Statistical Analysis Plan

Sponsor:	Opiant Pharmaceuticals, Inc.
Protocol No:	OPNT003-OOD-001
Protocol 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
PRA Project ID:	OPA20533-20533X
Version Date:	12-Apr-2022 (SAP Version 2)

2.0 Table of Contents

3.0 Introduction

This Statistical Analysis Plan (SAP) describes the statistical methods that will be used during the analysis and reporting of data collected under Opiant Pharmaceuticals, Inc. Protocol OPNT003-OOD-001.

This SAP should be read in conjunction with the study protocol and electronic case report form (eCRF). This version of the plan has been developed using the protocol dated 26-Aug-2021 (including all amendments up to this protocol date) and the final eCRF(s) for Part 1, Part 1 extension and Part 2 dated 07-Sep-2021.

An approved and signed SAP is a requirement for database lock.

This SAP only covers the results that will be processed by the PRA Early Development Services (EDS) Biostatistics Department.

PRA EDS will perform the pharmacokinetic (PK), pharmacodynamic (PD), and safety and tolerability evaluation.

This SAP supersedes the statistical considerations identified in the protocol; where considerations are substantially different, they will be so identified. Any post-hoc or unplanned analyses, or significant changes from the planned analysis in this SAP performed to provide results for inclusion in the clinical study report (CSR) but not included in this SAP, will be clearly identified in the CSR. Changes to planned analyses do not require an updated SAP but should be included in the CSR if significant.

4.0 Changes from Previous Version of Approved SAP

This is the second version of the SAP.

In section 17.1.3.1, the non-inferiority definition was updated to remove the individual criteria and only keep the criteria "the upper limit of the 95% confidence interval is less than 20% of the mean change in minute ventilation for naloxone" for the primary analysis.

As a consequence, in Section 17.1.5 the exploratory analysis was added to compare the proportions of subjects with the extreme responses to the two treatments.

Section 17.1.6 the sensitivity analysis was added to exclude the subjects with change in minute ventilation values out of the Interquartile Range (IQR) and rerun the primary analysis.

5.0 Study Objectives

5.1 Part 1 Objectives

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

5.1.1 Part 1 Endpoints

Primary

- Remifentanil dose required to achieve an approximate 40% or more suppression of CO_2-induced increases in minute ventilation

Secondary

- Time for minute ventilation to return to remifentanil-induced nadir after the mask is reapplied
- Maximum change in minute ventilation from remifentanil-induced nadir following brief mask removal
- Maximum change in minute ventilation from remifentanil-induced nadir after naloxone administration
- Time to maximum change in minute ventilation from remifentanil-induced nadir after naloxone administration
- Change in minute ventilation from remifentanil-induced nadir to 5 minutes after naloxone administration
- Change in minute ventilation from remifentanil-induced nadir to 120 minutes after naloxone administration
- Adverse events (AEs), vital signs, clinical laboratory results, 12-lead electrocardiograms (ECGs), cardiac telemetry, physical examination findings, and nasal cavity examinations

5.2 Part 2 Objectives

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

5.2.1 Part 2 Endpoints

Primary

- Change in minute ventilation from remifentanil-induced nadir to 5 minutes after study drug administration

Secondary

- Change in minute ventilation from remifentanil-induced nadir to 120 minutes after study drug administration
- Change in minute ventilation from the maximum change in minute ventilation from remifentanil-induced nadir to 120 minutes after study drug administration
- Change in minute ventilation from remifentanil-induced nadir to 90 minutes after study drug administration
- Change in minute ventilation from remifentanil-induced nadir to 60 minutes after study drug administration
- Maximum change in minute ventilation from remifentanil-induced nadir after study drug administration
- Time to maximum change in minute ventilation from remifentanil-induced nadir after study drug administration
- Change in minute ventilation from remifentanil-induced nadir to 20 minutes after study drug administration
- Change in minute ventilation from remifentanil-induced nadir to 15 minutes after study
- drug administration
- Change in minute ventilation from remifentanil-induced nadir to 10 minutes after study drug administration
- Change in minute ventilation from remifentanil-induced nadir to 7.5 minutes after study drug administration

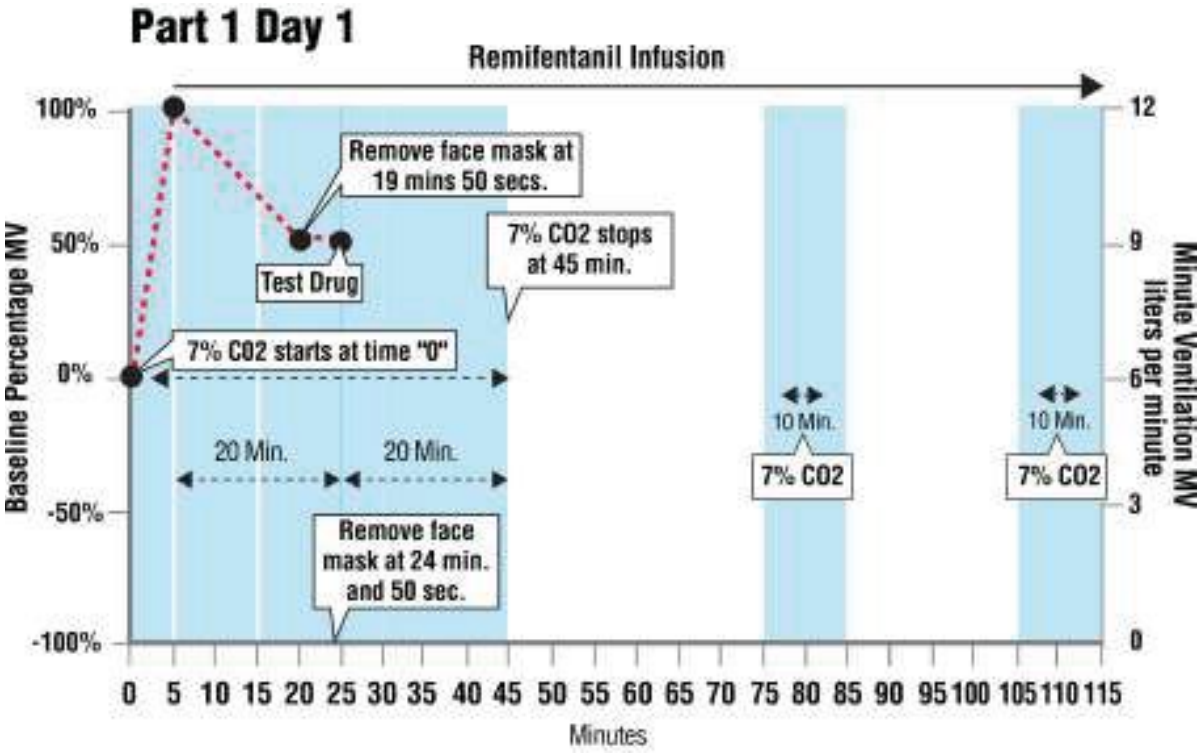
- Change in minute ventilation from remifentanil-induced nadir to 2.5 minutes after study drug administration
- AEs, vital signs, clinical laboratory results, ECGs, cardiac telemetry, physical examination findings, and nasal cavity examinations

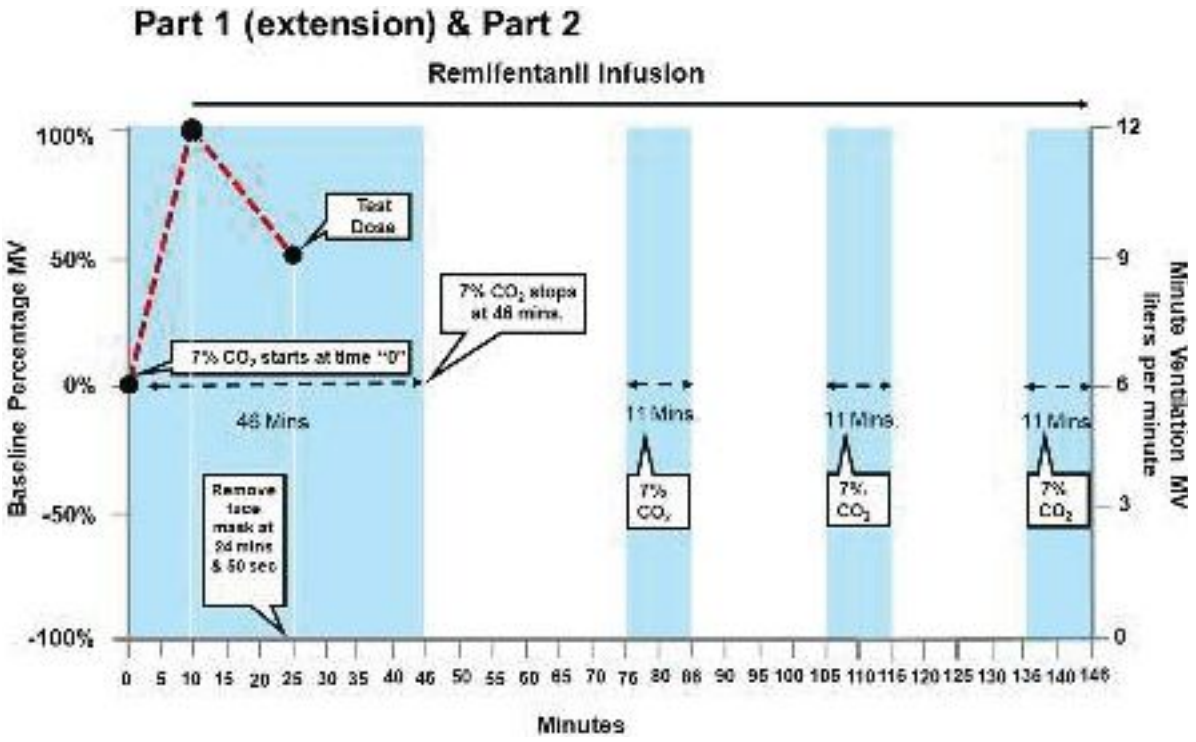
6.0 Study Design

6.1 Type of Study

This will be a single-center, 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 up to 16 healthy volunteers with prior opioid exposure. Part 2 will be a randomized, 2-period, 2-treatment crossover study to evaluate the 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 outpatient Screening Visits, an in-clinic Treatment Phase, and a Follow-Up Phone Call.

The study design is illustrated in figure below:





6.1.1 Screening Period

For Part 1 and Part 2, subjects will report to the medical screening facility/clinical site for the eligibility screening within 28 days prior to the first drug administration (ie, Day -28 to Day -2).

Subjects will sign the study specific informed consent form (ICF) prior to any study specific screening procedures being performed. The written informed consent will be obtained for all subjects, regardless of their eligibility for the study; the signed ICFs will be retained and archived at PRA and a copy will be provided to the subject.

Eligibility screening will consist of the assessments as presented in the Schedules of Assessments. Subjects will also be evaluated for ventilatory response to hypercapnia (VRH) tolerability and response to hypercapnia during screening. Subjects initially satisfying the inclusion and exclusion criteria during screening will be eligible for the Treatment Phase.

6.1.2 Naloxone Challenge Test (Day -1)

As shown in the Schedules of Assessments (Part 1 extension and Part 2), eligible subjects who successfully complete the Screening Visit will be admitted to the Clinical Research Unit (CRU) following eligibility review and complete the naloxone challenge test. The naloxone challenge test (Day -1) will be performed to ensure the subject is not physically dependent on opioids.

