SYNOPSIS

Study Title
A TWO-PART OPEN-LABEL STUDY OF THE PHARMACODYNAMIC EFFECTS OF INTRANASAL NALMEFENE COMPARED TO INTRANASAL NALOXONE IN HEALTHY VOLUNTEERS UNDER STEADY-STATE OPIOID AGONISM

Short Study Title
Effects of Intranasal Nalmefene in Healthy Volunteers with Opioid Exposure

Study Codes
Sponsor code : OPNT003-OOD-001
PRA code : OPA20533-20533X
IND number : 136851

Sponsor
Opiant Pharmaceuticals, Inc., 233 Wilshire Blvd., Suite 280, Santa Monica, CA 90401, US
Sponsor's contact : Mark Ellison, Chief Development Officer

Contract Research Organization
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Clinical Site
PRA-EDS, 1255 East 3900 South, Salt Lake City, UT 84124, US

Principal Investigator
Lynn Webster, MD

Objectives
Part 1
Primary : To determine the relationship between remifentanil dose and suppression of CO_2-induced increases in minute ventilation

Secondary : To determine the effect of brief mask removal on minute ventilation
To determine tolerability of intravenous (IV) remifentanil
To determine effects of intranasal (IN) naloxone on minute ventilation during steady-state remifentanil infusion

Part 2
Primary : To demonstrate noninferiority of IN nalmefene hydrochloride compared to IN naloxone hydrochloride on minute ventilation during steady-state remifentanil infusion

Secondary : To determine tolerability of administration of IN nalmefene hydrochloride during remifentanil infusion

Design and Treatments
This will be a single-center, randomized, open-label, 2-part study. Part 1 will be a pilot study to determine the relationship between remifentanil dose and suppression of CO_2-induced increases in minute ventilation in 6 healthy volunteers with prior opioid exposure. Part 2 will be a 2-period, 2-treatment crossover study to

evaluate the pharmacodynamic (PD) effects of IN nalmefene compared to IN naloxone to reverse remifentanil-induced suppression of CO_2-induced increases in minute ventilation, in 46 healthy volunteers with prior opioid exposure. Both Part 1 and Part 2 of the study will consist of an outpatient Screening Visit, an in-clinic Treatment Phase, and a Follow-Up Phone Call.

Part 1

Screening will occur up to 28 days before first drug administration. All subjects who have given their written informed consent and who satisfy all of the relevant inclusion and exclusion criteria will be screened for eligibility to participate in the study (Table 1).

On the day of clinic admission (Day -1), eligibility will be reviewed (Table 1) confirming all the relevant inclusion criteria and none of the exclusion criteria have been met.

On Days 1 and 2 prior to dosing and at scheduled time points specified (Table 1), respiratory volume of subjects will be monitored using the ExSpiron® device. Ventilatory response to hypercapnia (VRH) will also be monitored with the subjects breathing through a tightly sealed face mask in a bed at 45° recumbent.

On Day 1, 6 subjects, fasted for a period of at least 8 hours, will receive pretreatment (30 minutes to 1 hour prior to remifentanil infusion) with ondansetron (8 mg, oral) and sodium citrate (30 mL, oral). Subjects will start receiving a hypercapnic gas mixture (50% O_2, 43% N_2, 7% CO_2) using a VRH face mask at Time 0 minutes, followed by a remifentanil hydrochloride infusion at Time 5 minutes, at a rate of 0.05 µg/kg/min (estimated to produce a blood concentration of approximately 1.0 ng/mL), using an initial bolus (0.5 µg/kg) to achieve an expected steady-state. Minute ventilation will be continuously measured. To assess the effect of brief mask removal on minute ventilation, the VRH face mask will be removed at Time 19 minutes and 50 seconds for approximately 10 seconds. During that time, subjects will be asked to hold their breath; then the VRH face mask will be reapplied. After the VRH face mask is reapplied, each subject will receive 4 mg IN naloxone hydrochloride. To administer the IN naloxone hydrochloride, the VRH face mask will be removed at Time 24 minutes and 50 seconds for approximately 10 seconds. During that time, subjects will be asked to hold their breath and the IN naloxone hydrochloride will be administered at Time 25 minutes. After IN administration of naloxone hydrochloride, the VRH face mask will be reapplied to continue to assess the effect on respiration, and remifentanil infusion will continue for a further 90-minute period up to Time 115 minutes. The VRH face mask will be removed at Time 45 minutes (ie, 20 minutes after IN administration of naloxone hydrochloride). The VRH face mask will then be reapplied for 10 minutes at Time 75 minutes and at Time 105 minutes, respectively (ie, between 50 to 60 minutes and 80 to 90 minutes after IN administration of naloxone hydrochloride). The PD and safety assessments will be conducted as specified in the Schedule of Assessments (Table 1).

