Study Title: Clinical Study to investigate the effect of administration of selective serotonin reuptake inhibitors and an opioid on ventilation

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Statistical Analysis Plan

SCR-012: Clinical study to investigate the effect of administration of selective serotonin reuptake inhibitors and an opioid on ventilation

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Contents

Table of Abbreviation

Abbreviation	Definition
AE	adverse event
AUC	area under the concentration-time curve
CI	confidence interval
CV	coefficient of variation
C_{max}	maximum observed concentration
ECG	electrocardiogram
eCRFs	electronic case report forms
FDA	Food and Drug Administration
IV	intravenous
K_{el}	elimination rate
J-T_{peak}	early repolarization interval
O_2	oxygen
pCO_2	partial pressure of carbon dioxide
PD	pharmacodynamic
$P_{ET}CO_2$	end-tidal pCO_2
PK	pharmacokinetic
pO_2	partial pressure oxygen
QTc	heart rate-corrected QT interval
ΔQTc	change-from-baseline in QTc
ΔΔQTc	placebo adjusted change from baseline QTc
QTcF	heart rate-corrected QT interval using the Fridericia correction
SD	standard deviation
SOP	standard operating procedures
TEAE	treatment-emergent adverse event
T_{max}	time of maximum concentration (C_{max})
$t_{1/2}$	terminal half-life
VE55	minute ventilation at 55 mm Hg $P_{ET}CO_2$
ΔVE55	baseline-adjusted VE55
VRT	Ventilatory recruitment threshold

1. Introduction

This document outlines the proposed statistical methods for data analysis of data collection from Protocol 'SCR-012 Clinical study to investigate the effect of administration of selective serotonin reuptake inhibitors and an opioid on ventilation.

2. Study Objectives

2.1 Primary Objective

The two separate primary objectives include the following:

- To study ventilatory effects of selective serotonin reuptake inhibitors (SSRIs) (paroxetine or escitalopram) combined with oxycodone compared to oxycodone alone after 21 days of SSRI dosing.
- To study ventilatory effects of SSRIs (paroxetine or escitalopram) compared to placebo after 20 days of SSRI dosing.

2.2 Secondary Objectives

Secondary objectives include the following:

- To study ventilatory effects of SSRIs (paroxetine or escitalopram) combined with oxycodone compared to oxycodone alone after 6 and 12 days of SSRI dosing.
- To study ventilatory effects of SSRIs (paroxetine or escitalopram) compared to placebo after 5 and 11 days of SSRI dosing.

2.3 Exploratory Objectives

Exploratory objectives include the following:

- To study whether paroxetine or escitalopram affects the pharmacokinetics of oxycodone.
- To evaluate differences in hypercapnic ventilatory response under hyperoxic or hypoxic rebreathing.
- To study whether there is a direct pharmacodynamic interaction between paroxetine or escitalopram and oxycodone.
- To summarize additional pharmacokinetic parameters and pharmacodynamic measurements collected during the study.

3. Study Endpoints

3.1 Primary Endpoints

The following primary endpoints will be evaluated:

- Minute ventilation at 55mm Hg end tidal CO_2 (VE55) under hyperoxic conditions on day 21
- VE55 under hyperoxic conditions on day 20

3.2 Secondary Endpoints

The following secondary endpoints will be evaluated:

- VE55 under hyperoxic conditions on days 6 and 12
- VE55 under hyperoxic conditions on days 5 and 11

3.3 Exploratory Endpoints

The following exploratory PK parameters will be determined for oxycodone and oxymorphone (days 6, 12, and 21) and paroxetine, escitalopram, and escitalopram metabolites (days 2, 5, 6, 11, 12, 16, 20, and 21):

- Maximum observed plasma concentration (C_{max})
- Area under the plasma concentration time curve (AUC)
- Time at which C_{max} occurs (T_{max})
- Elimination rate constant (K_{el})
- Terminal half-life ($t_{1/2}$)
- Accumulation ratio (for escitalopram and paroxetine)

Additionally, the following exploratory PD markers may be evaluated:

Ventilation and Cardiovascular Assessments:

- During rebreathing stage
  - *VE55* (minute ventilation at the 55mm Hg end tidal CO_2 point)
  - *Baseline minute ventilation* when end tidal PCO_2 is less than the ventilatory recruitment threshold (represents non-chemoreflex drives to breathe)
  - *Ventilatory recruitment threshold* (end tidal PCO_2 above which minute ventilation starts to increase linearly with further increases in end tidal PCO_2)
  - *Slope of the minute ventilation / end-tidal partial pressure of carbon dioxide (end-tidal PCO_2) regression line* that reflects the increase in minute ventilation relative to the increase in end tidal PCO_2 (represents chemoreflex sensitivity)
  - *Extrapolated ventilation recruitment threshold* (intersection with x axis)
- During relaxation stage
  - Minute ventilation, tidal volume, respiratory rate, end-tidal PCO_2, end-tidal PO_2, oxygen saturation, and heart rate during the relaxation stage
  - Number of apneic events lasting > 10 s during the relaxation stage

Pupillary Assessments

- Maximum pupil diameter before constriction
- 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

ECG Assessments

- Change from baseline in QTc, PR, QRS, J-T_{peakC}, T_{peak}-T_{end} and heart rate.

