutaneous
SGOT	serum glutamic-oxaloacetic transaminase (also AST)
SGPT	serum glutamate pyruvate transaminase (also ALT)
SoA	Schedule of Assessments
SOC	System Organ Class
SOP	Standard Operation Practices
SpO2	hemoglobin-O_2 saturation determined by pulse oximetry

TCO2	transcutaneous CO2
THC	9-delta-tetrahydrocannabinol
TTO	time to onset
TV	tidal volume
uL	microliter(s)
ULN	upper limit of normal
US	United States
VAS	visual analog scale
WBC	white blood cell (count)
WHO	World Health Organization

1 Introduction

This statistical analysis plan (SAP) is designed to outline the statistical methods for the NAL1004 study regarding test drug of fentanyl, naloxone, nalmefene and to assess the change in minute ventilation at 5 minutes of intramuscular nalmefene and intranasal naloxone reversal from the opioid induced nadir.

This document has been prepared based on protocol Amendment 1 dated July 28, 2022. Details will be described in this analysis plan to ensure that the data listings, summary tables and figures which will be produced, and the statistical methodologies that will be used, are complete and appropriate to allow valid conclusions regarding the study objectives.

This analysis plan was finalized and approved by the sponsor [redacted] prior to the planned database lock.

This SAP supersedes the statistical considerations identified in the protocol; where considerations are substantially different, they will be identified, and justification will be provided. If additional analyses are required to supplement the planned analyses described in the SAP, they may be performed and will be identified in the clinical study report (CSR).

2 Study Design Overview

2.1 Overall Study Design

This will be a 2-part study. Part 1 is a Qualification Phase prior to Part 2 which will be conducted to identify subjects eligible to participate in the Treatment Phase. This will be based upon satisfying specified qualification criteria following completion of procedures and/or study drug administrations. Qualification may not be required if a subject has participated in Clinical Study NAL1003. Each Qualification cohort will have a screening, treatment, EOS and Follow-up. The qualification treatment phase will consist of 1 period and evaluate the pharmacodynamics (minute ventilation) of OIRD.

Part 2 will be a single-center, randomized, 4 period crossover, 2 treatment replicate study to evaluate the pharmacodynamic effects (change in minute ventilation from opioid induced nadir (OIRD)) of nalmefene (1.5 mg) when given as an autoinjector intramuscularly (IM; into the thigh) compared to naloxone (Narcan 4 mg) when given intranasally (IN; into the nose) to reverse opioid (fentanyl) -induced respiratory depression (OIRD) in healthy subjects with prior opioid exposure. The Treatment Phase will have a screening, treatment, EOS and Follow-up.

The two treatment sequences for first 2 periods:

- Sequence 1: Narcan 4 mg, IN - Nalmefene Autoinjector 1.5 mg, IM
- Sequence 2: Nalmefene Autoinjector 1.5 mg, IM - Narcan 4 mg, IN

Table 1 and Table 2 below present the complete schedule of activities for NAL1004.

Table 1 Part 1 Study Flow Chart - Overall Procedures

Phase	**Screening**	**Qualification**			**F/U**	**Notes**
Period	**Screening**	**Check-In**	**1**	**EOS**	**F/U**	
Study Day **Study Procedures**	**-29 - -2**	**-1**	**1**	**2**	**8-11**	
Informed consent	X					
Medical history	X					Including drug and alcohol use history
Demography	X					Including weight, height, and BMI
Physical exam	X	X		X		At check-in: Symptom-directed, at discretion of investigator or designee
Inclusion/exclusion	X	X				
DSM-IV-TR (dependence)	X					
Clinical laboratory	X	X		X		Chemistry, hematology and urinalysis At check-in: chemistry and hematology testing only
Vital signs and SpO_2 with HDYF?	X	X	X	X		Taken after 5 minutes seated (or during infusions when supine, seated, or semirecumbent): heart rate, blood pressure, respiratory rate, SpO_2 Screening: Additional standing (for ~2 minutes) vital signs (heart rate and blood pressure) Obtained predose before saline infusion During fentanyl infusion: Predose, 30, and 60 minutes End of fentanyl infusion: end of infusion, 2 and 4 hrs. Time points may be added, omitted, or adjusted. Vital signs also taken pre- and post-naloxone challenge test.
Oral temperature	X	X	X	X		For safety purposes, forehead temperature may be substituted for oral. Days 1: Predose, and 8-12 hrs post infusion
Serum pregnancy test (females only)	X			X		