Remifentanil dose for Day 2 will be determined based on the data from Day 1 by the Investigator and the Sponsor using the following criteria:

If the remifentanil infusion at a rate of 0.05 µg/kg/min (estimated to produce a blood concentration of approximately 1.0 ng/mL) on Day 1 achieves an approximate 40% suppression of CO_2-induced increases in minute ventilation, and naloxone has increased minute ventilation by >15% but <65% over remifentanil-induced nadir at 5 minutes following IN naloxone hydrochloride administration, then Part 1 of the study will be considered as completed and will proceed to Part 2 of the study.

If the minute ventilation was not increased by >15% over remifentanil-induced nadir at 5 minutes following IN naloxone hydrochloride administration, the remifentanil infusion will be reduced on Day 2.

If the remifentanil infusion at a rate of 0.05 µg/kg/min (estimated to produce a blood concentration of approximately 1.0 ng/mL) does not achieve an approximate 40% suppression of CO_2-induced increases in minute ventilation, and/or the minute ventilation has increased by >65% over remifentanil-induced nadir at 5 minutes following IN naloxone hydrochloride administration, the remifentanil infusion will be increased on Day 2, providing it is safe and well tolerated under these study conditions.

Day 2, if the remifentanil infusion will be decreased: On Day 2, 6 subjects, fasted for a period of at least 8 hours, will receive pretreatment (30 minutes to 1 hour prior to remifentanil infusion) with ondansetron (8 mg, oral) and sodium citrate (30 mL, oral). Subjects will start receiving a hypercapnic gas mixture (50% O_2, 43% N_2, 7% CO_2) using a VRH face mask at Time 0 minutes, followed by a remifentanil hydrochloride infusion at Time 5 minutes, at a rate, in the range of 0.025 µg/kg/min to 0.05 µg/kg/min (estimated to produce a blood concentration of up to approximately 1.0 ng/mL), using an initial bolus (0.5 µg/kg) to achieve an expected steady-state. Minute ventilation will be continuously measured. The remifentanil infusion rate can then be adjusted based on the % suppression of CO_2-induced increases in minute ventilation, and safety and tolerability.

Day 2, if the remifentanil infusion will be increased: On Day 2, 6 subjects, fasted for a period of at least 8 hours, will receive pretreatment (30 minutes to 1 hour prior to remifentanil infusion) with ondansetron (8 mg, oral) and sodium citrate (30 mL, oral). Subjects will start receiving a hypercapnic gas mixture (50% O_2, 43% N_2, 7% CO_2) using a VRH face mask at Time 0 minutes, followed by a remifentanil hydrochloride infusion at Time 5 minutes, at a rate, in the range of 0.05 µg/kg/min to 0.15 µg/kg/min (estimated to produce a blood concentration of up to approximately 2.5 ng/mL), using an initial bolus (0.5 µg/kg) to achieve an expected steady-state. Minute ventilation will be continuously measured. The remifentanil infusion rate can then be adjusted based on the % suppression of CO_2-induced increases in minute ventilation, and safety and tolerability.

