STATISTICAL ANALYSIS PLAN

Protocol Number: PRAX-114-214

Protocol Title: A Phase 2 Double-blind, Placebo-Controlled, Dose-Ranging Clinical Trial to Evaluate the Efficacy and Safety of PRAX-114 in Adjunctive and Monotherapy Treatment of Participants with Major Depressive Disorder and Inadequate Response to Antidepressant Treatment

Version Number: V1.0

Version Date: 24 June 2022

APPROVALS

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DocuSigned by: [redacted] Signer Name: [redacted] Signing Reason: I am the author of this document Signing Time: 24-Jun-2022 | 12.23.08 EDT [redacted] Author Biostatistician	24-JUN-2022
DocuSigned by: [redacted] Signer Name: [redacted] Signing Reason: I approve this document Signing Time: 24-Jun-2022 | 12:12:05 EDT [redacted] Reviewer Biostatistician	24-JUN-2022
Praxis Precision Medicines	
DocuSigned by: [redacted] Signer Name: [redacted] Signing Reason: I approve this document Signing Time: 24-Jun-2022 | 11:31:18 EDT [redacted] Statistician	24-JUN-2022
DocuSigned by: [redacted] Signer Name: [redacted] Signing Reason: I approve this document Signing Time: 24-Jun-2022 | 11.58.20 EDT [redacted] PhD, Executive Director	24-JUN-2022

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Definitions and Abbreviations

Abbreviation	Description
AE	Adverse Event
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical
ATRQ	Antidepressant Treatment Response Questionnaire
BLQ	Below the level of quantification
BMI	Body Mass index
BP	Blood pressure
BUN	Blood Urea Nitrogen
CGI-I	Clinical Global Impression-Improvement
CGI-S	Clinical Global Impression-Severity
CI	Confidence Interval
cm	centimeters
CRF	Case Report Form
CSR	Clinical Study Report
C-SSRS	Columbia-Suicide Severity Rating Scale
CV%	Coefficient of variation
DSM-5	Diagnostic and Statistical Manual of Mental Disorders. 5th Edition
[redacted]	[redacted]
eCRF	Electronic case report form
FDA	Food and Drug Administration
FSH	Follicle Stimulating Hormone
GEE	Generalized estimating equation
HAM-D17	17-Item Hamilton Depression Rating Scale
ICH	International Conference on Harmonization
IE	Intercurrent event
IRT	Interactive Response Technology
ITT	Intent-to-treat
kg	kilograms
MAR	Missing at Random
MCS	Mental health component summary
MDD	Major Depressive Disorder
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligrams
min	minutes

Abbreviation	Description
MINI	Mini International Neuropsychiatric Interview
MMRM	Mixed Model Repeated Measures
MNAR	Missing Not at Random
n	Number of participants
PCI	Potentially clinically important
PCS	Physical component summary
PGI-I	Patient Global Impression-Improvement
PK	Pharmacokinetic
PP	Per protocol
[redacted]	[redacted]
PT	Preferred Term
QHS	every night
RBC	Red Blood Cell
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System
SD	Standard Deviation
SDQ	Symptoms of Depression Questionnaire
SIGH-D	Structured Interview Guide for the Hamilton Depression Rating Scale
SoA	Schedule of Activities
SOC	System Organ Class
TEAE	Treatment Emergent Adverse Event
WBC	White Blood Cell
WHODrug	World Health Organization Drug Dictionary
WSAS	Work and Social Adjustment Scale

1 OVERVIEW

This Statistical Analysis Plan (SAP) describes the planned analysis and reporting for Protocol PRAX-114-214 (A Phase 2 Double-blind, Placebo-Controlled, Dose-Ranging Clinical Trial to Evaluate the Efficacy and Safety of PRAX-114 in Adjunctive and Monotherapy Treatment of Participants with Major Depressive Disorder and Inadequate Response to Antidepressant Treatment), Version 3.0 dated 22 November 2021.

The statistical analyses and summaries described in this SAP follow the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guideline for Statistical Principles for Clinical Trials (ICH E9 Expert Working Group, 1999) and will form the basis of the results sections of the clinical study report (CSR) in accordance with the ICH guideline for Structure and Content of Clinical Study Reports (ICH Harmonised Tripartite Guideline E3, 1995). This SAP will be finalized and fully executed before database lock and unblinding of treatment codes. Any changes to the analyses that are not included in this SAP will be documented in the CSR.

2 STUDY DETAILS

2.1 Study Objectives

The objective of this trial is to evaluate the efficacy and safety of 10, 20, 40 and 60 mg oral PRAX-114 as compared to placebo in the treatment of adults with MDD. The study will enroll participants on adjunctive treatment who had an inadequate response to their current antidepressant treatment and participants not currently being treated with pharmacotherapy for MDD. A sub-study to investigate the pharmacokinetics (PK) of PRAX-114 and metabolites when dosed in the evening in participants with MDD will be conducted in a subset of participants at selected research sites with serial PK sampling capabilities.

A list of the specific protocol objectives along with their respective endpoints are listed in the table below:

Objective	**Endpoint**
Primary	
• To assess the presence of a dose-response signal for PRAX-114 in MDD based on the HAM-D17	• Change from baseline in HAM-D17 total score at Day 15
Secondary	
• To assess the presence of a dose-response signal for PRAX-114 in MDD based on the HAM-D17 • To evaluate the efficacy for each dose of PRAX-114 in MDD compared to placebo based on the HAM-D17	• Change from baseline in HAM-D17 total score at Day 29 • Change from baseline in HAM-D17 total score at all time points
• To evaluate the efficacy for each dose of PRAX-114 in MDD compared to placebo based on additional depression outcomes	• Change from baseline in Clinical Global Impression-Severity (CGI-S) score at Day 15 and all other time points • Clinical Global Impression-Improvement (CGI-I) score at Day 15 and all other time points • HAM-D17 response (reduction from baseline score of ≥50%) at Day 15, Day 29, and all other time points • HAM-D17 remission (total score of ≤7) at Day 15, Day 29, and all other time points • Change from baseline in the Symptoms of Depression Questionnaire (SDQ) total and sub-scale scores at Day 15 and all other time points • Patient Global Impression-Improvement (PGI-I) score at Day 15 and all other time points
• To evaluate the efficacy of each dose of PRAX-114 in MDD compared to	• Change from baseline in the Change from baseline in the Work and Social

placebo based on additional symptoms and functional outcomes	Adjustment Scale (WSAS) at Day 15 and all other time points
Exploratory	
[redacted]	
Safety	
• To evaluate the tolerability and safety of PRAX-114 for each dose compared to placebo	• Incidence and severity of AEs [redacted] • Incidence of Columbia-Suicide Severity Rating Scale (C-SSRS) measured suicidal ideation or behavior

