Study Title: Clinical study to investigate the effect of the combination of psychotropic drugs and an opioid on ventilation

Document Title: Clinical Study Protocol – Study No. SCR-009

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CLINICAL STUDY PROTOCOL

Clinical study to investigate the effect of the combination of psychotropic drugs and an opioid on ventilation

PROTOCOL NO. SCR-009

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Version of Protocol:	8.0
Date of Protocol:	3 December 2020

CONFIDENTIAL

Sponsor Signature Page

This study will be conducted with the highest respect for the individual participants in accordance with the requirements of this clinical study protocol and also in accordance with the following:

- The ethical principles that have their origin in the Declaration of Helsinki;
- International Council for Harmonisation (ICH) harmonised tripartite guideline E6 (R2): Good Clinical Practice; and
- All applicable laws and regulations, including without limitation, data privacy laws and compliance with appropriate regulations, including human subject research requirements set forth by the Institutional Review Board (IRB).

Digitally signed by David Strauss -S
DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=David Strauss -S, 0.9.2342.19200300.100.1.1=2000507494
Date: 2020.12.16 17:25:31 -05'00'

David Strauss, MD, PhD
Director, Division of Applied Regulatory Science
U.S. Food and Drug Administration

Date

Investigator Signature Page

I confirm that I have read and that I understand this protocol, the investigator brochure, and other product information provided by the sponsor. I agree to conduct this study in accordance with the requirements of this protocol and also protect the rights, safety, privacy, and well-being of study subjects in accordance with the following:

- The ethical principles that have their origin in the Declaration of Helsinki;
- ICH harmonised tripartite guideline E6 (R2): Good Clinical Practice;
- All applicable laws and regulations, including without limitation data privacy laws and regulations;
- Human subject research requirements set forth by the IRB;
- Regulatory requirements for reporting of serious adverse events (SAEs) defined in Section 4.7.6.1 of this protocol; and
- Terms outlined in the Clinical Study Site Agreement.

I further authorize that my personal information may be processed and transferred in accordance with the uses contemplated in Section 6 of this protocol.

Carlos R. Sahabria, MD

Carlos Sahabria, MD
Principal Investigator

16 DEC 2020

Date

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Protocol Synopsis

Protocol Number:	SCR-009
Title:	Clinical study to investigate the effect of the combination of psychotropic drugs and an opioid on ventilation
Investigators:	Carlos Sanabria, MD
Study Phase:	1
Study Period:	This will be a single clinical study conducted in two parts for investigating the effects of study drugs. There will also be a screening period and a 4-day lead-in reproducibility assessment. The duration of Study Part 1 will be approximately 12 days, including check-in and check-out and four 1-day treatment periods with two washout days between each period. The duration of Study Part 2 will be approximately 31 days, including check-in and check-out and three 5-day treatment periods with 7 washout days between each period.
Study Site:	Spaulding Clinical Research Unit, West Bend, Wisconsin
Background and Motivation:	A well-known and potentially fatal adverse reaction associated with opioid administration, particularly in the scenario of misuse, abuse, or when coadministered with certain other drugs is that people 'stop breathing'. Research suggests this is caused by a reduced respiratory response to counteract increasing levels of systemic carbon dioxide (CO_2). Opioids contain a boxed warning for this adverse reaction (i.e., opioid-induced respiratory depression). In August 2016, FDA made safety labeling changes for benzodiazepines and opioids to include boxed warnings about increased potential for respiratory depression with their co-use. Following this action, concerns were raised that patients may be prescribed other sedative psychotropic drugs that may have similar effects when combined with opioids. In response to these concerns, FDA conducted non-clinical studies to fill gaps in knowledge. Fifteen drugs were evaluated and opioid (oxycodone)-induced respiratory depression was defined as increase in resting arterial partial pressure of carbon dioxide (pCO_2). At the dose studied, the benzodiazepine used (diazepam) did not increase pCO_2 on its own, however it did increase pCO_2 when combined with oxycodone compared to oxycodone alone, consistent with the new label warnings (Xu et al, 2020). Other study drugs, including paroxetine and quetiapine also increased pCO_2 when combined with oxycodone compared to oxycodone alone (manuscript in preparation). Existing clinical information was not adequate to define the impact of these two drugs (quetiapine and paroxetine) on respiration in patients. Consequently, FDA evaluated study designs that would allow a safe and controlled assessment of the effects of drug combinations on respiratory depression. A methodology was selected called the Read rebreathing procedure, where subjects re-breathe through a circuit with

increased level of O_2 and CO_2 (93% O_2 and 7% CO_2) (Read, 1967; Rebuck, 1976). The artificially increased levels of CO_2 trigger the subjects to increase ventilation. This "hypercapnic ventilatory response" can be decreased by opioids (Van der Schrier et al, 2017a; Van der Schrier et al, 2017b; Ladd et al, 2005; Bourke et al, 1989; Sarton et al, 1999; Rigg, 1978) and benzodiazepines (Forster et al, 1980; Power et al, 1983; Cohen et al, 1969; Geddes et al, 1976; Bailey et al, 1986), and potentially other drugs (Freye et al, 1985; Jarvis et al, 1992; Pavlin et al, 1996). Using this procedure, low doses of opioids or benzodiazepines can be administered that have minimal-to-no effects on respiration when subjects are going about normal activities breathing room air, however ventilation is decreased relative to the expected increase in ventilation as PCO_2 levels are artificially increased. Thus, there is minimal risk of true respiratory depression (i.e. inadequate gas exchange) and, if needed, the investigators can immediately halt the experiment (i.e., 'open' the circuit) to breathe room air or 100% O_2.

Using the Read rebreathing methodology, FDA designed the current clinical study to generate data characterizing changes in the ventilatory response to hypercapnia when administering quetiapine and paroxetine alone or in combination with oxycodone. This information will inform on the potential respiratory depression risk of combining quetiapine or paroxetine with opioids. In addition, findings from this study may demonstrate the utility of this study design and methodology as an approach for evaluating the effect of an investigational drug and drug combinations on ventilatory depression for investigational drug products. Thus, this study includes a lead-in reproducibility phase to carefully quantify the intra- and inter-subject variability within a day and between days. In addition, this study includes a positive control phase (Part 1) with relatively low doses of opioid alone, benzodiazepine alone and their combination to help assess assay sensitivity. While opioids and benzodiazepines have been studied alone and in combination with the rebreathing method previously (Ladd et al, 2005; Forster et al, 1980; Power et al, 1983; Cohen et al, 1969), many of these studies are older and it is important to define the reproducibility and effects of the drugs at relatively small doses. Together, these components of the study will help define the reproducibility and sensitivity of the methodology that could be applied to a broader range of investigational drugs in the future to assess their safety when combined with opioids.

In addition, pupillary measurements have been used to study the pharmacodynamic effects of opioids and opioid antagonists (Skulberg et al, 2018; Rollins et al, 2014) and there has been interest in expanding its use in clinical studies. However, there are limited data directly comparing pupillary changes to ventilatory changes and some drugs may influence the pupillary response to opioids (Kummer et al, 2011). As an exploratory endpoint, this study will obtain quantitative

pupillometry measurements before and after each rebreathing assessment to allow for comparisons of pupillary changes to ventilatory changes when subjects receive different drugs and drug combinations.

In summary, this study will 1) define the intra- and inter-subject variability within a day and between days of the Read rebreathing methodology, 2) assess whether relatively low doses of an opioid (oxycodone) and benzodiazepine (midazolam) decrease the ventilatory response to hypercapnia compared to an opioid alone to help determine assay sensitivity, and 3) assess whether two sedative psychotropic drugs (quetiapine and paroxetine), selected due to their effects in a nonclinical model, decrease the ventilatory response to hypercapnia compared to an opioid alone. The doses of oxycodone and midazolam were selected to be less than those previously administered in healthy volunteers undergoing the Read Rebreathing or similar respiratory circuit procedures that increase the inspired level of CO_2 (Forster et al, 1980; Power et al, 1983; van der Schrier et al, 2017a; van der Schrier et al, 2017b). The doses of quetiapine and paroxetine were selected to reach clinical steady-state levels.

Objectives and Endpoints:

Primary Objective(s)

The primary objective is to study whether combining psychotropic drugs (paroxetine, quetiapine and midazolam) with an opioid (oxycodone) decreases the ventilatory response to hypercapnia compared to an opioid alone.

Secondary Objective(s)

Secondary objectives include the following:

- To study whether each psychotropic drug (paroxetine, quetiapine, or midazolam) affects the pharmacokinetics of oxycodone.
- To study whether each drug (oxycodone, paroxetine, quetiapine, or midazolam) decreases the ventilatory response to hypercapnia on its own.

Exploratory Objective(s)

- To study whether there is a direct pharmacodynamic interaction between each psychotropic drug and oxycodone.
- To summarize additional pharmacokinetic parameters and pharmacodynamic measurements collected during the study.

Primary Endpoint

The primary endpoint is a comparison of the minute ventilation at the 55 mm Hg end tidal CO_2 point (VE55) of each psychotropic drug (paroxetine, quetiapine, or midazolam) combined with oxycodone vs. oxycodone alone. Part 2 will include a comparison at both day 1 and day 5 of treatment (multiple primary endpoints).

Secondary Endpoints

- VE55 of each psychotropic drug (oxycodone, paroxetine, quetiapine, or midazolam) on its own compared to placebo.
- The maximum observed plasma concentration (C_{max}) and area under the plasma concentration-time curve (AUC) of oxycodone alone vs. oxycodone in combination with a psychotropic drug (paroxetine or quetiapine or midazolam).

Exploratory Endpoints

- The PK/PD relationship for study drugs when administered alone vs. in combination.
- Additional pharmacokinetic and pharmacodynamic endpoints as specified in the protocol.

Study Design: The study will be divided into a lead-in reproducibility assessment phase and two main parts: Part 1 and Part 2.

Lead-in Reproducibility Assessment

The lead-in reproducibility assessment will have up to 10 healthy volunteer participants. Up to five participants will be enrolled, and data analysis will be performed. If reproducibility is acceptable after the first analysis, additional lead-in cohorts may not be needed. If any study procedure element changes are needed, they will be made at this point. Then, additional participants may be enrolled, and data will be analyzed.

For the reproducibility assessment, participants will check-in on Day -1, have PD assessments on Day 1 and Day 2 and check-out on Day 3. PD assessments will be performed on Day 1 and 2 at 0, 2, 3, 5, and 6 h. Participants will not be administered any drugs during the lead-in phase.

Study Part 1

The study Part 1 will assess the primary and secondary endpoints for oxycodone and midazolam and will inform oxycodone dose selection

for study Part 2. The study will be a 4-period randomized crossover study with up to 20 healthy volunteer participants with the following design:

Day 1	Days 2-3	Day 4	Days 5-6	Day 7	Days 8-9	Day 10
Period 1	Washout	Period 2	Washout	Period 3	Washout	Period 4

The following 4 treatments will be evaluated in a randomized order over the 4 study periods.

Treatment	Day 1	Day 2-3
A	Oxycodone + placebo IV	Wash-out
B	Oral placebo + midazolam IV	Wash-out
C	Oxycodone + midazolam IV	Wash-out
D	Oral placebo + placebo IV	Wash-out

Participants will enter the clinic on a staggered basis in cohorts of approximately five (i.e., only approximately five participants will undergo dosing, PK assessments, and PD assessments on any given day). Subjects will be staggered up to 15 minutes apart in order to allow for direct safety overview by medical staff during dosing and Read rebreathing.

Participants will check-in on Day -1 and receive dosing for the four respective periods on Days 1, 4, 7, and 10. There will be two days of washout between period. Participants will be confined in the study clinic from Day -1 until the morning of Day 11. On dosing days, the dosing, PK, and PD assessments will occur at the following time points:

- Dosing times: 0 h oxycodone, 115 min midazolam
- PK assessment: 0 (pre-dose), 1, 2, 3, 4, 6, 8, 12, 24 h
- PD assessment: 0 (pre-dose), 1, 2, 3, 4, 6, 24 h

The total number of participants to be enrolled is approximately 20. Interim analyses will be performed after approximately 5 participants and the doses of oxycodone and/or midazolam may be adjusted at that time. The oxycodone dose may be increased if the conditional power of concluding a decrease in oxycodone from placebo is less than 90%. Likewise, midazolam dose may be increased from 0.0375 mg/kg to 0.075 mg/kg if the conditional power of concluding a decrease of the combined effect of oxycodone and midazolam compared to oxycodone is less than 80%. The decision to dose escalate either drug will also consider clinical evaluations and whether stopping criteria was attained

in any of the participants. The remaining subjects will complete the study at the updated doses in staggered cohorts.

The goal with the oxycodone dose is to have a definable effect with oxycodone alone, but not too high (for safety considerations) as it will be combined with a benzodiazepine or other psychotropic drug. The goal with midazolam is to have a safe dose that decreases ventilatory response to hypercapnia when co-administered with oxycodone compared to oxycodone alone.

The primary analysis will occur at the 2 h timepoint. If dose adjustments are not needed, the analysis will be based on all subjects. Otherwise, the analysis will be limited to those subjects administered updated doses of oxycodone and/or midazolam.

Study Part 2

The study Part 2 will assess the primary and secondary endpoints for paroxetine and quetiapine with reference to oxycodone. The study will be a 3-period randomized crossover study in approximately 20 healthy volunteer participants. Each treatment period will have 5 days of dosing followed by 7 days of washout between periods. Participants will enter the clinic on a staggered basis in cohorts of approximately five (i.e., approximately five participants will undergo dosing, PK assessments, and PD assessments on any given day). Subjects will be staggered up to 15 minutes apart in order to allow for direct safety overview by medical staff during dosing and Read rebreathing. Participants will check-in on Day -1 and checkout on the morning of Day 6 for each period.

Days 1-5	Days 6-12	Days 13-17	Days 18-24	Day 25-29
Period 1	Washout	Period 2	Washout	Period 3

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

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5
E	Oxycodone + Placebo	Placebo	Placebo	Placebo	Oxycodone + Placebo
F	Paroxetine + Oxycodone	Paroxetine	Paroxetine	Paroxetine	Paroxetine + Oxycodone
G	Quetiapine + Oxycodone	Quetiapine	Quetiapine	Quetiapine	Quetiapine + Oxycodone

Primary assessments for the two psychotropic drugs (paroxetine or quetiapine) combined with oxycodone vs. oxycodone alone will occur on Days 1 and 5 (multiple primary endpoints). The comparisons for paroxetine and quetiapine vs. placebo will occur on Day 4.

Dosing of the 3 drugs will be staggered so that T_{max} occurs at 5 h for all drugs and the 5-h time point will be used for primary analyses. PD and PK assessments on Days 1, 4, and 5 of each treatment period will occur at the following time points. Paroxetine (or placebo) doses will be administered at time 0 on each day. The first daily dose of quetiapine (or placebo) and oxycodone dosing will occur at 3 h on dosing days; the second daily dose of quetiapine (or placebo) will be administered at approximately 14 h:

- PK assessment: 0 (pre-dose), 3, 4, 5, 6, 8, 9, 12, 24 h
- PD assessment:
  - Day 1 and 5 of each period: 0 (pre-dose), 4, **5**, 6, 8, 24 h
  - Day 4 of each period: 0 (pre-dose), 4, **5**, 6, 24* h

*Note: The 24h assessment on Day 4 and 0h (pre-dose) assessment on Day 5 will be the same assessment.

Subject Population:

Approximately 50 healthy subjects are planned for enrollment, of which up to 10 will be assigned to the Lead-in Reproducibility Assessment, approximately 20 will be assigned to Part 1, and approximately 20 will be assigned to Part 2. Up to 10 subjects may be qualified as replacements. Thus, a maximum of 60 subjects will be exposed to study drugs and procedures during the study. Every effort will be made to maintain an approximate 50:50 male to female sex distribution.

Recruitment materials (e.g., internet, radio, and print advertisements, social media posts) will be approved by the local Institutional Review Board (IRB, i.e., Advarra) before telephone screening. Subjects will be offered payment for Screening and participation in the study, but no special incentives are offered.

Study Drugs, Dosage, and Route of Administration:

In Part 1, oxycodone will be administered orally, and midazolam will be administered intravenously (IV). Subjects will be administered only a single-dose of each agent in each treatment period. In Part 2, all study drugs will be administered orally. Subjects in all treatment periods will receive multiple doses of oxycodone. Subjects will receive quetiapine, paroxetine or placebo for 5 days in each treatment period.

Part 1

On day 1 of each period, subjects receive one of the following treatments:

- Oral oxycodone immediate release (IR) tablets 10 mg and placebo IV
- Oral placebo and 0.0375 mg/kg midazolam IV
- Oral oxycodone IR tablets 10 mg and 0.0375 mg/kg midazolam IV

- Oral placebo and placebo IV

Oxycodone will be administered as 2 X 5 mg immediate release (IR) tablets at 0 h. Midazolam will be infused over 2-minutes starting at 115 minutes. All treatments will include matching oral or IV placebo, as needed. Participants will enter the clinic on a staggered basis in cohorts of approximately five (i.e., only approximately five participants will undergo dosing, PK assessments, and PD assessments on any given day). Subjects will be staggered up to 15 minutes apart in order to allow for direct safety overview by medical staff during dosing and Read rebreathing. Participants will also receive ondansetron 4 mg orally 30 min before oxycodone administration.

Based on assessment of approximately 5 subjects, either oxycodone or midazolam may be dose increased. If the oxycodone dose is increased, all subsequent subjects will be administered oxycodone as 3 X 5 mg (15 mg) immediate release tablets at 0 h. If the midazolam dose is increased, all subsequent subjects will be administered midazolam at 0.075 mg/kg as a 2-min infusion starting at 115 min.

Part 2

For Part 2, subjects will complete each of the 3 different treatments (above, E-G) and will receive the following drugs alone or in combination:

- Oxycodone (2-3 X 5mg tablets depending on findings from Part 1)
- Paroxetine 40 mg tablets on days 1, 2, 3, 4, and 5
- Quetiapine 50 mg tablets twice daily (BID) on day 1, followed by 100 mg (2 X 50 mg) BID on day 2, 150 mg BID (3 X 50 mg) on day 3, 200 mg (4 x 50 mg) BID on day 4 and 200 mg (4 x 50 mg) QD on day 5.
- Matching oral placebo for paroxetine and quetiapine for all periods

Additional details regarding study drug administration are as follows:

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5
E	0 h: Placebo	0 h: Placebo	0 h: Placebo	0 h: Placebo	0 h: Placebo
	3 h: Oxycodone, Placebo	3 h: Placebo (x2)	3 h: Placebo (x3)	3 h: Placebo (x4)	3 h: Oxycodone, Placebo (x4)
	14 h: Placebo	14 h: Placebo (x2)	14 h: Placebo (x3)	14 h: Placebo (x4)	--
F	0 h: Paroxetine	0 h: Paroxetine	0 h: Paroxetine	0 hr: Paroxetine	0 h: Paroxetine
	3 h: Oxycodone, Placebo	3 h: Placebo (x2)	3 h: Placebo (x3)	3 h: Placebo (x4)	3 h: Oxycodone, Placebo (x4)
	14 h: Placebo	14 h: Placebo (x2)	14 h: Placebo (x3)	14 h: Placebo (x4)	--

G	0 h: Placebo	0 h: Placebo	0 h: Placebo	0 h: Placebo	0 h: Placebo
	3 h: Quetiapine, Oxycodone	3 h: Quetiapine (x2)	3 h: Quetiapine (x3)	3 h: Quetiapine (x4)	3 h: Quetiapine (x4), Oxycodone
	14 h: Quetiapine	14 h: Quetiapine (x2)	14 h: Quetiapine (x3)	14 h: Quetiapine (x4)	--

Participants will also receive ondansetron 4 mg orally 30 min before oxycodone administration.