Day 2, study drug administration (if Day 2 is conducted): Each subject will receive 4 mg IN naloxone hydrochloride. To administer the IN naloxone hydrochloride, the VRH face mask will be removed at Time 24 minutes and 50 seconds for approximately 10 seconds. During that time, subjects will be asked to hold their breath and the IN naloxone hydrochloride will be administered at Time 25 minutes. After IN administration of naloxone hydrochloride, the VRH face mask will be reapplied to continue to assess the effect on respiration, and remifentanil infusion will continue for a further 90-minute period up to Time 115 minutes. The VRH face mask will be removed at Time 45 minutes (ie, 20 minutes after IN administration of naloxone hydrochloride). The VRH face mask will then be reapplied for 10 minutes at Time 75 minutes and at Time 105 minutes, respectively (ie, between 50 to 60 minutes and 80 to 90 minutes after IN administration of naloxone hydrochloride). The PD and safety assessments will be conducted as specified in the Schedule of Assessments (Table 1).

Part 2

Screening will occur up to 28 days before first drug administration. All subjects who have given their written informed consent and who satisfy all of the relevant inclusion and exclusion criteria will be screened for eligibility to participate in the study (Table 2).

On the day of clinic admission (Day -1), eligibility will be reviewed (Table 2) confirming all the relevant inclusion criteria and none of the exclusion criteria have been met.

Prior to dosing on days of study drug administration and at the scheduled time points, respiratory volume of subjects will be monitored using the ExSpiron® device. VRH will also be monitored with the subjects breathing through a tightly sealed face mask in a bed at 45° recumbent.

On days of study drug administration (Days 1 and 5), 46 subjects, fasted for a period of at least 8 hours, will receive pretreatment (30 minutes to 1 hour prior to remifentanil infusion) with ondansetron (8 mg, oral) and sodium citrate (30 mL, oral). Subjects will start receiving a hypercapnic gas mixture (50% O_2, 43% N_2, 7% CO_2) using a VRH face mask at Time 0 minutes, followed by a remifentanil hydrochloride infusion at Time 5 minutes, at the rate determined in Part 1, using an initial bolus (0.5 µg/kg) to achieve an expected steady-state. Minute ventilation will be continuously measured. Each subject will receive a single dose of either 3 mg IN nalmefene hydrochloride or 4 mg IN naloxone hydrochloride, in a randomized 2-period crossover manner, in accordance with the randomization schedule, with a 4-day washout period between doses. To administer the IN nalmefene hydrochloride and IN naloxone hydrochloride, the VRH face mask will be removed at Time 24 minutes and 50 seconds for approximately 10 seconds. During that time, subjects will be asked to hold their breath and IN nalmefene or naloxone will be administered at Time 25 minutes. After IN administration of nalmefene or naloxone, the VRH face mask will be reapplied to continue to assess the effect on respiration, and remifentanil infusion will continue for a further 90-minute period up to Time 115 minutes. The VRH face mask will be removed at Time 45 minutes (ie, 20 minutes after IN administration of nalmefene or naloxone). The VRH face mask will then be reapplied for 10 minutes at Time 75 minutes and at Time 105 minutes, respectively (ie, between 50 to 60 minutes and 80 to 90 minutes after IN administration of nalmefene or naloxone). The PD, pharmacokinetic (PK), and safety assessments will be conducted as specified in the Schedule of Assessments (Table 2).

The study design is illustrated in Figure 1.

Study Schedule

Screening	:	Day -28 and Day -2
Confinement Period	:	Part 1 - An anticipated period of 4 days in the clinic from Day -1 (admission) to Day 3 Part 2 - An anticipated period of 8 days in the clinic from Day -1 (admission) to Day 7
Follow-Up Phone Call	:	Part 1 - 3 to 7 days after discharge from the clinic Part 2 - 3 to 7 days after discharge from the clinic

Subjects

Part 1	:	6 healthy volunteers with prior opioid exposure
Part 2	:	46 healthy volunteers with prior opioid exposure