2.2 Study Design

This is a randomized, double-blind, placebo-controlled dose-ranging trial to assess the efficacy and safety of 10, 20, 40, and 60 mg PRAX-114 as monotherapy or adjunctive treatment in men and women aged 18 to 65 years with MDD. Participants will be randomized to receive 28 days of 10, 20, 40, or 60 mg PRAX-114 or placebo in a 1:1:1:1:1 ratio.

This clinical trial will enroll participants who meet a Diagnostic and Statistical Manual of Mental Disorders 5th edition (DSM-5) diagnosis of recurrent MDD as defined by the Mini-International Neuropsychiatric Interview (MINI) for DSM-5; who are experiencing a current major depressive episode (MDE) of at least 12 weeks and not more than 24 months in duration; and who have a 17-item Hamilton Depression Scale (HAM-D17) total score of ≥20 and CGI-S score of ≥4 at both Screening and Day 1 (Baseline). Adjunctive treatment participants must be on treatment with an antidepressant at a stable dose for at least 8 weeks prior to Day 1 (Baseline) and have demonstrated an insufficient clinical response to 1 to 2 adequate trials of standard of care antidepressant treatment in the current episode per the Antidepressant Treatment Response Questionnaire (ATRQ). Participants will be excluded if they have a lifetime history of bipolar disorder, a psychotic disorder, obsessive-compulsive disorder, or a history of a psychotic mood episode in the last 2 years; meet the definition of treatment resistant depression; have a history of substance use disorder within the past 12 months; are currently at risk for suicidal behavior; have an unstable medical condition; or have a history of seizures. A complete list of eligibility criteria can be found in the study protocol Sections 5.2 and 5.3.

The clinical trial will consist of 3 periods: Screening (up to 28 days, including a minimum 14-day observation period prior to Day 1 to verify symptom stability), Intervention (28 days), and Follow-up (14 days). To ensure collection of high-quality data from clinician administered rating scales, raters will be required to fulfill qualification and training requirements prior to rating any participant in this clinical trial.

Screening Period

The Screening Period will be up to 28 days in duration (Day -28 to Day -1). Prior to any clinical trial procedures, participants will provide written informed consent. Screening assessments will include psychiatric and medical history, including alcohol, tobacco and drug use, the MINI, ATRQ, HAM-D17, CGI-S, demographic characteristics, height and weight, human chorionic gonadotropin (hCG) test, hepatitis screen, physical examination, clinical laboratory evaluations, drug screen, blood sample for current antidepressant treatment (adjunctive treatment participants only), vital signs, 12-lead ECGs, and the C-SSRS as outlined in the Schedule of Activities (SoA) in the Appendix of this SAP.

External review and independent eligibility verification will be performed during Screening to ensure the enrollment of appropriate participants. Documented approval of participant eligibility must be received prior to randomization on Day 1 (Baseline).

Intervention Period

Participants will return to the clinic for the Day 1 (Baseline) assessments and for confirmation of eligibility as outlined in the SoA (See Appendix).

Participants who continue to meet all clinical trial entry criteria will be randomized in a 1:1:1:1:1 ratio to receive double-blind treatment with PRAX-114 10, 20, 40, 60 mg or placebo every night at bedtime (QHS) from Day 1 through Day 28. Randomization will be stratified by concomitant antidepressant pharmacotherapy (yes or no) to ensure balanced allocation to treatment groups. They will return to the clinic for clinical trial assessments and PK samples as outlined in the SoA (See Appendix) on Days 8, 15, 22, and 29. Day 4 clinical assessments will be conducted via telehealth procedures.

Participants who have completed screening procedures and meet eligibility criteria but who are not randomized within 29 days of the Screening date (28-day Screening period plus the 1-day window) may be considered for randomization after consultation with the Sponsor or designee if repeat safety laboratory assessments and any other assessments that may have changed indicate that the participant continues to meet all clinical trial entry criteria. The specific circumstances for each participant as discussed with the Sponsor or designee will determine which additional clinical trial assessments must be repeated.

Smartphone based technology to monitor daily study drug intake will be used to support and confirm participant adherence with study drug administration procedures (refer to study drug compliance, Protocol Section 6.4).

Follow-up Period

Participants will return to the clinic for efficacy and safety follow-up visits and blood sample collection for PK on Day 36 and Day 43, as outlined in the SoA (see Appendix).

PK Sub-study

A sub-study to investigate the PK of PRAX-114 and its metabolites dosed in the evening in participants with MDD will be conducted in a subset of participants at selected research sites with serial PK sampling capabilities. All study participants at the designated research sites will be offered participation in the PK sub-study, which will be conducted within 3 days of completing the Visit 7/Day 29 visit assessments (Table 3). To qualify for entrance into the PK sub-study, participants must have completed the treatment period of the parent study through Visit 7/Day 29. Participants will be admitted to the clinical research in-patient unit and remain in the unit until the last PK sample is collected (see Appendix). Sub-study participants will receive the same treatment to which they were assigned during the parent study. Sub-study participants will return for follow-up study procedures as defined in the main study SoA (See Appendix).