All subjects will receive naloxone (0.2 mg) as an IV bolus, followed by an assessment for signs of opioid withdrawal. If there are no signs of opioid withdrawal within 30 seconds after administration, a second IV bolus dose of 0.6 mg will be administered within 5 minutes of the first dose, followed by another assessment for signs of opioid withdrawal 5 minutes after the second naloxone dose. Only subjects who do not have signs and symptoms of opioid withdrawal, as assessed by the Clinical Opioid Withdrawal Scale (COWS score <5, Protocol Appendix 8.4) will be eligible to proceed to the Treatment Phase.

Vital signs will be recorded at predose (first naloxone dose) and at 5 minutes, 0.25, 0.5, 1, 1.5, and 2 hours following the second dose of naloxone. Vital signs will be recorded at nominal time points ± 5 minutes. COWS will be collected and recorded at predose, and at 30 seconds following the first naloxone dose and 5 minutes after the second dose is administered.

Any subject demonstrating evidence of withdrawal (COWS score ⩾5) will not be eligible for further participation in the trial. The subject will be released from the medical screening facility/clinical site when medically stable, as determined by the Investigator. Symptoms reported in the COWS as a consequence of opioid withdrawal will not be collected as AEs unless they meet the criteria for a new AE or an serious adverse event (SAE).

6.1.3 Treatment Phase – Part 1

Eligible subjects will be admitted to the CRU following eligibility review on Day -1 and will remain in the CRU for 7 days to complete the Treatment Phase of Part 1.

On Days 1 and 5 prior to dosing and at the time points specified in the Schedule of Assessments, 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.

Day 1:

On Day 1, 4 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, for the first 4 subjects this will be 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 at Time 25 minutes. 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 IN naloxone hydrochloride will be administered. 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.

The remifentanil infusion rate for the second 4 subjects will be at a rate as defined below and will follow the same procedure.

For the first 4 subjects, if naloxone has increased minute ventilation by >15% but <65% over remifentanil induced nadir at 5 minutes following IN naloxone hydrochloride administration, then the second 4 subjects will receive the same remifentanil infusion 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.

For the first 4 subjects, if the minute ventilation was not increased by >15% over remifentanil induced nadir at 5 minutes following IN naloxone hydrochloride administration, then the second 4 subjects can receive a reduced remifentanil dose, 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.

For the first 4 subjects, if the minute ventilation has increased by >65% over remifentanil induced nadir at 5 minutes following IN naloxone hydrochloride administration, then the second 4 subjects can receive an increased remifentanil dose, 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, providing it is safe and well tolerated under these study conditions.

A decision regarding dose administration for the second 4 subjects will be made by the Investigator and Opiant based on the results from the first 4 subjects and above mentioned criteria. If the data from first 4 subjects is inconsistent, the Investigator and Opiant will take a decision based on the available data.

There will be a 4-day washout period between doses.

Remifentanil Dose Selection for Day 5:

Remifentanil dose for Day 5 will be determined based on the data from Day 1 by the Investigator and the Sponsor. If the remifentanil hydrochloride 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 completed and will proceed to Part 2 of the study.

If the remifentanil infusion, 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) 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 repeat the lower infusion rate on Day 5.

If the remifentanil infusion, 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) 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 repeat the higher infusion rate on Day 5.

If naloxone has not increased minute ventilation by >15% but <65% over remifentanil induced nadir at 5 minutes following IN naloxone hydrochloride administration, at the remifentanil infusion rates administered on Day 1, the remifentanil dose will be further adjusted based on the following hierarchical approach:

1. 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 5.
2. If the remifentanil hydrochloride infusion 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 5, providing it is safe and well tolerated under these study conditions.

Day 5 – If Remifentanil Infusion Is Decreased From Previous Rate:

On Day 5, 4 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.

Dose administration for the second 4 subjects will follow the same process as mentioned above.

Day 5 – If Remifentanil Infusion Is Increased From Previous Rate:

On Day 5, if the remifentanil infusion is increased, 4 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.

Dose administration for the second 4 subjects will follow the same process as mentioned above.

Day 5 – Study Drug Administration (if Day 5 is conducted):

The first 4 subjects will receive 4 mg IN naloxone hydrochloride at Time 25 minutes. 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 IN naloxone hydrochloride will be administered. 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.

If naloxone has increased minute ventilation by >15% but <65% over remifentanil induced nadir at 5 minutes following IN naloxone hydrochloride administration, then the second 4 subjects will receive 4 mg IN naloxone hydrochloride.

If the minute ventilation was not increased by >15% over remifentanil induced nadir at 5 minutes following IN naloxone hydrochloride administration, then the second 4 subjects will receive 3 mg IN nalmefene hydrochloride at Time 25 minutes. To administer the IN nalmefene 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 hydrochloride will be administered. After IN administration of nalmefene 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 nalmefene 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 nalmefene hydrochloride). The PD and safety assessments will be conducted as specified in the Schedule of Assessments.

Subjects will be discharged following completion of the discharge procedures on Day 6. Subjects will be called 3 to 7 days after discharge to inquire concerning AEs and concomitant medications since discharge.

Progression to Part 2 will be based on the data from Part 1 and discussion between Investigator and the Sponsor using the following criteria:

If the remifentanil infusion rate on Day 5 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 remifentanil infusion rate on Day 5 achieves an approximate 40% suppression of CO_2-induced increases in minute ventilation, but minute ventilation was not increased by >15% over remifentanil induced nadir at 5 minutes following IN naloxone hydrochloride administration, however nalmefene has

increased minute ventilation by >15% over remifentanil induced nadir at 5 minutes following IN nalmefene 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 or IN nalmefene hydrochloride administration then the study will be stopped.

If the minute ventilation increased by >65% over remifentanil-induced nadir at 5 minutes following IN naloxone hydrochloride administration then Part 1 will be extended by enrolling additional subjects.

6.1.4 Treatment Phase – Part 1 (extension)

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 as presented in the Schedule of Assessments.

On the day of clinic admission (Day -1), a naloxone challenge test will be performed to ensure the subject is not physically dependent on opioids, and eligibility will be reviewed confirming all the relevant inclusion criteria and none of the exclusion criteria have been met.

On Days 1 and 5 prior to dosing and at scheduled time points specified in the Schedule of Assessments, 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 about a 45° recumbent position.

Day 1:

On Day 1, the first group (up to 4 subjects), fasted for a period of at least 8 hours, will receive pretreatment (30 minutes to 1 hour prior to remifentanil infusion) with famotidine (20 mg IV), 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 10 minutes, for the first group this will be at a rate of 0.175 µg/kg/min, using an initial bolus (0.5 µg/kg) to achieve an expected steady-state. Minute ventilation will be continuously measured. 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 121 minutes period up to Time 146 minutes. The VRH face mask will be removed at Time 46 minutes (ie, 21 minutes after IN administration of naloxone hydrochloride). The VRH face mask will then be reapplied for 11 minutes at Time 75 minutes, at Time 105 minutes and at Time 135 minutes, respectively (ie, between 50 to 61 minutes, 80 to 91 minutes and 110 to 121 minutes after IN administration of naloxone hydrochloride). The PD and safety assessments will be conducted as specified in the Schedule of Assessments.

The remifentanil infusion rate for the second group (up to 4 subjects) will be at a rate defined below and will follow the same procedure.

For the first group, if there is an approximate 40% or more suppression of CO2-induced increases in minute ventilation, and naloxone has increased minute ventilation by >15% but <80% over remifentanil-induced nadir at 5 minutes following IN naloxone hydrochloride administration, then the second group (up to 4 subjects) will also receive a remifentanil infusion, at a rate of 0.175 µg/kg/min, using an initial bolus (0.5 µg/kg) to achieve an expected steady-state, providing it is safe and well tolerated under these study conditions.

For the first group, if there is an approximate 40% or more suppression of CO2-induced increases in minute ventilation, but naloxone has increased minute ventilation by >80% over remifentanil-induced nadir at 5 minutes following IN naloxone hydrochloride administration, then the second group (up to 4 subjects) will receive an increased remifentanil infusion, at the rate 0.2 µg/kg/min, using an initial bolus

(0.5 µg/kg) to achieve an expected steady-state, providing it is safe and well tolerated under these study conditions.

For the first group, if minute ventilation was not increased by >15% over remifentanil-induced nadir at 5 minutes following IN naloxone hydrochloride administration or the remifentanil dose was not safe and well tolerated under these study conditions, then the second group (up to 4 subjects) will receive a reduced remifentanil dose, at a rate of 0.15 µg/kg/min, using an initial bolus (0.5 µg/kg) to achieve an expected steady-state.

A decision regarding dose administration for the second group (up to 4 subjects) will be made by the Investigator and Opiant based on the results from the first group and above mentioned criteria. If the data from first group is inconsistent, the Investigator and Opiant will take a decision based on the available data.

There will be an approximate 4-day washout period between doses.

Day 5:

Remifentanil dose for Day 5 will be determined based on the data from Day 1 by the Investigator and the Sponsor using the following criteria:

For the second group on Day 1, if the remifentanil infusion at a rate of 0.175 µg/kg/min achieves an approximate 40% or more suppression of CO2-induced increases in minute ventilation, and naloxone has increased minute ventilation by >15% over remifentanil-induced nadir at 5 minutes following IN naloxone hydrochloride administration, providing it is safe and well tolerated under these study conditions, then Part 1 (extension) of the study will be considered as completed and will proceed to Part 2 of the study.

For the second group on Day 1, if the remifentanil infusion, at a rate of 0.15 µg/kg/min achieves an approximate 40% or more suppression of CO2-induced increases in minute ventilation, and naloxone has increased minute ventilation by >15% over remifentanil-induced nadir at 5 minutes following IN naloxone hydrochloride administration, then Part 1 (extension) of the study will be considered as completed and will proceed to Part 2 of the study.

For the second group on Day 1, if the remifentanil infusion, at a rate of 0.175 µg/kg/min was not safe and well-tolerated or if the minute ventilation was not increased by >15% over remifentanil induced nadir at 5 minutes following IN naloxone hydrochloride administration, then for the first group (up to 4 subjects) on Day 5 the infusion rate will be reduced to 0.15 µg/kg/min, using an initial bolus (0.5 µg/kg) to achieve an expected steady-state and will follow the same procedure as Day 1.

For the second group on Day 1, if the remifentanil infusion, at a rate of 0.2 µg/kg/min was not safe and well-tolerated or if the minute ventilation was not increased by >15% over remifentanil induced nadir at 5 minutes following IN naloxone hydrochloride administration, then for the first group (up to 4 subjects) on Day 5 the infusion rate will be reduced to 0.175 µg/kg/min using an initial bolus (0.5 µg/kg) to achieve an expected steady-state and will follow the same procedure as Day 1.

For the second group on Day 1, if for the remifentanil infusion, at a rate of 0.2 µg/kg/min, there is an approximate 40% or more suppression of CO2-induced increases in minute ventilation, and naloxone has increased minute ventilation by >15% over remifentanil-induced nadir at 5 minutes following IN naloxone hydrochloride administration, then the first group (up to 4 subjects) on Day 5 will receive a remifentanil infusion, at the same rate of 0.2 µg/kg/min, using an initial bolus (0.5 µg/kg) to achieve an expected steady-state, and will follow the same procedure as Day 1, providing it is safe and well tolerated under these study conditions.

The remifentanil infusion rate for the second group (up to 4 subjects) on Day 5 will be at a rate defined below:

For the first group on Day 5, if the remifentanil infusion at a rate of 0.15 µg/kg/min achieves an approximate 40% or more suppression of CO2-induced increases in minute ventilation, and naloxone has increased minute ventilation by >15% over remifentanil-induced nadir at 5 minutes following IN naloxone hydrochloride administration, providing it is safe and well tolerated under these study conditions, then Part 1 (extension) of the study will be considered as completed and will proceed to Part 2 of the study.