Reference Drug, Dosage, and Route of Administration:

Part 1 Reference Drug

- Oral placebo and placebo IV

Part 2 Reference Drug

- Matching oral placebo for paroxetine and quetiapine for all periods

Inclusion Criteria: Subjects who meet all the following inclusion criteria will be eligible to participate in the study:

1. Subject signs an IRB approved written informed consent and privacy language as per national regulations (e.g., Health Insurance Portability and Accountability Act authorization) before any study related procedures are performed.
2. Subject is a healthy man or woman, 18 to 50 years of age, inclusive, who has a body mass index (BMI) of 18.5 to 29.9 kg/m2, inclusive, at Screening.
3. Subject has normal medical history findings, clinical laboratory results, vital sign measurements, 12 lead electrocardiogram (ECG) results, and physical examination findings at Screening or, if abnormal, the abnormality is not considered clinically significant (as determined and documented by the investigator or designee).
4. Subject must have a negative test result for alcohol and drugs of abuse at Screening and Check-in (Day -1).
5. Subject has no known or suspected allergies or sensitivities to any of the study drugs.
6. Female subjects must be of non-childbearing potential or, if they are of childbearing potential, they must: 1) have been strictly abstinent for 1 month before Check in (Day -1) and agree to remain strictly abstinent for the duration of the study and for at least 1 month after the last application of study drug; OR 2) be practicing 2 highly effective methods of birth control (as determined by the investigator or designee; one of the methods must be a barrier technique) from at least 1 month before Check in (Day -1) until at least 1 month after the last application of study drug.
7. Male subjects must agree to practice 2 highly effective methods of birth control (as determined by the investigator or designee;

one of the methods must be a barrier technique) from at least 1 month before Check in (Day -1) until at least 1 month after the last application of study drug.

8. Subject is highly likely (as determined by the investigator) to comply with the protocol defined procedures and to complete the study

Exclusion Criteria:

Subjects who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Subject has history of opioid or psychotropic drug use within 60 days of the start of the study.
2. Subject has non-reactive or misshapen pupil(s) or damaged orbit structure or surrounding soft tissue is edematous or has an open lesion.
3. Subject has a Mallampati intubation score of >2 (for Part 1 and 2 only).
4. Subject Read Rebreathing data is of poor quality or subject does not agree to remain clean-shaven for all days when the Read Rebreathing procedure is being performed.
5. Subject has used any prescription or nonprescription drugs (including aspirin or [non-steroidal anti-inflammatory drugs] NSAIDs and excluding oral contraceptives and acetaminophen) within 14 days or 5 half-lives (whichever is longer) or complementary and alternative medicines within 28 days before the first dose of study drug. This includes prescription or nonprescription ophthalmic drugs.
6. Subjects are currently participating in another clinical study of an investigational drug or are have been treated with any investigational drug within 30 days or 5 half-lives (whichever is longer) of the compound.
7. Subject has used nicotine-containing products (e.g., cigarettes, cigars, chewing tobacco, snuff) within 6 weeks of Screening.
8. Subject has consumed alcohol, xanthine containing products (e.g., tea, coffee, chocolate, cola), caffeine, grapefruit, or grapefruit juice within 48 h of dosing. Subjects must refrain from ingesting these throughout the study.
9. Subject has a history of sleep disorders, Panic Disorder, Panic Attacks, Generalized Anxiety Disorder, or any associated DSM diagnosis or condition.
10. Subject has any underlying disease or surgical or medical condition (e.g., cancer, human immunodeficiency virus [HIV], severe hepatic or renal impairment) that could put the subject at risk or would normally prevent participation in a clinical study. This includes subjects with any underlying medical conditions that the Investigator believes would put subjects at increased risk of severe illness from COVID-19 based on the Centers for

Disease Control and Prevention (CDC) guidelines. The CDC lists cancer, chronic kidney disease, chronic obstructive pulmonary disease, immunocompromised state from solid organ transplant, severe obesity, serious heart conditions, sickle cell disease, pregnancy, smoking and type 2 diabetes mellitus as conditions that put subjects at increased risk. Additionally, the CDC lists asthma (moderate-to-severe), cerebrovascular disease, cystic fibrosis, hypertension, immunocompromised state or immune deficiencies, neurologic conditions such as dementia, liver disease, pulmonary fibrosis, thalassemia, overweight, type 1 diabetes mellitus as conditions that might put subjects at increased risk.

11. Subject has any signs or symptoms that are consistent with COVID-19 per CDC recommendations. These include subjects with fever or chills, cough, shortness of breath or difficulty breathing, fatigue, muscle or body aches, headache, new loss of taste or smell, sore throat, congestion or runny nose, nausea or vomiting, or diarrhea may have COVID-19. In addition, the subject has any other findings suggestive of COVID-19 risk in the opinion of the investigator.
12. Subject tests positive for severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) by a molecular diagnostic test performed prior to admission.
13. Female subject is pregnant or lactating before enrollment in the study.
14. Subject has known or suspected allergies or sensitivities to any study drug.
15. Subject has clinical laboratory test results (hematology, serum chemistry) at Screening that are outside the reference ranges provided by the clinical laboratory and considered clinically significant by the investigator.
16. Subject has a positive test result at Screening for HIV 1 or 2 antibody, hepatitis C virus antibodies, or hepatitis B surface antigen.
17. Subject is unable or unwilling to undergo multiple venipunctures for blood sample collection because of poor tolerability or poor venous access.
18. Subject has a history of or currently has hypoventilation syndrome or sleep apnea and is on non-invasive ventilation.

Pharmacokinetic Assessments:

The PK blood samples (5 mL each) will be collected into tubes containing K2EDTA, inverted several times to mix the blood with the anticoagulant, and placed in an ice bath. Within 30 minutes of collection, the samples will be centrifuged for 10 minutes, at 3000 revolutions per minute, at 4°C, by a study team member.

The plasma will be separated using a disposable plastic pipette and equally aliquoted into duplicate cryotube vials labeled as Aliquot A (primary) and Aliquot B (backup). The plasma samples will be appropriately labeled and stored frozen at –70°C or below until shipment. Temperature monitoring logs should be maintained and accessible for review by the study monitor.

The Aliquot A samples (primary) will be shipped first, on dry ice, to the bioanalytical laboratory at FDA for processing when requested by the sponsor. The Aliquot B samples (backup) will be held for a second shipment at a time after the completion of all Aliquot A sample shipment(s) and the timing of the Aliquot B shipment communicated by the sponsor. None of the PK blood samples will be stored at clinical facility for future use, however the sponsor will store them for analytical purposes of this study only."

For each participant, the following oxycodone PK parameters will be determined as part of the secondary endpoints for the study:

- Maximum concentration (C_{max}) on the specified day
- AUC from time of drug administration to last sample of that day on day 1 for Part 1 and on days 1 and 5 for Part 2

AUC and C_{max} will be calculated for midazolam (Part 1, day 1) and quetiapine and paroxetine (Part 2, day 1, 4, and 5) as exploratory PK parameters. Additionally, the following exploratory PK parameters will be determined for oxycodone, midazolam, quetiapine, paroxetine, and metabolites on each specified day:

- Time at which C_{max} occurs (T_{max})
- Elimination rate constant (K_{el})
- Terminal half-life ($t_{1/2}$)

Pharmacodynamic Assessments:

Primary PD Biomarkers:

The primary PD biomarker is the minute ventilation (MV, L/min) at the 55 mm Hg $P_{ET}CO_2$ point on the MV / $P_{ET}CO2_{ET}$ regression line (VE55) of each psychotropic drug combined with oxycodone vs. oxycodone alone. The VE55 will be measured at the timepoints specified in the Study Design section.

Exploratory PD Biomarkers:

- Respiratory biomarkers:

- Slope of the MV / $P_{ET}CO_{2ET}$ regression line
- Number of apneic events lasting > 10 s
- Resting ventilation
- Resting tidal volume
- Resting respiratory rate
- Resting end-tidal P_{CO2}
- Resting oxygen saturation

- Pupillary biomarkers
  - Maximum pupil diameter before constriction
  - Dynamic pupillary measurements after a light stimulus
    - Minimum diameter at peak constriction
    - Percent change between min/max diameter
    - Latency of constriction
    - Average constriction velocity
    - Maximum constriction velocity
    - Dilation velocity after peak constriction
    - Time to reach 75% recovery of maximum diameter
- Sedation scores
  - Ramsey Sedation Scale
  - Visual Analogue Scale

Safety Assessments:

Safety will be evaluated in terms of adverse events (AE), vital sign measurements (blood pressure, heart rate, respiratory rate, oral body temperature, pulse oximetry), physical examination, and laboratory findings.

Other Assessments

Buffy coat samples will be collected and processed for genomic analyses. Maintenance of privacy of the genetic information of study subjects is a priority. All DNA samples will be de-identified. DNA may be stored and used for analyses for up to 10 years.

Sample Size and Threshold Determination:

Approximately 50 healthy participants are planned for enrollment. Up to ten healthy participants are planned for enrollment for the Lead-In Reproducibility Assessment. Approximately 20 healthy participants will be enrolled for the study Part 1 and approximately 20 healthy subjects for the study Part 2. The planned number of subjects allows for a 10% drop out rate during Part 1 and a 20% drop out rate during Part 2.

Part 1 is powered with consideration to both the effect of oxycodone versus placebo and oxycodone plus midazolam versus oxycodone. Assuming a -4 L/min effect size on VE55 and standard deviation of 5

L/min, there is greater than 90% power at a one-sided significance level. In the event that dose escalation of either oxycodone or midazolam are needed, the study has greater than 80% power at a one-sided significant level assuming these same effect sizes at the higher dose or doses. Part 1 is not designed to terminate early based on the interim analysis.

Part 2 is powered with consideration to the effect of oxycodone plus concomitant medication (quetiapine or paroxetine) at day 1 and day 5. Assuming a -4 L/min effect size on VE55 and standard deviation of 5 L/min, there is greater than 90% power at a one-side significance level. Both days will be considered as separate tests with an adjustment for multiplicity.

Statistical Methods:

For each subject, for each CO_2 re-breathing test, the regression line for the linear region of the plot of minute ventilation versus CO_2 concentration will be determined. See endpoints section for preliminary analysis plan.

- **Safety:** The safety population will include all subjects who receive at least 1 dose of any of the study drugs. Any AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). The incidence of AEs, organized by system organ class and preferred term, will be summarized with a focus on treatment-emergent AEs. Vital sign measurements will be summarized using descriptive statistics by time point. All values will be evaluated for clinically notable results. Data for additional safety parameters (e.g., physical examination findings) will be listed.

Date of Protocol: 3 December 2020

1. List of Abbreviations

Abbreviation	Definition
AE	adverse event
Ag/Ab	antigen/antibody
AUC	area under the plasma concentration-time curve
BID	twice daily
CFR	Code of Federal Regulations
C_{max}	maximum observed plasma concentration
COVID-19	coronavirus disease of 2019
CV	coefficient of variation
CYP2D6	cytochrome 2D6
CYP34A	cytochrome 34A
ECG	electrocardiogram
eCRF	electronic case report form
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HBsAg	hepatitis B surface antigen
HCl	hydrochloride
HepC	hepatitis C
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human immunodeficiency virus
ICH	International Council for Harmonisation
IR	immediate release
IRB	Institutional Review Board
IV	intravenous
NSAID	nonsteroidal anti-inflammatory drug
PBMC	peripheral blood mononuclear cell
PD	pharmacodynamic
PK	pharmacokinetic
QA	quality assurance
SAE	serious adverse event
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SD	standard deviation
TEAE	treatment-emergent adverse event
T_{max}	time of C_{max}

2. Introduction

2.1. General Background

A well-known and potentially fatal adverse reaction associated with opioid administration, particularly in the scenario of misuse, abuse, or when coadministered with certain other drugs is that people 'stop breathing'. Research suggests this is caused by a reduced respiratory response to counteract increasing levels of systemic carbon dioxide (CO_2). Opioids contain a boxed warning for this adverse reaction (i.e., opioid-induced respiratory depression). In August 2016, the FDA made safety labeling changes for benzodiazepines and opioids to include boxed warnings about increased potential for respiratory depression with their co-use. Following this action, concerns were raised that patients may be prescribed other sedative psychotropic drugs that may have similar effects when combined with opioids. In response to these concerns, FDA conducted non-clinical studies to fill gaps in knowledge. Fifteen drugs were evaluated and opioid (oxycodone)-induced respiratory depression was defined as increase in resting arterial partial pressure of carbon dioxide (pCO_2). At the dose studied, the benzodiazepine used (diazepam) did not increase pCO_2 on its own, however it did increase pCO_2 when combined with oxycodone compared to oxycodone alone, consistent with the new label warnings (Xu et al, 2020). Other study drugs, including paroxetine and quetiapine also increased pCO_2 when combined with oxycodone compared to oxycodone alone (manuscript in preparation).

Existing clinical information was not adequate to define the impact of these two drugs (quetiapine and paroxetine) on respiration in patients. Consequently, FDA evaluated study designs that would allow a safe and controlled assessment of the effects of drug combinations on respiratory depression. A methodology was selected called the Read rebreathing procedure, where subjects re-breathe through a circuit with increased level of O_2 and CO_2 (93% O_2 and 7% CO_2) (Read, 1967; Rebuck 1976). The artificially increased levels of CO_2 trigger the subjects to increase ventilation. This "hypercapnic ventilatory response" can be decreased by opioids (Van der Schrier et al, 2017a; Van der Schrier et al, 2017b; Ladd et al, 2005; Bourke et al, 1989; Sarton et al, 1999; Rigg, 1978) and benzodiazepines (Forster et al, 1980; Power et al, 1983; Cohen et al, 1969; Geddes et al, 1976; Bailey et al, 1986), and potentially other drugs (Freye et al, 1985; Jarvis et al, 1992; Pavlin et al, 1996). Using this procedure, low doses of opioids or benzodiazepines can be administered that have minimal-to-no effects on respiration when subjects are going about normal activities breathing room air, however ventilation is decreased relative to the expected increase in ventilation as PCO_2 levels are artificially increased. Thus, there is minimal risk of true respiratory depression (i.e. inadequate gas exchange) and, if needed, the investigators can immediately halt the experiment (i.e., 'open' the circuit) to breathe room air or 100% O_2.

Using the Read rebreathing methodology, FDA designed the current clinical study to generate data characterizing changes in the ventilatory response to hypercapnia when administering quetiapine and paroxetine alone or in combination with oxycodone. This information will inform on the potential respiratory depression risk of combining quetiapine or paroxetine with opioids. In addition, findings from this study may demonstrate the utility of this study design and methodology as an approach for evaluating the effect of an investigational drug and drug combinations on ventilatory depression for investigational drug products.

This study includes a lead-in reproducibility phase to carefully quantify the intra- and inter-subject variability within a day and between days and includes a positive control with relatively low doses of opioid alone, benzodiazepine alone and their combination to help assess assay sensitivity. While opioids and benzodiazepines have been studied alone and in combination with the rebreathing method previously (Ladd et al, 2005; Forster et al, 1980; Power et al, 1983; Cohen et al, 1969), many of these studies are older and it is important to define the reproducibility and effects of the drugs at relatively small doses. Together, these components of the study will help define the reproducibility and sensitivity of the methodology that could be applied to a broader range of investigational drugs in the future to assess their safety when combined with opioids.

In addition, pupil diameter measurements have been used to study the pharmacodynamic effects of opioids and opioid antagonists (Skulberg et al, 2018; Rollins et al, 2014) and there has been interest in using pupil diameter as a biomarker for opioid agonist/antagonist effects in clinical studies. However, there are limited data directly comparing pupillary changes to ventilatory changes and some drugs may influence the pupillary response to opioids (Kummer et al, 2011). As an exploratory endpoint, this study will obtain quantitative pupillometry measurements just before and after each rebreathing assessment to allow for comparisons of pupillary changes to ventilatory changes when subjects receive different drugs and drug combinations.

In summary, this study will 1) define the intra- and inter-subject variability within a day and between days of the Read rebreathing methodology, 2) study whether relatively low doses of an opioid (oxycodone) and benzodiazepine (midazolam) decrease the ventilatory response to hypercapnia compared to an opioid alone to help determine assay sensitivity, and 3) assess whether two sedative psychotropic drugs (quetiapine and paroxetine), selected due to their effects in a nonclinical model, decrease the ventilatory response to hypercapnia compared to an opioid alone. The doses of oxycodone and midazolam were selected to be less than those previously administered in healthy volunteers undergoing the Read Rebreathing or similar respiratory circuit procedures that increase the inspired level of CO_2 (Forster et al, 1980; Power et al, 1983; van der Schrier et al, 2017a; van der Schrier et al, 2017b). The doses of quetiapine and paroxetine were selected to reach clinical steady-state levels. The study will proceed in three stages as described below.

2.2. Lead-in Reproducibility Assessment

The objective of the lead-in reproducibility assessment is to determine the variability associated with the rebreathing method used to evaluate the ventilatory response to hypercapnia. Up to ten healthy volunteers will be enrolled. Initially data will be collected from up to 5 subjects with rebreathing assessments and pupillometry assessments before and after each rebreathing assessment. If reproducibility is acceptable after the first analysis, additional lead-in cohorts may not be needed. If procedural changes are necessary to reduce variability they will be implemented at this point. Subsequently, additional participants may be enrolled, and data analyzed. Participants will not be administered any drugs during the lead-in phase.

2.3. Part 1: Oxycodone and Midazolam

The objective of Part 1 is to assess the primary and secondary endpoints for oxycodone and midazolam. This study will be a 4-period randomized crossover study with approximately 20 healthy volunteers. Part 1 will also inform dose selection for Part 2. Interim analyses will be performed after approximately 5 participants complete Part 1 and if necessary, doses may be adjusted for the remaining participants.

Primary Endpoint

The primary endpoint is a comparison of minute ventilation at the 55 mm Hg end tidal CO2 point (VE55) of midazolam combined with oxycodone vs. oxycodone alone. Data will be analyzed in R using nonlinear regression of the minute ventilation versus $P_{ET}CO_2$ data and used to estimate VE55.

Secondary Endpoints

- A secondary endpoint is VE55 of oxycodone or midazolam on its own compared to placebo.
- A secondary endpoint is the C_{max} and AUC of oxycodone alone vs. oxycodone in combination with midazolam.
- A secondary endpoint is the PK and PD relationship between exposure of study drugs (oxycodone and midazolam) alone or in combination and minute ventilation.

Exploratory Endpoints

Midazolam C_{max} (on the specified day) and AUC (from time of drug administration to last sample) will be calculated as an exploratory parameter. In addition, the following exploratory PK parameters will be determined for oxycodone, midazolam, and metabolites over each treatment period:

- Time at which C_{max} occurs (T_{max})
- Elimination rate constant (K_{el})

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

Additionally, the following exploratory PD markers may be evaluated:

- Respiratory biomarkers
  - Slope of the MV / $P_{ET}CO_2$ regression line
  - Number of apneic events lasting > 10 s
  - Resting ventilation
  - Resting tidal volume
  - Resting respiratory rate
  - Resting end-tidal P_{CO2}
  - Resting oxygen saturation
- Pupillary biomarkers
  - Maximum pupil diameter before constriction
  - Dynamic pupillary measurements after a light stimulus
    - Minimum diameter at peak constriction
    - Percent change between min/max diameter
    - Latency of constriction
    - Average constriction velocity
    - Maximum constriction velocity
    - Dilation velocity after peak constriction
    - Time to reach 75% recovery of maximum diameter
- Sedation scores
  - Ramsey Sedation Scale
  - Visual Analogue Scale

An exploratory endpoint is the PK/PD relationship for study drugs when administered alone vs. in combination.