Main Criteria for Inclusion

Age	:	18 to 55 years, inclusive, at screening
Weight	:	≥50 kg, inclusive, at screening
Body mass index	:	18.0 to 32.0 kg/m^2, inclusive, at screening

Study Drug

Test preparation

Active substance	:	Nalmefene hydrochloride
Activity	:	Opioid antagonist
Indication/ In development for	:	Emergency treatment of known or suspected opioid overdose, as manifested by respiratory and/or central nervous system depression
Strength	:	3 mg (1 spray in 1 nostril delivers 0.1 mL of 30 mg/mL nalmefene hydrochloride)
Dosage form	:	Nasal spray
Manufacturer	:	Opiant Pharmaceuticals, Inc.

Reference medication (Narcan®)

Active substance	:	Naloxone hydrochloride
Activity	:	Opioid antagonist
Indication	:	Emergency treatment of known or suspected opioid overdose, as manifested by respiratory and/or central nervous system depression
Strength	:	4 mg (1 spray in 1 nostril delivers 0.1 mL of 40 mg/mL naloxone hydrochloride)
Dosage form	:	Nasal spray
Manufacturer	:	Adapt Pharma, Inc.; sourced by pharmacy at PRA

Variables

Safety	:	Adverse events (AEs), clinical laboratory, vital signs, 12-lead electrocardiogram (ECG), continuous cardiac monitoring, physical examination, nasal cavity examination, and continuous oxygen saturation.
Pharmacokinetics	:	Plasma concentrations of naloxone and nalmefene Plasma PK parameters of naloxone and nalmefene estimated using noncompartmental analysis (NCA), as appropriate: C_{max}, t_{max}, AUC_{0-t}, AUC_{0-inf}, and Partial AUCs ($AUC_{0-2.5\ min}$, $AUC_{0-5\ min}$, $AUC_{0-10\ min}$, $AUC_{0-15\ min}$, $AUC_{0-20\ min}$, $AUC_{10-20\ min}$).
Pharmacodynamics	:	PD parameters: minute ventilation, respiratory rate, flow rates, tidal volume, end-tidal CO_2 (ET_{CO2}) defined as below. Minute ventilation (expired minute volume, V_E; L/min): volume of gas exhaled per minute from the lungs) Respiratory rate (breaths/min) Flow rates (peak expired flow, PEF; L/min): maximum speed of expiration Tidal volume (expired tidal volume, V_T; mL): volume of gas displaced between normal inhalation and exhalation when extra effort is not applied End-tidal CO_2 (ET_{CO2}, mmHg): partial pressure of carbon dioxide at the end of an exhaled breath

Statistical Methods

Sample size calculation:		With the sample size of 46 subjects (in a crossover design), the coverage probability is 90% that the upper limit of a two-sided 95% confidence interval for the difference between the treatments (reference/naloxone – test/nalmefene) in mean change from nadir for minute ventilation will be less than the non-inferiority limit of 0.6 L/min (given alpha = 0.025 and a standard deviation of 1.75).
PD parameters	:	Part 1 All PD parameters and derived changes from baseline will be summarized using descriptive statistics (number of observations, arithmetic mean, standard deviation [SD], standard error [SE], coefficient of variation [%CV], minimum, median, and maximum, as well as quartiles [Q1 and Q3]) for each treatment group and time point. Linear plots of the mean (±SD) PD measurements over time will be provided for V_E, RR, PEF, V_T, and ET_{CO2} by treatment. All valid PD measurements will be listed by subject. Part 2 The primary endpoint of change in minute ventilation from remifentanil-induced nadir to 5 minutes after study drug administration will be analyzed using the linear model for a two-treatment, two-period crossover trial. The model will

include fixed effects for period and treatment. The mean difference (naloxone – nalmefene) of change in minute ventilation and corresponding 2-sided 95% confidence interval for the mean difference, as well as the mean change in minute ventilation of each treatment, will be estimated. Noninferiority will have been demonstrated if (1) the upper limit of the 95% confidence interval is less than 20% of the mean change in minute ventilation for naloxone, and (2) no individual subject has a change in minute ventilation on nalmefene that is less than 50% of the change in minute ventilation on naloxone.