2.3 Determination of Sample Size

Assuming a 1-sided test at an alpha level of 0.05, a sample size of 22 evaluable participants per group with Day 15 HAM-D17 data would provide >80% power to detect a dose-response relationship for PRAX-114 for the primary endpoint of change from baseline in HAM-D17 total score using a Multiple Comparison – Modelling (MCP-Mod) analysis (see Section 5.7). The sample size calculation assumes a monotonic dose-response relationship and an effect size of 0.59 at the 60 mg dose of PRAX-114; an effect size of 0.59 corresponds to a placebo adjusted difference of 4.7 points in the change from baseline in HAM-D17 total score at Day 15 with an assumed standard deviation of 8 points. By including 5 treatment groups and using a 1:1:1:1:1 randomization ratio, a total of 110 evaluable participants would be required. Assuming a maximum non-evaluability rate of 10%, approximately 125 participants are expected to be randomized. This sample size would also provide approximately 70% power for at least 1 candidate model assuming an effect size of 0.45.

2.4 Randomization and Blinding

Eligible participants will be randomized in a 1:1:1:1:1 ratio to either PRAX-114 or placebo. Participants will be randomized strictly sequentially (ie, in the order they are deemed eligible for randomization). Randomization will be stratified by concomitant antidepressant pharmacotherapy (yes or no) to ensure balanced allocation to treatment groups. If a participant discontinues from the clinical trial, the

participant's randomization number will not be reused, and the participant will not be allowed to re-enter the clinical trial.

The preparation, packaging, and labelling of study drug will be performed in a way to ensure blinding throughout the clinical trial.

All participants will be centrally assigned to randomized PRAX-114 or placebo using an Interactive Response Technology (IRT). Before the clinical trial is initiated, the telephone number and call-in directions for the IRT and/or the log in information and directions for the IRT will be provided to each site.

3 ANALYSIS SETS

An analysis data set will be created for each efficacy, safety, and PK assessment. In addition to raw data values, each analysis data set will include derived variables as specified in Section 4 of this SAP (eg, baseline, total, and change from baseline values). All analysis data sets will also include key demographic information (including but not limited to study, center, subject identifier, age, gender, race, ethnic group, randomization number, analysis set eligibility flags, study treatment start and stop dates, reason for treatment discontinuation, randomized treatment, and actual treatment received).

All data analyses will be performed using at least one of the below specified analysis sets. Patient eligibility for each analysis set will be finalized before database lock and unblinding of the data, where applicable.

3.1 Enrolled Analysis Set

The enrolled analysis set will include all participants who provide consent to participate in the study. This analysis set will be used for disposition analyses and listings.

3.2 Randomized Analysis Set

The randomized analysis set will include all participants who are randomized. Participants will be categorized according to randomized treatment. This analysis set will be used for dispositional analyses and listings.

3.3 Safety Analysis Set

The safety analysis set will include data from all participants who were randomized and received at least 1 dose of study drug. Participants will be classified according to actual treatment received. The safety analysis set will be used for all safety analyses.

3.4 Full Analysis Set

The full analysis set will include all randomized participants who receive at least one dose of study drug, have a valid baseline HAM-D17 assessment and at least one valid post-baseline HAM-D17 assessment. Participants will be classified according to randomized treatment. The full analysis set will be used for all efficacy analyses.

3.5 Per-protocol Analysis Set

The per-protocol (PP) analysis set is a subset of the full analysis set and will include all participants who do not have any significant protocol deviations. The PP analysis set will be defined prior to database lock. Participants will be categorized by randomized treatment. This analysis set may be used for additional analyses of the primary and key secondary efficacy variables.

3.6 Pharmacokinetic Analysis Set

The PK analysis set is a subset of the safety analysis set and will include all participants who have at least one reportable plasma concentration. This analysis set will be used for all plasma concentration analyses.

3.7 Pharmacokinetic Sub-study Analysis Set

The PK analysis set will include all participants who have enrolled into the PK sub-study and have at least one reportable plasma concentration. This analysis set will be used for all PK analyses associated with the sub-study.

4 DEFINITION OF STUDY VARIABLES

- Study Day 1 is defined as the date on which a participant is expected to take the first dose of study drug. Other study days are defined relative to Study Day 1.
- For assessments occurring on Day 1 or after, Study Day will be calculated as (date of event – date of expected first dose + 1). For assessments occurring before Day 1, Study Day will be calculated as (date of event – date of expected first dose).
- The number of days on treatment is calculated as (date of expected last dose – date of expected first dose) + 1.
- Baseline is defined as the last non-missing measurement obtained prior to the first dose of study drug.
- Change from baseline will be calculated as the value at the post-baseline visit minus the baseline value.
- Differences between treatment groups will be calculated as PRAX-114 minus placebo.

4.1 Participant Disposition

The participant number, informed consent date, protocol version at time of consent, randomization number, and randomization date will be recorded in the electronic case report form (eCRF).

Additionally, whether the patient was previously screened for the study and if so, their corresponding previous participant number as well as whether they consented to being added to the clinical trial registry or participating in the sub-study will be recorded in the eCRF. Consent and re-consent dates and protocol version at the time of reconsent will be recorded in the eCRF if applicable.

For patients not randomized, the screen fail date and reason not randomized ("Eligibility criteria not met", "Adverse event", "Lost to follow-up", "Withdrawal of consent", "Investigator decision", "Death", or "Other") will be collected in the CRF along with any specific eligibility criteria not met where applicable.

Treatment completion status (Yes/No) will also be collected on the eCRF, along with the primary reason for treatment discontinuation, adverse event or protocol deviation number (if applicable) and whether or not the subject will continue to the safety follow-up (Yes/No). Reasons for treatment discontinuation include: "Adverse event", Withdrawal of consent", "Lost to follow-up", "Investigator decision", "Non-compliance with study drug", "Significant protocol deviation", "Death", "Lack of efficacy", "Pregnancy" or "Other".