For the first group on Day 5, if the remifentanil infusion at a rate of 0.175 µg/kg/min achieves an approximate 40% or more suppression of CO2-induced increases in minute ventilation, and naloxone has increased minute ventilation by >15% over remifentanil-induced nadir at 5 minutes following IN naloxone hydrochloride administration, providing it is safe and well tolerated under these study conditions, then Part 1 (extension) of the study will be considered as completed and will proceed to Part 2 of the study.

For the first group on Day 5, if the remifentanil infusion at the rate of 0.2 µg/kg/min achieves an approximate 40% or more suppression of CO2-induced increases in minute ventilation, and naloxone has increased minute ventilation by >15% over remifentanil-induced nadir at 5 minutes following IN naloxone hydrochloride administration, providing it is safe and well tolerated under these study conditions, then Part 1 (extension) of the study will be considered as completed and will proceed to Part 2 of the study.

For the first group on Day 5, if the remifentanil infusion, at a rate of 0.2 µg/kg/min was not safe and well tolerated or if the minute ventilation was not increased by >15% over remifentanil induced nadir at 5 minutes following IN naloxone hydrochloride administration, then for the second group (up to 4 subjects) on Day 5 the infusion rate will be reduced to 0.175 µg/kg/min using an initial bolus (0.5 µg/kg) to achieve an expected steady state and will follow the same procedure as Day 1.

A decision regarding dose administration for the second group (up to 4 subjects) will be made by the Investigator and Opiant based on the results from the first group and above mentioned criteria. If the data from first group is inconsistent, the Investigator and Opiant will take a decision based on the available data.

Progression to Part 2 will be based on the data from Part 1 (extension) and discussion between Investigator and the Sponsor using the following criteria:

For the second group on Day 5, if the remifentanil infusion rate of 0.175 µg/kg/min achieves an approximate 40% or more suppression of CO2-induced increases in minute ventilation, and naloxone has increased minute ventilation by >15% over remifentanil-induced nadir at 5 minutes following IN naloxone hydrochloride administration, providing it is safe and well tolerated under these study conditions, then Part 1 (extension) of the study will be considered as completed and will proceed to Part 2 of the study.

6.1.5 Treatment Phase – Part 2

Eligible subjects will be admitted to the CRU following naloxone challenge test and eligibility review on Day -1 and will remain in the CRU for 7 days to complete the Treatment Phase of the Part 2. After naloxone challenge test, eligibility review and completion of admission procedures, each subject will be randomized to receive either IN nalmefene hydrochloride or IN naloxone hydrochloride in a 2 period crossover manner.

Prior to dosing on days of study drug administration and at the time points specified in the Schedule of Assessments, 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 about a 45° recumbent position.

On days of study drug administration (Days 1 and 5), a total of 46 subjects, fasted for a period of at least 8 hours, will receive pretreatment (30 minutes to 1 hour prior to remifentanil infusion) with famotidine (20 mg IV), 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 10 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 at Time 25 minutes, in a randomized 2 period crossover manner, in accordance with the randomization schedule, with an approximately 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. 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 121 minute period up to Time 146 minutes. The VRH face mask will be removed at Time 46 minutes (ie, 21 minutes after IN administration of nalmefene or naloxone). The VRH face mask will then be reapplied for 11 minutes at Time 75 minutes, at Time 105 minutes and at Time 135 minutes, respectively (ie, between 50 to 61 minutes, 80 to 91 and 110 to 121 minutes after IN administration of nalmefene or naloxone). The PD, PK, and safety assessments will be conducted as specified in the Schedule of Assessments.

6.1.6 Follow-Up

A safety Follow-Up Phone Call will be made approximately 3 to 7 days after discharge in Part 1 and Part 2 of the study.

Enquires during Follow-Up Phone Call will be performed as presented in the schedule(s) of assessments.

6.2 Sample Size Considerations

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).

The parameters for determination of sample size were calculated from the literature. Naloxone IV produced an approximate 3 L/min increase from the nadir of opioid induced depression of minute ventilation at 5 minutes post administration. A non-inferiority margin of 80% of the estimated reversal from nadir produced by naloxone under these conditions, was chosen, which represents a difference of 0.6 L/min between treatments. A difference of 20% or less would not likely represent a clinically meaningful difference between treatments. The standard deviation of differences in minute ventilation between IV doses of naloxone and nalmefene to reverse opioid induced depression of minute ventilation has been demonstrated to be 1.26 L/min. Based on this data, the standard deviation of treatment difference was estimated to 1.75 L/min. The sample size will be reviewed based on the data generated in Part 2 after 20 subjects and 46 subjects have been dosed. This will be based on the increase from nadir of opioid induced depression of minute ventilation following administration of IN naloxone hydrochloride and IN nalmefene hydrochloride and the standard deviation of the data.

6.3 Randomization

This will be a single-center, randomized, open-label, 2-part study. Part 1 and Part 1 (extension) will be not randomized. Subjects who enter Part 2 of the study will be randomized to 1 of 2 treatment sequences (XY and YX, where X = test medication and Y = reference medication) in a 1:1 ratio using a computer-generated randomization scheme produced by PRA. Subject randomization numbers will range from 1001 to 10XX with replacement numbers ranging from 2001 to 20XX.

7.0 Overview of Planned Analysis

7.1 Changes from Protocol

There are no changes from the protocol.

7.2 Interim Analysis and Key Results

There will be no interim analyses or summaries of data provided prior to the delivery of the full set of post-lock tables, figures and listings (TFLs).

7.3 Final Analysis

Draft TFLs will be provided after database lock. After Sponsor comments have been incorporated, the TFLs will be finalized and incorporated in the first draft CSR.

8.0 Data Review

8.1 Data Management

Data handling and transfer will take place under the PRA Data Management Plan for the study. All subjects who are screened will be entered in the database.

8.2 Acceptance of Data for Summarization

Programming of analysis datasets and TFLs may be ongoing during the data management of the study. However, programming of analysis datasets and TFLs will be completed and quality controlled (QC'd) after database lock. Only quality assured (QA'd) results released by the Safety Laboratory, Bioanalytical Laboratory, or other external data source will be used for the programming of analysis datasets and TFLs for the final report. Any data values requiring investigation or corrections that are identified while programming the analysis datasets and TFLs will be sent to the project Data Manager. If the issue affects the TFLs the Programmer or Statistician who identified the issue will follow it to resolution.

9.0 Definitions and General Analysis Methods

9.1 Analysis Data Presentation

9.1.1 Rounding

In listings data will be presented with the same precision as the original data. Derived data will be rounded for presentation purposes.

9.1.1.1 General

For summaries, the mean and median will be presented to one decimal place greater than the data, standard deviation to 2 greater than the data, and the minimum and maximum will be presented to the same number of decimal places as the data. Percentages will be presented with one decimal.

The above rule can be applied directly to collected data. For derived data rounding will occur prior to summarization (in the derived dataset as determined by the statistician) so a specific number of decimal places will have to be assumed to apply the above rounding rules for summary statistics.

P-values will be reported to four decimal places; p-value less than 0.0001 will be reported as <0.0001.

9.1.1.2 Pharmacokinetic/Pharmacodynamic

Concentration data will be presented in listings as received by the vendor. Summary statistics of concentration data will be presented to 4 significant digits with the exception of %CV which will be presented to 1 decimal place.

PK parameter data will be rounded in the listings to an appropriate number of decimal places for presentation purposes only. Unrounded values (left as received in analysis dataset) will be used for all calculations of summary statistics and analyses for the summary tables. The summary statistics will be presented to the precision listed in the table in section 16.2.2. When significant digits are used for precision, all summary statistics will be presented to the same precision. When decimal places are used for precision the rule outlined above in the General rounding section applies for summary statistics.

PD parameters will be rounded in the derived dataset as guided by the statistician and presented as is in the listings. Each parameter will have a fixed number of decimals. The statistician will use discretion when deciding the number of decimals for each parameter.

9.1.2 Imputation

Unless otherwise noted, data will not be imputed.

9.1.3 Daylight Savings Time Adjustments

On November 7th, 2021 at 2:00 am the clocks change to 1:00 am as Daylight Savings Time ends. All clinic procedures for the remainder of the treatment period will be moved back by one hour after daylight savings time ends. All duration (ie, AE duration, relative time from dosing for PK) calculations for times post-daylight savings time adjustment that will be relative to a time prior to daylight savings will need to be programmatically adjusted for the hour that was gained on the morning of November 7th.

9.1.4 Descriptive Statistics

Unless otherwise indicated, continuous variables will be summarized with the following descriptive statistics and nomenclature: n = number of observations or subjects, mean = arithmetic mean, SD = standard deviation, min = minimum value, median = median value, and max = maximum value.

Categorical data will be summarized and presented with the following nomenclature: n = frequency and % = percentage. Percentages by categories will be based on the number of subjects exposed within a treatment.

For categorical data, the categories will be presented in the tables exactly as they appear in the case report form (CRF) / Database.

9.1.5 Pooling

Not applicable.

9.1.6 Unscheduled Measurements

Unscheduled and early termination measurements will be included in the listings. With the exception of unscheduled measurements used for baseline, unscheduled and early termination measurements will be excluded from the descriptive statistics and statistical analysis.

9.2 Analysis Data Definitions

9.2.1 Baseline Definition

Unless otherwise stated, baseline is defined as the last observation recorded before any drug administration (pretreatment with ondansetron and sodium citrate). The last observation can be an unscheduled / repeated measurement.

9.2.2 Treatment/Subject Grouping

Label	Grouping
Study Drug	remifentanil, naloxone or nalmefene
Treatment Condition	**Part 1:** Hypercapnic Gas Mixture Only Hypercapnic Gas Mixture +Remifentanil Hypercapnic Gas Mixture +Remifentanil (Post Mask Removal) Hypercapnic Gas Mixture +Remifentanil+ Naloxone **Part 1 Extension:** Hypercapnic Gas Mixture Only Hypercapnic Gas Mixture +Remifentanil Hypercapnic Gas Mixture +Remifentanil+ Naloxone **Part 2:** Hypercapnic Gas Mixture Only Hypercapnic Gas Mixture Remifentanil+ Naloxone Hypercapnic Gas Mixture Remifentanil+ Nalmefene
Dose Level	remifentanil (To be decided) 3 mg nalmefene hydrochloride 4 mg naloxone hydrochloride

9.2.3 Common Variable Derivations

Variable	Data Type	Definition/Calculation
Change from Baseline	All	Postdose Observation minus Baseline Observation
Percent Change from Baseline	All	Change from Baseline/ Baseline Observation * 100
Analysis Study Day (Prior to Dose)	All	Date of Measurement minus Dose Date
Analysis Study Day (Post Dose)	All	Date of Measurement minus Dose Date +1
Hypercapnic Gas Mixture Only (Baseline)	MV	The mean of 12 x 5 second timepoints, covering -60 to 0 seconds prior to the start of the hypercapnic gas mixture
Hypercapnic Gas Mixture + Remifentanil (Baseline)	MV	The mean of 12 x 5 second timepoints, covering -60 to 0 seconds prior to the start of the remifentanil infusion
VRH Mask Removal (Baseline)	MV	The mean of 12 x 5 second timepoints, covering -120 to -60 seconds prior to the start of VRH mask removal
Remifentanil Induced Nadir (Baseline)	MV	The mean of 12 x 5 second timepoints, covering -120 to -60 seconds prior to the IN naloxone or IN nalmefene

		administration
Postdose Remifentanil Dose Minute Ventilation	MV	The mean of 12 x 5 second timepoints, covering 30 seconds either side of each selected postdose timepoint after study drug (remifentanil) administration
Postdose Minute Ventilation	MV	The mean of 12 x 5 second timepoints, covering 30 seconds either side of each selected postdose timepoint after study drug (naloxone or nalmefene) administration
$V_{E\ Change}$	MV	Post dose change in minute ventilation from remifentanil-induced nadir at selected timepoints at nominal time point 25, 27.5, 30, 32.5, 35, 40, 45, 55, 70, 85, 115, 125, 135, and 145 minutes and 155, 165, 175 minutes (Part 1 extension and Part 2 only) in relation to the start of the hypercapnic gas mixture (e.g.: nominal timepoint 27.5 corresponds to +2.5 minutes post naloxone or nalmefene study drug administration)
V_E E_{max}	MV	E_{max} is defined as the maximum effect after study drug administration (naloxone or nalmefene), obtained directly from the post-dose mean PD measurements. For V_E, the maximum effect is defined as the maximum change from remifentanil-induced nadir after study drug administration (naloxone or nalmefene).
TE_{max}	MV	Time to maximum effect E_{max}
TEAE	AE	AE is a TEAE if the AE Date/Time is greater than or equal to the Dose Date/Time

9.2.4 QC

The analysis datasets and the TFLs will be QC'd according to the PRA EDS QC plan.