An exploratory endpoint is the pharmacokinetic/pharmacodynamic (PK/PD) relationship for study drugs when administered alone versus in combination.

4. Study Overview

4.1 Study Design

This study will be a randomized, double blind, three period crossover study with approximately 25 healthy volunteer participants. This study will include three 21-day treatment periods with a 3-week washout between each period. Each subject will be randomized to 1 of 6 treatment sequences (i.e., ABC, ACB, BAC, BCA, CAB, CBA). FDA will prepare the randomization schedule.

Day -1	Days 1-21	Day 22	Days 23-41	Day 42	Days 43-63	Day 64	Days 64-83	Day 84	Days 85-105	Day 106
Check-in 1	Period 1	Check-out 1	Washout 1	Check-in 2	Period 2	Check-out 2	Washout 2	Check-in 3	Period 3	Check-out 3

Participants will enter the clinic on a staggered basis in cohorts of approximately five so that no more than five subjects are undergoing rebreathing on any given day. Subjects will be staggered to allow for direct safety overview by medical staff during Duffin Rebreathing procedure.

Participants will receive either placebo, paroxetine, or escitalopram on days 1-21 for each period (see below table). Oxycodone will be administered on days 6, 12, and 21 of each period. Dosing of the study drugs will occur at time 0 on each day. Oxycodone dosing will occur at 3 h so that maximum concentration occurs at approximately the same time for all drugs. Participants will also receive ondansetron 4 mg 30 min before oxycodone administration.

Participants will be confined to the study clinic from Day -1 the morning of Day 22 for each period. There will be a three-week washout between each period. Subjects will be discharged from the study after completion of all study procedures. If a subject discontinues from the study prematurely, all procedures scheduled for the end of the study will be performed. Meal timing and components, activity levels, and general conditions in the study clinic will be as similar as possible on the treatment days.

Upon return to clinic, on Days 42 & 84, eligibility criteria to continue study participation will be reviewed (see Schedule of Events for criteria listed on Days 42 & 84), any changes in medical history (including concomitant medications) will be documented.

Treatment	Day					
	1-5	6	7-11	12	13-20	21
A	Placebo	Placebo + 10 mg oxycodone	Placebo	Placebo + 10 mg oxycodone	Placebo	Placebo + 10 mg oxycodone
B	40 mg paroxetine	40 mg paroxetine + 10 mg oxycodone	60 mg paroxetine	60 mg paroxetine + 10 mg oxycodone	60 mg paroxetine	60 mg paroxetine + 10 mg oxycodone
C	20 mg escitalopram	20 mg escitalopram + 10 mg oxycodone	30 mg escitalopram	30 mg escitalopram + 10 mg oxycodone	30 mg escitalopram	30 mg escitalopram + 10 mg oxycodone

4.2 Sample Size

Approximately 25 healthy participants are planned for enrollment. The primary endpoint is a comparison of VE55 between two treatment on day 21 (oxycodone with the concomitant medication versus oxycodone alone) and on day 20 (concomitant medication versus placebo). The comparisons for day 20 and 21 will be considered as separate primary endpoints, each tested at a 0.025 significance level.

A previous study (20 participants) with paroxetine showed a decrease in 10.2 L/min in VE55 for paroxetine (40 mg) combined with oxycodone (10 mg) compared to oxycodone alone after 5 days of dosing with paroxetine (NCT04310579). Similarly, there was a 9.3 L/min decrease in paroxetine compared to placebo after 4 days of dosing with paroxetine. The standard deviation in assessments was approximately 6.5 L/min. Assuming a similar effect size with paroxetine and escitalopram at day 20 and 21, the planned number of participants allows for a 40% drop out rate while maintaining at least 90% power for separate one-sided tests at a 0.025 significance level with the multiple primary endpoints. Study sample size did not consider secondary endpoints.

5. Analysis Populations

The rebreathing analysis population will include all subjects who completed at least one rebreathing assessment on any day or at any timepoint.

For calculating the primary endpoint, the rebreathing analysis population will be subset to those subjects with data from at least two treatments and rebreathing assessments at the primary PD endpoint timepoint (5 h on day 20 and day 21, respectively). A subject who discontinues dosing after the primary timepoint on day 20 but before the primary timepoint on day 21, would still be part of the analysis population for the primary endpoint on day 20 but not for day 21.

Subjects in the rebreathing analysis population will be used for the planned primary, secondary, and exploratory analyses related to evaluating drugs effects on ventilation. 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.

A rebreathing assessment will be considered complete if the subject makes it through the entire procedure (e.g., subject makes it to the rebreathing stage with sufficient data collected to estimate VE55) and if there are no identifiable issues with how the procedure was conducted. Potential issues with how the procedure was conducted can include, 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), inaccurate readings from the pneumotach (e.g., non-physiologic baseline minute ventilation readings), or a subject suddenly increasing minute ventilation without subsequent increases in minute ventilation as end tidal pCO_2 increases (suggesting stress-associated or other non-CO_2 mediated hyperventilation confounding the ventilatory response to hypercapnia through $[H^+]$ chemoreceptors). Determination of the completeness of each rebreathing assessment and its individual components/measurements will be performed manually by two members blinded from treatment information (see Section 7.3).