Change in minute ventilation is defined as postdose minute ventilation minus nadir minute ventilation.

In addition to the measured PD parameters, the following derived PD parameter will be calculated: The V_E values will be plotted versus the ET_{CO2} values for each subject/treatment per time point, and the intercept and slope (S) will be determined by linear regression. S is the derived PD parameter, ie, the Hypercapnic Ventilatory Response (L/min per mmHg).

PD measurements and derived changes from baseline will be summarized by treatment and scheduled time point using descriptive statistics (n, mean, SD, SE, %CV, median, Q1, Q3, min, and max).

Linear plots of the mean (±SD) PD measurements over time will be provided for V_E, RR, PEF, V_T, and ET_{CO2} by treatment.

Individual plots of V_E versus ET_{CO2} will be provided by treatment and time point, with a regression line and corresponding regression equation displayed for each treatment.

All valid PD measurements will be listed by subject.

PK parameters : Individual and mean plasma concentrations for treatment group at each sampling time point will be presented by listings and descriptive summary statistics. Time profile plots, linear and semi-logarithmic plots of the mean (±SD) at each time measurement will be generated for plasma concentration data. All PK parameters will be presented by individual listings and summary statistics for each treatment.

Safety parameters : Statistical methods for the safety analyses will be primarily descriptive in nature. Safety data, including AEs, vital signs, ECGs, and laboratory values will be summarized. All safety data will be included in the data listings and all test values outside the normal range will be flagged.

Table 1 Schedule of Assessments – Part 1

Schedule of Assessments – Part 1						
	Screening	**Admission /Baseline**	**Treatment Phase**		**Discharge or Early Termination**	**Follow-Up Phone Call**
Study Day(s)	**-28 to -2**	**-1**	**1**	**2**	**3**	**+3 to 7 days after discharge**
Informed Consent	X					
Medical History (includes Smoking History)	X	X				
Demographics	X					
Eligibility (Inclusion/Exclusion)	X	X				
Physical Examination	X	X			X	
Nasal Cavity Examination	X	X	X[a]	X[a]		
ECG	X	X	X[b]	X[b]		
Continuous Cardiac Monitoring (Telemetry)			X[c]	X[c]		
Vital Signs	X[d]	X[d]	X[e]	X[e]		
Weight	X	X				
Height, BMI	X	X				
Clinical Chemistries & Coagulation Parameters[f]	X					
Hematology[g]	X					
Urinalysis[h]	X					
Serum FSH Levels (Postmenopausal Female Subjects)	X					
Serum Pregnancy Test (Female Subjects)	X					
Urine Pregnancy Test (Female Subjects)		X				
Urine Drug Screen[i]	X	X				
Urine Cotinine Screen	X	X				
Alcohol Breath Test	X	X				
Mallampati intubation score	X					
C-SSRS	X					
HIV, Hepatitis B and C	X					
Adverse Events	X	X	X[j]	X[j]	X	X
Concomitant Medications	X	X	X[k]	X[k]		X
Randomization			X			
VRH Test	X	X				
VRH			X[l]	X[l]		
Oxygen Saturation			X[m]	X[m]		
Respiration Rate			X[m]	X[m]		
Noninvasive Blood Pressure			X[m]	X[m]		
Minute Ventilation Measurement[n]			X	X		
Pretreatments[o]			X	X		
Remifentanil Infusion[p]			X	X		
Brief VRH Mask Removal[q]			X			
Study Drug Administration[r]			X	X		

Schedule of Assessments – Part 1						
	Screening	**Admission /Baseline**	**Treatment Phase**		**Discharge or Early Termination**	**Follow-Up Phone Call**
Study Day(s)	**-28 to -2**	**-1**	**1**	**2**	**3**	**+3 to 7 days after discharge**
Meals		X	X[s]	X[s]		