In addition, study completion status (Yes/No), the date of study completion or early withdrawal, reason for early withdrawal, and adverse event number or protocol deviation number (if applicable), will be recorded in the eCRF. Study completion status will be recorded as "Yes" or "No". The reason for early withdrawal will be recorded as "Adverse Event", "Withdrawal of consent", "Lost to follow-up", "Investigator decision", "Significant protocol deviation", "Death" or "Other".

4.2 Protocol Deviations

The failure to meet any eligibility criteria and the specific criteria not met will be recorded in the eCRF.

All participant data will be reviewed for the occurrence of protocol deviations. Protocol deviation information will be captured in the eCRF, including the date of the deviation, the deviation classification ("Major" or "Minor"), deviation category, deviation description, and if the deviation is tied to a specific assessment or visit.

Deviation categories captured on the eCRF include "Procedures/Assessments Outside Protocol Window", "Procedures/Assessments Not Done", "Incorrect Order of Procedures/Assessments", "Missed Visit", "Visit Outside Protocol Window", "Inclusion/Exclusion", "Study Drug Administration", "Documentation", "Study Restrictions", "Informed Consent", "COVID-19: Missed Visit", "COVID-19: Procedure/Assessment Not Done", "COVID-19: Procedure/Assessment Outside Protocol Window", "COVID-19: Other", and "Other".

The Sponsor project team will review all protocol deviations and their major/minor classifications prior to database lock. An impact assessment will be performed and those participants with confirmed "Major" protocol deviations that impact the primary endpoint will be excluded from the PP analysis set (see Section 3.5).

4.3 Demographic and Baseline Characteristics

4.3.1 Age

Age at the time of informed consent will be recorded in the eCRF.

4.3.2 Sex

Sex will be recorded in the eCRF as "Male" or "Female".

4.3.3 Childbearing Potential

If female, childbearing potential status will be recorded in the eCRF as "Yes" or "No".

4.3.4 Ethnicity

Ethnicity will be recorded in the eCRF as "Hispanic or Latino", "Not Hispanic or Latino", or "Not reported".

4.3.5 Race

Race will be recorded in the eCRF as "White", "Black or African American", "Asian", "American Indian or Alaska Native", "Hawaiian or Other Pacific Islander", "Other", or "Not reported".

4.3.6 Height

Height in centimeters (cm) will be recorded in the eCRF.

4.3.7 Weight

Weight in kilograms (kg) will be recorded in the eCRF.

4.3.8 Body Mass Index

Body mass index (BMI; kg/m^2) will be derived in the eCRF.

4.4 Baseline Disease Characteristics

The following psychiatric assessments will be collected on the eCRF and performed at Screening and/or prior to Randomization to determine participant eligibility or further characterize the participant's psychiatric diagnoses: MINI, and the DSM-5 MDD Anxious Distress Specifier.

The DSM-5 Anxious Distress Specifier includes 5 questions relating to anxiety and a severity rating depending on the number of affirmative responses to the questions. The severity categories are as follows: "N/A: ≤1 symptom", "Mild: Two symptoms", "Moderate: Three symptoms", "Moderate-Severe: Four to five symptoms", "Severe: Four or five symptoms and motor agitation". To meet the criteria for having the DSM-5 Anxious Distress Specifier, participants must have 2 or more affirmative responses.

The ATRQ is a self-report scale used to determine treatment resistance in MDD (Chandler et al., 2010) The ATRQ defines 6 weeks on an adequate dose of antidepressant medication as adequate duration of treatment. It also provides specific operational criteria for adequate dosage for each of the most commonly used antidepressants. Data collected on the eCRF include the specific medications taken for the current episode of MDD, if the minimally adequate dose was taken for 6 weeks and what percentage improvement in depression the patient felt when the medication was working at its best.

In addition, the MINI interview will be conducted at Screening only, used to confirm the primary diagnosis of MDD for inclusion into the trial, and the following recorded on eCRF: if the interview was completed, interviewer's initials, the date of the interview, the time the interview began and ended, any diagnoses and associated timeframe as well as the primary diagnosis. Total duration of the interview will be derived in EDC.

The HAM-D17 and CGI-S assessments (detailed descriptions in Sections 4.12.1 and 4.12.2 of this SAP, respectively) will be captured via Smartphone application, but are required to confirm eligibility prior to randomization.

The following variables will be presented for baseline disease characteristics:

- DSM-5 MDD Anxious Distress Specifier severity category and dichotomous representation
- HAM-D17 total score anxiety/somatisation factor, dichotomized anxiety/somatisation factor, and sleep factor

- CGI-S score
- ATRQ inadequate treatment response

4.5 Pharmacotherapy Status

Randomization will be stratified by concomitant antidepressant pharmacotherapy (yes or no). This variable will be used, as per randomized and in accordance with the stratification at time of randomization, in relevant efficacy analyses.

4.6 Medical History

For those participants reporting medical history, the medical condition, onset date, ongoing at Screening indicator, end date (if applicable), and treatment indicator (Yes/No) will be recorded in the eCRF. Each medical history condition will be coded using Medical Dictionary for Regulatory Affairs (MedDRA) version 24.0 or later.

4.7 Psychiatric History

Information regarding the participant's psychiatric history will be collected in the eCRF. Specifically, the date of diagnosis of MDD, the total number of depressive episodes in the participant's lifetime, the start date and duration of the current depressive episode, if the subject ever received psychotherapy for depression, whether or not the participant has been hospitalized at any time for depression and the number of times (if applicable) will be recorded. In addition, any biological family members with a history of psychiatric illness, the specific family member and associated diagnosis, as well as any childhood or recent traumatic events will be recorded on the eCRF. Traumatic events for childhood include "Death of a very close friend or family member", "Any upheaval between the parents (eg, divorce or separation)", "A traumatic sexual experience (eg, rape or molested)", "Victim of violence, mugged, assaulted other than sexual", "Extreme illness or injury". Recent traumatic events are the same for childhood traumatic events with one exception: the major upheaval refers to the patient and his/her spouse as opposed to the parents.