9.2.4.1 Critical Data

The QC plan requires datasets be classified as critical or non-critical. The datasets considered critical are subject level, pharmacokinetic, pharmacodynamic, and adverse events (ADSL, ADPC, ADPP, ADMV, ADVRH and ADAE). As these are related to the primary objectives these datasets will be double programmed per the QC Plan.

9.2.5 ADaM Datasets and Metadata

The analysis datasets will be generated in accordance with Clinical Data Interchange Standard Consortium (CDISC) Analysis Data Model (ADaM) Version 2.1.

ADaM compliant datasets will be delivered to the sponsor. A define.xml file version 2 with the corresponding metadata will be included. Analysis results metadata are excluded.

9.3 Software

The statistical analysis and reporting will be done using SAS® for Windows™ Version 9.4 or higher (SAS Institute, Inc.).

PK parameter calculations will primarily be done using Phoenix® WinNonlin® (WNL) version 8.1 or higher (Certara, L.P.). Additional PK computations may be performed in SAS®.

9.4 Statistical Methods

9.4.1 Statistical Outlier Determination

No statistical outlier analysis is planned.

9.4.2 Predetermined Covariates and Prognostic Factors

There are no predetermined covariates or prognostic factors.

9.5 TFL Layout

Report layout will be according to the PRA EDS – ICH E3 compliant – CSR Template. The layout of TFLs will be according to the PRA EDS standards.

Table shells are provided with and approved as part of this SAP. Small changes to shell layout due to the nature of the data may be required after lock at the discretion of the PRA project statistician. Other changes to the shells may be out of scope. The TFLs will be provided as a single document in Adobe PDF format (in Letter format), and as individual files for each table, figure and listing in Rich Text Format (.rtf).

10.0 Analysis Sets

The following subject level Analysis Sets (populations) will be used for summaries in the study.

10.1 Safety Set

The Safety Set will consist of subjects who receive at least one dose of any study drug. This set will be used for the safety data summaries, baseline characteristic summaries. This set will be analyzed as treated.

10.2 Intent to Treat Set

The intent to treat (ITT) set will consist of all subjects who are assigned a randomization number and who receive at least one dose of any study drug in the Treatment Phase of Part 2. This set will be used for the PD parameter summaries and analyses for Part 2. This set will be analyzed as randomized.

10.3 Pharmacokinetic Set

All subjects who receive at least 1 dose of test study drug and who have sufficient concentration-time data to calculate at least one PK parameter. This set will be used for the PK parameter summaries for Part 2.

11.0 Subject Disposition

The number and percentage of subjects dosed, randomized, and members of each analysis set will be presented. The number and percentage of subjects who completed and who withdrew from the study prematurely and a breakdown of the corresponding reasons for withdrawal will also be presented. Analysis set and study completion data will be listed by subject.

12.0 Protocol Deviations

Protocol deviations will be collected and reported per PRA's Protocol Deviation Management Standard Operating Procedure (SOP) and relevant Work Instruction (WI). Subject-level deviations will be extracted and pulled into the study tabulation model (SDTM) dataset from PRA's Clinical Trial Management. Deviations that have been reported and coded as "Important" will be listed by subject.

13.0 Demographic and Baseline Characteristics

13.1 Demographics

Subject demographics at screening will be summarized by treatment and overall. The summary will include the subjects' age (years), sex, race, ethnicity, weight (kg), height (cm), and body mass index (BMI) (kg/m^2). Demographics will be summarized for the Safety Set.

All demographic data as collected during the screening visit will be listed by subject.

13.2 Medical History

Medical history, categorized by preferred term according to MedDRA, will be listed by subject.

13.3 Other Baseline Characteristics

Smoking habit and alcohol consumption will be listed by subject.

Pregnancy test will be listed by subject.

Non-compliance to in- or exclusion criteria (if any) will be listed by subject.

Childbearing potential will be listed by subject.

14.0 Concomitant Medications

Concomitant medications collected on the eCRF as defined by the protocol will be categorized by medication group and subgroup according to WHO Drug Dictionary. All concomitant medications will be listed by subject. Medications with an end date prior to the first dose of study drug will be considered prior medications and will be noted in the listing. If the end date (e.g. partial or missing date) does not confirm that the medication was stopped prior to first dose the medication will not be flagged as prior.

15.0 Treatment Compliance and Exposure

The number of subjects receiving each dose of study drug in Part 1, Part 1 extension and Part 2 will be summarized.

Exposure data will be listed by subject.

Naloxone Challenge Dosing result will be listed by subject.

16.0 Pharmacokinetic Analyses

16.1 Pharmacokinetic Variables

Concentrations of naloxone and nalmefene will be collected in plasma for Part 2.

PK parameters of naloxone and nalmefene will be calculated for plasma for Part 2.

16.2 Plasma Pharmacokinetic Summaries

16.2.1 Plasma Concentrations

Plasma concentrations naloxone and nalmefene below the quantifiable limit (BQL) will be set to 0 in the computation of mean concentration values. Descriptive statistics (number of subjects, mean, geometric mean, SD, coefficient of variation [%CV], median, min, and max) will be used to summarize the plasma concentrations by treatment at each scheduled timepoint.

Linear (+/-SD) and semi-logarithmic (+SD) plots of the arithmetic mean plasma concentration by scheduled sampling time will be provided by treatment. These plots will show time in hours. The plots will present all calculated means and will include a reference line for the lower limit of quantification (LLOQ).

Linear and semi-logarithmic plots of the individual plasma concentration by actual sampling time will be provided by subject (one subject per page). These plots will show time in hours. Individual plots will use the BQL handling procedure described below for “Plasma Pharmacokinetic Parameters”.

All individual subject plasma concentration data will be listed by subject.

16.2.2 Plasma Pharmacokinetic Parameters

Plasma PK parameters for naloxone and nalmefene for part 2 will be estimated using non-compartmental methods with WinNonlin® using best fit regression. The PK parameters will be estimated from the concentration-time profiles, and AUCs will be calculated using linear up / log down method. In estimating the PK parameters, BQL values will be set to zero. Actual sampling times, rather than scheduled sampling times, will be used in all computations involving sampling times. If the actual time is missing, the scheduled time will be substituted and flagged.

The following flags will be used to include parameters that meet the predefined criteria for summary and analysis.

Criteria Name	Criteria
Extrapolation	$AUC_{\%Extrap}$ <= 20%
Regression	Adj Rsq >= 0.8
L_{z1}	Lz_Start >= 2*t_{max}
Span	Span > 2

Note: Flags will be applied to parameters prior to derivation of additional parameters in SAS and will be used to include derived parameters as well.

Parameter	Description	Analyte	SAS Programming Notes	Summary Statistic Reporting Precision*
C_{max}	Maximum plasma concentration. Observed peak analyte concentration obtained directly from the experimental data without interpolation, expressed in concentration units	naloxone nalmefene	C_{max} from WNL	3 significant digits

t_{max}	Time to maximum plasma concentration. First observed time to reach peak analyte concentration obtained directly from the experimental data without interpolation, expressed in time units.	naloxone nalmefene	T_{max} from WNL	2 decimal places
AUC_{0-t}	Area under the concentration-time curve (time 0 to time of last quantifiable concentration).	naloxone nalmefene	AUC_{last} from WNL	3 significant digits
AUC_{0-inf}	Area under the concentration-time curve from time 0 extrapolated to infinity.	naloxone nalmefene	AUC_{INF_obs} from WNL To be included in analysis/summaries if the following criteria are met: • Extrapolation, • Regression	3 significant digits
$AUC_{0-2.5}$	Area under the concentration-time curve from time 0 to 2.5 minutes postdose.	naloxone nalmefene	$AUC_{0-2.5}$ from WNL	3 significant digits
AUC_{0-5}	Area under the concentration-time curve from time 0 to 5 minutes postdose.	naloxone nalmefene	AUC_{0-5} from WNL	3 significant digits
AUC_{0-10}	Area under the concentration-time curve from time 0 to 10minutes postdose.	naloxone nalmefene	AUC_{0-10} from WNL	3 significant digits
AUC_{0-15}	Area under the concentration-time curve from time 0 to 15 minutes postdose.	naloxone nalmefene	AUC_{0-15} from WNL	3 significant digits
AUC_{0-20}	Area under the concentration-time curve from time 0 to 20 minutes postdose.	naloxone nalmefene	AUC_{0-20} from WNL	3 significant digits
AUC_{10-20}	Area under the concentration-time curve from time 10 to 20 minutes postdose.	naloxone nalmefene	AUC_{10-20} from WNL	3 significant digits

*Parameters with ‘decimal place’ precision will follow the General rule for summary statistics rounding with the number of decimal places noted as the starting point.

Descriptive statistics (number of subjects, mean, geometric mean, SD, %CV, median, min, and max) will be used to summarize the calculated PK parameters by treatment. For tmax, only median, min and max will be presented.

A scatter plot of individual (plus mean and median) PK parameters Cmax, AUC0-t and AUC0-inf by treatment will be provided.

All parameters will be listed by subject, parameters that meet the inclusion criteria will be accompanied by an indication that each is criteria met.

The following parameters are used for diagnostics and thus listed but not summarized.

Parameter	Description	SAS Programming Notes
$AUC_{\%Extrap}$	Percentage of AUCinf due to extrapolation from the last quantifiable concentration observed to infinity. AUC%Extrap = [AUCinf – AUClast]/AUCinf * 100	AUC_%Extrap_obs from WNL
Adj Rsq	Goodness of fit statistic for the log-linear terminal elimination phase of the concentration-time profile identified by least-squares linear regression and adjusted for the number of points (minimum of 3) used in the estimation of Lz.	Rsq_adjusted from WNL
L_z	Terminal phase rate constant calculated by linear regression of the terminal log-linear portion of the concentration vs. time curve. Using no weighting factor, the terminal log- linear phase of the concentration-time curve is identified by least-square linear regression of at least three data points that yielded a maximum G criteria, which is also referred to as adjusted R2. Lz is the absolute value of the slope of the terminal log-linear phase. Note: In Phoenix, use Best Fit method to determine regression.	Lambda_z from WNL
L_z_Start	L_z_Start is the start time used in the regression for the determination of Lz.	Lambda_z_lower from WNL
L_z_End	L_z_End is the end time used in the regression for the determination of Lz.	Lambda_z_upper from WNL
L_z_N	L_z_N is the number of points used in the regression for the determination of L_z.	No_points_lambda_z from WNL
Span	The minimum number of half-lives needed for the L_z range to be acceptable.	Span from WNL

17.0 Pharmacodynamic Analysis

17.1 Pharmacodynamic Assessment

All PD summaries will be performed using the Safety Set for Part 1 and Part 1 extension and ITT Set for Part 2. The primary measure is minute ventilation associated with treatment conditions. PD assessments will also include continuous measure minute ventilation (V_E), tidal volume (V_T), respiratory rate (RR) with noninvasive RVM, ExSpiron® device collected at each of the planned timepoints and listed in the Schedule of Assessments (See Appendix 2).