2.4. Part 2: Coadministration of Oxycodone with Paroxetine and Quetiapine

The objective of Part 2 is to assess the primary and secondary endpoints for paroxetine and quetiapine with reference to oxycodone. This study will be a 3-period randomized crossover study in approximately 20 healthy volunteers.

Primary Endpoint

The primary endpoint is a comparison of the minute ventilation at the VE55 of each psychotropic drug (paroxetine or quetiapine) combined with oxycodone vs. oxycodone alone. Part 2 will include a comparison at both day 1 and day 5 of treatment (multiple primary endpoints). Data will be analyzed in R using nonlinear regression of the minute ventilation versus $P_{ET}CO_2$ data and used to estimate VE55.

Secondary Endpoints

- A secondary endpoint is VE55 of oxycodone, paroxetine, or quetiapine on its own compared to placebo.
- A secondary endpoint is the C_{max} and AUC of oxycodone alone or in combination with a psychotropic drug (paroxetine or quetiapine) on days 1 and 5

Exploratory Endpoints

The following exploratory PK parameters will be determined for paroxetine and quetiapine on days 1, 4 and 5:

- C_{max} (on the specified day)
- AUC (from time of drug administration to last sample of that day)

The following exploratory PK parameters will be determined for paroxetine, quetiapine, and metabolites on days 1, 4 and 5 and for oxycodone and metabolites on days 1 and 5:

- Time at which C_{max} occurs (T_{max})
- Elimination rate constant (K_{el})
- Terminal half-life ($t_{1/2}$)

Additionally, the following exploratory PD markers may be evaluated:

- Respiratory biomarkers
    - Slope of the MV / $P_{ET}CO_2$ regression line
    - Number of apneic events lasting > 10 s
    - Resting ventilation
    - Resting tidal volume
    - Resting respiratory rate
    - Resting end-tidal P_{CO2}
    - Resting oxygen saturation
- Pupillary biomarkers
    - Maximum pupil diameter before constriction
    - Dynamic pupillary measurements after a light stimulus
        - Minimum diameter at peak constriction
        - Percent change between min/max diameter
        - Latency of constriction
        - Average constriction velocity
        - Maximum constriction velocity
        - Dilation velocity after peak constriction
        - Time to reach 75% recovery of maximum diameter
- Sedation scores
    - Ramsey Sedation Scale

- Visual analogue scale

An exploratory endpoint is the PK/PD relationship for study drugs when administered alone vs. in combination.

3. Study Objectives

3.1. Primary Objectives

The primary objective is to study whether combining psychotropic drugs (paroxetine, quetiapine and midazolam) with an opioid (oxycodone) decreases the ventilatory response to hypercapnia compared to an opioid alone.

3.2. Secondary Objective(s)

Secondary objectives include the following:

- To study whether each psychotropic drug (paroxetine, quetiapine, or midazolam) affects the pharmacokinetics of oxycodone.
- To study whether each drug (oxycodone, paroxetine, quetiapine, or midazolam) decreases the ventilatory response to hypercapnia on its own.

3.3. Exploratory Objectives

Exploratory objectives include the following:

- To study whether there is a direct pharmacodynamic interaction between each psychotropic drug and oxycodone.
- To summarize additional pharmacokinetic parameters and pharmacodynamic measures collected during the study.

4. Investigational Plan

4.1. Study Design

4.1.1 Lead-in Reproducibility Assessment

The lead-in reproducibility assessment will have up to 10 healthy volunteer participants. Up to five participants will be enrolled, and data analysis will be performed. If reproducibility is acceptable after the first analysis, additional lead-in cohorts may not be needed. If any study procedure element changes are needed, they will be made at this point. Then, additional participants may be enrolled, and data will be analyzed. For the reproducibility assessment, participants will check-in on Day -1, have PD assessments on Day 1 and Day 2 and check-out on Day 3. PD assessments will be performed on Day 1 and 2 at 0, 2, 3, 5, and 6 h. Participants will not be administered any drugs during the lead-in phase.

4.1.2 Part 1

The study Part 1 will assess the primary and secondary endpoints for oxycodone and midazolam and will inform oxycodone dose selection for study Part 2. The study will be a 4-period randomized crossover study with up to 20 healthy volunteer participants with the following design:

Table 4-1 Part 1 Study Design

Day 1	Days 2-3	Day 4	Days 5-6	Day 7	Days 8-9	Day 10
Period 1	Washout	Period 2	Washout	Period 3	Washout	Period 4

The following 4 treatments will be evaluated in a randomized order over the 4 study periods.

Table 4-2 Part 1 Treatments

Treatment	Day 1	Day 2-3
A	Oxycodone + placebo IV	Wash-out
B	Oral placebo + midazolam IV	Wash-out
C	Oxycodone + midazolam IV	Wash-out
D	Oral placebo + placebo IV	Wash-out

Participants will enter the clinic on a staggered basis in cohorts of approximately five participants (i.e., only approximately five participants will undergo dosing, PK assessments, and PD assessments on any given day). Subjects will be staggered up to 15 minutes apart in order to allow for direct safety overview by medical staff during dosing and Read rebreathing. Participants will check-in on Day -1 and receive dosing for the four respective periods on Days 1, 4, 7, and 10. There will be two days of washout between period. Participants will be confined in the study clinic from Day -1 until the morning of Day 11. On dosing days, the dosing, PK, and PD assessments will occur at the following time points:

- Dosing times: 0 h oxycodone, 115 min midazolam
- PK assessment: 0 (pre-dose), 1, 2, 3, 4, 6 h, 8 h, 12 h, 24 h
- PD assessment 0 (pre-dose), 1, 2, 3, 4, 6, 24 h

Participants will also receive ondansetron 4 mg 30 min before oxycodone or oral placebo administration. The total number of participants to be enrolled is approximately 20. Interim analyses will be performed after approximately 5 participants and the doses of oxycodone and/or midazolam may be adjusted at that time. The oxycodone dose may be increased from 10 mg to 15 mg if the conditional power to of concluding a decrease for oxycodone from placebo is less than 90% (difference of 12-18 L/min observed for 20 mg

oxycodone; van der Schrier 2017a; van der Schrier 2017b). Likewise, midazolam dose may be increased from 0.0375 mg/kg to 0.075 mg/kg if the conditional power of concluding a decrease of the combined effect of oxycodone and midazolam compared to oxycodone is less than 80%. The decision to escalate doses for either drug will also consider clinical evaluations and whether stopping criteria was attained in any of the participants. The remaining subjects will complete the study at the updated doses in cohorts of approximately five participants each.

The goal with the oxycodone dose is to have a definable effect (i.e. decreased ventilatory response to increasing CO_2) with oxycodone alone, but not too high (for safety considerations) as it will be combined with a benzodiazepine (Part 1) or other psychotropic drugs (Part 2). The goal with midazolam is to have a safe dose that decreases the ventilatory response to increasing CO_2 when co-administered with oxycodone compared to oxycodone alone. Additional details supporting oxycodone and midazolam dose selection is found in section 4.8.3 (Forster et al, 1980; Power et al, 1983; van der Schrier et al, 2017a; van der Schrier et al, 2017b).

The primary analysis will occur at the 2 h timepoint. If dose adjustments are not needed, the analysis will be based on all subjects with statistical adjustment for the interim analysis. Otherwise, the analysis will be limited to the subjects administered the higher doses of oxycodone and/or midazolam with no statistical adjustment.

4.1.3 Part 2

The study Part 2 will assess the primary and secondary endpoints for paroxetine and quetiapine with reference to oxycodone. The study will be a 3-period randomized crossover study in approximately 20 healthy volunteer participants. Each treatment period will have 5 days of dosing followed by 7 days of washout between periods. Participants will enter the clinic on a staggered basis in cohorts of approximately five (i.e., approximately five participants will undergo dosing, PK assessments, and PD assessments on any given day). Participants will check-in on Day -1 and checkout on the morning of Day 6 of each period.

Table 4-3 Part 2 Study Design

Days 1-5	Days 6-12	Days 13-17	Days 18-24	Day 25-29
Period 1	Washout	Period 2	Washout	Period 3

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

Table 4-4 Part 2 Treatments

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5
E	Oxycodone + Placebo	Placebo	Placebo	Placebo	Oxycodone + Placebo

F	Paroxetine + Oxycodone	Paroxetine	Paroxetine	Paroxetine	Paroxetine + Oxycodone
G	Quetiapine + Oxycodone	Quetiapine	Quetiapine	Quetiapine	Quetiapine + Oxycodone

Primary assessments for the two psychotropic drugs (paroxetine or quetiapine) combined with oxycodone vs. oxycodone alone will occur on Days 1 and 5 (multiple primary endpoints). The comparisons for paroxetine and quetiapine vs. placebo will occur on Day 4.

Dosing of the 3 drugs will be staggered so that T_{max} occurs at 5 h for all drugs such that the 5-h time point will be used for primary analyses. Section 4.1.5.2 contains additional details regarding dose timing for each treatment.

PD and PK assessments on Days 1, 4 and 5 of each treatment period will occur at the following time points.

- PK assessment: 0 (pre-dose), 3, 4, 5, 6, 8, 9, 12, 24 h
- PD assessment:

  - Day 1 and 5 of each period: 0 (pre-dose), 4, **5**, 6, 8, 24 h
  - Day 4 of each period: 0 (pre-dose), 4, **5**, 6, 24* h

*Note: The 24h assessment on Day 4 and 0h (pre-dose) assessment on Day 5 will be the same assessment.

4.1.4 Common Procedures

To maintain the study blind, subjects will be blindfolded during study drug administration. The rebreathing analysis will be blinded to treatment, time, and study day/subject identifiers.

At the study clinic (Spaulding Clinical Research unit in West Bend, Wisconsin), subjects will be screened for study eligibility from Day –28 to Day –2. On Day -1, subjects will enter the study clinic for check-in procedures the day before study drug administration (or procedures without study drug during the Lead-in phase). During the Screening visit, the inclusion and exclusion criteria will be reviewed to ensure the subject is appropriate for the study. The informed consent form will be reviewed with the subject by a member of the study team and the subject will be encouraged to ask questions to ensure the subject has a good understanding of the study. If the subject is eligible and agrees to participate, the subject will be asked to sign the informed consent form before any study-specific procedure is performed, including randomization. Additionally, during Screening subjects will participate in training for the Read Rebreathing Procedure and undergo the procedure two times either at Screening or Check-in to ensure that subjects are able to tolerate the procedure.

After the consent process is complete, demographic data, medical history, and concomitant medications (including over-the-counter and complimentary/alternative supplements) will be recorded. A physical examination will be performed by a study team member. Clinical laboratory tests (hematology, serum chemistry, and urinalysis), vital signs and pulse oximetry will be performed. Female subjects must have a negative pregnancy test result. Any values outside the reference range will be evaluated for clinical significance. If a value is determined to be clinically significant or the subject has a positive pregnancy test result, the subject will be instructed to follow-up with his or her personal physician. Screening tests will be performed within 28 days of and no later than 2 days before Day 1. Screening procedures will be performed by clinic staff, and all screening results will be evaluated by the study clinician/investigator against the inclusion/exclusion criteria to confirm subject eligibility.

At check-in, eligibility criteria will be reviewed, any changes in medical history (including concomitant medications) will be documented, vital sign measurements, pulse oximetry and a 12-lead ECG will be performed, urinalysis, clinical laboratory (including estrogen and progesterone for females), drug and alcohol, and pregnancy tests (for females) will be performed, and an intravenous (IV) catheter may be inserted into the subject's forearm region for blood collection (if needed). Additionally, subjects will undergo the Read Rebreathing Procedure training at check-in to refamiliarize them with the process and undergo the procedure two times (if not performed at Screening) to ensure they are able to tolerate the procedure.

Subject assigned to the Lead-in Reproducibility Assessment will not receive any drug treatments and will only participate in the Read Rebreathing and pupillometry procedures for staff training purposes and to better inform the investigation team about variability in the method. Total study length for the Reproducibility Assessment is 3 days.

The FDA project biostatistician will prepare the randomization schedule, and subjects that are in Part 1 or Part 2 will be randomly assigned to a treatment:

- Subjects assigned to Part 1 will participate in each of the 4 treatments (see Study Design), randomized over the 4 study periods. Total study length of Part 1 is 12 days (including check-in and check-out).
- Subjects assigned to Part 2 will participate in each of the 3 treatments (see Study Design), randomized over 3 study periods. Total study length of Part 2 is 31 days (including check-in and check-out).

Subjects will enter the study clinic for check-in procedures the day before study initiation (Day -1). On Day 1 of each period for Part 1 subjects will receive their assigned treatment according to the randomization schedule. On Days 1-5 of each period for Part 2 subjects will receive their assigned treatment according to the randomization schedule.

In the study clinic, study drugs will be administered on Day 1 per period for Part 1 or Days 1-5 per period for Part 2 as described in the dosing schedule in Section 4.1.5. For Part 1, continuous telemetry and pulse oximetry will be recorded for approximately 24 h on each of the drug dosing days. For Part 2, continuous telemetry will be recorded for 24 h on days when oxycodone is given (Days 1 and 5 of each period) and continuous pulse oximetry will be performed on Days 1, 4, and 5. For Part 1, subjects will stay in the study clinic overnight during each period, and blood samples for PK analysis will be collected at a set of pre-specified time points on Day 1 of each period. For Part 2, subjects will stay in the study clinic during treatment periods only and will be discharged from the clinic for each washout period. Blood samples for PK analysis will be collected at a set of pre-specified time points for Part 2 on Days 1, 4, and 5 of each period. For the Lead-in phase and Part 1 of the study, subjects will be required to fast until the completion of all PD assessments (i.e., for the Lead-in phase subjects will receive their first meal after the 6 h PD assessment and for Part 1 subjects will receive their first meal after the 6 h PD assessment). For Part 2, on Days 1 & 5 subjects will receive a light snack after the 6 h PD assessment and their first meal after the 8 h PD assessment whereas on Day 4 subjects will receive their first meal after the 6 h assessment. Subjects will be given a second meal later in the evening and a snack prior to bed.

All sampling times are relative to first dose administered in the period. Preparatory steps for the Read Rebreathing procedure (i.e., relaxation, hyperventilation) should begin prior to the scheduled sample time. Timing of sample collection is planned so that the PD assessment (i.e. the Read Rebreathing procedure) begins at the specified time and a 3- to 15-minute window is planned for completion of the procedure. Pupillometry will be performed before and after rebreathing. Collection of some of the PK samples will be time-matched to the PD assessments. For the time-matched PK/PD assessments, the PK sample will be collected after the rebreathing procedure to avoid interference with the rebreathing assessment results. A 5-minute window for PK sample collection is allowed after the rebreathing procedure (see Table 8-1).

Vital signs will be performed daily on treatment days for Parts 1 and 2 (see Table 8-1). Continuous pulse oximetry monitoring will be performed on all treatment days for Part 1 and on days 1, 4, and 5 of each period for Part 2. Pulse oximetry will also be checked at Screening, Check-in and Check-out. Telemetry will be performed on treatment days in Part 1 and on Days 1 and 5 in Part 2. If an adverse event (AE) is reported the medical monitor will be notified as quickly as possible (see Section 4.7.6.1).

Meal timing and components, activity levels, and general conditions in the study clinic will be as similar as possible on the treatment days. Subjects will fast during the intensive PD assessment portions of the Lead-In, Part 1, and Part 2. Subjects will be provided their first meal after the 6 h PD assessment for the Lead-in and for Part 1 of the study. For Part 2 on Days 1 & 5 subjects will be provided with a light snack after the 6 h PD assessment

and their first meal after 8 h PD assessment. For Part 2 on Day 4 subjects will be provided their first meal after the 6 h PD assessment. Subjects will have a second meal in the late evening, and a snack prior to bed.

Subjects assigned to the Lead-in Reproducibility Assessment will be discharged from the Study on Day 3. Subjects assigned to Part 1 will be discharged from the study on Day 11 and subjects assigned to Part 2 will be discharged from the study on Day 30 after completion of all study procedures and removal of IV catheter (if applicable). If a subject discontinues from the study prematurely, all procedures scheduled for discharge day (i.e., Day 11 for Part 1 and Day 30 for Part 2) will be performed.

Safety will be evaluated in terms of AEs, clinical laboratory results (hematology, serum chemistry, and urinalysis), vital sign measurements (blood pressure, heart rate, respiratory rate, pulse oximetry, and oral body temperature), telemetry, ECG results, and physical examination findings (see Section 4.7.6).

4.1.5 Dosing Schedule

4.1.5.1 Part 1

On day 1 of each period, subjects will receive one of the following treatments:

- Oral oxycodone 10 mg and placebo IV
- Oral placebo and 0.0375 mg/kg midazolam IV
- Oral oxycodone 10 mg and 0.0375 mg/kg midazolam IV
- Oral placebo and placebo IV

Oxycodone will be administered as 2 X 5 mg immediate release tablets at 0 h. Midazolam will be infused over 2-minutes starting at 115 minutes. All treatments will include matching oral or IV placebo, as needed. Based on assessment of approximately 5 subjects, either oxycodone or midazolam may be dose increased. If the oxycodone dose is increased, all subsequent subjects will be administered oxycodone as 3 X 5 mg (15 mg) immediate release tablets at 0 h. If the midazolam dose is increased, all subsequent subjects will be administered midazolam at 0.075 mg/kg as a 2-min infusion starting at 115 minutes.

4.1.5.2 Part 2

For Part 2, subjects will complete each of the 3 different treatments (Table 4-4, E-G) and will receive the following drugs alone or in combination:

- Oxycodone (2-3 X 5mg tablets depending on findings from Part 1) of each period
- Paroxetine 40 mg tablets on days 1, 2, 3, 4, and 5

- Quetiapine 50 mg tablets twice daily (BID) on day 1, followed by 100 mg (2 X 50 mg) BID on day 2, 150 mg BID (3 X 50 mg) on day 3, 200 mg (4 x 50 mg) BID on day 4 and 200 mg (4 x 50 mg) QD on day 5.
- Matching oral placebo for paroxetine and quetiapine for all periods

Dose timing details for Part 2 can be found in Table 4-5.

Table 4-5 Part 2 Dose Timing

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5
E	0 h: Placebo	0 h: Placebo	0 h: Placebo	0 h: Placebo	0 h: Placebo
	3 h: Oxycodone, Placebo	3 h: Placebo (x2)	3 h: Placebo (x3)	3 h: Placebo (x4)	3 h: Oxycodone, Placebo (x4)
	14 h: Placebo	14 h: Placebo (x2)	14 h: Placebo (x3)	14 h: Placebo (x4)	--
F	0 h: Paroxetine	0 h: Paroxetine	0 h: Paroxetine	0 hr: Paroxetine	0 h: Paroxetine
	3 h: Oxycodone, Placebo	3 h: Placebo (x2)	3 h: Placebo (x3)	3 h: Placebo (x4)	3 h: Oxycodone, Placebo (x4)
	14 h: Placebo	14 h: Placebo (x2)	14 h: Placebo (x3)	14 h: Placebo (x4)	--
G	0 h: Placebo	0 h: Placebo	0 h: Placebo	0 h: Placebo	0 h: Placebo
	3 h: Quetiapine, Oxycodone	3 h: Quetiapine (x2)	3 h: Quetiapine (x3)	3 h: Quetiapine (x4)	3 h: Quetiapine (x4), Oxycodone
	14 h: Quetiapine	14 h: Quetiapine (x2)	14 h: Quetiapine (x3)	14 h: Quetiapine (x4)	--

Participants will also receive ondansetron 4 mg 30 min before oxycodone administration.

Paroxetine (or placebo) doses will be administered at time 0 on each day. The first daily dose of quetiapine (or placebo) and oxycodone dosing will occur at 3 h on dosing days; the second daily dose of quetiapine (or placebo) will be administered at approximately 14 h.