The time in months from the date of diagnosis of MDD to date of informed consent will be calculated using SAS as:

- INTCK("month", diagnosis date of MDD, informed consent date);

If the MDD diagnosis date is completely unknown (ie, the day, month, and year are all missing), then the diagnosis date will not be imputed (and consequently time from the date of diagnosis will also be missing).

For partial MDD diagnosis dates, the following conventions will be used for imputing the dates:

- If the year is present and the month and day are missing, then the month and day will be set to January 1.
- If the year and day are present and the month is missing, then the month will be set to January.
- If the year and month are present and the day is missing, then the day will be set to the first day of the month.

4.8 Prior and Concomitant Medications

For each prior and concomitant medication, the medication/therapy name, indication ("Treatment of AE", "Medical History Condition", "Prophylaxis/Supplement", or "Other"), dose, frequency, route, start date, ongoing indicator, and stop date (for those medications not ongoing) will be recorded in the eCRF. Each medication will be coded using the World Health Organization Drug Dictionary (WHODrug), Global B3 March 2021 version. If reason performed was "Treatment of AE" or "Medical History Condition", the relevant AE number or medical history number will also be collected.

Each medication will be classified according to the study period in which its use occurred (ie, Prior, Concomitant, Post-treatment). Prior medications are those that started and ended before the initiation of study drug. Concomitant medications are (i) those that started before initiation of study drug and continued after initiation of study drug or (ii) those that started after initiation of study drug. Any medications starting on the same day as the initiation of study drug will be considered concomitant. Post-treatment medications will be defined as any medication starting after the date of the last dose of study drug (ie, during the Follow-up period).

If the start date (or stop date) of a medication is completely unknown (ie, the day, month, and year are all missing) or only the day is known, then the start date (or stop date) will not be imputed. Unless the stop date is before the start date of study drug, then the medication will be considered concomitant.

For a partial start date of medication, the following conventions will be used for imputing the start date;

- If the year is present and the month and day are missing, then the month and day will be set to January 1.
- If the year and day are present and the month is missing, then the month will be set to January.
- If the year and month are present and the day is missing, then the day will be set to the first day of the month.
- If the imputed start date of medication is after the non-imputed end date of medication, then the start date will be set to the stop date of medication.

For a partial stop date of medication, the following conventions will be used for imputing the stop date:

- If the year is present and the month and day are missing, then the month and day will be set to December 31.
- If the year and day are present and the month is missing, then the month will be set to December.
- If the year and month are present and the day is missing, then the day will be set to the last day of the month.

4.9 Prior and Concomitant Procedures

For each participant with any surgical, therapeutic or diagnostic procedure, the following will be collected on the eCRF: name of the procedure, the reason it was performed ("Treatment of AE", "Medical History Condition", "Prophylaxis", or "Other"); start date, ongoing at the end of study indicator, and stop date. If reason performed was "Treatment of AE" or "Medical History Condition", the relevant AE number or medical history number will also be collected.

4.10 Study Drug Exposure

Study drug administration will be captured on a daily basis with Smartphone based technology throughout the Intervention Period. For each participant, the date of expected first dose is the evening of the day when study drug is first dispensed. For completers, the date of expected last dose is the evening prior to the actual Day 29 study visit. For participants that discontinue treatment, the date of expected last dose is the evening prior to the Early Termination study visit. In the event that an Early Termination study visit is not obtained, the date of expected last dose is based on the amount of drug dispensed. If the last visit is prior to drug dispensation at the Day 8 study visit, the date of expected last dose is study day 10; if the last visit is prior to the Day 15 study visit, the date of expected last dose is study day 20; and if the last visit is the Day 15 study visit or later, the date of expected last dose is study day 28.

The duration (in days) on study treatment will be calculated for each participant as:

- Date of last reported dose – date of first reported dose + 1

The expected duration (in days) on study treatment will be calculated for each participant as:

- Date of expected last dose – date of expected first dose + 1

The number of tablets reported as taken will be calculated as:

- Number of tablets reported as taken by the Smartphone based technology

Since the number of active study drug tablets varied in both mg and counts across treatment groups, the total dose (in mg) of study drug received by each participant will be calculated as:

- 10 mg: Number of days with at least 1 tablet reported as taken x 10 mg
- 20 mg: Number of days with at least 1 tablet reported as taken x 20 mg
- 40 mg: Number of days with at least 2 tablets reported as taken x 40 mg + the number of days with at least 1 tablet taken x 20 mg
- 60 mg: Number of tablets taken x 20 mg

And total dose for placebo will be 0 mg.

4.11 Treatment Compliance

Smartphone based technology will also be used to monitor daily study drug intake to confirm participant adherence to study drug administration procedures. For each tablet taken, participants are instructed to use the smartphone device to collect visual confirmation of the ingestion of study drug. For tablets taken without visual confirmation, participants will be instructed to enter dose time in the smartphone application.

For each visual confirmation using the Smartphone based technology, a green/yellow/orange/red rating scale is applied, with green indicating a confirmation of proper study drug ingestion and

yellow/orange/red indicating degrees of worse performance of study drug ingestion. Instances of red grades are indicative of not ingesting study drug therefore, for the purpose of calculating the confirmed number of tablets taken, a grade of red is considered the same as missing the dose.