The PK population will include all subjects who receive study drug and have at least 1 estimable PK parameter after dosing.

The ECG exposure-response population will include all subjects who receive at least 1 dose of any of the study drugs and have digital ECG (QTc and J-T_{peakC}) data for the treatment period collected before dosing and at 1 or more time points after dosing as well as plasma concentration data (except for the placebo arm) from the same time points after dosing. Subjects in this population will be used for the ECG exposure-response analysis.

The safety population will include all subjects who receive at least 1 dose of any of the study drugs.

6. Data Screening and Acceptance

6.1 Handling of Missing or Incomplete Data

The following will be performed:

- PK measurements below the quantification limits will be considered equal to zero for all analyses.
- Missing PK or PD data (e.g., subject discontinued from study; subject could not successfully complete rebreathing at a time point) will not be imputed. Data that are excluded from the descriptive or inferential analyses will be included in the subject data listings.
- Pupillometry data collected from the PLR®-3000 pupillometer device flagged as being anomalous based on device error codes will be excluded from analyses.

7. General Statistical Considerations

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

7.1 Subject Disposition

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

7.2 Demographic and Baseline Characteristics

Continuous demographic and baseline characteristic variables (e.g., age, height, weight, and body mass index) will be summarized by study part and by treatment using descriptive statistics (number of subjects, mean, SD, median, minimum, maximum, and interquartile range). The number and percentage of subjects in each class of categorical demographic and baseline characteristic variables will also be summarized.

7.3 Pharmacodynamic Analyses

7.3.1 Rebreathing Analyses

Figure 7-1 below shows representative time-course results from the rebreathing procedure, with minute ventilation, oxygen percentage, and end-tidal PCO_2 shown from top to bottom. Throughout the procedure subjects will undergo different steps designed to reduce noise and variability. Initially, subjects breathe room air for 5 minutes and relax as much as possible. Around the 5-minute mark, the subject is instructed to begin hyperventilating (primarily through deep breathing) for 5 min to achieve an end tidal PCO_2 of approximately 20-25 mmHg prior to rebreathing. During rebreathing procedures, subjects will be maintained at one of 2 different isoxic end tidal PO_2. The isoxia at a hyperoxic (150 mmHg) or hypoxic (50 mmHg) end tidal PO_2 will be maintained by providing a computer-controlled flow of 100% O_2 to the rebreathing bag. The hyperoxic rebreathing procedures will be performed at 0, 4, and 5 h on days 5, 6, 11, 12, 20, and 21. The hypoxic rebreathing procedure will be performed on the same days at 5 h immediately following the hyperoxic rebreathing assessment.

Each of these steps can be noted on the example figure below (7-1). In this example, relaxation while breathing room air was performed for 5-minutes. From 5 to 10 minutes, the test subject began hyperventilation and slight decreases in O_2% and PCO_2 with an increase in minute ventilation are observed. Finally, the test subject begins rebreathing at 10 minutes. After taking deep breaths, PCO_2 in the rebreathing circuit and the lungs equilibrate and then PCO_2 increases linearly to approximately 55 mm Hg at the end of rebreathing in this subject. An increase in

minute ventilation is triggered at a certain point during rebreathing (ventilatory recruitment threshold), and both measures increase until completion of the procedure based on subject tolerability or until other procedure stopping criteria are reached (see protocol and rebreathing SOP for full list). If the subject's data does not follow these trends due to a leak in the apparatus (e.g., incomplete seal between the mask and subject's face), inaccurate readings from the pneumotach, issues in operator recording of the data, or the subject not having a linear increase in minute ventilation as end tidal PCO_2 increases prior to reaching PCO_2 of 55 mm Hg, the run will not be considered completed and will be excluded from subsequent data analysis that is dependent on the minute ventilation/end tidal PCO_2 regression line.