Phase	**Screening**	**Qualification**			**F/U**	**Notes**
Period	**Screening**	**Check-In**	**1**	**EOS**	**F/U**	
Study Day **Study Procedures**	**-29 - -2**	**-1**	**1**	**2**	**8-11**	
Serum FSH (post-menopausal females only)	X					
Urine pregnancy test (females only)		X				
Conventional 12-lead ECG	X	X		X		
Drug/alcohol screen	X	X				
Serology (HBsAg and HCV)	X					
Naloxone challenge test		X				Performed at least approximately 12 hours prior to first dose in Period 1. Includes Objective Opioid Withdrawal Scale (OOWS). Does not need to be performed if conducted during a previous check-in visit within 60 days.
RVM/capnography/ PD training and practice session	X	X				May be performed at screening or Check-in day -1 or both.
Study drug dosing			X			Time = 0, relative to other procedures. [redacted]
Respiratory volume monitoring and capnography			X			Procedure will be performed pre (Baseline) and post IV study drug administration. Predose (baseline) and continuous RVM throughout dosing and for 15 minutes after the end of the infusion for remifentanil and for 1 hr after the end of the infusion for fentanyl. Time interval may be adjusted or separate time points may be examined.
Transcutaneous CO_2 monitoring			X			Obtained prior to the start of the saline infusion, every 15 minutes during saline infusion and at the end of the saline infusion, every 5 minutes during the fentanyl Infusion, and at the end of the fentanyl Infusion. Time points may be added and adjusted.

Phase	**Screening**	**Qualification**			**F/U**	**Notes**
Period	**Screening**	**Check-In**	**1**	**EOS**	**F/U**	
Study Day **Study Procedures**	**-29 - -2**	**-1**	**1**	**2**	**8-11**	
Continuous SpO_2 monitoring			X			Relative to IV dosing: Predose to 8-12 hours for fentanyl Note: For fentanyl dosings, subjects can be taken off the pulse oximetry monitoring for up to a half hour each day to shower
Follow-up phone call					X	A follow-up phone call will be made to (or received from) each subject 7 to 10 days after Day 1 or after early withdrawal from the study.
Confined to study unit		◀----------		----------▶		
Concomitant therapies	◀----------			----------▶		
Adverse events	◀----------			----------▶		

EOQ: End of Qualification; F/U: Follow-up; HYDF?=How do you feel question; IV=Intravenous; RVM= respiratory volume monitor(ing);

SpO_2=Hemoglobin-O_2 saturation determined by pulse oximetry

Table 2 Part 2 Study Flow Chart - Overall Procedures

Phase	**Screen-ing**	**Treatment**						**F/U**	**Notes**
Period	**Screen-ing**	**Check-In**	**1**	**2**	**3**	**4**	**EOS**	**F/U**	
Study Day	**-29 - -2**	**-1**	**1**	**4**	**7**	**10**	**11**	**18-21**	
Study Procedures									
Informed consent	X								
Medical history	X								Including drug and alcohol use history
Demography	X								Including weight, height, and BMI
Physical exam	X	X					X		At check-in: Symptom-directed, at discretion of investigator or designee
Inclusion/exclusion	X	X							
DSM-IV-TR (dependence)	X								
Clinical laboratory	X	X					X		Chemistry, hematology and urinalysis At check-in: chemistry and hematology testing only Testing for COVID-19 may also be done at any time
Serum pregnancy test (females only)	X						X		
Serum FSH (post-menopausal females only)	X								
Urine pregnancy test (females only)		X							
Conventional 12-lead ECG	X	X					X		
Drug/alcohol screen	X	X							