4.1.6 Risk/Benefit

Subjects will be informed that participation in a human PK-PD study like the present one cannot be of benefit to healthy volunteers. Nevertheless, the information from the physical examination, vital sign measurements, and ECG results may be shared with the subject's personal physician if this is the subject's choice. Subjects will be informed that it is also their choice to inform their personal physician that they are participating in this research study.

Subjects will be informed that their contribution to the study is of major importance to agencies like the U.S. FDA for helping this agency better evaluate the potential for respiratory depression with coadministration of psychotropic drugs and opioids.

However, since this is a study involving healthy volunteers, subjects will be informed that they have the alternative not to participate.

Subjects will be informed that opioids have a box warning for life-threatening respiratory depression and that benzodiazepines and opioids have a box warning with concomitant use. Subjects will be informed that the current study includes a treatment where oxycodone (opioid) and midazolam (benzodiazepine) will be co-administered at doses that have been shown to have a minimal effect on respiration. Given previous study results with opioids and benzodiazepines and using a similar procedure, it is not anticipated that severe respiratory depression will occur with the doses used in this study. To mitigate potential events, subjects will undergo telemetry and continuous pulse oximetry monitoring, there will be stopping rules for the treatment, rescue medications for oxycodone and midazolam will be present, and a safety management plan for staff will be implemented during the study which includes a requirement to have 1:1 advanced cardiac life support staff to subjects in addition to the study investigator.

Subjects will be informed that they may be exposed to risks associated with the pharmacological properties of the investigational product and the study procedures. The following summary of potential AEs for the study drugs will be provided to and discussed with the subjects:

1. Oral Oxycodone 10 or 15 mg: The most common adverse events include typical opioid-related adverse reactions: nausea, constipation, vomiting, headache, pruritus, insomnia, dizziness, asthenia, agitation, anxiety, hallucinations, nightmares, and somnolence. Serious adverse reactions include: respiratory depression, respiratory arrest, circulatory depression, cardiac arrest, hypotension, and/or shock.
2. IV Midazolam 0.0375 or 0.075 mg/kg: The most common adverse events include: non-life threatening decreased tidal volume, respiratory rate decrease with combined therapeutic doses, aspiration, and apnea as well as minor variations in blood pressure and pulse rate. Additional adverse reactions included: hiccoughs, coughing, over sedation, drowsiness, nausea, vomiting, and headache. Local effects at the IV site include: tenderness, pain during injection, redness, induration, or phlebitis. The majority of serious adverse effects, particularly those associated with oxygenation and ventilation, were reported when midazolam is administered with other medications capable of depressing the central nervous system.
3. Oral Paroxetine 40 mg IR: The most common adverse events include: somnolence, insomnia, agitation, tremor, anxiety, dizziness, constipation, nausea, diarrhea, dry mouth, vomiting, flatulence, asthenia, erectile dysfunction, delayed ejaculation, vaginal paresthesia, itching, and discharge, yawning, infection, sweating, decreased appetite, nervousness, impotence, and libido decreased.

4. Quetiapine 50-200 mg tablets: The most common adverse events include: somnolence, dry mouth, dizziness, constipation, nausea, vomiting, asthenia, abdominal pain, postural hypotension, pharyngitis, weight gain, lethargy, ALT increased, and dyspepsia.
5. Ondansetron 4 mg tablets given prior to oxycodone for nausea/vomiting and 4 mg IV as needed: The most common adverse events include: diarrhea, headache, fever, malaise, fatigue, constipation, and hypoxia. Additional adverse events include: hypersensitivity reactions, including anaphylaxis and bronchospasm, QT interval prolongation and Torsade de Pointes, and serotonin syndrome (usually when prescribed with another serotoninergic medication such as select serotonin reuptake inhibitors and serotonin-norepinephrine reuptake inhibitors).
6. Naloxone 0.4 mg IV: The most common adverse events include nausea, vomiting, sweating, tachycardia, hypertension, dyspnea, tremulousness, seizures, body aches, fever, sweating, restlessness, irritability, agitation, and abdominal cramps. Additional adverse events include ventricular tachycardia, fibrillation, and pulmonary edema. Naloxone will be administered as a rescue medication for subjects with severe respiratory depression meeting study stopping rules or at the discretion of the investigator.
7. Flumazenil 0.2 mg IV: The most common adverse events include injection site irritation, agitation, tremors, flushing, dizziness, excessive sweating, headaches, blurred vision, fatigue, nausea, vomiting, abnormal sensations, seizures, arrhythmia, and tinnitus. Flumazenil will be administered as a rescue medication for subjects with severe respiratory depression meeting study stopping rules, or at the discretion of the investigator, to counter side effects of the benzodiazepine.

Selected doses of individual drugs are within the FDA approved labeled doses. As drug combinations are being studied, the doses of oxycodone and midazolam are less than that which has been used in prior studies in opioid naïve healthy volunteers undergoing the Read rebreathing or other similar procedures (see below).

- Oxycodone -- A prior study of the effects of decreased ventilatory response to hypercapnia of oxycodone with (van der Schrier et al, 2017a) and without ethanol (van der Schrier et al, 2017b) in opioid naïve healthy volunteers (young and elderly participants) used a dose of oxycodone 20 mg. An additional ongoing study (unpublished) is using a dose of oxycodone 40 mg in opioid naïve healthy volunteers. The present study will use an initial dose of oxycodone 10 mg. The oxycodone dose will be increased from 10 mg to 15 mg if the conditional power to of concluding a decrease for oxycodone from placebo is less than 90% (difference of 12-18 L/min observed for 20 mg oxycodone; van der Schrier et al 2017a; van der Schrier et al, 2017b) after the interim analysis in Part 1.

- Midazolam – Two prior studies safely investigated the effect of midazolam on decreased ventilatory response to hypercapnia in healthy volunteers. The first study (Power et al, 1983) used a dose of midazolam 0.075 mg/kg and saw decreases in the slope of the ventilatory response curve that were not significant in the overall analysis but did show significant decreases from baseline in the slope at 3 and 15 min based on paired t-tests. The second study (Forster et al, 1980) used a dose of midazolam 0.15 mg/kg and did see a decreased ventilatory response to hypercapnia. The present study will use an initial dose of midazolam 0.0375 mg/kg and increase the dose to 0.075 mg/kg if the conditional power of concluding a decrease of the combined effect of oxycodone and midazolam compared to oxycodone is less than 80% after the interim analysis in Part 1.
- Paroxetine – Paroxetine will be administered at 40 mg/day for 5 days. This is within the maximal labeled dose of paroxetine (60 mg/day). The dose is higher than the typical starting dose of 20 mg/day that is titrated up over 1-week intervals, however starting doses up to 60 mg have been given safely to healthy volunteers (Heydorn et al, 1999).
- Quetiapine – Quetiapine will be administered according to the labeled dose for Bipolar I Disorder, Mania: Day 1: 100 mg/day divided twice a day; Day 2: 200 mg/day divided twice a day; Day 3 300 mg/day divided twice a day; Day 4 400 mg/day divided twice a day; Day 5 200 mg QD.
- Ondansetron - Ondansetron will be administered as a single 4 mg oral dose in all treatment periods prior to administration of oxycodone and will be available as 4 mg IV as needed for nausea and vomiting. A dose of 4 mg is less than the highest labeled dosing for other ondansetron indications (24 mg as a single dose or 8 mg twice a day).
- Naloxone – Naloxone will be administered intravenously at the labeled dose of 0.4 mg for opioid overdose. Naloxone will only be administered as a rescue medication for subjects with severe respiratory depression meeting study stopping rules or at the discretion of the investigator.
- Flumazenil – Flumazenil will be administered intravenously at the labeled dose of 0.2 for reversal of the sedative effects of benzodiazepines. Flumazenil will only be administered as a rescue medication for subjects with severe respiratory depression meeting study stopping rules or at the discretion of the investigator.

The study drugs will not be administered to anyone who is pregnant. All women must take a pregnancy test before receiving any study drug in this study. All women of childbearing potential enrolled on this study will be informed that they must use effective birth control methods (abstinence, intrauterine device, and contraceptive foam and a condom [i.e., double-barrier method]) during treatment. Subjects will be informed that

they must notify the investigator if they or their female partners become pregnant during the course of the study. If a subject becomes pregnant, she will be informed that neither Spaulding Clinical Research nor the sponsor will be responsible for the cost of any obstetric or related care, or for the child's care.

Subjects will be informed that they will be participating in the Read Rebreathing procedure where they will breathe a mixture of oxygen and carbon dioxide through a tight-fitting face mask. The mixture of oxygen and carbon dioxide will be higher than is normally found in room air – 93% oxygen (normally 21% in room air) and 7% carbon dioxide (normally 0.4% in room air). Subjects will be informed that the higher level of carbon dioxide breathed in may result in a feeling of needing to breathe faster. Subjects will be informed that this procedure has been used in previous studies and has been proven to be safe. Subjects will also be shown the Read Rebreathing equipment and will be trained how to use it to understand if the procedure is tolerable.

Subjects will be informed that insertion of an IV catheter may be required for blood sample collection and, during insertion of the catheter, soreness, bruising, or infection at the insertion site are possible but unlikely. Subjects will also be informed that dizziness and lightheadedness may occur during direct venipuncture, insertion of the IV catheter, or during blood collection.

Subjects will be informed that they may eat only meals and snacks that are provided during periods of their stay in the study clinic, and that they must consume all of each meal that is served at a reasonable pace (within 25 minutes).

Subjects will be informed that blood samples will be collected for genetic testing to explore how a person's genes affect the way the body and drug interact. Testing for CYP2D6 and CYP3A4 will be performed at Screening using available laboratory tests.

Subjects will be informed that extra precautions will be put in place that will minimize the risk of exposure to COVID-19. Precautions will be documented in a COVID-19 risk management plan. Currently, this includes phone screening to prevent symptomatic participants or those with known COVID-19 exposure from entering the clinic; triage of all potential study subjects entering the building at Screening and Check-in for potential contacts with COVID-19, signs and symptoms, temperature monitoring and serology screening for severe acute respiratory syndrome corona virus 2 (SARS CoV-2); SARS CoV-2 molecular testing just prior to or at check-in for admission to the study floor; all study participants and staff wearing masks except when in a private room or for a limited time for a study procedure (e.g. study drug administration); staff wearing personal protective equipment; social distancing during screening and in-house stays including 1 subject per room for overnight stays; extra hand sanitation stations with hand washing and sanitation policies per CDC recommendations; closing common areas and serving food at subjects' room resulting in subjects spending most of their time in their rooms with the exception of specified times for walking in the halls; daily temperature

screening; and separate staff for confined vs. not-confined participants whenever possible. Designated isolation rooms will be set up to segregate any participant(s) that develop any symptoms of concern while housed in the unit and COVID-19 testing will be done when deemed necessary by the Investigator. Subjects will be informed that despite the extra precautions there is still a risk of them contracting COVID-19. Any changes to the COVID-19 precautions (e.g. due to updated CDC recommendations or new testing becoming available) will be documented in the COVID-19 risk mitigation plan.

Subjects will be informed that the confidentiality of their data will be respected at all times according to state law, and the study personnel handling their study data are bound by confidentiality agreements.

Subjects will be informed that the study drug and all tests, procedures, and visits required by the study are provided at no cost to them. If subjects become ill or physically injured because of participation in this study, they will be informed that costs of treatment will not be covered by the sponsor.

4.2. Selection of Study Population

Subjects will be screened and the data collected will be reviewed by the principal investigator. Only those subjects who meet all of the eligibility criteria will be enrolled. Approximately 50 healthy subjects are planned for enrollment, of which up to 10 will be assigned to the Lead-in Reproducibility Assessment, approximately 20 will be assigned to Part 1, and approximately 20 will be assigned to Part 2 at randomization. Up to 10 subjects may be qualified as replacements as described in Section 4.5.2. Thus, a maximum of 60 subjects will be exposed to study drugs and procedures during the study. Every effort will be made to maintain an approximate 50:50 male to female sex distribution within each part of the study.

4.2.1 Inclusion Criteria

Subjects who meet all of the following inclusion criteria will be eligible to participate in the study:

1. Subject signs an IRB-approved written informed consent and privacy language as per national regulations (e.g., Health Insurance Portability and Accountability Act authorization) before any study-related procedures are performed.
2. Subject is a healthy man or woman, 18 to 50 years of age, inclusive, who has a body mass index (BMI) of 18.5 to 29.9 kg/m2, inclusive, at Screening.
3. Subject has normal medical history findings, clinical laboratory results, vital sign measurements, 12-lead electrocardiogram (ECG) results, and physical examination findings at Screening or, if abnormal, the abnormality is not considered clinically significant (as determined and documented by the investigator or designee).

4. Subject must have a negative test result for alcohol and drugs of abuse at screening and Check-in (Day -1).
5. Subject has no known or suspected allergies or sensitivities to any of the study drugs.
6. Female subjects must be of non-childbearing potential or, if they are of childbearing potential, they must: 1) have been strictly abstinent for 1 month before Check in (Day -1) and agree to remain strictly abstinent for the duration of the study and for at least 1 month after the last application of study drug; OR 2) be practicing 2 highly effective methods of birth control (as determined by the investigator or designee; one of the methods must be a barrier technique) from at least 1 month before Check in (Day -1) until at least 1 month after the last application of study drug.
7. Male subjects must agree to practice 2 highly effective methods of birth control (as determined by the investigator or designee; one of the methods must be a barrier technique) from at least 1 month before Check in (Day -1) until at least 1 month after the last application of study drug.
8. Subject is highly likely (as determined by the investigator) to comply with the protocol defined procedures and to complete the study

4.2.2 Exclusion Criteria

Subjects who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Subject has history of opioid or psychotropic drug use within 60 days of the start of the study.
2. Subject has non-reactive or misshapen pupil(s) or damaged orbit structure or surrounding soft tissue is edematous or has an open lesion.
3. Subject has a Mallampati intubation score of >2 (for Part 1 and 2 only).
4. Subject Read Rebreathing data is of poor quality or subject does not agree to remain clean-shaven for all days when the Read Rebreathing procedure is being performed.
5. Subject has used any prescription or nonprescription drugs (including aspirin or NSAIDs and excluding oral contraceptives and acetaminophen) within 14 days or 5 half-lives (whichever is longer) or complementary and alternative medicines within 28 days before the first dose of study drug. This includes prescription or nonprescription ophthalmic drugs.
6. Subjects are currently participating in another clinical study of an investigational drug or are have been treated with any investigational drug within 30 days or 5 half-lives (whichever is longer) of the compound.
7. Subject has used nicotine-containing products (e.g., cigarettes, cigars, chewing tobacco, snuff) within 6 weeks of Screening.

8. Subject has consumed alcohol, xanthine-containing products (e.g., tea, coffee, chocolate, cola), caffeine, grapefruit, or grapefruit juice within 48 h of dosing. Subjects must refrain from ingesting these throughout the study.
9. Subject has a history of sleep disorders, Panic Disorder, Panic Attacks, Generalized Anxiety Disorder, or any associated DSM diagnosis or condition.
10. Subject has any underlying disease or surgical or medical condition (e.g., cancer, human immunodeficiency virus [HIV], severe hepatic or renal impairment) that could put the subject at risk or would normally prevent participation in a clinical study. This includes subjects with any underlying medical conditions that the Investigator believes would put subjects at increased risk of severe illness from COVID-19 based on the Centers for Disease Control and Prevention (CDC) guidelines. The CDC lists cancer, chronic kidney disease, chronic obstructive pulmonary disease, immunocompromised state from solid organ transplant, severe obesity, serious heart conditions, sickle cell disease, pregnancy, smoking and type 2 diabetes mellitus as conditions that put subjects at increased risk. Additionally, the CDC lists asthma (moderate-to-severe), cerebrovascular disease, cystic fibrosis, hypertension, immunocompromised state or immune deficiencies, neurologic conditions such as dementia, liver disease, pulmonary fibrosis, thalassemia, overweight, type 1 diabetes mellitus as conditions that might put subjects at increased risk.
11. Subject has any signs or symptoms that are consistent with COVID-19 per CDC recommendations. These include subjects with fever or chills, cough, shortness of breath or difficulty breathing, fatigue, muscle or body aches, headache, new loss of taste or smell, sore throat, congestion or runny nose, nausea or vomiting, or diarrhea may have COVID-19. In addition, the subject has any other findings suggestive of COVID-19 risk in the opinion of the investigator.
12. Subject tests positive for SARS-CoV-2 by a molecular diagnostic test performed prior to admission.
13. Female subject is pregnant or lactating before enrollment in the study.
14. Subject has known or suspected allergies or sensitivities to any study drug.
15. Subject has clinical laboratory test results (hematology, serum chemistry) at screening that are outside the reference ranges provided by the clinical laboratory and considered clinically significant by the investigator.
16. Subject has a positive test result at Screening for HIV 1 or 2 antibody, hepatitis C virus antibodies, or hepatitis B surface antigen.
17. Subject is unable or unwilling to undergo multiple venipunctures for blood sample collection because of poor tolerability or poor venous access.

18. Subject has a history of or currently has hypoventilation syndrome or sleep apnea and is on non-invasive ventilation

4.3. Screening Failures

Subjects who sign and date the informed consent form but who fail to meet the inclusion and exclusion criteria are defined as screening failures. A screening log, which documents the subject initials and reason(s) for screening failure, will be maintained by the investigator for all screening failures. A copy of the log should be retained in the investigator's study files.

If a subject fails the screening process because of an abnormal laboratory result, they can receive a copy of the results upon request. The investigator will determine if follow-up for the abnormal laboratory result is needed and will encourage the subject to follow-up with his or her personal physician as appropriate. All subjects will be informed as to the reason(s) they are excluded from study participation, even if follow-up is not required. If a subject fails the screening process because of a positive test result for human immunodeficiency virus or hepatitis, the positive result will be reported to local health authorities as required by law.

4.4. Termination of Study or Investigational Site

4.4.1 Criteria for Termination of the Study

The study will be completed as planned unless one of the following criteria is satisfied that requires early termination of the study.

- New information regarding the safety or efficacy of the study drug(s) that indicates a change in the known risk profile for the study drug(s), such that the risk is no longer acceptable for subjects participating in the study.
- Significant violation of Good Clinical Practice (GCP) that compromises the ability to achieve the primary study objective or compromises subject safety.

4.4.2 Criteria for Termination of Investigational Site

The study site may be terminated if the site (including the investigator) is found in significant violation of GCP, the protocol, the contractual agreement, or is unable to ensure adequate performance of the study.

In the event that the sponsor elects to terminate the study or the investigational site, a study-specific procedure for early termination will be provided by the sponsor; the procedure will be followed by the applicable investigational site during the course of termination.

4.5. Criteria for Subject Withdrawal

Subjects may withdraw from the study at any time at their own request, or they may be withdrawn by the investigator without the approval of the subject based on the investigator's clinical judgment. A subject is not required to provide a written request to withdraw from the study; however, a written request is required if a subject withdraws consent for his or her personal data to be used for study-related purposes.

A subject may be discontinued for any of the following reasons:

- AE: The subject has experienced an AE that, in the opinion of the investigator, requires early termination. The appropriate electronic case report form (eCRF) must be completed for each AE. If a subject is discontinued from the study due to an AE, the investigator is required to follow-up with the subject until the event resolves or becomes stable. If a subject dies during the study, the cause of death must be reported as a serious AE (SAE), with an outcome of death noted in the eCRF.
- Ventilation will be monitored with the respiratory system in the research unit. The respiratory system measures the end-tidal CO_2 and O_2, inspiratory CO_2 and O_2, respiratory rate and minute ventilation. Subjects will be discontinued from treatment if any of the following stopping criteria are achieved and the appropriate rescue medication may be administered at the physician's discretion:
    - Apnea defined as discontinuation of rhythmic breathing for > 90 sec
    - $pCO_2 > 67.5$ mmHg
    - O_2 saturation < 85% lasting more than 2 minutes
- Protocol Violation: The subject failed to meet protocol entry criteria or did not adhere to protocol requirements, and continued participation poses an unnecessary risk to the subject's health.
- Withdrawal by Subject: The subject (or other responsible individual [e.g., caregiver]) wishes to withdraw from the study in the absence of a medical need.