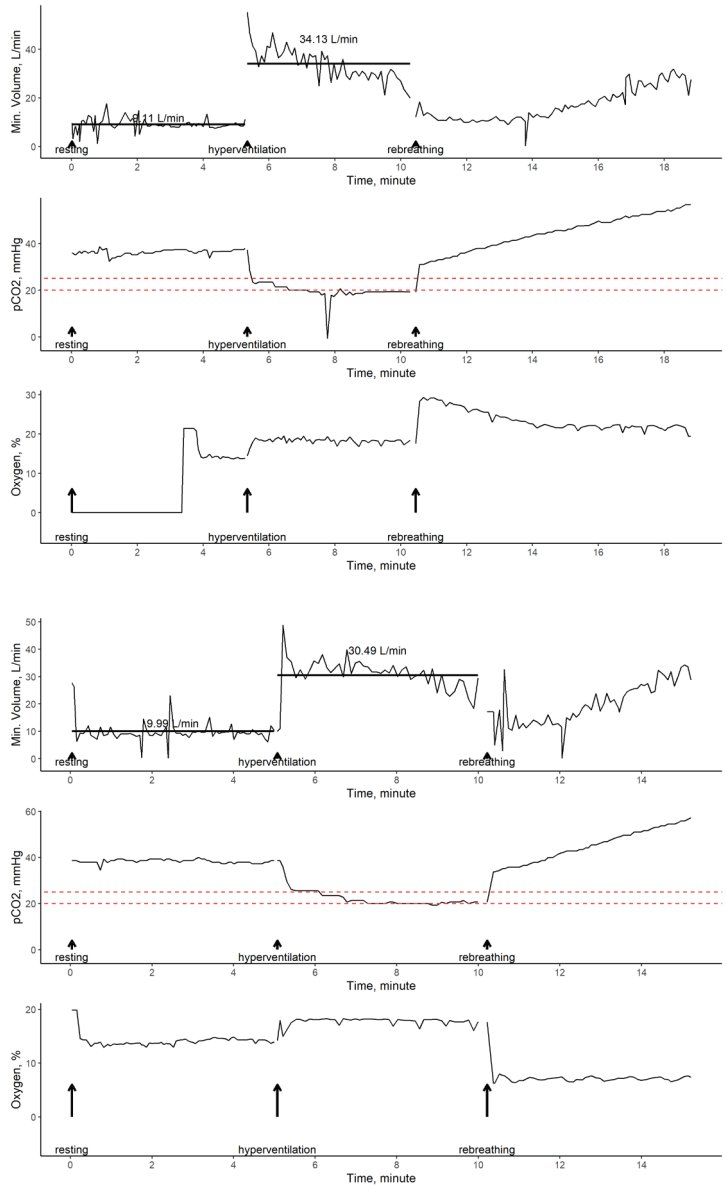

Figure 7-1: Sample data from a test subject during relaxation, hyperventilation, and rebreathing, with minute ventilation, O_2 percentage, and PCO_2 mmHg over time during these experimental steps. A representative hyperoxic test is shown at the top (oxygen percentage maintained at ~21% during rebreathing) and a representative hypoxic test is shown at the bottom (oxygen percentage maintained at ~7.1% during rebreathing).

From these results, minute ventilation and end tidal PCO_2 levels over time will be matched. End tidal PCO_2 will be calculated as % CO_2 • (barometric pressure – 47 mmHg [water vapor]). Separate piecewise linear and nonlinear (hockey stick model) regressions will be fit to the experiment data. The nonlinear and piecewise linear regressions will be fit to data from the rebreathing stage of the rebreathing procedure (i.e., during isoxic hypercapnia). This functional relationship for the nonlinear model assumes a constant relationship between minute ventilation and end tidal PCO_2 up until a threshold value is reached, after which minute ventilation and end tidal PCO_2 both increase linearly. Example code for the nonlinear fit can be found in the Appendix, and a sample result from the test case can be seen in Figure 7-2. Because of potential inconsistencies in when the stage transition may have been flagged by the device operator (pressed while hyperventilation was still ongoing) and as the isoxic setpoint is being achieved, the first 15 to 30 seconds of data from the rebreathing stage (based on visual inspection) will be removed when calculating the regressions. In addition, all other exploratory pharmacodynamic endpoints will be automatically calculated.

Because of the potential for outlying data points in the minute ventilation and/or end-tidal PCO_2 signal at each individual breath, it is sometimes necessary to exclude outlying data points from the regression. Such outlying data points can be introduced by subject postural changes, sighing, hiccups, talking, or faulty sensors. In addition, there may be a need to remove additional data at the beginning of rebreathing or to remove data at the end of rebreathing (e.g., nonlinearity as subject approaches or exceeds end tidal PCO_2 55 mmHg).

Outlying values for the regressions will be identified automatically through evaluation of standardized residuals after performing the initial regression (i.e., standardized residuals > 2). Then, the data analysis team will be provided with time course plots from the full assessment (all rebreathing procedure stages with stage transitions marked) and regression results using data from the rebreathing stage. The data analysis team will evaluate the completeness of the rebreathing assessment and visually assess the automatically calculated values in comparison to the underlying data. Any automatically calculated values that do not accurately capture the underlying data will be flagged, additional outliers will be removed, and the automated calculations will be re-run. A subject may have incomplete rebreathing data not supporting calculation of one or more primary endpoints, but other endpoint measures will still be used for analyses.

If the nonlinear regression converges, the regression results will be used to derive ventilation for that subject at that assessment. Baseline minute ventilation when end tidal PCO2 is less than the ventilatory recruitment threshold is the V_0 parameter from the regression. Likewise, the slope of the PCO2-ventilatory response curve is the s_{slope} parameter from the regression. The ventilatory recruitment threshold is calculated as the intersection between the two line segments. The extrapolated ventilatory recruitment threshold is calculated as the intersection of the PCO2-ventilatory response curve and the x-axis. If the nonlinear regression does not converge, results from the piecewise linear regression will be used to determine the slope of the PCO2-ventilatory response curve and data from the relaxation stage will be used to determine baseline minute ventilation. The ventilatory recruitment threshold and the intersection of the PCO2-ventilatory response curve and the x-axis will then be calculated as described above. All modeling analyses for the regressions will be performed in statistical software.