Phase	**Screen-ing**	**Treatment**						**F/U**	**Notes**
Period	**Screen-ing**	**Check-In**	**1**	**2**	**3**	**4**	**EOS**	**F/U**	
Study Day **Study Procedures**	**-29 - -2**	**-1**	**1**	**4**	**7**	**10**	**11**	**18-21**	
Serology (HBsAg and HCV)	X								
Naloxone challenge test		X							Performed at least approximately 12 hours prior to first dose in Period 1. Includes Objective Opioid Withdrawal Scale (OOWS). Does not need to be performed if conducted during Part 1 and subject enters Part 2 within 60 days
Vital signs and SpO_2 with HDYF?	X	X	X	X	X	X	X		Taken after 5 minutes seated (or during infusions when supine, seated, or semirecumbent): heart rate, blood pressure, respiratory rate, SpO_2 Screening: Additional standing (for ~2 minutes) vital signs (heart rate and blood pressure) Days 1, 4, 7, 10: During fentanyl infusion: Predose (-15 minutes), end of infusion, 2 and 4 hrs post end of infusion. Time points may be added, omitted, or adjusted. Vital signs also taken pre- and post-naloxone challenge test.
Oral temperature	X	X	X	X	X	X	X		For safety purposes, forehead temperature may be substituted for oral. Days 1, 4, 7, 10: Predose, and 8-12 hrs post infusion. Time points may be added, omitted, or adjusted.
Respiratory monitoring/PD training and practice session	X	X							May be performed at screening or Check-in Day -1 or both.
Randomization			X						Prior to study drug dosing in Period 1 only

Phase	**Screen-ing**	**Treatment**						**F/U**	**Notes**
Period	**Screen-ing**	**Check-In**	**1**	**2**	**3**	**4**	**EOS**	**F/U**	
Study Day **Study Procedures**	**-29 - -2**	**-1**	**1**	**4**	**7**	**10**	**11**	**18-21**	
Fentanyl Infusion administration			X	X	X	X			Time is relative to start of IV infusion. 3-step fentanyl infusion: first infusion at an initial rate of 5.0 mcg/min, a second infusion for 20 min and a third infusion up to 90 min, see Table 7.
Nalmefene or Naloxone Study drug dosing			X	X	X	X			Nalmefene or Naloxone will be administered approximately 10 min following the start of Fentanyl Infusion #2. Time = 0, relative to other procedures.
Local Tolerability			X	X	X	X	X		Nalmefene Treatment only, Days 1, 4, 7, 10: Prior to the start of IV infusion Relative to Nalmefene administration: 15, 30 minutes, 1 hr and 24 hr after nalmefene administration.
Blood sample for drug concentration			X	X	X	X			Relative to Nalmefene/Naloxone administration: 2.5, 5, 10, 15, 20, and 30 minutes and at 1, 2, 4, 8, 12, and 24 hours after nalmefene/naloxone administration.
Respiratory volume monitoring and capnography			X	X	X	X			Relative to fentanyl infusion, Predose (baseline) and continuous RVM throughout dosing and for 1 hr after the end of the infusion for fentanyl. Time interval may be adjusted, or specific time points or multiple shorter intervals may be selected.
Transcutaneous CO_2 monitoring			X	X	X	X			Relative to fentanyl infusion, Predose (baseline) and continuous throughout dosing and for 1 hr after the end of the infusion for fentanyl. Time interval may be adjusted, or specific time points or multiple shorter intervals may be selected.

Phase	**Screen-ing**	**Treatment**						**F/U**	**Notes**
Period	**Screen-ing**	**Check-In**	**1**	**2**	**3**	**4**	**EOS**	**F/U**	
Study Day **Study Procedures**	**-29 - -2**	**-1**	**1**	**4**	**7**	**10**	**11**	**18-21**	
Continuous SpO_2 monitoring			◀----------				▶		Relative to fentanyl infusion: Predose to 8-12 hrs post dose Time interval may be adjusted, or specific time points or multiple shorter intervals may be selected dependent on the agonist administered. Note: Subjects can be taken off the pulse oximetry monitoring for up to a half hour each day to shower.
Device deficiencies			◀----------				▶		
Follow-up phone call								X	A follow-up phone call will be made to (or received from) each subject 7 to 10 days after EOS or after early withdrawal from the study.
Confined to study unit		◀----------					▶		
Concomitant therapies	◀----------						▶		
Adverse events	◀----------						▶		

EOS: End of study; F/U: Follow-up; HYDF?=How do you feel question; IV=Intravenous; RVM=respiratory volume monitor(ing);

SpO_2=Hemoglobin-O_2 saturation determined by pulse oximetry

2.2 Sample Size Justification

Part 1: A sufficient number of subjects will be screened and entered into Part 1 to provide the number of subjects required in Part 2.