  NOTE: Withdrawal due to an AE should not be recorded in the "voluntary withdrawal" category.
- Study Terminated by Sponsor: The sponsor, IRB, FDA, or other regulatory agency terminates the study.
- Pregnancy: The subject is found to be pregnant.

  NOTE: If the subject is found to be pregnant, the subject must be withdrawn immediately. The pregnancy will be followed-up to term, and the outcome, including any premature termination will be recorded. All live births must be followed for a minimum of 30 days or until the first well-baby visit.

- Other.

  NOTE: This category records withdrawals caused by an accidental or a medical emergency, unblinding, and other rare cases. The specific reason should be recorded in the comment space of the eCRF.

4.5.1 Handling of Withdrawals

The investigator may terminate a subject's study participation at any time during the study when the subject meets the criteria described in Section 4.5. In addition, a subject may discontinue his or her participation without giving a reason at any time during the study. Subjects will be informed that their participation in the study is voluntary, that refusal to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled, and that the subject may discontinue participation at any time without penalty or loss of benefits to which the subject is otherwise entitled.

Should a subject's participation be discontinued, the primary reason for termination must be recorded. In addition, efforts should be made to perform all procedures scheduled for the early termination visit. Any data and samples collected before subject withdrawal will become the property of the sponsor.

4.5.2 Replacement Subjects

Approximately 50 healthy subjects are planned for enrollment, of which up to 10 will be assigned to the Lead-in Reproducibility Assessment, approximately 20 will be assigned to Part 1, and approximately 20 will be assigned to Part 2 at randomization. Up to 10 subjects may be qualified as replacements for all parts of the study combined. Thus, a maximum of 60 subjects will be exposed to study drugs and procedures during the study.

Lead-in Reproducibility Assessment

It is anticipated that up to 10 subjects will be needed to complete the Lead-in Reproducibility Assessment. Up to five subjects will be enrolled initially and complete the Read Rebreathing Procedures five times on Days 1 and 2. If reproducibility is acceptable after the first analysis, additional lead-in cohorts may not be needed. If data analysis reveals that there are issues with the procedure, or variability exceeds expectations for the experimental setup, modifications may be made and then additional subjects will be enrolled and their data analyzed.

Part 1

Part 1 will be a 4-period crossover study with approximately 20 healthy volunteer subjects. An initial cohort of approximately 5 subjects will participate in the study and interim analyses will be performed to understand if doses need be adjusted. If needed,

doses may be adjusted, and the remaining subjects will complete the study at the updated doses. Subjects are expected to complete all 4 treatments of the study.

A replacement algorithm will be pre-specified in the subject replacement plan to guide the unblinded Spaulding pharmacist to determine this while the rest of the staff and sponsor remain blinded. A maximum of 5 replacement subjects may be enrolled in Part 1 of the study, and replacement subjects (if needed) must complete the treatment period. A replacement subject will receive the same treatment as the subject being replaced. During Part 1 of the study, a maximum of 25 subjects will be exposed to study drugs and procedures.

If a subject vomits after dosing, a PK blood sample will be collected and, if the subject vomits immediately after dosing, the sponsor will be consulted regarding a decision to continue the subject in the study. Under no circumstances will a dose of any of the study drugs be repeated. If the subject does not appear to be tolerating the rebreathing procedures, the sponsor will be consulted regarding a decision to continue the subject in the study.

Part 2

Part 2 will be a 3-period randomized crossover study in approximately 20 healthy volunteer subjects. Subjects are expected to complete all 3 periods of the study.

A maximum of 5 replacement subjects may be enrolled in Part 2 of the study, and replacement subjects (if needed) must would complete the treatment period. A replacement subject will receive the same treatment as the subject being replaced. During Part 2 of the study, a maximum of 25 subjects will be exposed to study drugs and procedures.

4.6. Study Visits

4.6.1 Recruitment

Recruitment materials (e.g., internet, radio, and print advertisements, social media posts) will be approved by the local IRB before telephone screening. The sponsor is responsible for registration of the study on clinicaltrials.gov. Recruitment may not occur until the study is fully registered on clinicaltrials.gov.

4.6.2 Compensation

Subjects will be offered payment for Screening; however, if the results of their alcohol and drug screening tests are positive, they will not be compensated. Subjects who complete the entire study (Day -1 to Day 3 [Lead-in] or Day -1 to Day 11 [Part 1] or Day 30 [Part 2]) will receive payment according to the schedule provided in the informed

consent form. No special incentives are offered. Final payment will not be released until all follow-up procedures have been completed and accepted by the investigator.

If a subject chooses to withdraw from the study prematurely, he or she will only be compensated for completed days. If subjects are withdrawn for medical reasons or if the study is halted temporarily or permanently, the subjects will receive compensation proportional to the time spent in the study. No compensation will be provided if a subject is dismissed from the study for noncompliance (e.g., improper conduct, ingesting alcohol and/or drugs [including recreational drugs], tampering with the study drug, consuming any prohibited foods or beverages).

If subjects are required to stay in the clinic for a longer period for safety reasons, they will be compensated at a rate proportional to the entire compensation for the study. If a subject becomes ill or physically injured because of participation in this study, the subject will be referred for treatment.

4.6.3 Screening

The following procedures and assessments will be performed at Screening (Day –28 to Day –2):

- Obtain informed consent/HIPAA authorization (The informed consent process will be performed by a clinical research nurse in a private room. The subject will be given unlimited time to ask questions regarding study participation, and each subject will be questioned to ensure their understanding.)

After informed consent is obtained:

- Review inclusion/exclusion criteria to confirm subject eligibility
- Record demographic information
- Measure height, weight, and calculate body mass index
- Perform serology screening (HIV antigen/antibody Combo 1/2, HepC antibody, HBsAg)
- Record medical history
- Perform alcohol and drug screening (amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, alcohol, opiates, phencyclidine, propoxyphene, and methadone)
- Perform a serum pregnancy test (female subjects only)
- Record prior medications
- Monitor for AEs

- Perform clinical laboratory tests (hematology, serum chemistry, and urinalysis)
- Measure vital signs (blood pressure, heart rate, respiratory rate, pulse oximetry and oral body temperature)
- Perform a safety 12-lead ECG
- Perform a complete physical examination
- Examine pupils for shape and reactivity
- Complete Read Rebreathing training
- Collect whole blood sample for CYP2D6 and CYP3A4 screening

4.6.4 Study Periods

The Lead-in Reproducibility Assessment has a single treatment period. Part 1 of this study has a crossover design with 4 treatment periods. Subjects will be kept in the study clinic between the 4 periods. Part 2 of this study has a crossover design with 3 treatment periods. Subjects will be not kept in the study clinic between the 3 treatment periods.

4.6.4.1 Check-in

The following procedures and assessments will be performed at Check-in (Day -1):

- Perform/review results from SARS-CoV-2 molecular test (may be performed ~2 days before check-in to allow time for results)
- Review inclusion/exclusion criteria to confirm subject eligibility
- Review medical history
- Perform alcohol and drug screening (amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, alcohol, opiates, phencyclidine, propoxyphene, and methadone)
- Perform a serum pregnancy test (female subjects only)
- Complete Read Rebreathing training
- Admit subject to the study clinic
- Randomization (after completion of check-in procedures on Day -1 or just before dosing on Day 1)
- Record concomitant medications
- Monitor for AEs
- Measure vital signs (blood pressure, heart rate, respiratory rate, pulse oximetry and oral body temperature)

- Perform a safety 12-lead ECG
- Perform a complete physical examination

4.6.4.2 Treatment

The following procedures and assessments will be performed during the treatment period according to the Schedules of Events (Table 8-1):

- Record concomitant medications
- Monitor for AEs
- Perform clinical laboratory tests at time points indicated in Table 8-1
- Collect samples for estrogen and progesterone at time points indicated in Table 8-1 (female subjects only)
- Administer study drug according to the randomization schedule (Part 1 and Part 2 only)
- Measure vital signs (blood pressure, heart rate, respiratory rate, pulse oximetry, and oral body temperature) at the time points specified in Table 8-1. If vital signs are scheduled at the same time as another event, vital signs will be measured after the ECG, but before blood sample collection.
- Perform a safety 12-lead ECG at the time points specified in Table 8-1. If scheduled for the same time, safety 12-lead ECGs will always be performed before vital sign measurement and blood sample collection.
- Perform continuous telemetry monitoring on all dosing days in Part 1 and on days 1 and 5 in Part 2.
- Perform pulse oximetry monitoring on all dosing days in Part 1 and on days 1, 4, and 5 in Part 2.
- Collect PK blood samples (5 mL) at the time points specified in Sections 4.1.2 and 4.1.3.
- Conduct pupillometry assessments before and after each rebreathing assessment.
- Perform Read rebreathing procedure at the time points specified in Sections 4.1.2 and 4.1.3. Preparatory steps for the Read Rebreathing procedure (i.e., relaxation, hyperventilation) should begin prior to the scheduled sample time. Timing of sample collection is planned so that the PD assessment begins at the specified time and a 3- to 15-minute window is planned for completion of the procedure.
- Perform sedation assessments as described in Section 4.7.3 (Ramsey Sedation Scale and Visual Analogue Scale) (Part 1 and Part 2 only).

- Collect buffy coat for genomic analysis (Part 1 and Part 2 only).

4.6.4.3 Washout

For Part 1, there will be 2 washout days (72 h from dose to dose) between the dosing days. For Part 2, there will be a 7-day washout period (192 h from dose to dose) between the last dose of the period and the first dose of the next period.

4.6.5 Discharge (or Early Termination)

The following procedures and assessments will be performed before the subject is discharged from the study or at early termination:

- Perform a serum pregnancy test (female subjects only)
- Record concomitant medications
- Monitor for AEs
- Perform clinical laboratory tests (hematology, serum chemistry, and urinalysis)
- Measure vital signs (blood pressure, heart rate, respiratory rate, pulse oximetry and oral body temperature)
- Assess for pre-discharge sedation
- Perform a safety 12-lead ECG
- Perform a complete physical examination
- Remove IV catheter (if applicable)
- Discharge subject from the study clinic after completion of all study procedures

4.7. Study Procedures

4.7.1 Read Rebreathing Assessments

Training on the Read Rebreathing procedure will be performed at screening and at check-in and the procedure will be administered two times either at screening or check-in to exclude any subjects who cannot tolerate the procedure.

Prior to conducting assessments, subjects will be fit to the appropriate size headgear and mask face piece. Subjects will be placed in a semi-supine position on a bed and [redacted] connected to the respiration measurement set up. Subjects will breathe from a bag with a specified gas mixture with an elevated level of CO_2 (7% CO_2 in 93% oxygen) which will stimulate increased ventilation. Once the proper set-up has been confirmed, the entire procedure should take approximately 13-15 minutes. Data collected throughout the

procedure includes respiratory flow, ventilation, percent gas compositions of O_2 and CO_2, and $P_{ET}O_2$ and $P_{ET}CO_2$.

During the Lead-in Reproducibility Phase, a tolerable variability level will be determined by conducting assessments with up to 10 health volunteer participants. Assessments will be performed 5 times at specified time points on Day 1 and Day 2. Data will be acquired and analyzed using the measurement and data collection system. After up to 5 subjects have completed the 2-day lead-in study, data will be analyzed, and the sponsor will be consulted to see if procedural changes are needed. If reproducibility is acceptable after the first analysis, additional lead-in cohorts may not be needed. If the variability level is not acceptable, adjustments will be made to the procedure and the additional subjects will complete the lead-in study.

For Part 1 and Part 2, the procedure will be completed according to the schedule marked in Table 8-1. Similarly to the lead-in phase, subjects will be fit to ensure appropriate fit of the equipment. The clock on the rebreathing recorder will be confirmed to be consistent with the study clock at the clinical site; this will be confirmed on each treatment day. Preparatory steps for the Read Rebreathing procedure (i.e., relaxation, hyperventilation) should begin prior to the scheduled sample time. Timing of sample collection is planned so that the PD assessment begins at the specified time and a 3- to 15-minute window is planned for completion of the procedure. The rebreathing results will be reviewed in a blinded manner by FDA staff. Some of the rebreathing assessments are matched to PK assessments and in such cases, the PK blood draws will occur after the rebreathing procedure is complete.

Additional details on the Read Rebreathing Assessments are described in a separate SOP.

4.7.2 Pupillary Assessments

At Screening, subjects will be evaluated to ensure they meet requirements necessary for pupillary assessments (e.g., pupils are reactive and not misshapen). During the study, a pupillometer (a hand-held optical scanner) will be used to measure pupil size and dynamics in response to a light stimulus. Pupillary assessments will be performed before and after each rebreathing period while the ambient light is kept to a consistent level. The rubber cup of the pupillometer will be placed sequentially on the right eye followed by the left eye. The actual data recording only takes seconds on each eye.

4.7.3 Sedation Assessments

The Ramsey sedation scale will be completed by an observer prior to the start of rebreathing assessment.

- Level 1: Patient awake, anxious and agitated or restless, or both
- Level 2: Awake, cooperative, orientated, and tranquil

- Level 3: Patient awake and responds to commands only
- Level 4: Asleep, brisk response to light glabellar tap or loud auditory stimulus
- Level 5: Asleep, sluggish response to light glabellar tap or loud auditory stimulus
- Level 6: Asleep, no response to light glabellar tap or loud auditory stimulus

At the same time as performing the Ramsey sedation scale, a visual analog scale will be administered to subjects by having subjects mark on a 100 mm perpendicular line how sedated they are from 'awake and alert' to 'very sedated' (i.e. 0 and 100 on the scale, respectively). The distance from the left end of the line to the perpendicular mark will be measured in mm.

4.7.4 Pharmacokinetic Assessments

4.7.4.1 Pharmacokinetic Sample Collection

For Part 1, pharmacokinetic blood samples will be collected on Day 1 of each period (i.e., dosing days). Blood samples will be collected by direct venipuncture or by inserting an IV catheter into the subject's forearm region. A single PK blood sample (5 mL) will be collected at the following time points within each period: 0 (i.e. immediately before dosing), 1, 2, 3, 4, 6, 8, 12, and 24 h after dosing on Day 1 (Table 8-1).

For Part 2, pharmacokinetic blood samples will be collected on Days 1, 4, and 5 of each period. Blood samples will be collected by direct venipuncture or by inserting an IV catheter into the subject's forearm region. A single blood sample (5 ml) will be collected at the following timepoints: 0 (i.e., immediately before dosing), 3, 4, 5, 6, 8, 9, 12, and 24 h on Days 1, 4, and 5 of each period (Table 8-1).

Each blood sample will be labeled with subject number, study number, study day, time point, event, and a barcode that matches that belonging to the subject.

A 5-minute window for PK sample collection is allowed after the rebreathing procedure.

4.7.4.2 Pharmacokinetic Specimen Handling

The PK blood samples (5 mL each) will be collected into tubes containing K_2EDTA, inverted several times to mix the blood with the anticoagulant, and placed in an ice bath. Within 30 minutes of collection, the samples will be centrifuged for 10 minutes, at 3000 revolutions per minute, at 4°C, by a study team member.

The plasma will be separated using a disposable plastic pipette and equally aliquoted into duplicate cryotube vials labeled as Aliquot A (primary) and Aliquot B (backup). Plasma samples will be appropriately labeled and stored frozen at –70°C or below until shipment. Temperature monitoring logs should be maintained and accessible for review by the study monitor.

The Aliquot A samples (primary) will be shipped first, on dry ice, to the bioanalytical laboratory at FDA for processing when requested by the sponsor. The Aliquot B samples (backup) will be held for a second shipment at a time after the completion of all Aliquot A sample shipment(s) and the timing of the Aliquot B shipment communicated by the sponsor. None of the PK blood samples will be stored at clinical facility for future use, however the sponsor will store them for analytical purposes of this study only.

Plasma concentrations of oxycodone, midazolam, paroxetine, and quetiapine and key metabolites will be assayed using a validated liquid chromatography with tandem mass spectrometry method.

4.7.4.3 Pharmacokinetic Parameters

Due to the nature of this study, C_{max} will be computed for oxycodone on the specified days (i.e., day 1 for Part 1 and days 1 and 5 for Part 2) as part of the secondary endpoints for this study. AUC from time of drug administration to the last sample of the day on day 1 for Part 1 and day 1 and 5 for Part 2 will also be computed. AUC and C_{max} will be calculated for midazolam (Part 1, day 1) and quetiapine and paroxetine (Part 2, day 1, 4, and 5) as exploratory PK parameters. Additionally, the following exploratory PK parameters will be determined for oxycodone, midazolam, quetiapine, paroxetine, and metabolites on each specified day:

- Time at which C_{max} occurs (T_{max})
- Elimination rate constant (K_{el})
- Terminal half-life ($t_{1/2}$)

4.7.5 Additional Assessments

4.7.5.1 Sample Collection

Samples for genomic biomarker assessment will be collected at the following time points:

- Buffy coat: Day 1 (Pre-dose) for Part 1 and Part 2

These samples will be used for analyses that will be considered hypothesis-generating and exploratory.

4.7.5.2 Specimen Handling

A buffy coat from each study participant will be used for exploratory genomic analyses. On the specified collection day/time (see Table 8-1), 5 mL of whole blood sample will be processed for plasma and the upper plasma phase will be transferred to a new sterile tube without disturbing the intermediate buffy coat layer. The buffy coat layer will then be transferred to a sterile 2 mL Eppendorf (safe-lock) tube (on wet ice). Buffy coat samples should be immediately frozen in a dry ice/ethanol bath (for ~ 40 seconds) and then transferred to a -80°C freezer. Only sterile (nuclease-free) plastic pipettes, pipette tips and

tubes should be used. All barcoded buffy coat Eppendorf tubes should be frozen and stored at -80°C as described above until appropriately transported to the FDA for long-term storage. Sample analysis will be performed by DARS research staff.

4.7.6 Safety Assessments

Safety will be evaluated in terms of AEs, clinical laboratory results (hematology, serum chemistry, and urinalysis), vital sign measurements (blood pressure, heart rate, respiratory rate, pulse oximetry, and oral body temperature), telemetry, and physical examination findings.

4.7.6.1 Adverse Events

4.7.6.1.1 Adverse Event Definitions

An AE is defined as any untoward and/or unintended sign, including an abnormal clinical laboratory finding, symptom, or disease temporally associated with the use of a study drug, whether or not considered related to the study drug. Events or conditions that increase in frequency or severity during or as a consequence of use of a drug in human clinical trials will also be considered AEs.

A treatment-emergent adverse event (TEAE) is defined as an AE that begins after study drug administration.

An unexpected AE is any AE having a specificity or severity not consistent with the current investigator's brochure for the study drug(s).

An SAE is defined as any AE occurring at any dose that meets the following criteria:

- Results in death,
- Is life threatening,
- Requires hospitalization or prolongation of existing hospitalization,
- Results in persistent or significant disability or incapacity,
- Is a congenital anomaly/birth defect, or
- Is an important medical event that may not meet the previous criteria but, based upon appropriate medical judgment, jeopardizes the subject or requires medical or surgical intervention to prevent one of the outcomes listed previously.