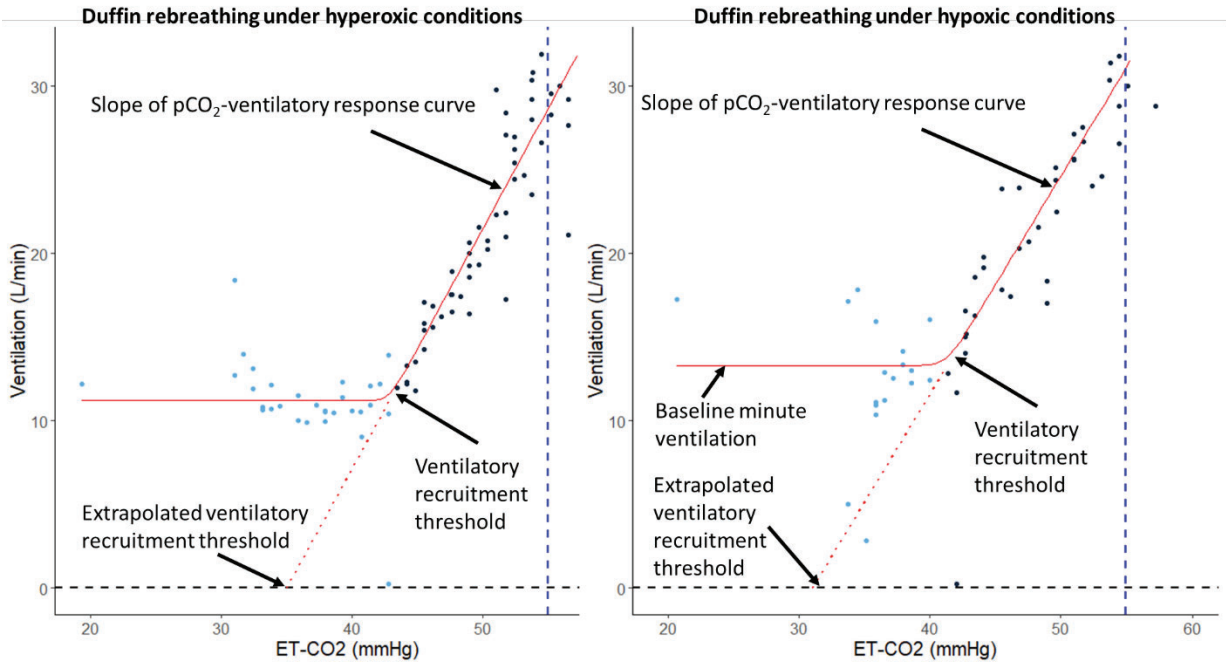

Figure 7-2: Sample nonlinear least squares fit between minute ventilation and end tidal PCO_2 for test subject under hyperoxic (left) or hypoxic (right) conditions. Different hypercapnic ventilation endpoints of interest are annotated on each figure.

The estimated VE55 from the regression will be used for primary endpoint analyses. All modeling analyses for the regressions will be performed in statistical software. This model will be used to predict minute ventilation at pCO_2 of 55 mmHg (i.e. VE55). If a subject does not reach 55 mmHg, the model will be extrapolated to predict the on-treatment minute ventilation at 55 mmHg. Baseline-adjusted VE55 (ΔVE55) will be calculated for each subject at each time

point by subtracting on-treatment VE55 from baseline (i.e., hyperoxic and hypoxic rebreathing assessments performed on day -1).

7.3.1.1 Primary Analyses

Minute ventilation and end-tidal pCO_2 ($P_{ET}CO_2$) data from the rebreathing stage of the Duffin Rebreathing procedure will be analyzed at the specified primary timepoints using nonlinear and piecewise linear regressions. The regressions will be used to predict VE55 as described in Section 7.3.1.

Multiple primary analyses are planned for each drug. The first primary analysis will be VE55 on day 21 at 5 h (hyperoxic) between oxycodone with paroxetine or escitalopram compared to oxycodone alone. The second primary analysis will be VE55 on day 20 at 5 h (hyperoxic) between paroxetine or escitalopram compared to placebo. Since Day 20 and 21 are considered multiple primary endpoints, comparisons will have an adjustment for multiplicity using a Bonferroni correction (each day will be tested at a 0.025 significance level). No adjustments will be made for separate testing of paroxetine or escitalopram since the treatments are considered separate interventions in the study.

A linear mixed effects model will be developed using all study data from hyperoxic assessments. Fixed effects will include treatment, period, sequence, and baseline VE55 (i.e., VE55 obtained at 0 h on Day -1 of each treatment period). Subject will be included as a random effect on the intercept. If a subject's baseline VE55 for a treatment period is not available, then the baseline value for that period will be calculated based on the median of all other baseline values for that subject from other study periods. If a subject does not have any completed baseline VE55 data, the subject will be assigned a baseline VE55 equal to the median of the population.