The Primary PD assessment will be performed on the data with ExSpiron® device. The VRH data will be captured for potential future use, but no analysis of PD measurements is planned at this point with the exception of End-tidal CO_2 (ET_{CO2}).

17.1.1 Pharmacodynamic Measurements

The following PD parameters will be derived for each PD measurement, per subject, per treatment condition, per timepoint (See table below for calculation of derived PD measurements).

Pharmacodynamic Measurements		
Measurement	**Description**	**Data Source**
V_E	Minute ventilation. Volume of gas exhaled per minute from the lungs, expressed in L/min. Calculated as the mean V_E measurement ("MV" from file) per subject, per treatment, per timepoint.	ExSpiron® device
RR	Respiratory rate, expressed in breaths/min. Calculated as the mean RR measurement ["RR (spont)" from file] per subject, per treatment, per timepoint, excluding zeros.	ExSpiron® device
V_T	Tidal volume. Volume of gas displaced between normal inhalation and exhalation when extra effort is not applied, expressed in mL. Calculated as the mean [sum of inspiratory and expiratory tidal volumes ("Tvi" + "Tve" from file)] per subject, per treatment, per timepoint, excluding zeros.	ExSpiron® device
ET_{CO2}	End tidal CO_2. Partial pressure of carbon dioxide at the end of an exhaled breath. Calculated as the mean $ETCO_2$ measurement per subject, per treatment, per timepoint, excluding zeros.	VRH device

17.1.2 ExSpiron® Device Measurement for the PD Summaries

Descriptive statistics (n, mean, SD, standard error [SE], %CV, median, min, max, as well as quartiles [Q1 and Q3]) will be used to summarize the ExSpiron® device measurement for both Part 1, Part 1 extension and Part 2.

Part 1

Summary tables will be provided of PD measurements (V_E, RR, and V_T) changes and percent changes immediately following each treatment condition (hypercapnic gas mixture only, hypercapnic gas mixture + remifentanil infusion, hypercapnic gas mixture + remifentanil infusion + mask removal, hypercapnic gas mixture + remifentanil infusion + naloxone administration) for selected time intervals postdose will be provided. The Baseline definitions have been specified for each table.

A summary table of PD measurements (V_E, RR, and V_T), changes and percent changes from Baseline by scheduled time (at each minute for 5 minutes post after start of hypercapnic gas mixture) will be provided. The baseline (Hypercapnic Gas Mixture Only Baseline) is defined as the mean of 12 x 5 second timepoints, covering -60 to 0 seconds prior to the start of the hypercapnic gas mixture. All the subjects for Part 1 will be pooled.

A summary table of PD measurements (V_E, RR, and V_T), changes and percent changes from Baseline by scheduled time (at 5, 10, 15, and 20 minutes relative to the start of hypercapnic gas mixture) will be provided. The baseline (Hypercapnic Gas Mixture + Remifentanil Baseline) is defined as the mean of 12 x 5 second timepoints, covering -60 to 0 seconds prior to the start of the remifentanil infusion. The table will be presented by remifentanil dose and overall. Postdose remifentanil dose minute ventilation will be

calculated as the mean of 12 x 5 second timepoints, covering 30 seconds either side of each of the selected postdose timepoints.

A similar summary table of PD measurements (V_E, RR, and V_T), changes and percent changes from Baseline by scheduled time (each minute for 5 minutes post after the start of the mask removal) will be provided. The baseline (VRH Mask Removal Baseline) is defined as the mean of 12 x 5 second timepoints, covering -120 to -60 seconds prior to the start of VRH mask removal. The table will be presented by remifentanil dose and overall.

A summary table of PD measurements (V_E, RR, and V_T), changes and percent changes from Remifentanil-Induced Nadir by scheduled time (at 25, 27.5, 30, 32.5, 35, 40, 45, 55, 70, 85, 115, 125, 135, and 145 minutes relative to the start of the hypercapnic gas mixture) will be provided. Note that the nominal timepoint of 27.5 minutes corresponds to +2.5 minutes post naloxone administration. The baseline (Remifentanil Induced Nadir) is defined as the mean of 12 x 5 second timepoints, covering -120 to -60 seconds prior to the IN naloxone administration. Postdose minute ventilation will be calculated as the mean of 12 x 5 second timepoints, covering 30 seconds either side of each of the selected postdose timepoints. The table will be presented by remifentanil dose and overall.

For all valid ExSpiron® Device PD measurements (V_E, RR, and V_T), the average of each 30 second interval for each parameter will be derived for each subject at every timepoint over the course of the study. The average 30 second parameter value will be presented in the data listing for each subject and timepoint.

Linear plots of the mean (±SD) for ExSpiron® Device PD measurements (V_E, RR, and V_T) over time (from the start of the hypercapnic gas mixture 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) will be provided. These plots will show time in minutes.

Part 1 Extension

Summary tables will be provided of PD measurements (V_E, RR, and V_T) changes and percent changes immediately following each treatment condition (hypercapnic gas mixture only, hypercapnic gas mixture + remifentanil infusion, hypercapnic gas mixture + remifentanil infusion + naloxone administration) for selected time intervals postdose will be provided. The Baseline definitions have been specified for each table.

A summary table of PD measurements (V_E, RR, and V_T), changes and percent changes from Baseline by scheduled time (each minute for 10 minutes post start of hypercapnic gas mixture) will be provided, The baseline (Hypercapnic Gas Mixture Only Baseline) is defined as the mean of 12 x 5 second timepoints, covering -60 to 0 seconds prior to the start of the hypercapnic gas mixture. All the subjects for Part 1 extension will be pooled.

A summary table of PD measurements (V_E, RR, and V_T), changes and percent changes from Baseline by scheduled time (at 10, 15, 20 minutes relative to the start of hypercapnic gas mixture) will be provided. The baseline (Hypercapnic Gas Mixture + Remifentanil Baseline) is defined as the mean of 12 x 5 second timepoints, covering -60 to 0 seconds prior to the start of the remifentanil infusion. The table will be presented by remifentanil dose and overall. Postdose remifentanil dose minute ventilation will be calculated as the mean of 12 x 5 second timepoints, covering 30 seconds either side of each of the selected postdose timepoints. The table will be presented by remifentanil dose and overall.

A summary table of PD measurements (V_E, RR, and V_T), changes and percent changes from Remifentanil-Induced Nadir by scheduled time (at 25, 27.5, 30, 32.5, 35, 40, 45, 55, 70, 85, 115, 125, 135, 145, 155 165 and 175 minutes relative to the start of the hypercapnic gas mixture) will be provided. Note that the nominal timepoint of 27.5 minutes corresponds to +2.5 minutes post naloxone administration. The baseline (Remifentanil Induced Nadir) is defined as the mean of 12 x 5 second timepoints, covering -120 to -60 seconds prior to the IN naloxone administration. Postdose minute ventilation will be calculated as the mean of 12 x 5 second timepoints, covering 30 seconds either side of each of the selected postdose timepoints. The table will be presented by remifentanil dose and overall.

For all valid ExSpiron® Device PD measurements (V_E, RR, and V_T), the average of each 30 second interval for each parameter will be derived for each subject at every timepoint over the course of the study. The average 30 second parameter value will be presented in the data listing for each subject and timepoint.

Linear plots of the mean (±SD) for ExSpiron® Device PD measurements (V_E, RR, and V_T) over time (from the start of the hypercapnic gas mixture at time 0, 5, 10, 15, 20, 25, 27.5, 30, 32.5, 35, 40, 45, 55, 70, 85, 115, 125, 135, 145, 155 165 and 175 minutes) will be provided. These plots will show time in minutes.

Part 2

A summary table of PD measurements (V_E, RR, and V_T), changes and percent changes from Baseline by scheduled time (each minute for 10 minutes post start of hypercapnic gas mixture) will be provided, The baseline (Hypercapnic Gas Mixture Only Baseline) is defined as the mean of 12 x 5 second timepoints, covering -60 to 0 seconds prior to the start of the hypercapnic gas mixture. All the subjects for Part 1 will be pooled.

A summary table of PD measurements (V_E, RR, and V_T), changes and percent changes from Baseline by scheduled time (at 10, 15 and 20 minutes relative to the start of hypercapnic gas mixture) will be provided. The baseline (Hypercapnic Gas Mixture + Remifentanil Baseline) is defined as the mean of 12 x 5 second timepoints, covering -60 to 0 seconds prior to the start of the remifentanil infusion. The table will be presented by remifentanil dose and overall. Postdose remifentanil dose minute ventilation will be calculated as the mean of 12 x 5 second timepoints, covering 30 seconds either side of each of the selected postdose timepoints. The table will be presented by remifentanil dose and overall.

A summary table of PD measurements (V_E, RR, and V_T), changes and percent changes from Remifentanil-Induced Nadir by scheduled time (at 25, 27.5, 30, 32.5, 35, 40, 45, 55, 70, 85, 115, 125, 135, 145, 155, 165 and 175 minutes in relation to the start of the hypercapnic gas mixture) will be provided. The baseline (Remifentanil Induced Nadir) is defined as the mean of 12 x 5 second timepoints, covering -120 to -60 seconds prior to the IN naloxone or IN nalmefene administration. Postdose minute ventilation will be calculated as the mean of 12 x 5 second timepoints, covering 30 seconds either side of the selected postdose timepoint.

For all valid ExSpiron® Device PD measurements (V_E, RR, and V_T), the average of each 30 second interval for each parameter will be derived for each subject at every timepoint over the course of the study. The average 30 second parameter value will be presented in the data listing for each subject and timepoint.

Linear plots of the mean (±SD) for ExSpiron® Device PD measurements (V_E, RR, and V_T) over time (from the start of the hypercapnic gas mixture at time 0, 5, 10, 15, 20, 25, 27.5, 30, 32.5, 35, 40, 45, 55, 70, 85, 115, 125, 135, 145, 155, 165 and 175 minutes) will be provided. These plots will show time in minutes.

Pharmacodynamic Parameters

The PD parameter $V_{E\ Change}$, V_E E_{max} and V_E TE_{max} have been defined in section 9.2.3. PD Parameter V_E E_{max} and V_E TE_{max} values will be summarized for all parts. For the V_E TE_{max}, only median, min and max will be presented. All PD parameters will be listed by subject.

A summary table of change in minute ventilation from the maximum change in minute ventilation from remifentanil-induced nadir (V_E E_{max}) to 120 minutes after study drug administration will be provided by treatment for Part 2.

The ExSpiron® Device start and end time will be listed by subject.

17.1.3 Statistical Analysis of Pharmacodynamic Parameters

17.1.3.1 Noninferiority Analysis

The primary endpoint of interest is the $V_{E\ Change}$ at 5 minutes post study drug administration (naloxone or nalmefene) in Part 2. The statistical analyses of noninferiority will be performed using the ITT set for Part

2 with ExSpiron® Device. The primary endpoint of $V_{E\ Change}$ at 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 treatment, period and sequence as fixed effects, and subject nested within sequence as a random effect. The least squares (LS) means and the mean difference (naloxone – nalmefene) of change in minute ventilation and corresponding 2-sided 95% confidence interval (CI) will be estimated. Noninferiority will have been demonstrated if:

- the upper limit of the 95% confidence interval is less than 20% of the mean change in minute ventilation for naloxone

The following SAS PROC MIXED pseudo-code may be used:

```
proc mixed data = adMV;
   class treatment period sequence subject;
   model aval = treatment period sequence/ddfm=kr;
   random subject(sequence);
   lsmeans treatment / alpha = 0.1;
   estimate "naloxone vs nalmefene" treatment -1 1 /e cl alpha=0.05;
run;
```

17.1.4 VRH Device PD Measurements

ET_{CO2} with VRH device will be summarized using descriptive statistics (n, mean, SD, standard error [SE], %CV, median, min, max, as well as quartiles [Q1 and Q3]) by the timepoint (from the start of the hypercapnic gas mixture at time 0, 5, 10, 15, 20, 25, 27.5, 30, 32.5, 35, 40, 45 minutes).