4.7.6.1.2 Adverse Event Reporting

The recording of AEs will begin after the subject signs the informed consent form and will continue until discharge (or early termination). All AEs, whether serious or nonserious and whether or not related to the study drug, must be recorded in the eCRF. Study subjects will be instructed to warn study staff if he or she has any unexpected

symptoms. In addition, all subjects will receive a reminder telephone call approximately 24 h before Check-in.

Any SAE (whether expected or unexpected) must be entered into the eCRF system and reported by facsimile to the medical monitor or designee using the SAE Reporting Form within 24 h of the investigator or study clinic staff becoming aware of the event. It is the responsibility of the investigator to report all SAEs to the medical monitor, to provide the most complete report possible, and to assess each SAE for its relationship to the study drug. The investigator is responsible for obtaining follow-up information on all SAEs and submitting follow-up SAE data. Any unexpected SAEs must be reported promptly to the investigator's IRB as per the IRB's requirements.

In the event of a fatal or life-threatening SAE, the sponsor will notify the appropriate FDA authorities by telephone or facsimile within 7 calendar days of receipt of the report. The sponsor will follow all 7-day alert reports with a written report within 10 working days of receipt of the case. Serious AE cases that concern nonfatal, nonlife-threatening events that are unexpected and at least possibly related to the study drug will be submitted in writing to the FDA within 10 working days of receipt.

Furthermore, any AEs that are not expected, occur at a higher frequency, or would require modification of the study protocol and/or informed consent must be reported to the FDA within 10 working days.

Adverse events that are assessed by the investigator as possibly or probably related to the study drug will be followed until they resolve or stabilize. All SAEs will be followed until resolution.

4.7.6.1.3 Assessment of Severity

The investigator will assess the severity of each AE using the following scale:

- Mild: The subject is aware of the AE but is still able to perform all activities; minimal or no medical intervention or therapy is required.
- Moderate: The subject has to discontinue some activities due to the AE; minimal or no medical intervention or therapy is required.
- Severe: The subject is incapacitated by the AE and is unable to perform normal activities; significant medical intervention or therapy is required and hospitalization is possible.

4.7.6.1.4 Assessment of Causality

The investigator will assess the causal relationship/relatedness of each AE to the study drug using the following scale:

- Not Related: Onset of the AE has no reasonable temporal relationship to administration of the study drug, a causal relationship to administration of the study drug is biologically implausible, or the event is attributed to an alternative etiology.
- Unlikely Related: Onset of the AE has a reasonable temporal relationship to study drug administration and although a causal relationship is unlikely, it is biologically plausible.
- Possibly Related: Onset of the AE has a strong temporal relationship to administration of the study drug, cannot be explained by the subject's clinical state or other factors, and a causal relationship is biologically plausible.
- Probably Related: Onset of the AE shows a distinct temporal relationship to administration of the study drug that cannot be explained by the subject's clinical state or other factors, the AE is a known reaction to the product or chemical group, or can be predicted by the product's pharmacology.

4.7.6.1.5 Pregnancy

A serum pregnancy test will be performed for female subjects at the time points presented in the Schedules of Events (Section **Error! Reference source not found.**). If a subject becomes pregnant while on the study, this should be reported immediately to the investigator, the subject will be withdrawn from the study and the medical monitor and the subject will be instructed to follow-up with his or her personal physician. All pregnancies are to be reported as an AE and followed for outcome.

4.7.6.2 Clinical Laboratory Tests

Clinical laboratory and diagnostic screening tests will be performed at the time points presented in the Schedules of Events (Table 8-1) and will be collected in accordance with acceptable laboratory procedures. Clinical laboratory testing will be performed by Spaulding Clinical Laboratory, West Bend, Wisconsin, and Quest Diagnostics. The clinical laboratory tests that will be performed are presented in Table 4-6. Unused clinical laboratory test samples will not be stored for future use.

Table 4-6 Clinical Laboratory Tests and Diagnostic Screening Tests

Hematology	Serum Chemistry	Urinalysis
Hematocrit Hemoglobin Platelet count Red blood cell count White blood cell count (with automated differential)	Alanine aminotransferase Albumin Alkaline phosphatase Aspartate aminotransferase Bicarbonate Bilirubin (total, direct, and indirect) Blood urea nitrogen Calcium Chloride Creatinine (including calculated creatinine clearance) Glucose Lactate dehydrogenase Magnesium Phosphorus Potassium Sodium Total protein Uric acid	Appearance Bilirubin Blood Color Glucose Ketones Leukocyte esterase Microscopic examination: red blood cells, white blood cells, epithelial cells, bacteria, crystals, and casts (if present) Nitrite pH Protein Specific gravity Urobilinogen
Diagnostic Screening Tests:		
Serum	**Urine**	**Whole Blood**
Serology (human immunodeficiency virus Ag/Ab Combo 1/2, hepatitis C virus antibody, and hepatitis B surface antigen) **Female Subjects Only** Human chorionic gonadotropin (for pregnancy) Estrogen Progesterone	Drug screen including: amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, alcohol, opiates, phencyclidine, propoxyphene, and methadone	Cytochrome P450 2D6 genotype Cytochrome P450 3A4 genotype
Other		
SARS-CoV2 molecular test		

Clinical laboratory results will be reviewed by the investigator or designee together with data in the eCRF. Any values outside the reference range will be evaluated for clinical significance. If a value is determined to be clinically significant, the subject will be instructed to follow-up with his or her personal physician. The investigator or designee may repeat the clinical laboratory tests if deemed appropriate. The investigator will maintain a copy of the laboratory accreditation and the reference ranges for the laboratory used.

4.7.6.3 Vital Sign Measurements

Vital signs (blood pressure, heart rate, respiratory rate, pulse oximetry and oral body temperature) will be measured using an automated device at the time points presented in the Schedules of Events (Section **Error! Reference source not found.**). The subject

should be in a supine position, if possible, for a minimum of 5 minutes before vital signs are measured.

4.7.6.4 Pulse Oximetry

Continuous pulse oximetry monitoring will be performed on treatment days for Part 1 and on Days 1, 4 and 5 of each treatment period in Part 2 as specified in the Schedule of Events (Table 8-1).

4.7.6.5 Telemetry

Telemetry will be performed on treatment days for Part 1 and when oxycodone is administered in Part 2 (Days 1 and 5 of each period) as specified in the Schedule of Events (Table 8-1).

4.7.6.6 12-lead Electrocardiograms

Safety 12-lead ECGs will be performed at check-in and check-out.

4.7.6.7 Physical Examinations

A complete physical examination will be performed at the time points presented in the Schedules of Events (Section **Error! Reference source not found.**).

The complete physical examination will include, but not be limited to, assessments of the head, eyes, ears, nose, throat, skin, thyroid, nervous system, respiratory system, cardiovascular system, abdomen (liver and spleen), lymph nodes, and extremities. Height, weight (without shoes and wearing the lightest possible clothing), and calculation of body mass index will be performed at Screening only.

If a clinically significant abnormality is observed upon physical examination, the subject will be instructed to follow-up with his or her personal physician.

4.7.7 Demographics and Medical History

Demographic data (date of birth, gender, race, and ethnicity) will be collected at Screening.

Each subject will provide a complete medical history at Screening that will be reviewed at Check-in. Specific information relating to any prior or existing medical conditions/surgical procedures will be recorded in the subject's eCRF.

4.8. Study Treatments

4.8.1 Dose Strategy

Part 1

Prior data with oxycodone alone using 20 mg doses, showed significant decreases in the ventilatory response to hypercapnia (VE55) from baseline (van der Schrier 2017a; van der Schrier 2017b). Previous studies have been conducted with midazolam doses of 0.075 mg/kg and 0.15 mg/kg using the procedure, but the effect on VE55 was not reported (Forster et al, 1980; Power et al, 1983). The goal with the oxycodone dose is to have a definable effect on the ventilatory response to hypercapnia with oxycodone alone, but not too high (for safety considerations) as it will be combined with a benzodiazepine or other psychotropic drug. The goal with midazolam is to have a safe dose that reduces the ventilatory response to hypercapnia when co-administered with oxycodone compared to oxycodone alone.

Approximately twenty participants are planned for enrollment. Participants will enter the clinic on a staggered basis in cohorts of approximately five (i.e., approximately five participants will undergo dosing, PK assessments, and PD assessments on any given day). Subjects will be staggered up to 15 minutes apart in order to allow for direct safety overview by medical staff during dosing and Read rebreathing. Interim analyses will be performed after approximately 5 participants have completed procedures and the doses of oxycodone and/or midazolam may be adjusted at that time. The oxycodone dose may be increased from 10 mg to 15 mg if the conditional power to of concluding a decrease for oxycodone from placebo is less than 90% (difference of 12-18 L/min observed for 20 mg oxycodone; van der Schrier et al, 2017a; van der Schrier et al, 2017b). Likewise, midazolam dose may be increased from 0.0375 mg/kg to 0.075 mg/kg if the conditional power of concluding a decrease of the combined effect of oxycodone and midazolam compared to oxycodone is less than 80%. The decision to dose escalate either drug will also consider clinical evaluations and whether stopping criteria was attained in any of the participants. The remaining subjects will complete the study at the updated doses in cohorts of approximately five participants each.

The primary analysis will occur at the 2 h timepoint. If dose adjustments are not needed, the analysis will be based on all subjects with statistical adjustment for the interim analysis. Otherwise, the analysis will be limited to the subjects administered the higher doses of oxycodone and/or midazolam with no statistical adjustment.

Part 2

Approximately twenty participants are planned for enrollment. Participants will enter the clinic on a staggered basis in cohorts of approximately five (i.e., approximately five participants will undergo dosing, PK assessments, and PD assessments on any given day). Subjects will be staggered up to 15 minutes apart in order to allow for direct safety overview by medical staff during dosing and Read rebreathing. For each period, subjects are administered oxycodone (either 10 mg or 15 mg depending on findings from Part 1) on Day 1 and Day 5 at time zero. Timing of dosing for paroxetine and quetiapine and appropriate placebos will be staggered so that T_{max}

occurs at 5 h for all drugs. Paroxetine (or placebo) doses will be administered at time 0 on each day. The first daily dose of quetiapine (or placebo) and oxycodone dosing will occur at 3 h on dosing days; the second daily dose of quetiapine (or placebo) will be administered at approximately 14 h.

Quetiapine dosing was selected to follow labeled dosing for Bipolar I Disorder, Mania. Paroxetine dosing is titrated from 20 mg up to a maximum of 60 mg; dosing was selected to evaluate potential interactions at a 40 mg dose.

4.8.2 Treatments Administered

Subjects will receive each of the following treatments according to the randomization schedule. For Part 1, drug administration will occur on Day 1 for each of the four periods. For Part 2, drug administration will occur on Days 1, 2, 3, 4, and 5 for each of the three periods according to the treatments described below.

Part 1:

- Treatment A: Oxycodone (10 or 15 mg IR) + placebo IV
- Treatment B: Oral placebo + midazolam IV (0.0375 or 0.075 mg/kg IV infusion over 2 minutes)
- Treatment C: Oxycodone (10 or 15 mg IR) + midazolam IV (0.0375 or 0.075 mg/kg IV infusion over 2 minutes)
- Treatment D: Oral placebo + placebo IV

Ondansetron 4 mg will occur at -30 min. Oxycodone or oral placebo dosing will occur at time 0 and midazolam or placebo IV dosing will occur 115 minutes after oxycodone or oral placebo administration.

Part 2:

- Treatment E: Oxycodone (10 or 15 mg IR) + Placebo on Days 1 and 5; Placebo on Days 2-4
- Treatment F: Paroxetine (40 mg IR) + Oxycodone (10 or 15 mg IR) on Day 1; Paroxetine (40 mg IR) on Days 2-4; Paroxetine (40 mg IR) + Oxycodone (10 or 15 mg IR) on Day 5
- Treatment G: Quetiapine (50 mg BID) + Oxycodone (10 or 15 mg IR) on Day 1; Quetiapine (100 mg BID) on Day 2; Quetiapine (150 mg BID) on Day 3; Quetiapine (200 mg BID) on Day and 4; Quetiapine (200 mg) + Oxycodone (10 or 15 mg IR) on Day 5

Paroxetine (or placebo) doses will be administered at time 0 on each day. The first daily dose of quetiapine (or placebo) and oxycodone dosing will occur at 3 h on dosing days; the second daily dose of quetiapine (or placebo) will be administered at

approximately 14 h. Participants will also receive ondansetron 4 mg 30 min before oxycodone administration on day 1 and day 5. For additional details, see Table 4-5.

Study drugs will be administered by a clinical research nurse on the study clinic floor at the subject's bedside. The pharmacist and investigator will be available if needed during study drug administration. Oral study drugs will be administered with 240 mL of room-temperature water.

4.8.3 Dose Selection

4.8.3.1 Oxycodone

The initial dose selected for oxycodone is 10 mg IR. If required based on the dose escalation study in Part 1 (see Section 4.8), the dose will be increased to 15 mg IR. Oxycodone dose selection is based on previous published results where oxycodone was administered alone or in combination using rebreathing procedures in healthy volunteers. Two studies by van der Schrier and others evaluated oxycodone 20 mg alone or in combination with ethanol. Sedation levels in the ethanol study in particular, which included a higher dose of oxycodone and was performed in the young and elderly, were moderate and well addressed by mild stimulation of the subjects. Likewise, varying dose combinations of oxycodone and morphine (total combined doses of 15 mg) have been safely evaluated using a similar system (Ladd et al, 2005). Based on these results, oxycodone 10 mg is expected to have minimal-to-no effects on ventilation when breathing room air, and a small but measurable decrease in ventilatory response to hypercapnia during rebreathing.

4.8.3.2 Midazolam

The initial dose selected for midazolam is 0.0375 mg/kg IV. If required based on the dose escalation study in Part 1 (see Section 4.8), the dose will be increased to 0.075 mg/kg IV. Midazolam dose selection is based on previous published results where midazolam was administered alone or in combination with other drugs using rebreathing procedures in healthy volunteers. Midazolam doses up to 0.15 mg/kg have been safely evaluated and published using this experimental procedure (Forster et al, 1980). Other doses of midazolam have been evaluated alone (Power, 1983 – 0.075 mg/kg) or in combination with nalbuphine (Sury et al, 1988 – midazolam 0.05 mg/kg with nalbuphine 0.05, 0.10, or 0.20 mg/kg) or fentanyl with flumazenil for reversal (Mora et al, 1995). Based on these results, midazolam is expected to have minimal-to-no effects on ventilation when breathing room air, and a small but measurable decrease in ventilatory response to hypercapnia during rebreathing.

4.8.3.3 Paroxetine

The dose selected for paroxetine is 40 mg IR for Days 1-5. Paroxetine dose selection was based on available safety data from the original development program and to achieve typical steady state exposures of paroxetine by the morning of day 5 for the study. Target dosing range for paroxetine is 20-60 mg/day depending on the indication. Initial starting doses in labeling for general anxiety disorder, social anxiety disorder, posttraumatic stress disorder, obsessive compulsive disorder, and major depressive disorder are 20 mg/day with dose increases of 10-mg/day made in intervals of 1 week. Paroxetine has been administered at 30 mg/day for 30-days in healthy volunteers and up to 60-mg as a single dose in healthy volunteers (Heydorn et al, 1999).

4.8.3.4 Quetiapine

The dose selected for quetiapine is 50 mg BID on Day 1, 100 mg BID on Day 2, 150 mg BID on Days 3, 200 mg BID on Day 4 and 200 mg QD on Day 5. Quetiapine dose selection was based on approved product labeling for Bipolar I Disorder, Mania.

4.8.3.5 Ondansetron

The dose selected for ondansetron is 4 mg orally given 30 minutes before oxycodone administration. Up to two additional doses of IV ondansetron 4 mg can be given in a day for nausea/vomiting. The selected ondansetron dose is less than the recommended dosing for labeled indications of nausea and vomiting associated with highly or moderately emetogenic cancer chemotherapy, radiotherapy, or postoperative nausea or vomiting.

4.8.3.6 Naloxone

The dose selected for naloxone is 0.4 mg administered intravenously, as needed, for respiratory depression as deemed necessary by the investigator. Naloxone may be administered as a rescue medication for opioid-induced respiratory depression.

4.8.3.7 Flumazenil

The dose selected for flumazenil is 0.2 mg (2 mL) administered over 15 seconds. If the desired level of consciousness is not obtained after waiting an additional 45 seconds, a second dose of 0.2 mg (2 mL) can be injected and repeated at 60 second intervals where necessary to a maximum total of 1 mg. Flumazenil may be administered as a rescue medication for sedative effects from benzodiazepines in the current study.

4.8.4 Method of Assigning Subjects to Treatment Sequence

4.8.4.1 Randomization Process

The project biostatistician will create the specifications that will be used to generate the randomization schedule. The specifications will be based on the protocol requirements

and appropriate statistical programming with consideration for study design, number of treatments, number of subjects planned for enrollment, stratification, and blocking.

Based on these specifications, the project biostatistician (or designee) will generate a dummy randomization schedule. The schedule is generated using R.

The project biostatistician (or designee) distributes the 'dummy' randomization schedule to specified personnel for review. Any change (e.g., change in block size, change in stratification levels) that requires an update to the specifications will reset this process. Minor changes (e.g., display formatting) will not require a change to the specifications.

After the approval of the 'dummy' randomization schedule, the project biostatistician (or designee) transfers the program used to generate the 'dummy' schedule to the randomization biostatistician (unblinded), who is an independent party and will not be participating in any programming or statistical decisions for the study before breaking the blind. No transfer is necessary if the unblinded randomization biostatistician also created the 'dummy' randomization.

The randomization biostatistician is responsible for generating the final randomization schedule. The output is sent only to designated unblinded recipients, who will maintain a secured digital and printed copy for their use.

Archival of the programs and output is accomplished by the creation of an encrypted, password-protected ZIP file containing the program and output file(s).The ZIP file is copied to a secure storage drive on the sponsor's site. Randomization will occur after informed consent is obtained, either after completion of check-in procedures on Day -1 or just before dosing on Day 1. Approximately 50 healthy male and female subjects are planned for enrollment, of which up to 10 will be assigned to the Lead-in Reproducibility Assessment, up to 20 will be assigned to Part 1, and up to 20 will be assigned to Part 2 at randomization. Up to 10 subjects may be qualified as replacements as described in Section 4.5.2. Thus, a maximum of 60 subjects will be exposed to study drugs and procedures during the study.

Enrolled subjects will be assigned to 1 of 3 different parts. Subsequently:

- Subjects assigned to the Lead-in Reproducibility Assessment will not receive any drug treatments but will participate in the Read Rebreathing Assessments. After up to 5 subjects complete the 3-day Reproducibility Assessment study, the data will be analyzed. If reproducibility is acceptable after the first analysis, additional lead-in cohorts may not be needed. If any study procedure element changes are needed, they will be made at this point. Then, additional participants may be enrolled, and data will be analyzed. Replacement subjects will be assigned to the group if necessary to complete the assessments.

- Subjects assigned to Part 1 will be randomly assigned to the order in which they will complete each of the 4 treatments over the 4 periods. Thus each subject will receive each of the treatments presented in Table 4-7 below.
- Subjects assigned to Part 2 will be randomly assigned to the order in which they will complete each of the 3 treatments over the 3 periods. Thus each subject will receive each of the treatments described in Table 4-8 below.

Table 4-7 Study Part 1 Treatment Groups

Subjects (n)	Treatment	Day 1 Treatment	Day 2-3
20	A	Oxycodone + placebo IV	Wash-out
	B	Oral placebo + midazolam IV	Wash-out
	C	Oxycodone + midazolam IV	Wash-out
	D	Oral placebo + placebo IV	Wash-out

Table 4-8 Study Part 2 Treatment Groups

Subjects (n)	Treatment	Day 1	Day 2	Day 3	Day 4	Day 5
20	E	Oxycodone + Placebo	Placebo	Placebo	Placebo	Oxycodone + Placebo
	F	Paroxetine + Oxycodone	Paroxetine	Paroxetine	Paroxetine	Paroxetine + Oxycodone
	G	Quetiapine + Oxycodone	Quetiapine	Quetiapine	Quetiapine	Quetiapine + Oxycodone

All randomization information will be secured and housed in a locked storage area, accessible only by the randomization personnel and the assigned pharmacist and his or her verifier.