Part 2: A total of up to 22 subjects will be randomized to complete approximately 20.

In a non-inferiority test on MV at 5min from a 4 period, 2 treatment replicate cross-over design, a total sample size of 20 would achieve >90% power at a 2.5% significance level (1-sided) when the true difference between the means is 0.8L/min, the non-inferiority margin is -0.5L/min, and the within-subject standard deviation is 1.2L/min.

In a superiority test on MV at 5min from a 4 period, 2 treatment replicate cross-over design, a total sample size of 20 would achieve 83% power at a 2.5% significance level (1-sided) when the true difference between the means is 0.8L/min and the within-subject standard deviation is 1.2L/min.

The planned sample size is adequate for sequential testing of noninferiority followed by superiority.

3 Study Objectives

3.1 Primary Objectives

- To assess the change in minute ventilation at 5 minutes of intramuscular nalmefene and intranasal naloxone reversal from the opioid induced nadir

3.2 Secondary

- To assess the time course of changes in minute ventilation of intramuscular nalmefene and intranasal naloxone reversal from the opioid induced nadir
- To evaluate the pharmacokinetics of nalmefene and naloxone following various routes of administration
- To assess the safety and tolerability of fentanyl when co-administered with and without nalmefene or naloxone

3.3 Exploratory

- To characterize the time course (Magnitude of change in minute ventilation) of intramuscular nalmefene and intranasal naloxone reversal from the opioid induced nadir

4 Study Endpoints and Evaluations

4.1 Pharmacokinetic Endpoints and Evaluations

Pharmacokinetic concentration and endpoint will be included in a separate data analysis plan (DAP) and will not be included in this SAP.

4.2 Pharmacodynamics Evaluations

Pharmacodynamic (PD) evaluations will be performed for the following time frames: prior to fentanyl, after fentanyl and before any antagonists, and after antagonist administration (nalmefene, naloxone). The PD variables include:

- Minute Ventilation (MV) in L/min - calculated as the product of tidal volume and respiratory rate, determined in a continuous fashion by noninvasive respiratory volume monitor
- Transcutaneous CO_2 (TCO_2) in mmHg – a lagging measure of respiratory status in OIRD, it is an exploratory supplement endpoint to MV

4.3 Safety Endpoints and Evaluations

Safety will be assessed using

- Treatment emergent adverse events (TEAEs)
- Clinical laboratory test results
- Vital signs, including SpO_2
- Physical examinations
- Conventional 12-lead ECGs
- Local tolerability

4.4 Other Evaluations

Other evaluations include:

- Demographics and baseline characteristics
- Disposition
- Study drug dosing

- Medical history
- Drug screen
- Pregnancy test
- Medications (prior and concomitant)

5 Analysis Populations and Applications

For purposes of analysis, the following analysis populations are defined:

5.1 Enrolled Population

Enrolled population includes all subjects who signed informed consent.

5.2 Randomized Safety Population

Randomized safety population, also known as the safety population, includes subjects who are randomized and receive at least 1 dose of the study drug.

5.3 Eligible Analysis Set (EAS)

Twenty subjects will be expected to complete the study, each of whom will receive nalmefene or naloxone in different order after fentanyl -induced respiratory depression (OIRD).

For each period, eligible analysis set (EAS) include subjects/periods who are randomized, receive study drug, have at least 1 valid PD measurement, and their OIRDs are worse than baseline for MV and TCO_2. Note: baseline and OIRD for MV and TCO_2 may be different. Therefore, eligible analysis set for each PD parameter and period may be different.

To evaluate the treatment difference between nalmefene and naloxone, the same treatment under different periods will be pooled by averaging PD parameters from eligible data for each subject.

This analysis set will be used as primary analysis.

5.4 Full Analysis Set (FAS)

Full analysis set (FAS) includes subjects/periods who are randomized, receive study drug, and have at least 1 valid PD measurement for one individual period.

To evaluate the treatment difference between nalmefene and naloxone, the same treatment under different periods will be pooled by averaging PD parameters from eligible data for each subject.