The confirmed number of tablets taken will be calculated as:

- Number of tablets reported as taken that are not associated with a grade of red

It is expected that three tablets are taken on each dosing day. Therefore, using the definition of expected duration (described in Section 4.10), the number of tablets expected to be taken will be calculated as:

- Expected duration x 3

Overall Compliance (%) will be calculated as:

- (Number of tablets reported as taken/ Number of tablets expected to be taken) x 100

Confirmed overall compliance (%) will be calculated as:

- (Confirmed number of tablets taken / Number of tablets expected to be taken) x 100

Overall treatment compliance and confirmed treatment compliance will be categorized into the following categories:

- Less than 80%
- 80% to 120%
- More than 120%

4.12 Efficacy Variables

For each efficacy assessment, study day will be calculated as: the date of assessment – the date of expected first dose of study drug + 1. As deviations are expected in the number of days from the date of expected first dose (ie, study day 1) to the study day that planned assessments actually occur, visit windows will be used to derive visit numbers for use in efficacy analyses. Efficacy variable visit windows and their associated derived visit numbers and labels are defined in Table 1. Except for the SDQ administered remotely, all efficacy measures will be assigned to visit windows. In cases where more than one assessment is assigned to a specific visit window, the assessment closest to the scheduled day will be used for analysis purposes. If the assessments are equally close to the scheduled day, then the later assessment will be used for analytic purposes.

Table 1: Visit Windows for Efficacy Variables

Derived Visit Number	Visit Label	Scheduled Day		Visit Window (days) for each efficacy measure			
			HAM-D17, CGI-S	CGI-I, PGI-I	SDQ*	[redacted]	WSAS, [redacted]
1	Screening	0	<=0		<=0		<=0
2	Day 1 (Baseline)	1	1		1		1
3	Day 4	4	2 to 5	1 to 5			
4	Day 8	8	6 to 10	6 to 10	6 to 10		
5	Day 15	15	11 to 18	11 to 18	11 to 18		2 to 22
6	Day 22	22	19 to 25	19 to 25	19 to 25		
7	Day 29	29	26 to 32	26 to 32	26 to 32		23 to 36
8	Day 36	36	33 to 39	33 to 39	33 to 39		
9	Day 43	43	40+	40+	40+		37+

*Analysis visit windows apply only to SDQ assessments associated with visits expected to be performed in-clinic

For all efficacy variables, baseline will be defined as the last assessment prior to the expected first dose of study drug. Change from baseline values will be calculated as the assessment value minus the baseline value.

4.12.1 Hamilton Depression Rating Scale

The HAM-D17 assessment will be conducted utilizing a tablet-based assessment platform that follows the Structured Interview Guide for the Hamilton Depression Rating Scale (SIGH-D).

The HAM-D17 is a depression rating scale consisting of 17 items; 9 items are scored on a 5-point scale (ranging from 0 to 4) and 8 items are scored on a 3-point scale (ranging from 0 to 2). The total score is the sum of the 17 items and ranges from 0 to 52, with higher scores indicating greater depression. Therefore, a decrease in the total score or on individual item scores indicates improvement. (Hamilton, 1960).

For the HAM-D17 assessment, if two or more item scores are missing, then the total score will also be considered missing. If one item score is missing, the total score will be imputed using the following:

- If the missing item is scored on a 0 to 4 scale (ie, item 1, 2, 3, 7, 8, 9, 10, 11, or 15), then the HAM-D17 total score = (52/48) x $\sum$ (non-missing items)

- If the missing item is scored on a 0 to 2 scale (ie, item 4, 5, 6, 12, 13,14, 16, or 17),

  then the HAM-D17 total score = (52/50) x ∑ (non-missing items)

Responses of 3="NA" on question 16 will be considered as 0 for the calculation of HAM-D total score.

The anxiety/somatisation and the sleep factors of the of HAM-D17 will also be summarized. The anxiety/somatisation factor consists of six items (anxiety psychic, anxiety somatic, gastrointestinal somatic, general somatic, hypochondriasis, and insight) and the sleep factor consists of three items (insomnia early, insomnia middle, and insomnia late). The factor scores are the sum of the factor-specific items. For the anxiety/somatisation and sleep factors, if one or more item scores are missing, the factor score will be considered as missing. Additionally, the anxiety/somatisation factor will be dichotomized into two categories to describe anxious depression: <7 and >=7.

The change from baseline for the HAM-D17 total score, anxiety/somatisation factor, and sleep factor will be calculated at each post-baseline assessment.

4.12.1.1 HAMD-17 Response

For each post-baseline assessment, an indicator of HAM-D17 response will be calculated as:

- IF the percent reduction from baseline in the HAM-D17 total score is ≥50%, THEN set the HAM-D17 response indicator equal to 1.
- IF the percent reduction is <50%, THEN set the HAM-D17 response indicator equal to 0.

4.12.1.2 HAMD-17 Remission

For each post-baseline assessment, an indicator of HAM-D17 remission will be calculated as:

- IF the HAM-D17 total score is ≤7, THEN set the HAM-D17 remission indicator equal to 1.
- IF the HAM-D17 total score is >7, THEN set the HAM-D17 remission indicator equal to 0.

4.12.1.3 Anxiety/Somatisation Dichotomized

For each post-baseline assessment, an indicator of HAM-D17 anxious depression will be calculated as:

- IF the HAM-D17 anxiety/somatisation factor score is <7, THEN set the HAM-D17 anxiety/somatisation dichotomized factor equal to 1.
- IF the HAM-D17 anxiety/somatisation factor score is >=7, THEN set the HAM-D17 anxiety/somatisation dichotomized factor equal to 0.

4.12.2 Clinical Global Impression

The Clinical Global Impression (CGI; Guy, 1976) scale is a clinician-rated scale consisting of 3 items. Only 2 of the CGI items will be used in this trial: the CGI – Severity (CGI-S) and CGI – Improvement (CGI-I). Both CGI items will be performed using a tablet-based assessment platform.

The CGI-S item is used to assess the clinician's impression of the participant's current depression symptoms and is rated on a 7-point scale from 1 = "Normal, not at all ill" to 7 = "Among the most severely ill patients".

The CGI-I item is used to assess the participant's improvement (or worsening) and is rated on a 7-point scale from 1 = "Very much improved" to 7 = "Very much worse".

A CGI-S or CGI-I score of "0" will be considered as missing as this score denotes "Not assessed".

The change from baseline in CGI-S will be calculated at each post-baseline assessment.