Abbreviations: ALT=alanine aminotransferase; AST=aspartate aminotransferase; BMI=body mass index; CK=creatine kinase; C-SSRS=Columbia-Suicide Severity Rating Scale; COVID-19=coronavirus disease 2019; ECG=12-lead electrocardiogram; FSH=follicle stimulating hormone; HDL=high density lipoprotein; HIV=human immunodeficiency virus; IN=intranasal; LDL=low density lipoprotein; RBC=red blood cell; SARS-CoV-2=severe acute respiratory syndrome coronavirus 2; THC=tetrahydrocannabinol; TSH=thyroid stimulating hormone; VRH=ventilatory response to hypercapnia; WBC=white blood cell.

Note: Additional COVID–19-related precautions and procedures (including SARS-CoV-2 testing/screening) may be implemented based on the prevailing situation during study conduct, at the Investigator's discretion; the instructions will be provided in a separate document. Any procedure implemented will be in accordance with the local and national regulations and shall be documented appropriately. If a subject is tested positive for SARS-CoV-2, the subject will be discontinued from the study and all required measures with regards to subject safety and study conduct will be taken.

[a] Nasal cavity examination predose, and at approximately 2 hours postdose.
[b] ECG assessment will be conducted predose and at approximately 4 hours postdose.
[c] Continuous cardiac monitoring will be performed from at least 1 hour pre-remifentanil infusion and will continue until at least 1 hour post-remifentanil infusion.
[d] Sitting (5 minutes) blood pressure, heart rate, respiration rate, and temperature.
[e] Sitting (5 minutes) blood pressure, heart rate, respiration rate, and temperature predose and at approximately 2 hours postdose.
[f] Chemistry parameters include sodium, potassium, chloride, bicarbonate, CO_2, creatinine, CK, amylase, lipase, glucose (fasting), urea, albumin, blood urea nitrogen, calcium, magnesium, inorganic phosphorus, alkaline phosphatase, AST, ALT, total bilirubin, indirect and direct bilirubin, total protein, total cholesterol, HDL, LDL, triglycerides, uric acid, creatinine clearance, and TSH. Coagulation parameters include PT and aPTT.
[g] Hematology includes RBCs, WBCs with differential (neutrophils, eosinophils, lymphocytes, monocytes, and basophils), hemoglobin, hematocrit, and platelet count.
[h] Urinalysis includes specific gravity, ketones, nitrites, bilirubin, leukocyte esterase, protein, blood, glucose, WBCs, and pH.
[i] Urine drug screen for opiates, cocaine, amphetamines/methamphetamine, benzodiazepines, cannabinoids/THC, barbiturates.
[j] Adverse events reviewed at approximately 2 hours postdose.
[k] Concomitant medications reviewed at approximately 2 hours postdose.
[l] Subjects will start receiving a hypercapnic gas mixture using a VRH face mask at Time 0 minutes, which will be removed at Time 45 minutes and then be reapplied for 10 minutes at Time 75 minutes and at Time 105 minutes, respectively.
[m] Conducted during the remifentanil infusion (predose; at dosing; after 2.5, 5, 10, 15, 20, 25, 30 minutes postdose; then every 5 minutes until 20 minutes after remifentanil is discontinued; and then every 15 minutes thereafter for 90 minutes).
[n] Minute ventilation will be determined at Time 0, 5, 10, 15, 20, 25, 27.5, 30, 32.5, 35, 40, 45, 55, 70, 85, 115, 125, 135, and 145 minutes (ie, at -25, -20, -15, -10, -5, 0, 2.5, 5, 7.5, 10, 15, 20, 30, 45, 60, 90, 100, 110, and 120 minutes in relation to study drug administration), using the ExSpiron® device.
[o] Pretreatment with ondansetron (8 mg, oral) and sodium citrate (30 mL, oral) will be administered 30 minutes to 1 hour prior to remifentanil infusion.
[p] Remifentanil hydrochloride infusion will be administered at Time 5 minutes using a syringe pump, delivering an initial bolus (0.5 µg/kg) to achieve the approximate target steady-state.
[q] To assess the effect of brief mask removal on minute ventilation, the VRH face mask will be removed at Time 19 minutes and 50 seconds for approximately 10 seconds. During that time, subjects will be asked to hold their breath; then the VRH face mask will be reapplied.
[r] Subjects will receive a 4 mg naloxone hydrochloride IN dose at Time 25 minutes.
[s] Subjects will fast for a period of 8 hours before remifentanil dosing until one hour after dosing. Water will be provided *ad libitum*.