4.8.5 Identity of Study Drugs

Oxycodone HCl (referred to throughout this document as oxycodone) is indicated for the management of pain severe enough to require an opioid analgesic and for which alternative treatments are inadequate. Oxycodone tablets are available as 5, 10, 15, 20, or 30 mg strengths. The tablets are designed for immediate release of the drug where about 60% to 87% of the drug reaches systemic circulation in comparison to a parenteral dose. Oxycodone HCl has a molecular weight of 351.82 and the molecular formula $C_{18}H_{21}NO_4 \bullet HCl$. The physical form is a white odorless crystalline powder derived from the opium alkaloid, thebaine. Oxycodone dissolves in water (1 g in 6 to 7 mL) and is considered slightly soluble in alcohol. (SpecGX LLC prescribing information, 2018)

Midazolam HCl (referred to throughout this document as midazolam) has several indications as a sedative agent: preoperative sedation; sedation/anxiolysis/amnesia prior to or during diagnostic, therapeutic, or endoscopic procedures; induction of general anesthesia, before administration of other anesthetic agents; or continuous infusion for sedation of intubated and mechanically ventilated patients as a component of anesthesia or during treatment in a critical care setting. Midazolam HCl is a water-soluble benzodiazepine available as a sterile, nonpyrogenic parenteral dosage form for intravenous or intramuscular injection. Midazolam is a white to light yellow crystalline compound, insoluble in water. The hydrochloride sale of midazolam, formed *in situ*, is soluble in aqueous solutions. The molecular weight of Midazolam HCl is 362.25 and the molecular formula is $C_{18}H_{13}ClFN_3 \bullet HCl$. Midazolam Injection, USP pharmacy bulk package is available as 500mg midazolam in a 100 mL vial. (Fresenius Kabi USA package insert, 2017)

Paroxetine HCl (referred to throughout this document as paroxetine) is indicated for the treatment of major depressive disorder. Paroxetine HCl has a molecular weight of 374.8 and the empirical formula is $C_{19}H_{20}FNO_3 \bullet HCl \bullet 1/2H_2O$. Paroxetine HCl is an odorless, off-white powder with a solubility of 5.4 mg/mL in water. Tablets are available for oral administration in 10, 20, 30, and 40 mg strengths. (Apotex Technologies prescribing information, 2017)

Quetiapine fumarate (referred to throughout this document as quetiapine) is indicated for the treatment of schizophrenia and bipolar disorder. Quetiapine fumarate has a molecular weight of 883.11 and the molecular formula is $C_{42}H_{50}N_6O_4S_2 \bullet C_4H_4O_4$. Quetiapine fumarate is a white to off-white crystalline powder which is moderately soluble in water. Quetiapine fumarate is available for oral administration as 25, 50, 100, 200, 300, and 400 mg tablets. (Astrazeneca Pharms prescribing information, 2018)

Ondansetron is indicated for the prevention of nausea and vomiting associated with highly emetogenic cancer chemotherapy, including cisplatin greater than or equal to 50 mg/m2, initial and repeat courses of moderately emetogenic cancer chemotherapy, radiotherapy in patients receiving either total body irradiation, single high-dose fraction to the abdomen, or daily fractions to the abdomen, and prevention of postoperative nausea and/or vomiting. The empirical formula is C18H19N3O•HCl•2H2O, representing a molecular weight of 365.9. Ondansetron hydrochloride dihydrate is a white to off-white powder that is soluble in water and normal saline. Ondansetron is available for oral administration as 4 and 8 mg tablets. (Novartis, prescribing information, 2017). Ondansetron hydrochloride is supplied as 20 mL multi-dose vials, 2 mg/mL. (Mylan Institutional LLC, prescribing information, 2017)

Naloxone hydrochloride injection is indicated for the complete or partial reversal of opioid depression, including respiratory depression, induced by natural and synthetic opioids. Naloxone hydrochloride is also indicated for the diagnosis of suspected or

known acute opioid overdosage. Naloxone hydrochloride, an opioid antagonist, is a synthetic congener of oxymorphone, with a molecular weight of 363.84 and molecular formula of C19H21NO4 • HCl. Naloxone hydrochloride injection may be diluted for intravenous infusion in 0.9% sodium chloride injection or 5% dextrose injection. The addition of 2 mg of naloxone hydrochloride in 500 mL of either solution provides a concentration of 0.004 mg/mL. Mixtures should be used within 24 h. After 24 h, the remaining unused solution must be discarded. Naloxone hydrochloride injection is supplied as 10 mL multi-dose vials, 4 mg/10 mL (0.4 mg/mL). (West-Ward prescribing information, 2018)

Flumazenil injection is indicated for reversal of the sedative effects of benzodiazepines administered conscious sedation, general anesthesia, or management of a known or suspected benzodiazepine overdose. Flumazenil injection is a benzodiazepine receptor antagonist. Chemically, flumazenil is ethyl 8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5-a](1,4) benzodiazepine-3-carboxylate. Flumazenil has an imidazobenzodiazepine structure, a calculated molecular weight of 303.3. Flumazenil is a white to off-white crystalline compound with an octanol:buffer partition coefficient of 14 to 1 at pH 7.4. It is insoluble in water but slightly soluble in acidic aqueous solutions. Flumazenil is available as a sterile parenteral dosage form for intravenous administration. Flumazenil is supplied as either 5- or 10-mL multi-dose vials. (Akorn-Strides, LLC prescribing information, 2008)

Placebo capsules containing only cornstarch will be used as the placebo oral control in this study.

Placebo IV containing saline will be used as the placebo IV control in this study.

4.8.6 Management of Clinical Supplies

4.8.6.1 Study Drug Packaging and Storage

The active study drugs will be obtained from commercial sources. Storage instructions for the active study drugs are as follows:

- Oxycodone tablets should be stored at 25°C (77°F) with excursions permitted from 15° to 30°C (59° to 86°F) and should be protected from moisture. (Roxane Laboratories, Inc. prescribing information, 2018)
- Midazolam IV should be stored at 20° to 25°C (68° to 77°F). (Fresenius Kabi USA prescribing information, 2017)
- Paroxetine tablets should be stored between 15° and 30°C (59° and 86°F). (Apotex Technologies prescribing information, 2014 and 2017)

- Quetiapine tablets should be stored at 25°C (77°F) with excursions permitted from 15° to 30°C (59° to 86°F). (Astrazeneca Pharms prescribing information, 2018)
- Naloxone hydrochloride injection should be stored at 20° to 25°C (68° to 77°F). (West-Ward prescribing information, 2018)
- Flumazenil injection should be stored at 20° to 25°C (68° to 77°F). (Akorn-Strides, LLC prescribing information, 2008)
- Ondansetron tablets should be stored at 2° to 30°C (36° to 86°F). (Novartis, prescribing information, 2017)
- Ondansetron injection should be stored at 20° to 25°C (68° to 77°F). (Mylan Institutional LLC, 2017)

Placebo capsules and placebo IV will be supplied by the clinical site, stored at controlled room temperature (15°C to 30°C; 59°F to 86°F), and protected from light and moisture.

4.8.6.2 Study Drug Accountability

Good clinical documentation practices will be employed to record the receipt, storage conditions, accountability, and use or return of the study drug. The study drug will be stored in a secure location with access to the study personnel who will be managing the storage, dispensing, and accountability of the study drug.

Upon completion or termination of the study, final accountability review by the study monitor, and written authorization from the sponsor, all unused and/or partially used study drug should be returned or destroyed at the study clinic. It is the investigator's responsibility to ensure that the sponsor has provided written authorization for study drug disposal, the disposal process follows the study clinic's standard operating procedures, and appropriate records of the disposal are documented and maintained. No unused study drug may be disposed until fully accounted for by the study monitor (or designee). Documentation of unused study drug should include subject number, medication identity (medication #, period #), date, and quantity of study drug used.

4.8.7 Blinding

The study will be double-blind and the blind will be maintained through a randomization schedule held by the dispensing pharmacist. In addition, subjects will be blindfolded during study drug administration and the Read Rebreathing data analysis will be blinded to treatment. The pharmacist (and designated staff member responsible for confirmation of study drug dose) will be unblinded to subject treatment assignment; however, the pharmacist will not perform any study procedures other than study drug preparation and dispensing.

Additional details regarding blinding can be found in the Spaulding Blinding SOP which will be followed to ensure the blind is maintained throughout the study.

4.8.7.1 Breaking the Blind

The study drug blind will not be broken by the investigator or designee unless information concerning the study drug is necessary for the medical treatment of the subject. For unblinding a subject, the randomization information for unblinding can be obtained by contacting the dispensing pharmacist. The sponsor or medical monitor must be notified immediately if the study drug blind is broken. The date, time, and reason that the blind was broken will be recorded in the source documents. If the blind is broken by the investigator or designee, the study drug must be stopped immediately and the subject must be withdrawn from the study. Data or specimens already collected from subjects who discontinue prematurely and for whom the blind is broken will be made available for analysis if needed.

4.8.8 Treatment Compliance

At Screening, as part of the inclusion criteria, it will be confirmed that subjects are able to comply with the protocol-defined procedure of ingesting oral study drug or receiving IV infusions. All doses of the study drug will be administered in the study clinic either under direct observation of or administered by clinic personnel, and recorded in the eCRF. Clinic personnel will perform a hand and mouth check to confirm and document that the subject has ingested the entire dose of study drug. If a subject vomits after dosing, the event will be documented as an AE. The decision to replace any subject who vomits after dosing will be made as described in Section 4.5.2.

4.8.9 Prior and Concomitant Medications

Subjects are prohibited from using any prescription or nonprescription drugs (including aspirin or NSAIDs and excluding oral contraceptives and acetaminophen) within 14 days or 5 half-lives (whichever is longer), or complementary and alternative medicines within 28 days before the first dose of study drug. Subjects are prohibited from using any opioid or psychotropic drugs within 60 days of the start of the study.

Subjects are also prohibited from currently participating in another clinical study of an investigational drug and may not have been treated with any investigational drug within 30 days or 5 half-lives (whichever is longer) of the compound.

Subjects must be instructed not to take any medications, including over-the-counter products, without first consulting with the investigator.

4.8.10 Subject Restrictions

Subjects are not allowed to use nicotine-containing products (e.g., cigarettes, cigars, chewing tobacco, snuff) within 6 weeks before Screening. In addition, subjects are not allowed to ingest alcohol, xanthine-containing products (e.g., tea, coffee, chocolate, cola), caffeine, grapefruit, or grapefruit juice for 48 h before dosing and throughout the study. Subjects are not allowed to use aspirin or NSAIDs within 14 days before the first dose of study drug. Subjects will be asked if they have used any of these substances and their responses will be recorded on the eCRF.

Subjects must be able to tolerate a controlled, quiet study conduct environment, including avoidance of music, television, movies, games, and activities that may cause excitement, emotional tension, or arousal during prespecified times (e.g., before and during rebreathing assessment windows) throughout the duration of the study.

Subjects must be willing to comply with study rules, including the meal schedule, attempting to void at specified times (e.g., before rebreathing assessment windows), remaining quiet, awake, undistracted, motionless, and seated during specified times, and avoiding vigorous exercise as directed throughout the duration of the study. Subjects will not be allowed to sleep during any rebreathing assessment periods.

Standardized meals will be served at consistent times relative to dosing, and no food or fluids will be served containing caffeine. Subjects will fast during the intensive PD assessment portions of the Lead-In, Part 1, and Part 2. Subjects will be provided their first meal after the 6 h PD assessment for the Lead-in and for Part 1 of the study. For Part 2 on Days 1 & 5 subjects will be provided with a light snack after the 6 h PD assessment and their first meal after the 8 h PD assessment. For Part 2 on Day 4 subjects will be provided their first meal after the 6 h PD assessment. Subjects will have a second meal in the late evening, and a snack prior to bed. Outside of meal times, the subjects will only be allowed to intake water, which will be available ad libitum.

4.9. Statistical Methods

4.9.1 Sample Size

Up to 60 healthy subjects will be enrolled (including 10 potential replacement subjects).

4.9.1.1 Lead-in Reproducibility Assessment

For the Lead-in Reproducibility Assessment, the sample size of up to 10 subjects (up to 5 subjects assessed initially, followed by additional subjects if needed) was selected empirically to acquire experience for the research staff with use of the rebreathing equipment and to assess performance over repeated assessments in a participant over multiple days. Based on prior publications, it is expected that the Lead-In will have a VE55 variability of approximately 5 L/min.

4.9.1.2 Part 1

For Part 1, the sample size of approximately 20 subjects in a crossover design was selected with consideration to both the effect of oxycodone versus placebo and oxycodone plus midazolam versus oxycodone. Assuming a -4 L/min effect size on VE55 and standard deviation of 5 L/min, there is greater than 90% power at a one-sided significance level. In the event that dose escalation of either oxycodone or midazolam are needed, the study has greater than 80% power at a one-sided significant level assuming these same effect sizes at the higher dose or doses. Part 1 is not designed to terminate early based on assessment of approximately 5 subjects at the starting dose.

4.9.1.3 Part 2

For Part 2, the sample size of 20 subjects in a crossover design was selected with consideration to the effect of oxycodone plus concomitant medication (quetiapine or paroxetine) at day 1 and day 5. Assuming a -4 L/min effect size on VE55 and standard deviation of 5 L/min, there is greater than 90% power at a one-side significance level. Both days will be considered as separate tests with an adjustment for multiplicity.

4.9.2 Analysis Populations

The analysis population will be defined differently for Lead-In, Part 1, and Part 2. For Lead-In, the analysis population will include all subjects who completed at least one rebreathing assessment.

For Part 1 and Part 2, the pharmacodynamic analysis population will include all subjects with data from at least two treatments, including receiving all doses of study drug and completing rebreathing assessments at the specified primary timepoint (2 h for Part 1 and 5 h for Part 2). A rebreathing assessment will be considered complete if the subject makes it through the entire procedure at a specific timepoint, if there are no identifiable issues with how the procedure was conducted, and if the VE55 regression converges. Potential issues with how the procedure was conducted can included, but are not limited to, a leak from the system (e.g., substantially decreasing O_2 during rebreathing or no evidence of an increase in CO_2 during rebreathing) or inaccurate readings from the pneumotach (e.g., baseline minute ventilation readings less than 5 L/min).

Subjects in this population will be used for the planned primary and secondary analyses related to evaluating drugs effects on the ventilatory response to hypercapnia. If a subject does not contribute data from all treatments due to early discontinuations or other reasons, only those comparisons where the subject has all required data will be performed. For example, to be included in the primary analysis from Part 1 it is necessary that a subject have a completed rebreathing assessment at 2 h for oxycodone with midazolam and oxycodone alone.

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

The safety population will include all subjects who receive at least one dose of any of the study drugs.

4.9.3 General Statistical Considerations

All data will be presented in data listings. Data from subjects excluded from an analysis population will be presented in the data listings, but not included in the calculation of summary statistics.

4.9.4 Subject Disposition

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

4.9.5 Demographics and Baseline Characteristics

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

4.9.6 Rebreathing Analyses

4.9.6.1 Primary Analysis

Part 1

Part 1 of the study will assess the primary objective for oxycodone and midazolam. The primary objective of Part 1 is to study whether combining midazolam with oxycodone decreases ventilatory response to hypercapnia compared to oxycodone alone. The primary endpoint for the analysis will be a comparison of the minute ventilation at the 55 mm Hg end tidal CO_2 point (VE55) for midazolam co-administered with oxycodone vs. oxycodone alone.

Part 2

Part 2 of the study will assess the primary objective for paroxetine and quetiapine with reference to oxycodone. The primary objective of Part 2 is to study whether combining paroxetine or quetiapine with oxycodone decreases ventilatory response to hypercapnia compared to oxycodone alone. Primary assessments for the two psychotropic drugs combined with oxycodone vs oxycodone alone will occur on Days 1 and 5 (multiple

primary endpoints). The primary endpoint for the analysis will be a comparison of the minute ventilation at the 55 mm Hg end tidal CO_2 point (VE55) for paroxetine or quetiapine co-administered with oxycodone vs. oxycodone alone.

4.9.6.2 Data Analysis

Baseline and on-treatment minute ventilation versus $P_{ET}CO_2$ data will be analyzed using nonlinear regression at the specified primary timepoints. The regressions will be used to predict minute ventilation at $P_{ET}CO_2$ of 55 mm Hg (VE55). Individual baseline-adjusted values will be calculated for each subject by subtracting on-treatment VE55 from baseline. VE55 will be compared between treatments for Part 1 and Part 2. For Part 1 the comparisons will be between oxycodone versus placebo, oxycodone + midazolam versus oxycodone, and midazolam versus placebo. VE55 will be compared between treatments using a linear mixed effects model. No adjustment for multiplicity will be included.

For Part 2, the comparisons will be between oxycodone and quetiapine/paroxetine versus oxycodone on day 1 and day 5. Secondary assessments will include a comparison between quetiapine/paroxetine versus placebo on day 4. VE55 will be compared between treatments using a linear mixed effects model. Both days will be considered as separate tests with an adjustment for multiplicity.

4.9.7 PK/PD Modeling

A nonlinear-mixed effect pharmacokinetic/pharmacodynamic model will be developed for VE55 and baseline-adjusted VE55 versus time for all treatments. The model will be developed using NONMEM modeling software, version 7.3.

The PK/PD analysis will be sequential, first developing pharmacokinetic models for each of the four drugs evaluated in this study. These will be one- or two compartment models with linear absorption, as appropriate. The PK modeling will not include covariate exploration but will attempt to model key metabolites for oxycodone and quetiapine. As there is extensive experience with all of these compounds, models from the literature will serve as starting points for model evaluation.

The PD modeling will use predictions from the PK modeling as input. Different model structures will be evaluated, including an effect compartment coupled to an indirect response model or a modified Bateman function. No covariate exploration will be performed for the PD model, though stand-alone interactions between drug effects will be evaluated.

4.9.8 Pharmacokinetic Analyses

The PK parameters C_{max}, AUC_{0-t}, T_{max}, and K_{el} will be summarized using descriptive statistics (number of subjects, geometric mean, coefficient of variation [CV], mean, SD, median, minimum, and maximum) on Day 1 of each period for Part 1 and Days 1, 4, and

5 of each period for Part 2 for each active drug. The PK parameters will be analyzed using noncompartmental methods based on actual sampling times. All parameters will be calculated using SAS or R software. Geometric mean and individual concentration-time profiles will be presented in graphs.

4.9.9 Additional Analyses

4.9.9.1 Exploratory Respiratory Analyses

Other respiratory measures will be calculated and summarized from information captured during the scheduled Read Rebreathing procedure and from continuous pulse oximetry data collected during study days. Data collected during the Read Rebreathing procedure will be used to determine the slope of the MV / PETCO2 regression line. Resting ventilation, tidal volume end-tidal PCO2, and oxygen saturation will be determined using data from the relaxation portion of the Read Rebreathing procedure. Apneic events lasting > 10 s will be determined using data collected during relaxation and preparation portion of the Read Rebreathing procedure. An event is defined as the absence of inspiratory flow (as measured by the pneumotachograph) for at least 10 s during this period. These parameters will be summarized using descriptive statistics, and time courses summaries of both scores will be generated for all treatment groups. These analyses will be considered hypothesis-generating and exploratory.