The Kenward-Roger approximation will be used to estimate denominator degrees of freedom for tests of fixed effects. Normality assumption will be verified using the Shapiro-Wilke test for normality. Homogeneity of variances will be verified using Levene's test. If the data in its original or transformed form does not satisfy assumptions for normality and homogeneity, a Wilcoxon signed-rank test will be used for all comparisons rather than a linear mixed effect model. I

To demonstrate an effect when an SSRI (paroxetine or escitalopram) is combined with oxycodone compared to oxycodone alone, the upper bound of the one-sided 97.5% CI of the least-square mean VE55 difference between treatments at day 21 must not overlap 0 L/min. Similarly, to demonstrate an effect of paroxetine (or escitalopram) compared to placebo, the upper bound of the one-sided 97.5% CI of the least-square mean VE55 difference between treatments at day 20 must not overlap 0 L/min. Each comparison will be performed separately (i.e., 'trt' as 'paroxetine' or 'escitalopram'):

Day 21:

- H_0: $VE55_{oxy+trt} - VE55_{oxy} \geq 0$ L/min

•H_A: $VE55_{oxy+trt} - VE55_{oxy} < 0$ L/min

Day 20:

•H_0: $VE55_{trt} - VE55_{placebo} \geq 0$ L/min

•H_A: $VE55_{trt} - VE55_{placebo} < 0$ L/min

7.3.1.2 Secondary Analyses

Two separate sets of secondary endpoints are planned for the study. Effects of SSRIs (paroxetine or escitalopram) with oxycodone compared to oxycodone alone will be performed on day 6 and day 12 at the 5 h (hyperoxic) timepoint. As additional secondary endpoints, effects of SSRIs (paroxetine or escitalopram) alone compared to placebo will be determined on day 5 and 11 at the 5 h (hyperoxic) timepoint. The linear mixed effect model developed for the primary analyses will be used for these comparisons.

Testing for the secondary endpoints will be performed in a hierarchical manner. If a significant difference is observed at day 21 between treatment combined with oxycodone compared with oxycodone alone, then comparisons between treatment combined with oxycodone compared with oxycodone alone will be performed at a 0.025 significance level for day 12. To demonstrate an effect when a treatment is combined with oxycodone compared to oxycodone alone, the upper bound of the one-sided 97.5% CI of the least-square mean VE55 difference between treatments at day 12 must not overlap 0 L/min. If a significant difference is observed for day 12, then similar testing will be performed at day 6. If either the original primary or first secondary comparison fails, then comparisons will still be performed and the one-sided 97.5% CI of the least-square mean VE55 difference between treatments will be reported without a p-value.

A similar approach will be utilized for comparisons between treatments and placebo. If a significant difference is observed at day 20 between treatment compared with placebo, then comparisons testing will be performed at a 0.025 significance level for day 11. To demonstrate an effect with treatment compared to placebo, the upper bound of the one-sided 97.5% CI of the least-square mean VE55 difference between treatments at day 11 must not overlap 0 L/min. If day 11 passes, then similar testing will be performed at day 5. If either the original primary or first secondary comparison fails, then comparisons will still be performed and the one-sided 97.5% CI of the least-square mean VE55 difference between treatments will be reported without a p-value.

7.3.2 Exploratory Ventilation Measures

In addition to planned VE55 primary and secondary analyses, data will be summarized by treatment, treatment day, and time point using descriptive statistics (number of subjects, mean, SD, median, minimum, maximum, confidence intervals). Both VE55 and ΔVE55 time course profiles will be calculated and plotted for the overall treatments and for individuals. Additional comparisons may be performed at other study time points to determine consistency of responses observed at the 5-h timepoint. Comparisons between hypercapnic ventilatory response measures

from hyperoxic or hypoxic rebreathing assessments at the 5-h timepoint will be performed using paired t-tests. These analyses will be considered hypothesis--generating and exploratory.

Other exploratory respiratory measures will be calculated and summarized from information collected during planned rebreathing procedures (see Section 3.3). Baseline minute ventilation, ventilatory recruitment threshold, slope of the PCO_2-ventilatory response curve, and the extrapolated ventilatory recruitment threshold will be calculated for each subject at each rebreathing assessment. Data collected during the Duffin Rebreathing procedure will be used to determine the slope of the minute ventilation / $P_{ET}CO_2$ regression line. Resting ventilation, tidal volume, end-tidal PCO_2, and oxygen saturation will be determined using data from the relaxation portion of the Duffin Rebreathing procedure. All measures will be summarized by treatment, day, and time using descriptive statistics (number of subjects, mean, SD, median, minimum, and maximum, confidence intervals). Comparisons may be performed across treatment groups or by subgroups (e.g., demographic variables) to further explore treatment results. Time course profiles for all measures will be calculated and plotted. Comparisons between hypercapnic ventilatory response measures from hyperoxic or hypoxic rebreathing assessments at the 5-h timepoint will be performed using paired t-tests. These analyses will be considered both -hypothesis generating and exploratory.

Apneic events lasting > 10 s will be determined using data collected during relaxation and preparation portion of the Duffin 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 (number of subjects, number of events) by study part, treatment, day, and time, as appropriate.

7.3.3 Electrocardiogram Analyses

The QT interval will be corrected for heart rate using Fridericia's formula ($QTc=QT/RR^{1/3}$). The J-T_{peak} interval will be corrected for heart rate using the formula ($J\text{-}T_{peakC} = J\text{-}T_{peakC}/RR^{0.58}$). Baseline will be the mean of the 3 predose ECG extractions of Day 1.

Exposure-response analyses will be performed for the change-from-baseline in QTc (ΔQTc) and change from baseline in other ECG measurements, where the mean of the 3 predose ECG readings on Day 1 will be used as the baseline. The concentration of the drug will be used as a covariate. Exposure-response analysis will be done following most recent best practices in concentration-QTc modeling.