This set will be used as sensitivity analyses. However, if it is determined that EAS is very similar to FAS, only EAS will be performed.

6 Statistical Considerations

All summaries and statistical analysis will be performed by SAS version 9.4 or later.

Following each administration of Nalmefene Autoinjector or Narcan, the reversal time course of the fentanyl induced respiratory depression for MV and TCO_2 will be evaluated.

The following data will be provided in the summary tables:

- Disposition
- Demographics and baseline characteristics
- Medical history
- Concomitant medications
- Treatment exposure
- Treatment emergent adverse events
- Derived pharmacodynamic (PD) parameters based on PD assessment data
    - Change in MV from opioid induced nadir (Opioid Induced Respiratory Depression (OIRD))
    - Maximum of reversal in MV
    - Time to maximum of reversal in MV

  The summary figures/tables for PD assessment will include

    - Individual profiles
    - Treatment mean profiles
    - Change from baseline and percent change from baseline in MV and TCO2 from opioid induced nadir
    - Change in MV from opioid induced nadir (Opioid Induced Respiratory Depression (OIRD))
    - Maximum of reversal in MV
    - Time to maximum of reversal in MV
    - Time to onset
- Vital signs
- Physical examinations

The following data will be provided in data listings:

- Demographics and baseline characteristics
- Treatment exposure
- Medical history
- Prior and concomitant medication/therapy

- Treatment emergent adverse events
- Derived pharmacodynamic (PD) parameters
- Vital signs
- Physical examinations
- Electrocardiogram

6.1 General Statistical Procedures

Frequency distributions for categorical variables will be provided as number and percentage of subjects with a response in the category. Unless otherwise specified, percentages will be based on number of subjects in the given population as noted. Percentages will be reported to one decimal place.

The descriptive statistics for continuous variables will be number of subjects, mean, standard deviation (SD), median, minimum and maximum. Mean and median will be reported to 1 more decimal place than the raw data, while the SD will be reported to 2 more decimal places than the raw data. Minimum and maximum will be reported the same as the original data.

Unless otherwise stated, all statistical summary will be performed separately for each treatment in each part.

In general, all listings will be ordered by Part, subject ID and visit for available data unless otherwise specified in the footnote.

6.2 Statistical Hypotheses

Hypotheses #1: **Non-inferiority Test**

The null hypothesis is that Nalmefene (μT) is inferior to reference treatment (μR).

The alternative hypothesis is that Nalmefene is not inferior to reference treatment.

$$H0: \mu T - \mu R \leq -0.5 \quad vs. \quad H1: \mu T - \mu R > -0.5$$

Hypothesis #2: **Superiority Test**

The null hypothesis is that Nalmefene (μT) is not superior to reference treatment (μR).

The alternative hypothesis is that Nalmefene is superior to reference treatment.

$$H0: \mu T - \mu R \leq 0 \quad vs. \quad H1: \mu T - \mu R > 0$$

Statistical analysis will be performed for hypothesis #1 first, if noninferiority is met the analysis will proceed to hypothesis #2.

6.2.1 Multiplicity Adjustment

For the primary endpoint, the non-inferiority test will be performed first. If non-inferiority is achieved, superiority will be tested sequentially. Using this hierarchical test procedure, the comparison of the two treatments will be tested based on a one-sided alpha = 0.025.

The secondary endpoints will not be adjusted for multiplicity.

6.3 Subject Enrollment and Disposition

Number of subjects will be provided overall for Enrolled population for both Qualification Phase and Treatment Phase as follows:

Number of subjects enrolled

Number of subjects in randomized safety population

Number of subjects in full analysis population

Number of subjects completed study

Number of subjects completed treatment phase

Number of subjects discontinued (including screen failures)

Reasons for discontinuation

For the Randomized Safety population, the denominators for percentages are based on number of Enrolled population.

Enrollment information and disposition will be provided in a data listing.

6.4 Demographic and Other Baseline Characteristics

The following variables will be summarized overall using Enrolled population and randomized safety population.