Linear plots of the mean (±SD) ET_{CO2} by timepoint (up to the first 45 minutes) specified in the protocol will be provided. These plots will show time in minutes.

All valid PD measurements with the VRH device will be listed by subject.

17.1.5 Exploratory Analysis

In order to compare the proportion of subjects with the extreme responses to the two treatments, the Pearson chi-square test will be employed to test the proportion of the subjects with a change in minute ventilation on nalmefene that is less than 50% of the change in minute ventilation on naloxone vs the proportion of the subjects with a change in minute ventilation on naloxone that is less than 50% of the change in minute ventilation on nalmefene.

H0: the two proportions are equal.

HA: the two proportions are unequal.

The small p-value for the test ($p<0.05$) indicates that the null hypothesis of equal proportions can be rejected and that the proportions are unequal.

The following PROC PREQ statements compare the proportion of subjects with extreme responses may be used:

```
proc freq order=data;
        weight Count;
        table outcome* Response / chisq riskdiff;
        run;
```

For the chi-square test to be valid, all cell counts should be at least 5. When some cell counts are less than 5, then Fisher's exact test will be used.

17.1.6 Sensitivity Analysis

As a sensitivity analysis, the primary analysis will be rerun excluding the subjects for which the change in minute ventilation from remifentanil-induced nadir to 5 minutes that are more than 1.5 IQR below first percentile (Q1) or more than 1.5 IQR above third percentile (Q3).

18.0 Safety Analyses

18.1 Safety Variables

- Adverse Events (AEs)
- Clinical Laboratory Evaluations
    - Clinical Chemistry
    - Hematology
    - Urinalysis
    - Coagulation
- Vital Signs
    - Semi-recumbent Blood Pressure
        - Systolic Blood Pressure
        - Diastolic Blood Pressure
    - Pulse rate
    - Oral body temperature
    - Respiratory rate
- Electrocardiograms (ECG)
    - Heart Rate
    - PR Interval
    - QRS-Duration
    - QT Interval
    - QTc (Friderica) Interval
- Continuous Cardiac Monitoring (telemetry)
- Columbia-Suicide Severity Rating Scale (C-SSRS)
- Physical Examination
- Nasal Cavity Examination
- Continuous Oxygen Saturation

18.1.1 Adverse Events

Treatment emergence will be evaluated for all AEs. Treatment-emergent adverse events (TEAE) are those that occur after the first dose of study drug.

TEAEs occurring following dosing in a specific period but before dosing in the next period will be attributed to the treatment in that period. If the time is missing for an AE on a dosing day then the AE will be attributed to the treatment given on that day.

The following missing data will be imputed as defined (for calculations/summary tables only and will not be presented in listings):

- Missing AE start and / or end times for the calculation of onset and duration will be assumed to be at 00:01 for a start time and 23:59 for end times
- Missing AE severity or relationship will be assumed to be severe (Grade 3 if Common Terminology Criteria for Adverse Events [CTCAE]) or related, respectively
- Missing AE start times for the determination of treatment emergence will be assumed to occur after treatment unless partial date documents the AE as happening prior to treatment
- Missing AE start times for the determination of treatment assignment will be assumed to occur after treatment on the recorded date one minute after dosing

- Missing AE start date will be assumed to be after treatment for the determination of TEAE and on treatment but will not be attributed to a specific treatment

All the AE tables will be provide for Part 1, Part 1 extension and Part 2. A summary of number and percentage of subjects reporting TEAEs, TEAEs by severity and relationship, serious AEs (SAEs), and subjects who discontinued study drug due to an AE will be provided.

A summary of the number and percentage of subjects reporting each TEAE, categorized by system organ class and preferred term coded according to the MedDRA, will be presented by treatment and overall. Counting will be done by subject only, not by event; subjects will only be counted once within each body system or preferred term.

A summary of the number and percentage of subjects reporting each TEAE will be presented by relationship to study drug (as recorded on the eCRF). Subjects with multiple events within a system organ class or preferred term will be counted under the category of their most drug-related event within that system organ class or preferred term.

A summary of the number and percentage of subjects reporting each TEAE will be presented by severity (as recorded on eCRF). Subjects with multiple events within a system organ class or preferred term will be counted under the category of their most severe event within that system organ class or preferred term.

A summary of the number and percentage of subjects reporting each TEAE will be presented by toxicity grade (as recorded on eCRF). Subjects with multiple events within a system organ class or preferred term will be counted under the category of their largest grade level within that system organ class or preferred term.

All AEs (including non-treatment-emergent events) recorded on the eCRF will be listed by subject.

A separate listing of AEs leading to study drug discontinuation will be provided.

18.1.2 Deaths and Serious Adverse Events

A listing of deaths and other SAEs will be provided by subject.

18.1.3 Laboratory Data

Clinical laboratory data will be presented using units from SDTM Controlled Terminology.

Descriptive statistics summarizing continuous laboratory results of clinical chemistry, hematology, and urinalysis at baseline will be provided for Part 1 and Part 1 extension.

Descriptive statistics summarizing continuous laboratory results of clinical chemistry, hematology, and urinalysis at baseline and Day 7 will be provided for Part 2.

All laboratory data will be listed by subject, including laboratory tests not listed in the protocol. A separate listing of out-of-range values will also be provided. Normal ranges will be used directly from the clinical laboratory and will be included in the listings for reference.

18.1.4 Vital Signs

Descriptive statistics summarizing vital signs and changes from predose by dosing day and scheduled time will be provided for Part 1 and Part 1 extension.

Descriptive statistics summarizing vital signs and changes from predose by treatment and scheduled time will be provided for Part 2.

All vital signs will be listed by subject.

18.1.5 Electrocardiograms

Descriptive statistics summarizing ECG parameters and changes from predose by dosing day and scheduled time will be provided for Part 1 and Part 1 extension.

Descriptive statistics summarizing ECG parameters and changes from predose by treatment and scheduled time will be provided for Part 2.

All ECG parameters and the corresponding abnormalities will be listed by subject.

18.1.6 Noninvasive Blood Pressure and Oxygen Saturation

Descriptive statistics summarizing noninvasive blood pressure and oxygen saturation data by dosing day and scheduled time will be provided for Part 1 and Part 1 extension.

Descriptive statistics summarizing noninvasive blood pressure and oxygen saturation data by treatment and scheduled time will be provided for Part 2.

All noninvasive blood pressure and oxygen saturation data will be listed by subject.

18.1.7 Nasal Cavity Examination

A summary of nasal cavity examination data will be presented as the number and percentage of findings (Normal, Abnormal Not Clinically Significant (NCS), Abnormal Clinically Significant [CS] and Not Examined as recorded on the eCRF) and nasal irritation scale reported by visit and timepoint for Part 1 and Part 1 extension.

A summary of nasal cavity examination data will be presented as the number and percentage of findings (Normal, Abnormal NCS, Abnormal CS and Not Examined as recorded on the eCRF) and nasal irritation scale reported by treatment and timepoint for Part 2.

All nasal cavity examination data will be listed by subject.

18.1.8 Mallampati Score

A summary of mallampati intubation score will be presented as the number and percentage at screening for all parts.

Mallampati intubation score will be listed by subject.

18.1.9 Cardiac Telemetry

A listing of the start and stop times for cardiac telemetry will be provided. Any clinically significant abnormal objective test findings will be recorded as AEs.

18.1.10 Columbia-Suicide Severity Rating Scale

C-SSRS data will be listed by subject.

18.1.11 Clinical Opiate Withdrawal Scale

COWS results during the naloxone challenge test for Part 1 extension and Part 2 will be listed by subject.

19.0 References

SAS Institute, Inc., SAS® Version 9.4 software, Cary, NC.

Clinical Study Protocol. 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. Version 7, 26 Aug2021.

Appendix 1: Glossary of Abbreviations

Glossary of Abbreviations:	
AE	Adverse event
ADaM	Analysis data model
ANOVA	Analysis of variance
BMI	Body mass index
BQL	Below the quantifiable limit
CDISC	Clinical Data Interchange Standard Consortium
CI	Confidence interval
COWS	Clinical Opioid Withdrawal Scale
CRF	Case Report Form
CRU	Clinical Research Unit
CSR	Clinical study report
CS	Clinically significant
C-SSRS	Columbia-Suicide Severity Rating Scale
CTCAE	Common Terminology Criteria for Adverse Events
CV	Coefficient of variation
ECG	Electrocardiogram
eCRF	Electronic case report form
EDS	Early Development Services
ICF	Informed Consent Form
ICH	The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
IN	Intranasal
IQR	Interquartile range
ITT	Intent to treat
IV	Intravenous
LLOQ	Lower limit of quantification
LS	Least Squares
MedDRA	Medical Dictionary for Regulatory Activities
NCS	Not clinically significant
PD	Pharmacodynamic
PK	Pharmacokinetic
QA'd	Quality assured

Q1	First percentile
Q3	Third percentile
QC'd	Quality controlled
SAP	Statistical analysis plan
SAE	Serious adverse event
SD	Standard deviation
SDTM	Study data tabulation model
SE	Standard Error
SOP	Standard operating procedure
TEAE	Treatment-emergent adverse event
TFL(s)	Tables, figures and listings
VRH	Ventilatory response to hypercapnia
WI	Work Instruction
WNL	WinNonlin

Appendix 2: Protocol Schedule of Assessments – Part 1

Schedule of Assessments – Part 1									
	Screening	**Admission /Baseline**	**Dose Day 1**	**Washout**			**Dose Day 2**	**Discharge or Early Termination**	**Follow-Up Phone Call**
Study Day(s)	**-28 to -2**	**-1**	**1**	**2**	**3**	**4**	**5**	**6**	**+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			X[a]	X	
ECG	X	X	X[b]			X	X[b]		
Continuous Cardiac Monitoring (Telemetry)			X[c]				X[c]		
Vital Signs	X[d]	X[d]	X[e]	X			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				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	X	X	X[j]	X	X
Concomitant Medications	X	X	X[k]	X	X	X	X[k]		X
Enrollment			X						
VRH Test	X	X							
VRH			X[l]				X[l]		
Oxygen Saturation			X[m]				X[m]		
Respiration Rate (using ExSpiron® device)			X[m]				X[m]		

Schedule of Assessments – Part 1									
	Screening	**Admission /Baseline**	**Dose Day 1**	**Washout**			**Dose Day 2**	**Discharge or Early Termination**	**Follow-Up Phone Call**
Study Day(s)	**-28 to -2**	**-1**	**1**	**2**	**3**	**4**	**5**	**6**	**+3 to 7 days after discharge**
Noninvasive Blood Pressure (Semi-recumbent)			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		
Meals		X	X[s]	X	X	X	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 prior to intranasal dosing, and at approximately 2 hours post intranasal dosing.
b. ECG assessment will be conducted prior to intranasal dosing and remifentanil infusion and at approximately 4 hours post intranasal dosing.
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. Semi-recumbent blood pressure, heart rate, respiration rate, and temperature.
e. Semi-recumbent blood pressure, heart rate, respiration rate, and temperature prior to remifentanil infusion and at approximately 2 hours post intranasal dosing.
f. Chemistry parameters include sodium, potassium, chlorideCO_2, creatinine, CK, amylase, lipase, glucose (fasting), 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/WBCs, protein, blood, glucose, and pH.
i. Urine drug screen for opiates, cocaine, amphetamines/methamphetamine, benzodiazepines, cannabinoids/THC, barbiturates.
j. Adverse events reviewed at approximately 2 hours post intranasal dosing.
k. Concomitant medications reviewed at approximately 2 hours post intranasal dosing.
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 (pre-remifentanil infusion; at intranasal dosing; after 2.5, 5, 10, 15, 20, 25, 30 minutes post intranasal dosing; 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 following the start of the hypercapnic gas mixture 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. On Dose Day 1, subjects will receive a 4 mg naloxone hydrochloride IN dose at Time 25 minutes. On Dose Day 2, the first 4 subjects will receive a 4 mg naloxone hydrochloride IN dose. If naloxone has increased minute ventilation by >15% but <65% over remifentanil-induced nadir at 5 minutes following IN naloxone hydrochloride administration, then the second 4 subjects will receive 4 mg IN naloxone hydrochloride at Time 25 minutes. If the minute ventilation was not increased by >15% over remifentanil-induced nadir at 5 minutes following IN naloxone hydrochloride administration, then the second 4 subjects will receive 3 mg IN nalmefene hydrochloride 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.