To assess the appropriateness of a linear model, normal QQ-plots for the weighted residuals versus concentration and weighted residuals versus fitted values will be produced. A model with a quadratic term in concentration will be fitted and the quadratic term will be tested on the 2-sided 5% alpha level. In case of a significant quadratic term, nonlinear models, such as a

loglinear model and an E_{max} model, will be investigated and the primary model will be selected based on the Akaike Information Criterion and plausibility arguments.

Unless the prespecified test procedure for linearity indicates otherwise, the ECG analysis will be based on a linear mixed effects model implemented in R software, with ΔQTc as the dependent variable, drug plasma concentration and baseline QTc as continuous covariates, treatment and time point as categorical factors, and subject specific random effects for the intercept and slope. All postdose data will be used. The degrees of freedom for the model estimates will be determined by the Kenward-Rogers method. From the model, the slope (i.e., the regression parameter for the concentration) and the treatment effect will be estimated together with 2-sided 90% CIs.

The predicted mean placebo-adjusted change from baseline QTc (ΔΔQTc) at the observed geometric mean C_{max} (i.e., the product with the slope estimate + treatment effect [$\Delta QTc_{active} - \Delta QTc_{placebo}$]) and the 2--sided 90% CI of the estimate will be calculated for days 2 and 16.

Exposure response analysis, similar to that described for QTc will be applied to JT_{peakC}, PR, QRS, and T_{peak}-T_{end} for data from days 2 and 16.

For each time point, an analysis of variance model will be fitted with ΔQTc as the dependent variable, treatment (active or placebo) as the factor, and baseline QTc as a covariate. From this model, the difference ($\Delta QTc_{active} - \Delta QTc_{placebo}$) will be estimated with a 2-sided 90% CI. Separate models will be fitted for each treatment, all of them using the same placebo data. Change from Baseline in heart rate, JT_{peakC}, PR, QRS, and $T_{peak}T_{end}$ will be calculated using descriptive summary statistics.

7.3.4 Pupillometry Analyses

Maximum pupil diameter before constriction and dynamic pupillary measurements after a light stimulus will be measured before and after each rebreathing assessment (except in between the hyperoxic and hypoxic paired assessments). These exploratory parameters (see Section 3.3) will be summarized using descriptive statistics (number of subjects, mean, SD, median, minimum, maximum, confidence intervals). Time course summaries of each pupillary measurement will be generated for all treatment groups. Pupillary changes will be compared to baseline measurements 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.

7.3.5 Sedation Score Analyses

The Ramsay Sedation Scale is an observer-based assessment of sedation, with a scale of 1-6, that will be collected for each subject during the relaxation period of each Duffin Rebreathing procedure. In addition, subjects will be asked to provide their own assessment using the Visual

Analog Scale for sedation (a perpendicular mark on a 100 mm line with a scale of 0 ['awake and alert'] to 100 ['very sedated'] mm) 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 sedated the subject is during the study. These exploratory parameters will be summarized using descriptive statistics, and time course summaries of both scores will be generated for all treatments.

7.4 Pharmacokinetic Analyses

7.4.1 Plasma Pharmacokinetics

PK parameters will be calculated for the different drugs and metabolites and study days based on data collected from the time of drug administration to the 24-h sample on the specified day. C_{max} and AUC (i.e. AUC_{3-24} for oxycodone and oxymorphone, AUC_{0-24} for paroxetine, escitalopram, and escitalopram metabolites) will be computed on the specified days (i.e., day 6, 12, and 21 for oxycodone and oxymorphone; day 5, 6, 11, 12, 20, and 21 for paroxetine, escitalopram, and escitalopram metabolites) as part of the exploratory endpoints for this study. Additionally, the following exploratory PK parameters will be determined for oxycodone, oxymorphone, paroxetine, escitalopram, and escitalopram metabolites on each specified day:

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

The PK parameters will be analyzed using noncompartmental methods based on actual sampling times. All parameters will be calculated using statistical software. Serum concentrations below the limits of quantification will be set to zero for the purpose of this analysis (see Section 6). K_{el} and $t_{1/2}$ for each analyte and subject will only be included if the subject has 3 or more concentration values on the terminal portion of the pharmacokinetic curve and the linear regression of the log-concentration time curve has an adjusted coefficient of determination (R^2) greater than 0.80.

Oxycodone and oxymorphone C_{max} and AUC will be log-transformed and the values from oxycodone alone versus oxycodone with either paroxetine or escitalopram will be compared using a linear mixed effects model (i.e., treatment as fixed effects and subject as random effects) to determine if oxycodone exposures differ between the two treatments on days 6, 12, and 21. The geometric mean ratio will be determined and no difference in exposure will be concluded if the two-sided 95% confidence interval includes 1.

The PK parameters C_{max}, AUC_{x-24}, T_{max}, and K_{el} will be summarized using descriptive statistics (number of subjects, geometric mean, mean, SD, coefficient of variation [CV], median, minimum, maximum, and interquartile range). Geometric mean and individual

concentration-time profiles will be presented in graphs. Descriptive statistics and comparisons between subgroups may be performed with respect to demographic variables (e.g., age, sex, weight, metabolizer phenotype).