Table 2 Schedule of Assessments – Part 2

Schedule of Assessments – Part 2										
	Screening	**Admission /Baseline**	**Period 1**	**Washout**			**Period 2**	**Washout**	**Discharge or Early Termination**	**Follow-Up Phone Call**
Study Day(s)	**-28 to -2**	**-1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**+3 to 7 days after discharge**
Informed Consent	X									
Medical History (includes Smoking History)	X	X								
Demographics	X									
Eligibility (Inclusion/Exclusion)	X	X								
Physical Examination	X	X							X	
Nasal Cavity Examination	X	X	X[a]	X[b]			X[a]	X[b]	X	
ECG	X	X	X[c]			X	X[c]		X	
Continuous Cardiac Monitoring (Telemetry)			X[d]				X[d]			
Vital Signs	X[e]	X[e]	X[f]	X[g]			X[f]	X[g]	X[e]	
Weight	X	X							X	
Height, BMI	X	X								
Clinical Chemistries & Coagulation Parameters [h]	X								X	
Hematology [i]	X								X	
Urinalysis [j]	X								X	
Serum FSH Levels (Postmenopausal Female Subjects)	X									
Serum Pregnancy Test (Female Subjects)	X								X	
Urine Pregnancy Test (Female Subjects)		X				X				
Urine Drug Screen [k]	X	X								
Urine Cotinine Screen	X	X								
Alcohol Breath Test	X	X								
Mallampati intubation score	X									
C-SSRS	X									
HIV, Hepatitis B and C	X									
Adverse Events	X	X	X[l]	X[m]	X	X	X[l]	X[m]	X	X
Concomitant Medications	X	X	X[n]	X[o]	X	X	X[n]	X[o]	X	X
Randomization			X							
VRH Test	X	X								
VRH			X[p]				X[p]			
Oxygen Saturation			X[q]				X[q]			
Respiration Rate			X[q]				X[q]			
Noninvasive Blood Pressure			X[q]				X[q]			
Minute Ventilation Measurement [r]			X				X			
Pretreatments [s]			X				X			
Remifentanil Infusion [t]			X				X			
PK Blood Sampling [u]			X				X			

Schedule of Assessments – Part 2										
	Screening	**Admission /Baseline**	**Period 1**	**Washout**			**Period 2**	**Washout**	**Discharge or Early Termination**	**Follow-Up Phone Call**
Study Day(s)	**-28 to -2**	**-1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**+3 to 7 days after discharge**
Study Drug Administration [v]			X				X			
Meals		X	X[w]	X	X	X	X[w]	X		

Abbreviations: ALT=alanine aminotransferase; AST=aspartate aminotransferase; BMI=body mass index; CK=creatine kinase; C-SSRS=Columbia-Suicide Severity Rating Scale; COVID-19=coronavirus disease 2019; ECG=12-lead electrocardiogram; FSH=follicle stimulating hormone; HDL=high density lipoprotein; HIV=human immunodeficiency virus; IN=intranasal; LDL=low density lipoprotein; PK=pharmacokinetic(s); RBC=red blood cell; SARS-CoV-2=severe acute respiratory syndrome coronavirus 2; THC=tetrahydrocannabinol; TSH=thyroid stimulating hormone; VRH=ventilatory response to hypercapnia; WBC=white blood cell.