Appendix 3: Protocol Schedule of Assessments – Part 1 (extension)

Schedule of Assessments – Part 1 (extension)									
	Screening	**Admission /Baseline**	**Dose Day 1**	**Washout**			**Dose Day 2**	**Discharge or Early Termination**	**Follow-Up Phone Call**
Study Day(s)	**-28 to -2**	**-1**	**1**	**2**	**3**	**4**	**5**	**6**	**+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			X[a]	X	
ECG	X	X	X[b]			X	X[b]		
Continuous Cardiac Monitoring (Telemetry)			X[c]				X[c]		
Vital Signs	X[d]	X[d]	X[e]	X			X[e]		
Weight, BMI	X	X							
Height	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				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								
Naloxone Challenge Test[s]		X							
Adverse Events	X	X	X[j]	X	X	X	X[j]	X	X
Concomitant Medications	X	X	X[k]	X	X	X	X[k]		X
Enrollment			X						
VRH Test (10 minutes)	X	X							
VRH			X[l]				X[l]		
Oxygen Saturation			X[m]				X[m]		

Schedule of Assessments – Part 1 (extension)									
	Screening	**Admission /Baseline**	**Dose Day 1**	**Washout**			**Dose Day 2**	**Discharge or Early Termination**	**Follow-Up Phone Call**
Study Day(s)	**-28 to -2**	**-1**	**1**	**2**	**3**	**4**	**5**	**6**	**+3 to 7 days after discharge**
Respiration Rate (using ExSpiron® device)			X[m]				X[m]		
Noninvasive Blood Pressure (Semi-recumbent)			X[m]				X[m]		
Minute Ventilation Measurement [n]			X				X		
Pretreatments [o]			X				X		
Remifentanil Infusion [p]			X				X		
Study Drug Administration [q]			X				X		
Meals		X	X[r]	X	X	X	X[r]		

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; COWS=Clinical Opioid Withdrawal Scale; ECG=12-lead electrocardiogram; FSH=follicle stimulating hormone; HDL=high density lipoprotein; HIV=human immunodeficiency virus; IN=intranasal; IV=intravenous; 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 prior to intranasal dosing, and at approximately 2 hours post intranasal dosing.
b. ECG assessment will be conducted prior to intranasal dosing and remifentanil infusion and at approximately 4 hours post intranasal dosing.
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. Semi-recumbent blood pressure, heart rate, respiration rate, and temperature.
e. Semi-recumbent blood pressure, heart rate, respiration rate, and temperature prior to remifentanil infusion and at approximately 2 hours post intranasal dosing.
f. Chemistry parameters include sodium, potassium, chloride, CO2, creatinine, CK, amylase, lipase, glucose (fasting), 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/WBCs, protein, blood, glucose, and pH.
i. Urine drug screen for opiates, cocaine, amphetamines/methamphetamine, benzodiazepines, cannabinoids/THC, barbiturates.
j. Adverse events reviewed at approximately 2 hours post intranasal dosing.
k. Concomitant medications reviewed at approximately 2 hours post intranasal dosing.
l. Subjects will start receiving a hypercapnic gas mixture using a VRH face mask at Time 0 minutes, which will be removed at Time 46 minutes and then be reapplied for 11 minutes at Time 75 minutes, at Time 105 minutes and at Time 135 minutes, respectively.

m. Conducted during the remifentanil infusion (pre-remifentanil infusion; at intranasal dosing; after 2.5, 5, 10, 15, 20, 25, 30 minutes post intranasal dosing; 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 prior to the start of the hypercapnic gas mixture and following the start of the hypercapnic gas mixture at Time 0, 5, 10, 15, 20, 25, 27.5, 30, 32.5, 35, 40, 45, 55, 70, 85, 115, 125, 135, 145, 155, 165, 175 minutes (ie, at -25, -20, -15, -10, -5, 0, 2.5, 5, 7.5, 10, 15, 20, 30, 45, 60, 90, 100, 110, 120, 130, 140, 150 minutes in relation to study drug administration), using the ExSpiron® device.
o. Pretreatment with famotidine (20 mg IV), 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 10 minutes using a syringe pump, delivering an initial bolus (0.5 µg/kg) to achieve the approximate target steady-state.
q. On Dose Day 1, subjects will receive a 4 mg naloxone hydrochloride IN dose at Time 25 minutes. On Dose Day 2, subjects will receive a 4 mg naloxone hydrochloride IN dose at Time 25 minutes.
r. Subjects will fast for a period of 8 hours before remifentanil dosing until one hour after dosing. Water will be provided ad libitum.
s. Naloxone (0.2 mg IV) is administered first. If there are no signs of withdrawal apparent within 30 seconds after administration, another 0.6 mg naloxone IV is administered. Vital signs will be recorded at predose (first naloxone dose) and at 5 minutes, 0.25, 0.5, 1, 1.5, and 2 hours following the second dose of naloxone. Vital signs will be recorded at nominal time points ± 5 minutes. COWS will be collected and recorded at predose, and at 30 seconds following the first naloxone dose and 5 minutes after the second dose is administered. Symptoms of withdrawal following naloxone administration (Naloxone Challenge Test) will not be collected as adverse events unless they meet the criteria for a new adverse event or an serious adverse event.

Appendix 4: Protocol 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, BMI	X	X							X	
Height	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									
Naloxone Challenge Test[x]		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 (10 minutes)	X	X								
VRH			X[p]				X[p]			

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**
Oxygen Saturation			X[q]				X[q]			
Respiration Rate (using ExSpiron® device)			X[q]				X[q]			
Noninvasive Blood Pressure (Semi-recumbent)			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			
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; COWS=Clinical Opioid Withdrawal Scale; ECG=12-lead electrocardiogram; FSH=follicle stimulating hormone; HDL=high density lipoprotein; HIV=human immunodeficiency virus; IN=intranasal; IV=intravenous; 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 prior to intranasal dosing, and at approximately 2 hours post intranasal dosing.
b. Nasal cavity examination at approximately 24 hours post intranasal dosing.
c. ECG assessment will be conducted prior to intranasal dosing and remifentanil infusion and at approximately 4 hours post intranasal dosing.
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. Semi-recumbent blood pressure, heart rate, respiration rate, and temperature.
f. Semi-recumbent blood pressure, heart rate, respiration rate, and temperature prior to remifentanil infusion and at approximately 2 hours post intranasal dosing.
g. Semi-recumbent blood pressure, heart rate, respiration rate, and temperature at approximately 24 hours post intranasal dosing.
h. Chemistry parameters include sodium, potassium, chloride, CO_2, creatinine, CK, amylase, lipase, glucose (fasting), 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/WBCs, protein, blood, glucose, and pH.

k. Urine drug screen for opiates, cocaine, amphetamines/methamphetamine, benzodiazepines, cannabinoids/THC, barbiturates.
l. Adverse events reviewed at approximately 2 hours post intranasal dosing.
m. Adverse events reviewed at approximately 24 hours post intranasal dosing.
n. Concomitant medications reviewed at approximately 2 hours post intranasal dosing.
o. Concomitant medications reviewed at approximately 24 hours post intranasal dosing.
p. Subjects will start receiving a hypercapnic gas mixture using a VRH face mask at Time 0 minutes, which will be removed at Time 46 minutes and then be reapplied for 11 minutes at Time 75 minutes, at Time 105 minutes and at Time 135 minutes, respectively.
q. Conducted during the remifentanil infusion (pre-remifentanil infusion; at intranasal dosing; after 2.5, 5, 10, 15, 20, 25, 30 minutes post intranasal dosing; 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 following the start of the hypercapnic gas mixture at Time 0, 5, 10, 15, 20, 25, 27.5, 30, 32.5, 35, 40, 45, 55, 70, 85, 115, 125, 135, 145, 155, 165, 175 minutes (ie, at -25, -20, -15, -10, -5, 0, 2.5, 5, 7.5, 10, 15, 20, 30, 45, 60, 90, 100, 110, 120, 130, 140, 150 minutes in relation to study drug administration), using the ExSpiron® device.
s. Pretreatment with famotidine (20 mg IV), 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 10 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, 90, 120 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.
x. Naloxone (0.2 mg IV) is administered first. If there are no signs of withdrawal apparent within 30 seconds after administration, another 0.6 mg naloxone IV is administered. Vital signs will be recorded at predose (first naloxone dose) and at 5 minutes, 0.25, 0.5, 1, 1.5, and 2 hours following the second dose of naloxone. Vital signs will be recorded at nominal time points ± 5 minutes. COWS will be collected and recorded at predose, and at 30 seconds following the first naloxone dose and 5 minutes after the second dose is administered. Symptoms of withdrawal following naloxone administration (Naloxone Challenge Test) will not be collected as adverse events unless they meet the criteria for a new adverse event or an serious adverse event.