- Age (years) at screening. If not reported, calculated as integer of (date of informed consent signed – date of birth)/365.25.
- Age category ($18 \leq age < 35$, $35 \leq age < 50$, $50 \leq age < 55$)
- Sex
- Race
- Ethnicity
- Height (cm)
- Weight (kg)
- BMI (body mass index, kg/m^2, calculated as Weight (kg) / $(Height\ (m))^2$)
- Child-bearing potential (Yes, No, Not Applicable: Subject is Male)

Conversions for height and weight are as follows:

Height (cm) = Height (inches) × 2.54

Weight (kg) = Weight (lb) × 0.4536

The above demographics and baseline characteristics will be summarized for the Qualification Phase using the enrolled population and the Treatment Phase using randomized safety population.

Demographic characteristics will also be listed.

7 Efficacy Analysis

Not applicable.

8 Safety Analysis

8.1 Adverse Events

Adverse events (AEs) will be presented separately by treatment assignment. AEs will be coded using the most current version of MedDRA and will be classified by SOC and PT of MedDRA. Severity of AEs will be assessed by investigators according to CTCAE.

Treatment-emergent adverse events are defined as AEs that:

- Emerge during treatment, having been absent at pretreatment; or
- Reemerge during treatment, having been present at pre-treatment but stopped prior to treatment; or
- Worsen in intensity during treatment relative to the pre-treatment state, when the AE is continuous.

An AE is treatment emergent if the start date/time of the event occurs any time on or after the first dose received on Day 1.

Adverse events that start within seven days after the last dose of study drug will be considered TEAEs.

The following types of summary will be provided by treatment using Randomized Safety population for each treatment phase separately:

1. Overview of TEAE
2. TEAEs by SOC, PT, and Maximum Severity
3. TEAEs by SOC and PT
4. Drug-Related TEAEs by SOC and PT
5. Drug-Related TEAEs by SOC, PT and Maximum Severity
6. TEAEs by Preferred Term in Descending Order

If a PT or SOC was reported more than once for a subject, the subject would only be counted once in the incidence for that PT or SOC.

In tabulation by severity grade,

- For a given SOC, only the most severe SOC for each subject will be included.
- For a given PT, only the most severe PT for each subject will be included.

In tabulations by relationship to study drug,

- For a given SOC, only the most related SOC for each subject will be included.
- For a given PT, only the most related PT for each subject will be included.

All AEs, TEAEs, AEs leading to treatment discontinuation will be provided in listings.

8.2 Vital signs

For each continuous vital sign parameter, descriptive summary statistics for values observed at Baseline and at each scheduled timepoint, and for the change from Baseline will be presented. For this summary, the Baseline is the last non-missing value prior to the start of Fentanyl treatment. This summary will be presented in the randomized safety population.

Vital sign data will be provided in a listing for subjects in Randomized Safety population.

8.3 Electrocardiogram (ECG)

For each continuous ECG parameter, descriptive summary statistics for values observed at Baseline and at each scheduled timepoint, and for the change from Baseline will be presented. For this summary, the Baseline is the last non-missing value prior to the start of Fentanyl treatment. This summary will be presented in the randomized safety population.

ECG data will be provided in a data listing for subjects in Randomized Safety population.

9 Pharmacodynamic Analysis (Part 2 Only)

9.1 Pharmacodynamics Over Time Profiles

The mean profile of smoothed data of MV and raw data of TCO_2 for each treatment (i.e., each pooled period) will be plotted with overlay of individual subject's replicated treatments. In addition, mean profiles for each treatment will be overlaid in one figure.

Individual and mean profiles will also be provided for change from baseline and % Change from Baseline based on the smoothed data of MV and raw data of TCO_2.

Minute Ventilation (MV): Individual time profiles of MV raw data are generally highly variable. To better characterize the magnitude and time course of opioid agonist/antagonist effects on ventilation, medians will be moved over a 4-minute centered window continuously to smooth the raw data. In the earlier NAL1003 study, this 4-minute window was determined to be the shortest window that adequately reduced the moment-moment variation while accurately preserving time course differences.

To avoid any carryover effects from the previous drug, the smoothing algorithm will be applied in a piece-wise fashion, as follows:

- Data before any fentanyl (without any opioid agonist) administration
- Data from beginning of fentanyl administration to antagonist administration (only the effect from fentanyl)
- Data on and after antagonist administration with continuing fentanyl administration (the effect of naloxone and any antagonist)

Transcutaneous CO_2: Individual time profiles of TCO_2 raw data will be used as is.