7.4.2 PK/PD Analyses

A nonlinear-mixed effect PK/PD model will be developed for ΔVE55 versus time for all treatments. The model will be employed using statistical modeling software.

PK models may be developed for each of the three drugs and metabolites evaluated in this study. Model structures will be selected based on previously published literature examples, visual inspection of the PK time course data, and diagnostic plots and objective function values from model fitting. The PK modeling may consider covariate exploration as well as fitting of metabolites for oxycodone and escitalopram that are analyzed.

The PD modeling may use either predictions from the PK modeling or observed drug concentration data as input. Different PD model structures will be evaluated (e.g., effect compartment coupled to an indirect response model). Covariate exploration may be performed for the PD model based on demographic variables. A separate modeling analysis plan will be developed for the planned PK/PD modeling analyses.

7.5 Safety Analyses

7.5.1 Adverse Events

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

7.5.2 Clinical Laboratory Tests

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

7.5.3 Vital Sign Measurements

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

7.5.4 Safety 12-lead Electrocardiograms

Safety 12-lead ECG data will be summarized using descriptive statistics (number of subjects, mean, SD, minimum, median, and maximum) by treatment and time point.

7.5.5 Pulse Oximetry and Telemetry

Continuous telemetry monitoring will be performed on days 5, 6, 11, 12, 20, and 21. Events requiring intervention from the staff or discontinuation from the study will be recorded in appropriate logs.

Oxygen saturation measurements obtained from 24-h pulse oximeters recordings will be used to plot time course profiles of oxygen saturation for treatments from days 5, 6, 11, 12, 20, and 21.

7.5.6 Physical Examinations

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

7.5.7 Other Safety Data

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

8. Data Quality Assurance

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

Furthermore, the investigator retains full responsibility for the accuracy and authenticity of all data entered into the electronic data capture system. The completed dataset and their associated files are the sole property of the sponsor and should not be made available in any form to third

parties, except for appropriate governmental health or regulatory authorities, without written permission of the sponsor.

9. Appendices

9.1 Randomization Schedule

The study is a 3-period, double-blind, crossover study where subjects will be enrolled in cohorts of approximately 5. Subjects will be randomized to one of six sequences (i.e., ABC, ACB, BAC, BCA, CAB, CBA) where treatment codes and interventions are summarized in the table below. Subjects will be randomized in blocks of 6. The remaining subject (i.e., 25th subject) will be assigned on of the six sequences randomly.

The project biostatistician created the specifications for generating the randomization schedule as described above. A dummy randomization schedule generated in R. The project biostatistician will transfer 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. The randomization biostatistician will be responsible for generating the final randomization schedule. The output will be sent only to designated unblinded recipients.

Table 1: Treatment Codes and Treatment Names for SCR-012

Treatment Code	Treatment Name
A	Oxycodone/placebo
B	Oxycodone/paroxetine
C	Oxycodone/escitalopram

9.2 Nonlinear Regression Model

The rebreathing procedure consists of three separate periods: i) breathing room air, termed 'relaxation' period; ii) hyperventilation (primarily through deep breathing); and iii) rebreathing at one of 2 different isoxic end tidal PO_2 ($PetO_2$) (i.e., at 50 mm Hg and 150 mm Hg). For the purposes of data analysis and determination of the primary endpoints, data from iii) will be used to estimate the ventilatory response to hypercapnia curve. This relationship, which is characterized by a flat portion between minute ventilation and end-tidal PCO_2 followed by a period of linear increase, can be represented using a hockey-stick function. For purposes of these analyses, the following equation is pre-specified to describe data from the procedure:

$V_i = V_0$ (if $PCO_2 \leq$ inflection point)

$V_i = V_0 * (1 + ((PCO_{2,i} - PCO_{2,inflection}) * s_{slope} / V_0)^g)^{1/g}$ (if $PCO_2 >$ inflection point)

Here, V_i is the minute ventilation that corresponds to an end-tidal CO_2 of $PCO_{2,i,}$ V_0 is baseline minute ventilation rate on the flat portion of the curve, $PCO_{2,inflection}$ is the end-tidal CO_2 where increase from baseline minute ventilation begins, s_{slope} is the slope of the curve, and g is a shape parameter in the hockey stick function that is set to 20 to provide the desired curvature. Outlier measures, introduced by subject postural changes, sighing, talking, hiccups and other subject behaviors, will be identified based on evaluation of standardized residuals from the nonlinear regression (i.e., standardized residuals > 2 SD). Coding for the nonlinear function will be performed in R. An example of the coded function is provided below.

```
ff <- function(p, v0, b, s) {
  vs <- (p-b)*s
  vs[which(vs<0)] <- 0
  a <- 0.95
  g <- 1/(1-a)
  v <- v0*(1 + (vs/v0)**g)**(1/g)
}

fits<-function (x){
  nls(v ~ ff(p,v0,b,s),
      lower=list(v0=5,  b=0,  s=0),
      start=list(v0=8,  b=30,  s=1),
      upper=list(v0=15, b=100, s=5),
      trace=F, algorithm="port", data=x)
}
```

10. References

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