Note: Additional COVID–19-related precautions and procedures (including SARS-CoV-2 testing/screening) may be implemented based on the prevailing situation during study conduct, at the Investigator's discretion; the instructions will be provided in a separate document. Any procedure implemented will be in accordance with the local and national regulations and shall be documented appropriately. If a subject is tested positive for SARS-CoV-2, the subject will be discontinued from the study and all required measures with regards to subject safety and study conduct will be taken.

[a] Nasal cavity examination predose, and at approximately 2 hours postdose.
[b] Nasal cavity examination at approximately 24 hours postdose.
[c] ECG assessment will be conducted predose and at approximately 4 hours postdose.
[d] Continuous cardiac monitoring will be performed from at least 1 hour pre-remifentanil infusion and will continue until at least 1 hour post-remifentanil infusion.
[e] Sitting (5 minutes) blood pressure, heart rate, respiration rate, and temperature.
[f] Sitting (5 minutes) blood pressure, heart rate, respiration rate, and temperature predose and at approximately 2 hours postdose.
[g] Sitting (5 minutes) blood pressure, heart rate, respiration rate, and temperature at approximately 24 hours postdose.
[h] Chemistry parameters include sodium, potassium, chloride, bicarbonate, CO_2, creatinine, CK, amylase, lipase, glucose (fasting), urea, albumin, blood urea nitrogen, calcium, magnesium, inorganic phosphorus, alkaline phosphatase, AST, ALT, total bilirubin, indirect and direct bilirubin, total protein, total cholesterol, HDL, LDL, triglycerides, and uric acid. Creatinine clearance and TSH will be measured at screening only. Coagulation parameters include PT and aPTT.
[i] Hematology includes RBCs, WBCs with differential (neutrophils, eosinophils, lymphocytes, monocytes, and basophils), hemoglobin, hematocrit, and platelet count.
[j] Urinalysis includes specific gravity, ketones, nitrites, bilirubin, leukocyte esterase, protein, blood, glucose, WBCs, and pH.
[k] Urine drug screen for opiates, cocaine, amphetamines/methamphetamine, benzodiazepines, cannabinoids/THC, barbiturates.
[l] Adverse events reviewed at approximately 2 hours postdose.
[m] Adverse events reviewed at approximately 24 hours postdose.
[n] Concomitant medications reviewed at approximately 2 hours postdose.
[o] Concomitant medications reviewed at approximately 24 hours postdose.
[p] Subjects will start receiving a hypercapnic gas mixture using a VRH face mask at Time 0 minutes, which will be removed at Time 45 minutes and then be reapplied for 10 minutes at Time 75 minutes and at Time 105 minutes, respectively.
[q] Conducted during the remifentanil infusion (predose; at dosing; after 2.5, 5, 10, 15, 20, 25, 30 minutes postdose; then every 5 minutes until 20 minutes after remifentanil is discontinued; and then every 15 minutes thereafter for 90 minutes).
[r] Minute ventilation will be determined at Time 0, 5, 10, 15, 20, 25, 27.5, 30, 32.5, 35, 40, 45, 55, 70, 85, 115, 125, 135, and 145 minutes (ie, at -25, -20, -15, -10, -5, 0, 2.5, 5, 7.5, 10, 15, 20, 30, 45, 60, 90, 100, 110, and 120 minutes in relation to study drug administration), using the ExSpiron® device.
[s] Pretreatment with ondansetron (8 mg, oral) and sodium citrate (30 mL, oral) will be administered 30 minutes to 1 hour prior to remifentanil infusion.
[t] Remifentanil hydrochloride infusion will be administered at Time 5 minutes using a syringe pump, delivering an initial bolus (0.5 µg/kg) to achieve the approximate target steady-state.
[u] Predose (within 15 minutes) and approximately 2.5, 5, 7.5, 10, 15, 20, 30, 45, 60, and 90 minutes after study drug administration.

[v] Subjects will receive either a 3 mg nalmefene hydrochloride IN dose or a 4 mg naloxone hydrochloride IN dose at Time 25 minutes.
[w] Subjects will fast for a period of 8 hours before remifentanil dosing until one hour after dosing. Water will be provided *ad libitum*.