4.12.2.1 CGI-I Response

For each post-baseline assessment, an indicator of CGI-I response will be calculated as:

- IF the CGI-I score is ≤2 (ie, "very much improved" or "much improved"), THEN set the CGI-I response indicator equal to 1.
- IF the CGI-I score is >2, THEN set the CGI-I response indicator equal to 0.

4.12.3 Symptoms of Depression Questionnaire

The Symptoms of Depression Questionnaire (SDQ) is a 44-item, self-report scale assessing the severity of symptoms across several subtypes of depression. The SDQ will be administered both in-person at the in-clinic visits and remotely. SDQ item responses will be collected using a smartphone application.

The 44 items are rated on a 6-point scale (1 to 6). Each item is rated based on a participant's perception of what is normal for the individual (item score=2), what is better than normal (item score=1), and what is worse than normal (item scores=3 to 6). There are 5 subscales defined for the SDQ. Each subscale score is calculated as the sum of the items comprising the scale. Table 2 defines each subscale and provides the items included and the scoring range.

Table 2: SDQ Subscale Definitions

SDQ Subscale	Individual items included	Subscale score range
SDQ-1 (Lassitude, mood, cognitive functioning)	2, 3, 5, 7, 16, 17, 18, 19, 20, 22, 35, 36, 37, 38, 39, 40, 41, 42	18 to 108
SDQ-2 (Anxiety, agitation, irritability, anger)	4, 6, 8, 21, 23, 24, 25, 26, 27, 32, 33, 34, 43	13 to 78
SDQ-3 (Suicidal ideation)	1, 9, 10, 11, 12, 44	6 to 36
SDQ-4 (Sleep quality)	13, 14, 15	3 to 18
SDQ-5 (Changes in appetite and weight)	28, 29, 30, 31	4 to 24

The SDQ total score is the sum of all 44 item scores and ranges from 44 to 264, with higher scores indicating greater depression.

For the SDQ total score, if two or more item scores are, then the total score will also be considered missing. If one item score is missing, the total score will be imputed using the following:

- SDQ total score = (264/258) x $\sum$ (non-missing items)

Subscale scores will be set to missing if there are any missing item scores.

The change from baseline will be calculated at each post-baseline timepoint for both the total score as well as each of the subscale scores.

4.12.4 Patient Global Impression of Improvement

The Patient Global Impression of Improvement scale (PGI-I) is a global self-assessment used to rate the response of a participant's condition to therapy or intervention. It consists of 1 question that asks the participant to rate their current condition compared to how it was prior to beginning treatment on a scale of 1 ("Very much better") to 7 ("Very much worse").

The PGI-I will be collected using a smartphone application.

4.12.5 Changes in Sexual Functioning Questionnaire

The CSFQ-14 is a structured self-reported questionnaire designed to measure illness- and medication-related changes in sexual functioning that consists of 14 items (Clayton et al., 1997). Separate versions are administered to females and males. In both versions, each item is scored on a scale from 1 to 5 with lower scores associated with worse sexual functioning. The CSFQ-14 will be collected on paper and entered into EDC.

The CSFQ-14 total score is calculated as the sum of the 14 item scores. A CSFQ-14 responder is defined as a participant that experiences an increase from baseline by at least 3 points in the CSFQ-14 total score. Normal sexual functioning status is determined separately for females and males. For females, sexual functioning is demonstrated by a score that is greater than or equal to 41 while the score must be greater than or equal to 47 for males. Additionally, the CSFQ-14 provides scoring for the 5 subscales of pleasure (1 item), desire/frequency (2 items), desire/interest (3 items), arousal (3 items), and orgasm (3 items). Two items are included in the total score, but do not map to a specific subscale. The total CSFQ-14 score ranges from 14 to 70.

Table 3: CSFQ-14 Subscale Definitions

CSFQ-14 Subscale	Individual items included	Subscale score range
Pleasure	1	1 to 5

Desire/Frequency	2, 3	2 to 10
Desire/Interest	4, 5, 6	3 to 15
Arousal/Excitement	7, 8, 9	3 to 15
Orgasm/Completion	11, 12, 13	3 to 15

Note: Items 10 and 14 are included in the Total CSFQ Score, but do not map to any subscale.

For the CSFQ-14 total score, if two or more item scores are missing, then the total score will also be considered missing. If one item score is missing, the total score will be imputed using the following:

- CSFQ-14 total score = (70/65) x ∑ (non-missing items)

The change from baseline will be calculated at each post-baseline timepoint.

4.12.5.1 CSFQ-14 Response

For each post-baseline assessment, an indicator of CSFQ-14 response will be calculated as:

- IF the change from baseline in the CSFQ-14 total score is ≥3, THEN set the CSFQ-14 response indicator equal to 1.
- IF the change from baseline is <3, THEN set the CSFQ-14 response indicator equal to 0.

4.12.5.2 CSFQ-14 Sexual Functioning Status

For each assessment, an indicator of CSFQ-14 normal sexual functioning status will be calculated as:

- IF the participant is female AND the score is ≥41, THEN set the CSFQ-14 normal sexual functioning status indicator equal to 1.
- IF the participant is female AND the score is <41, THEN set the CSFQ-14 normal sexual functioning status indicator equal to 0.
- IF the participant is male AND the score is ≥47, THEN set the CSFQ-14 normal sexual functioning status indicator equal to 1.
- IF the participant is male AND the score is <47, THEN set the CSFQ-14 normal sexual functioning status indicator equal to 0.