Parameters (Prior to Antagonist Administration Phase)

For MV and TCO_2, the following parameters will be calculated to evaluate the action of OIRD for each subject and each period, during the period of fentanyl only administration:

- Baseline (Pre-treatment value) - Median value of the PD data during the 10 minutes before the start of Fentanyl
- OIRD value - Median value of the PD data between -10 and -5 minutes before the start of the antagonist

9.2 Primary Endpoint(s) Analysis

Change in MV from opioid induced nadir (Opioid Induced Respiratory Depression (OIRD))

- Pre-specified Time Point: 5 min after administration of antagonist

The change in MV from OIRD at 5 min after administration of antagonist will be provided by treatment in tables and figures with 95% confidence interval presented.

9.3 Secondary Endpoint(s) Analysis

Change in MV from opioid induced nadir (Opioid Induced Respiratory Depression (OIRD)

- Pre-specified Time Point: 2.5 min, 10 min, 15 min, 20 min, 90 min after administration of antagonist

The change in MV from OIRD will be provided by treatment in tables and figures with 95% confidence interval presented.

Maximum of Reversal in MV - The highest MV achieved after administration of antagonist

- Time frames: 0-2.5 min, 0-5 min, 0-10 min, 0-15 min, 0-20 min, 0-90 min from administration of antagonist

The maximum reversal on MV (L/min) will be summarized by treatment in tables with 95% confidence interval provided.

Time to Maximum of Reversal in MV - Time from administration of antagonist to Maximum reversal (change in MV)

- Time frames: 0-2.5 min, 0-5 min, 0-10 min, 0-15 min, 0-20 min, 0-90 min from administration of antagonist

The time to maximum reversal on MV (L/min) will be summarized by treatment in tables with 95% confidence interval provided.

To reduce the moment-to-moment variation, additional smoothing windows may be applied to assess the robustness of PD results. For example:

Approach 1: Based on 1-minute window smoothed MV data (Smooth every 1 min of the raw data with center smoothing).

Approach 2: Based on 4-minutes window smoothed MV data (Smooth every 4 mins of the raw data with center smoothing)

9.4 Exploratory Endpoint(s) Analysis

Time to Onset (TTO) - onset of reversal

In the setting of opioid overdose, onset of reversal is expected within minutes following antagonist administration. A 30-minute window was selected to ensure the initial onset is fully captured. If TTO for X% of reversal is not achieved, then it will be considered a reversal failure and will be imputed to 30 minutes.

Onset of reversal for each treatment will include:

- TTO: Time from administration of antagonist (Nalmefene, Narcan) to X% of baseline reversal, where baseline is defined as median value of the PD of interest during the 10 minutes before the start of Fentanyl

Thresholds (X%) of reversal is defined as X% of baseline reversal and calculated as X %) * (Baseline – OIRD) + OIRD, where X are 25, 33, 50, 67, 75, 90, and 100.From the onset of reversal from individual periods of each treatment, observed onset of reversal for a pooled period is calculated based on:

- Eligible analysis set - the observed onset of reversal for a pooled treatment period will be the average of onset from eligible periods

Bar charts, scatter plots and overlay boxplots of time to onset of reversal will be plotted by treatment and pooled treatment periods if data warrants.

10 Definitions and Conventions for Data Handling

10.1 Definition of Baseline

For all evaluations unless otherwise noted, baseline is defined as the most recent non-missing measurement prior to or on the first administration of Fentanyl for qualification phase or each period during treatment phase, including unscheduled measurements. Baseline can be the same date as first dose, given the measurement is expected prior to first dose when only date information is available.

10.2 Definition of Study Days

Unless otherwise noted, study days of an evaluation are defined as number of days relative to the first dosing date of Fentanyl of qualification or treatment phase which is designated as Day 1, and the preceding day is Day -1, the day before that is Day -2, etc.

- If evaluation date is on or after first dose date, then study days are calculated as
  - Evaluation date minus - first dose date of study drug + 1
- If evaluation date is before first dose date, then relative study days are calculated as
  - Evaluation date - first dose date of study drug

10.3 First Dose Date of Study Treatment

The date of first dose of study treatment is defined as the earliest dose date of study drugs in the treatment period.

11 REFERENCES

None.