Appendix 5: List of End of Text Outputs

List of End of Text Tables and Figures:		
Output	**Title**	**Analysis Set**
Section 14.1 – Disposition and Demographic Data		
Table 14.1.1	Summary of Analysis Sets	All Subjects
Table 14.1.2	Summary of Subject Disposition	Safety
Table 14.1.3	Summary of Demographics	Safety
Table 14.1.4.1	Summary of Study Drug Administration – Part 1	Safety
Table 14.1.4.2	Summary of Study Drug Administration – Part 1 Extension	Safety
Table 14.1.4.3	Summary of Study Drug Administration – Part 2	Safety
Section 14.2.1 – PD Data		
Table 14.2.1.1.1	Summary of ExSpiron® Device PD Measurements Immediately Following Treatment Condition Hypercapnic Gas Mixture – Part 1	Safety
Table 14.2.1.1.2	Summary of ExSpiron® Device PD Measurements Immediately Following Treatment Condition Remifentanil Infusion – Part 1	Safety
Table 14.2.1.1.3	Summary of ExSpiron® Device PD Measurements Immediately Following Treatment Condition VRH Mask Removal – Part 1	Safety
Table 14.2.1.1.4	Summary of ExSpiron® Device PD Measurements Immediately Following Treatment Condition IN Naloxone Administration – Part 1	Safety
Table 14.2.1.1.5	Summary of ExSpiron® Device Minute Ventilation PD Parameter – Part 1	Safety
Figure 14.2.1.1.6	Plot of Mean (+/-SD) ExSpiron® Device PD measurements Versus Time – Part 1	Safety
Table 14.2.1.1.7	Summary of VRH ET_{CO2} – Part 1	Safety
Figure 14.2.1.1.8	Plot of Mean (+/-SD) VRH ET_{CO2} Versus Time – Part 1	Safety
Table 14.2.1.2.1	Summary of ExSpiron® Device PD Measurements Immediately Following Treatment Condition Hypercapnic Gas Mixture – Part 1 Extension	Safety
Table 14.2.1.2.2	Summary of ExSpiron® Device PD Measurements Immediately Following Treatment Condition Remifentanil Infusion – Part 1 Extension	Safety
Table 14.2.1.2.3	Summary of ExSpiron® Device PD Measurements Immediately Following Treatment Condition IN Naloxone Administration – Part 1 Extension	Safety
Table 14.2.1.2.4	Summary of ExSpiron® Device Minute Ventilation PD Parameter – Part 1 Extension	Safety
Figure 14.2.1.2.5	Plot of Mean (+/-SD) ExSpiron® Device PD measurements Versus Time – Part 1 Extension	Safety

Table 14.2.1.2.6	Summary of VRH ET_{CO2} – Part 1 Extension	Safety
Figure 14.2.1.2.7	Plot of Mean (+/-SD) VRH ET_{CO2} Versus Time – Part 1 Extension	Safety
Table 14.2.1.3.1	Summary of ExSpiron® Device PD Measurements Immediately Following Treatment Condition Hypercapnic Gas Mixture – Part 2	ITT
Table 14.2.1.3.2	Summary of ExSpiron® Device PD Measurements Immediately Following Treatment Condition Remifentanil Infusion – Part 2	ITT
Table 14.2.1.3.3	Summary of ExSpiron® Device PD Measurements Immediately Following Treatment Condition IN Naloxone Administration – Part 2	ITT
Table 14.2.1.3.4	Summary of ExSpiron® Device Minute Ventilation PD Parameter – Part 2	ITT
Table 14.2.1.3.5	Summary of ExSpiron® Device Minute Ventilation Change from Emax – Part 2	ITT
Table 14.2.1.3.6	Statistical Analysis of Noninferiority Analysis of Nalmefene Versus Naloxone – Part 2	ITT
Table 14.2.1.3.7	Statistical Analysis of comparing the proportions of subject with the extreme responses	ITT
Table 14.2.1.3.8	Sensitivity Analysis: Statistical Analysis of Noninferiority Analysis of Nalmefene Versus Naloxone – Part 2	ITT
Figure 14.2.1.3.9	Plot of Mean (+/-SD) ExSpiron® Device PD Measurements Versus Time – Part 2	ITT
Table 14.2.1.3.10	Summary of VRH ET_{CO2} – Part 2	ITT
Figure 14.2.1.3.11	Plot of Mean (+/-SD) VRH ET_{CO2} Versus Time – Part 2	ITT
Section 14.2.2 – PK Data		
Table 14.2.2.1	Summary of Naloxone Plasma Concentrations – Part 2	PK
Table 14.2.2.2	Summary of Nalmefene Plasma Concentrations – Part 2	PK
Table 14.2.2.3	Summary of Naloxone Plasma Pharmacokinetic Parameters – Part 2	PK
Table 14.2.2.4	Summary of Nalmefene Plasma Pharmacokinetic Parameters – Part 2	PK
Figure 14.2.2.5.1	Plot of Mean (± SD) Naloxone Plasma Concentrations Versus Time on a Linear Scale – Part 2	PK
Figure 14.2.2.5.2	Plot of Mean (+SD) Naloxone Plasma Concentrations Versus Time on a Semi-Log Scale – Part 2	PK
Figure 14.2.2.6.1	Plot of Individual Naloxone Plasma Concentrations Versus Time on a Linear Scale – Part 2	PK
Figure 14.2.2.6.2	Plot of Individual Naloxone Plasma Concentrations Versus Time on a Semi-Log Scale – Part 2	PK
Figure 14.2.2.7.1	Plot of Mean (± SD) Nalmefene Plasma Concentrations Versus Time on a Linear Scale – Part 2	PK
Figure 14.2.2.7.2	Plot of Mean (+SD) Nalmefene Plasma Concentrations Versus Time on a Semi-Log Scale – Part 2	PK
Figure 14.2.2.8.1	Plot of Individual Nalmefene Plasma Concentrations Versus Time on a	PK

	Linear Scale – Part 2	
Figure 14.2.2.8.2	Plot of Individual Nalmefene Plasma Concentrations Versus Time on a Semi-Log Scale – Part 2	PK
Section 14.3 – Safety Data		
Table 14.3.1.1.1	Summary of Adverse Events – Part 1	Safety
Table 14.3.1.1.2	Summary of Treatment Emergent Adverse Events by System Organ Class and Preferred Term – Part 1	Safety
Table 14.3.1.1.3	Summary of Treatment Emergent Adverse Events by Relationship to Study Drug – Part 1	Safety
Table 14.3.1.1.4	Summary of Treatment Emergent Adverse Events by Severity – Part 1	Safety
Table 14.3.1.2.1	Summary of Adverse Events – Part 1 Extension	Safety
Table 14.3.1.2.2	Summary of Treatment Emergent Adverse Events by System Organ Class and Preferred Term – Part 1 Extension	Safety
Table 14.3.1.2.3	Summary of Treatment Emergent Adverse Events by Relationship to Study Drug – Part 1 Extension	Safety
Table 14.3.1.2.4	Summary of Treatment Emergent Adverse Events by Severity – Part 1 Extension	Safety
Table 14.3.1.3.1	Summary of Adverse Events – Part 2	Safety
Table 14.3.1.3.2	Summary of Treatment Emergent Adverse Events by System Organ Class and Preferred Term – Part 2	Safety
Table 14.3.1.3.3	Summary of Treatment Emergent Adverse Events by Relationship to Study Drug – Part 2	Safety
Table 14.3.1.3.4	Summary of Treatment Emergent Adverse Events by Severity – Part 2	Safety
Table 14.3.2	Listing of Deaths and Other Serious Adverse Events	All Subjects
Table 14.3.3	Not part of TFL – Reserved for Narratives in CSR	
Table 14.3.4	Listing of Abnormal Laboratory Values	All Subjects
Table 14.3.5.1	Summary of Laboratory Results – Part 1	Safety
Table 14.3.5.2	Summary of Laboratory Results – Part 1 Extension	Safety
Table 14.3.5.3	Summary of Laboratory Results – Part 2	Safety
Table 14.3.6.1	Summary of Vital Signs – Part 1	Safety
Table 14.3.6.2	Summary of Vital Signs – Part 1 Extension	Safety
Table 14.3.6.3	Summary of Vital Signs – Part 2	Safety
Table 14.3.7.1	Summary of 12-Lead Electrocardiogram Results – Part 1	Safety
Table 14.3.7.2	Summary of 12-Lead Electrocardiogram Results – Part 1 Extension	Safety
Table 14.3.7.3	Summary of 12-Lead Electrocardiogram Results – Part 2	Safety
Table 14.3.8.1	Summary of Noninvasive Blood Pressure and Oxygen Saturation – Part 1	Safety

Table 14.3.8.2	Summary of Noninvasive Blood Pressure and Oxygen Saturation – Part 1 Extension	Safety
Table 14.3.8.3	Summary of Noninvasive Blood Pressure and Oxygen Saturation – Part 2	Safety
Table 14.3.9.1	Summary of Nasal Cavity Examination – Part 1	Safety
Table 14.3.9.2	Summary of Nasal Cavity Examination – Part 1 Extension	Safety
Table 14.3.9.3	Summary of Nasal Cavity Examination – Part 2	Safety
Table 14.3.10.1	Summary of Mallampati Intubation Score – Part 1	Safety
Table 14.3.10.2	Summary of Mallampati Intubation Score – Part 1 Extension	Safety
Table 14.3.10.3	Summary of Mallampati Intubation Score – Part 2	Safety

List of End of Text Listings:	
Output	**Title**
Section 16.2.1 – Disposition	
Listing 16.2.1.1	Subject Disposition
Listing 16.2.1.2	Eligibility Criteria
Section 16.2.2 – Protocol Deviations	
Listing 16.2.2	Important Protocol Deviations
Section 16.2.3 – Excluded Subjects	
Listing 16.2.3	Analysis Sets
Section 16.2.4 – Demographics and Baseline Characteristics	
Listing 16.2.4.1	Subject Demographics
Listing 16.2.4.2	Medical History
Listing 16.2.4.3	Prior and Concomitant Medications
Listing 16.2.4.4	Telephone Contact
Listing 16.2.4.5	Naloxone Challenge Dosing
Listing 16.2.4.6	Clinical Opiate Withdrawal Scale Results
Section 16.2.5 – Compliance	
Listing 16.2.5.1	Study Drug Administration – Active Drug
Listing 16.2.5.2	Study Drug Administration – Remifentanil
Listing 16.2.5.3	Study Drug Administration – Pretreatment
Section 16.2.6 – Response Data	
Listing 16.2.6.1	ExSpiron® Device Measurements
Listing 16.2.6.2	ExSpiron® Device Parameters
Listing 16.2.6.3	VRH Measurements

Listing 16.2.6.4	Naloxone Plasma Concentrations
Listing 16.2.6.5	Nalmefene Plasma Concentrations
Listing 16.2.6.6	Naloxone Plasma Pharmacokinetic Parameters
Listing 16.2.6.7	Nalmefene Plasma Pharmacokinetic Parameters
Listing 16.2.6.8	Naloxone Plasma Pharmacokinetic Diagnostic Parameters
Listing 16.2.6.9	Nalmefene Plasma Pharmacokinetic Diagnostic Parameters
Section 16.2.7 – Adverse Events Data	
Listing 16.2.7.1	Adverse Events
Listing 16.2.7.2	Adverse Events Leading to Study Drug Discontinuation
Section 16.2.8 – Laboratory Data	
Listing 16.2.8.1	Clinical Laboratory Results – Chemistry
Listing 16.2.8.2	Clinical Laboratory Results – Hematology
Listing 16.2.8.3	Clinical Laboratory Results – Urinalysis
Listing 16.2.8.4	Clinical Laboratory Results – Additional Assessments
Listing 16.2.8.5	Clinical Laboratory Results – Pregnancy
Listing 16.2.8.6	Clinical Laboratory Results – Urine Drug and Alcohol Screen
Section 16.2.9 Onward – Other Safety Data	
Listing 16.2.9	Vital Signs
Listing 16.2.10	12-Lead Electrocardiogram Results
Listing 16.2.11	Physical Examinations
Listing 16.2.12	Columbia-Suicide Severity Rating Scale Results
Listing 16.2.13	Mallampati Intubation Score
Listing 16.2.14	Noninvasive Blood Pressure and Oxygen Saturation
Listing 16.2.15	Nasal Cavity Examination
Listing 16.2.16	Childbearing Potential
Listing 16.2.17	Smoking and Alcohol Habits
Listing 16.2.18	VRH Removal and Application Time
Listing 16.2.19	ExSpiron® Device Time
Listing 16.2.20	Cardiac Telemetry Time

Other Appendix Outputs:	
Output	**Title**
Appendix 16.1.9.2	Statistical Appendices

Appendix 6: Shells for Post-Text Tables, Figures and Listings

Shells are provided in a separate document.

20.0 Document History

Version Date	Modified/Reviewed By	Brief Summary of Changes (if created from a template, include template code)
08-Sep-2021	Cindy Wang Jacqueline Cater Nirmal Patel Ali Keshavarz Heather Jordan	Initially created.
12-Apr-2022	Cindy Wang Jacqueline Cater	Modified from final document dated 08-Sep-2021. See Section 4 for a description of the changes