4.9.9.2 Pupillometry Analyses

Maximum pupil diameter before constriction and dynamic pupillary measurements after a light stimulus will be measured before and after each Read rebreathing assessment in both eyes. These parameters will be summarized using descriptive statistics, and time courses summaries of both scores will be generated for all treatment groups. Pupillary changes will be compared to baseline measurements and between treatments to evaluate the effect of different interventions on pupillary changes. In addition, time course pupillary changes will be compared to time course ventilatory changes across treatments to evaluate concordance between these measures when subjects receive different drugs and drug combinations. These analyses will be considered hypothesis-generating and exploratory.

4.9.9.3 Sedation Scores Analyses

Ramsay Sedation Scale is an observer-based assessment of sedation that will be collected for each subject as during the relaxation period of each Read Rebreathing procedure. In addition, subjects will be asked to provide their own assessment of sedation using the Visual Analog Scale during the same relaxation period. These are two measures for assessing an individual's level of sedation and will provide a subjective assessment of how sedation the subject is experiencing during the study. These parameters will be

summarized using descriptive statistics, and time courses summaries of both scores will be generated for all treatment groups. These analyses will be considered hypothesis-generating and exploratory.

4.9.9.4 Genomic Analyses

Gene (genotype) variances may be explored that may contribute to the PK or PD of the study drugs. These analyses will be considered hypothesis-generating and exploratory.

4.9.9.5 Sex Hormone Analyses

Estrogen and progesterone levels will be collected at the start of all study days with a Read Rebreathing assessment and may be used to explore variation in ventilation and the response to study drugs across the menstrual cycle. These analyses will be considered hypothesis-generating and exploratory.

4.9.10 Safety Analyses

4.9.10.1 Adverse Events

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

4.9.10.2 Clinical Laboratory Tests

Clinical laboratory results will be reviewed by the investigator or designee together with data in the eCRF. Any values outside the reference range will be evaluated for clinical significance. If a value is determined to be clinically significant, the subject will be instructed to follow-up with his or her personal physician. The investigator or designee may repeat the clinical laboratory tests if deemed appropriate. The investigator will maintain a copy of the laboratory accreditation and the reference ranges for the laboratory used.

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

4.9.10.3 Vital Sign Measurements

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

4.9.10.4 Pulse Oximetry and Telemetry

Continuous pulse oximetry and telemetry monitoring will be performed on treatment days for Part 1. For Part 2, telemetry monitoring will be performed on Days 1 and 5 and continuous pulse oximetry will be performed on Days 1, 4, and 5 of each period. Events requiring intervention from the staff or discontinuation from the study will be recorded in appropriate logs.

4.9.10.5 Safety 12-lead Electrocardiograms

12-lead ECGs will be obtained with the subjects in the supine position for a minimum of 5 minutes before recording. ECGs will be overread by a physician. If an abnormality is observed, the subject will be instructed to follow-up with his or her personal physician.

4.9.10.6 Physical Examinations

Physical examination findings will be presented in a data listing, and abnormal physical examination findings will be recorded as AEs.

4.9.10.7 Other Safety Data

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

4.9.11 Interim Analyses

In Part 1, the total number of participants to be enrolled is approximately 20. Interim analyses will be performed after approximately 5 participants have completed procedures, and the doses of oxycodone and/or midazolam may be adjusted at that time. The oxycodone dose may be increased if the conditional power of concluding a decrease in oxycodone from placebo is less than 90%. Likewise, midazolam dose may be increased from 0.0375 mg/kg to 0.075 mg/kg if the conditional power of concluding a decrease of the combined effect of oxycodone and midazolam compared to oxycodone is less than 80%. The remaining subjects will complete the study at the updated doses.

4.9.12 Missing Data

Missing data will not be imputed. Data that are excluded from the descriptive or inferential analyses will be included in the subject data listings. This will include data from subjects not in the particular analysis population, measurements from unscheduled

visits, or extra measurements that may arise from 2 or more analyses of a plasma sample at the same time point. Details on the handling of missing data will be further described in the Statistical Analysis Plan.

4.10. Data Quality Assurance

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

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

5. Ethical Considerations

5.1. Ethical Conduct of the Study

This study will be performed in accordance with the recommendations guiding physicians in biomedical research involving human subjects adopted by the 18th World Medical Association General Assembly, Helsinki, Finland, 1964 and later revisions, as well as, United States Title 45 Code of Federal Regulations (CFR) Part 46 GCP, and International Council for Harmonisation (ICH) guidelines describing technical requirements for registration of pharmaceuticals for human use.

5.2. Institutional Review Board (IRB)

After the protocol is approved by FDA, FDA staff with primary responsibility for the FDA's involvement with the project (i.e., the FDA Project Lead) will submit the protocol and associated documentation to FDA's Office of the Chief Scientist to facilitate an Institutional Review Board Authorization Agreement (IAA) with FDA as the relying institution. The FDA Project Lead or investigator will provide the local IRB (i.e., Advarra) with all required documents, including the study protocol and informed consent form. The study will not be initiated until appropriate IRB approval is obtained from the local IRB. The investigator will provide the FDA Project Lead with copies of the approval documents for the protocol, informed consent form, and all recruiting materials.

The local IRB will also receive copies of any original or amended information sheets or pamphlets given to the study subject in support of the informed consent process and any advertisements or other recruitment material. Such materials will not be employed in the study before approval by the local IRB.

Subjects will be informed that they have the right to contact the local IRB or Office for Human Research Protections if they have any questions, concerns, complaints, or believe they have been harmed by the participation in this research study as a result of investigator negligence. Subjects will be given the address and phone number of the local IRB.

6. Administrative Procedures

6.1. Responsibilities of the Investigator

The following administrative items are meant to guide the investigator in the conduct of the study but may be subject to change based on industry and government standard operating procedures, working practice documents, or guidelines. Changes may be reported to the IRB but will not result in protocol amendments.

6.1.1 Form FDA 1572

The investigator will complete and sign the Form FDA 1572.

6.1.2 Adherence to Protocol

The investigator agrees to conduct the study as outlined in this protocol in accordance with the ICH E6(R2) and all applicable guidelines and regulations.

6.1.3 Reporting Requirements

By participating in this study, the investigator agrees to submit reports of SAEs according to the time line and method outlined in the protocol (Section 4.7.6.1.2). In addition, the investigator agrees to submit reports to the IRB as appropriate. The investigator also agrees to provide the sponsor with an adequate report shortly after completion of the investigator's participation in the study.

6.1.4 Source Documentation

By participating in this study, the investigator agrees to maintain adequate case histories for the subjects treated as part of the research under this protocol. The investigator agrees to maintain accurate eCRFs and source documentation as part of the case histories.

6.1.5 Retention of Records

The investigator agrees to keep the records stipulated in this protocol and those documents that include (but are not limited to) the study-specific documents, identification log of all participating subjects, medical records, source worksheets, all original signed and dated informed consent forms, subject authorization forms regarding the use of personal health information (if separate from the informed consent form), copies of all eCRFs, query responses, and detailed records of drug disposition, to enable evaluations or audits from regulatory authorities, the sponsor, or its designees.

Furthermore, ICH 4.9.5 requires the investigator to retain essential documents specified in ICH E6(R2) (Section 8) until at least 2 years after the last approval of a marketing application for a specified drug indication being investigated or, if an application is not approved, until at least 2 years after the investigation is discontinued and regulatory authorities are notified. In addition, ICH 4.9.5 states that the study records should be retained until an amount of time specified by applicable regulatory requirements or for a time specified in the clinical study site agreement between the investigator and sponsor.

Refer to the clinical study site agreement for the sponsor's requirements on record retention. The investigator should contact and receive written approval from the sponsor before disposing of any such documents.

6.1.6 Financial Disclosure and Obligations

The investigator is required to provide financial disclosure information to allow the sponsor to submit the complete and accurate certification or disclosure statements required under 45 CFR 46. In addition, the investigator must provide to the sponsor a commitment to update this information promptly if any relevant changes occur during the course of the investigation and for 1 year after the completion of the study.

Neither the sponsor nor the study clinic is financially responsible for further testing or treatment of any medical condition that may be detected during the screening process.

6.2. Confidentiality and Disclosure of Data

All subjects will sign a HIPAA-compliant authorization form containing the mandated core elements and requirements before participation in this clinical study. The sponsor and designees affirm and uphold the principle of the subject's right to protection against invasion of privacy. Throughout this study, a subject's source data will only be linked to the sponsor's electronic data capture system database or documentation via a unique identification number. As permitted by all applicable laws and regulations, limited subject attributes such as gender, age or date of birth, and subject initials may be used to verify the subject and accuracy of the subject's unique identification number.

To comply with ICH Guidelines for GCP and to verify compliance with this protocol, the sponsor requires that the investigator allow review of the subject's original medical records (source data or documents) by the study monitor, representatives from any regulatory authority (e.g., FDA), the sponsor's designated auditors, and the appropriate IRB. These medical records will include, but will not be limited to, clinical laboratory test result reports, ECG reports, admission and discharge summaries for hospital admissions occurring during a subject's study participation, and autopsy reports. Access to a subject's original medical records requires the specific authorization of the subject as part of the informed consent process.

Copies of any subject source documents that are provided to the sponsor must have certain personally identifiable information removed (i.e., subject name, address, and other identifier fields not collected in the subject's eCRF).

Data will be maintained and backed up in the electronic data capture system. All access to the data is protected by username and password, and each staff member and all sponsor staff will have separate access that requires a separate username and password. Access is only given to site staff and requested sponsor staff who have completed the appropriate training.

6.3. Subject Consent

Written informed consent in compliance with 45 CFR 46 will be obtained from each subject before entering the study or performing any unusual or nonroutine procedure that involves risk to the subject. An informed consent template may be provided by the sponsor to the study clinic. If any institution-specific modifications to study-related procedures are proposed or made by the study clinic, the consent should be reviewed by the sponsor or its designee or both before IRB submission. Once reviewed, the consent will be submitted by the investigator to the IRB for review and approval before the start of the study. If the informed consent form is revised during the course of the study, all active participating subjects must sign the revised form.

Before enrollment, each prospective subject will be given a full explanation of the study and be allowed to read the approved informed consent form. The informed consent process will be performed by a clinical research nurse in a private room. The subject will be given unlimited time to ask questions regarding study participation, and each subject will be questioned to ensure their understanding. Once the investigator is assured that the subject understands the implications of participating in the study, the subject will be asked to give consent to participate in the study by signing the informed consent form.

The investigator will provide a copy of the signed informed consent form to the subject. The original form will be maintained in the subject's medical records at the site.

6.4. Data Collection

Full details of procedures for data collection and handling will be documented in the data management plan, which is initiated with the final protocol receipt. The data management plan is a changing document that evolves over the course of the study and is finalized by database lock.

6.5. Publications

No information related to or generated by this study will be released to the public until it has been reviewed by the sponsor. The sponsor shall own intellectual rights for the data and analysis resulting from this study. Authorship on publications will be determined by standard journal requirements.

7. Study Management

7.1. Release of Study Drug to the Study Clinic

Before the study drug can be released to the study clinic, the following documents will be collected from the study clinic by the clinical research organization, retained in the trial master file, and a study drug shipment approval form will be completed by the clinical research organization:

- Protocol signature page signed by the investigator
- IRB approval of the protocol and informed consent form and IRB membership list
- Completed Form FDA 1572, curriculum vitae, and medical licenses from each investigator
- Financial disclosure and debarment certification from each investigator
- Executed contract with investigator and study clinic

7.2. Monitoring

The sponsor or its designee will monitor the study to ensure that it is being conducted according to the protocol, GCP standards, and applicable region-specific requirements, and to ensure that study initiation, conduct, and closure are adequate. The investigators and the study clinic staff will be expected to cooperate fully with the study monitors and personnel or agents of the sponsor, and be available during monitoring visits to answer questions sufficiently and to provide any missing information. The investigators and their institutions will permit direct access to source data/documents for study-related monitoring activities, audits, IRB reviews, and regulatory inspections.

During any on-site visits, the study monitor will:

- Check and assess the progress of the study

- Review all informed consent forms
- Review study data collected
- Conduct source document verification
- Identify any issues and address their resolution
- Verify that the facility remains acceptable
- Conduct study drug accountability

These monitoring activities will be done in order to verify that the:

- Data are authentic, accurate, and complete.
- The safety and rights of the subjects are being protected.
- The study is being conducted in accordance with the currently approved protocol (including any amendments), GCP, and all applicable regulatory requirements.

In addition, the sponsor, designated auditors, and government inspectors must be allowed access to eCRFs, source documents, and other study files that may be required to evaluate the conduct of the study.

7.3. Management of Protocol Amendments and Deviations

7.3.1 Modification of the Protocol

Any changes in this research activity, except those necessary to remove an apparent immediate hazard to the subject, must be submitted to the sponsor or designee and reviewed and approved by the local IRB before implementation. Amendments to the protocol must be submitted in writing to the investigator's IRB for approval before subjects are enrolled into an amended protocol.

7.3.2 Protocol Violations and Deviations

Any significant protocol deviations that the investigator or study clinic staff believes are of major importance (e.g., incorrect randomizations, subject enrolled but not eligible) should be reported to the sponsor and the investigator's IRB as soon as possible. Significant protocol deviations may include the following:

- Deviations from the inclusion/exclusion criteria that may affect subject safety
- Deviations (omission or delay) of safety monitoring procedures
- Deviations in the administration of the study drug
- Deviations in obtaining informed consent

All subjects who are enrolled and receive the study drug, regardless of whether they have a major protocol violation, must continue to be followed for safety for all follow-up study visits.

8. Appendix

Table 8-1 Overall Schedule of Events

Screening

Clinical Study 4 - Opioids Screening SoE	
	Screening Period
Study Week	-4 to -1
Study Day	-28 to -2
In house residency	
Outpatient visit	X
Recruitment/Screening – healthy	X
Informed consent process	X
Medical history – healthy	X
Prior/concomitant meds assessment	X
Physical Exam	X
Vital signs (including pulse oximetry)	X
ECG - single[1]	X
Telemetry	
Chemistry/hematology[1]	X
Urinalysis	X
CYP2D6 and 3A4 Screening	X
Urine drug screen + alcohol screen	X
Pregnancy test[2]	X
HIV test	X
Hepatitis test	X
Study Agent preparation & administration - Oral	
Plasma PK draw	
Read Rebreathing Training	X
AE assessment	

Lead-in Reproducibility Assessment

Clinical Study 4 - Opioids Lead-In Reproducibility Assessment SoE					
	Check-In and Baseline	Treatment Period		Check-out	Follow-Up
Study Week	0	1			
Study Day	-1	1	2	3	
In house residency	X	X	X		
Outpatient visit					
Standard Assessments					
Recruitment/Screening – healthy					
Informed consent process					
Medical history – healthy	X				
Prior/concomitant meds assessment	X				
Comprehensive physical exam	X			X	
Vital signs (including pulse oximetry)	X			X	
ECG - single[1]	X			X	
Telemetry					
Chemistry/hematology[1]	X			X	
Urinalysis	X			X	
Urine drug screen + alcohol screen	X				
Pregnancy test[2]	X			X	
SARS-CoV-2 Molecular Test	X				
HIV test					
Hepatitis test					
Study Agent preparation & administration - Oral					
Plasma PK draw					
Estrogen and progesterone		X	X		
Genomics (buffy coat)					
Read Rebreathing Training	X				
Read Rebreathing Procedure (See protocol for time points)		5	5		
Pupillometry Assessments (See protocol for time points)		10	10		
AE assessment		X	X		

1 Clinically notable results are repeated

2 Serum test

Part 1

Clinical Study 4 - Opioids Part 1 SoE													
	Check-In and Baseline	Treatment Period										Check-out	Follow-Up
Study Week	0	1							2				
Study Day	-1	1	2	3	4	5	6	7	8	9	10	11	
In house residency	X	X	X	X	X	X	X	X	X	X	X		
Outpatient visit													
Recruitment/Screening – healthy													
Informed consent process													
Medical history – healthy													
Prior/concomitant meds assessment	X		X	X		X	X		X	X		X	
Comprehensive physical exam	X											X	
Vital signs (including pulse oximetry)	X	X			X			X			X	X	
Pre-discharge Sedation Assessments												X	
ECG - single[1]	X											X	
Telemetry		X			X			X			X		
Chemistry/hematology[1]	X											X	
Urinalysis	X											X	
Urine drug screen + alcohol screen	X												
Pregnancy test[2]	X											X	
HIV test													
Hepatitis test													
SARS-CoV-2 Molecular Test	X												
Read Rebreathing Training	X												
Study Agent preparation & administration - Oral		X			X			X			X		
Study Agent preparation & administration - IV		X			X			X			X		
Read Rebreathing Procedure (See protocol for time points)		7			7			7			7		
Pupillometry Assessments (See Protocol for time points)		14			14			14			14		
Plasma PK draw (See protocol for time points)		9			9			9			9		
Estrogen and progesterone		X			X			X			X		
Genomics (buffy coat)		X											
Sedation Assessment (See protocol for time points)		7			7			7			7		
Continuous Pulse Oximetry		X			X			X			X		
AE assessment		X	X	X	X	X	X	X	X	X	X	X	
		Dosing Day	Wash-out Day	Wash-out Day	Dosing Day	Wash-out Day	Wash-out Day	Dosing Day	Wash-out Day	Wash-out Day	Dosing Day		

1 Clinically notable results are repeated

2 Serum test

Part 2

Clinical Study 4 – Opioids Part 2 SoE																							
	Check-in and Baseline	Treatment Period																				Check-out	Follow-Up
Study Week	0	1					2								3					4			
Study Day	-1	1	2	3	4	5	6-11	12	13	14	15	16	17	18-23	24	25	26	27	28	29	30		
In house residency	X	X	X	X	X	X		X	X	X	X	X	X		X	X	X	X	X	X			
Outpatient visit																							
Recruitment/Screening – healthy																							
Informed consent process																							
Medical history – healthy																							
Prior/concomitant meds assessment	X							X							X						X		
Comprehensive physical exam	X							X							X						X		
Targeted Physical Exam							X							X									
Vital signs (including pulse oximetry)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Pre-discharge Sedation Assessment							X							X							X		
ECG – single[1]	X																				X		
Telemetry		X				X			X				X			X				X			
Chemistry/hematology[1]	X							X							X						X		
Urinalysis	X							X							X						X		
Urine drug screen + alcohol screen	X							X							X								
Pregnancy test[2]	X							X							X						X		
HIV test																							
Hepatitis test																							
SARS-CoV-2 Molecular Test	X							X							X								
Read Rebreathing Training	X																						
Study Agent preparation & administration - Oral (see protocol for details)		X	X	X	X	X			X	X	X	X	X			X	X	X	X	X			
Read Rebreathing Procedure (See protocol for time points)		6			6	6			6			6	6			6			5	6			
Pupillometry Assessment (See protocol for time points)		12			10	12			12			10	12			12			10	12			
Plasma PK draw (See protocol for time points)		9			9	9			9			9	9			9			9	9			
Estrogen and progesterone		X			X	X			X			X	X			X			X	X			
Genomics (buffy coat)		X																					
Sedation Assessment (see protocol for time points)		6			6	6			6			5	6			6			5	6			
Continuous Pulse oximetry		X			X	X			X			X	X			X			X	X			
AE assessment		X	X	X	X	X	X		X	X	X	X	X	X		X	X	X	X	X	X		
		Dosing Day	Dosing Day	Dosing Day	Dosing Day	Dosing Day	Wash-out Week (check-in Day 12)		Dosing Day	Dosing Day	Dosing Day	Dosing Day	Dosing Day	Wash-out Week (check-in Day 24)		Dosing Day	Dosing Day	Dosing Day	Dosing Day	Dosing Day			

1 Clinically notable results are repeated
2 Serum test

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