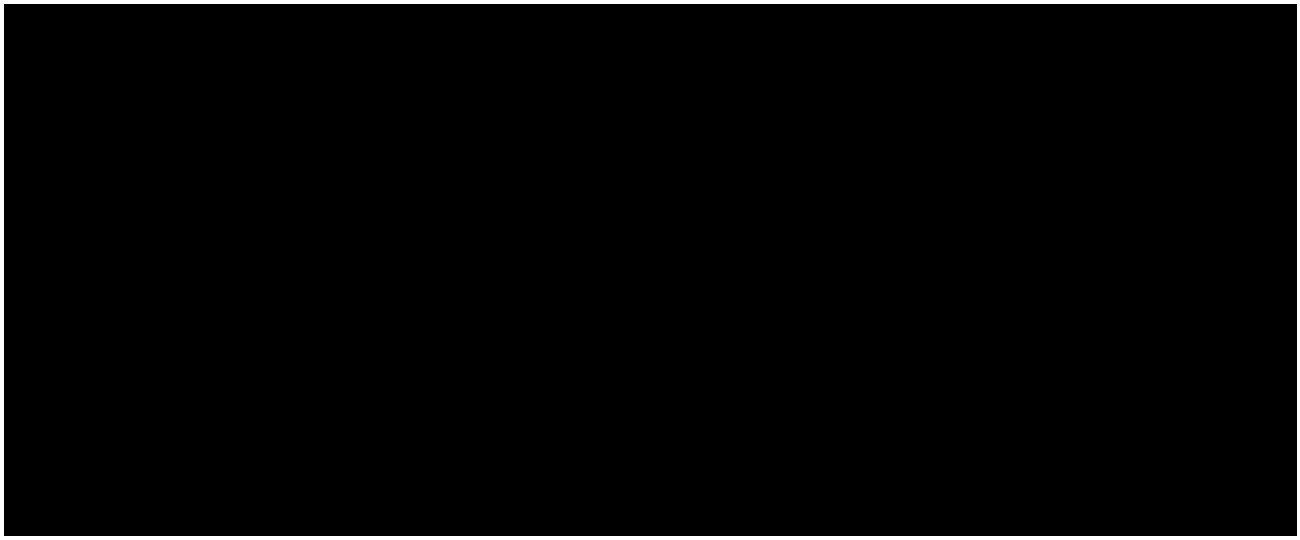
4.12.6 Work and Social Adjustment Scale

The Work and Social Adjustment Scale (WSAS) assesses the degree to which mental health problems interfere with day-to-day functioning in 5 domains: work, social leisure activities, private leisure activities, home- management, and personal relationships (Mundt et al., 2002). The measure provides an assessment of the experiential impact of mental health symptoms from the sufferer's point of view. The WSAS will be collected using a smartphone application.

Each domain includes one item with each item rated on a scale of 0 to 8, where 0 = "Not at all impaired" and 8 = "Very severely impaired". The WSAS total score is the sum of the 5 item scores and ranges from 0 to 40 with higher scores indicating poorer adjustment.

The WSAS total score will be set to missing if there are any missing item scores.

The change from baseline will be calculated at each post-baseline timepoint.



4.13 Safety Variables

For all safety variables, the baseline value will be derived as the last observation prior to the start of study drug. Change from baseline values will be calculated as the assessment value minus the baseline value.

4.13.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a patient or clinical trial participant, temporally associated with the use of study drug, whether or not considered related to the study drug. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study drug.

AEs are collected from the time the informed consent form is signed until study participation is completed. The investigator will be responsible for the follow up of ongoing AEs until the event or its sequelae, resolve or stabilize, or 30 days post-last dose of study drug.

For each AE, the following data will be recorded in the eCRF: verbatim text, start date and time (if collected), stop date and time (if collected), investigator's assessment of severity, relationship to disease under study, relationship to study procedures, relationship to study drug, action taken with study drug,

administration of concomitant medication, any procedures performed, outcome, assessment of seriousness and serious criteria, if applicable. The verbatim text will be coded using MedDRA version 24.0 or later.

- Action taken with study drug will be recorded as “None”, “Study drug interrupted”, “Study drug withdrawn”, or “Not applicable”;
- Outcome will be recorded as “Recovered/Resolved”, “Recovered/Resolved with Sequelae”, “Not Recovered/Not Resolved”, “Fatal”, or “Unknown”;
- Relationship to disease under study, study procedures, and study drug will be recorded as “Unrelated” or “Related”;
- Investigator’s assessment of severity will be recorded as “Mild”; “Moderate”; or “Severe”;
- Assessment of seriousness will be recorded as “Y” or “N”.
- If applicable, serious criteria will be recorded as “Results in Death”, “Date of Death”, “Cause of Death”, “Life Threatening”, “Requires or Prolongs Existing Hospitalization”, “Date of Admission”, “Date of Discharge”, “Results in Persistent or Significant Disability/Incapacity”, “Congenital Anomaly/Birth Defect”, or “Other Important Medical Event”

A flag for AEs leading to study drug withdrawal will be calculated as:

- IF the action taken with study drug is “Study drug withdrawn”, THEN set the AE leading to study drug withdrawal flag equal to “Y”.
- OTHERWISE, set the AE leading to study drug withdrawal flag equal to “N”.

Treatment emergent AEs (TEAEs) are defined as those AEs occurring or worsening after the first dose of study drug. If an AE start date is completely missing (ie, in which the day, month, and year are all unknown), then the AE start date will be set to the date of first dose of study drug. For a partial AE start date, the following conventions will be used for imputing the AE start date:

When the year is present and the month and day are missing:

- If the year of AE start = the year of first dose of study drug, then the month and day will be set to the month and day of first dose of study drug.
- If the year of AE start < the year of first dose of study drug, then the month and day will be set to December 31st.
- If the year of AE start > the year of first dose of study drug, then month and day will be set to January 1st.
- When the year and day are present and the month is missing:
- If the year of AE start = the year of first dose of study drug, then the month will be set to the month of first dose of study drug.
- If the year of AE start < the year of first dose of study drug, then the month will be set to December.
- If the year of AE start > the year of first dose of study drug, then the month will be set to January.

When the month and year are present and the day is missing:

- If the year of AE start = the year of first dose of study drug and
- the month of AE start = the month of first dose of study drug, then the day will be set to the day of first dose of study drug.

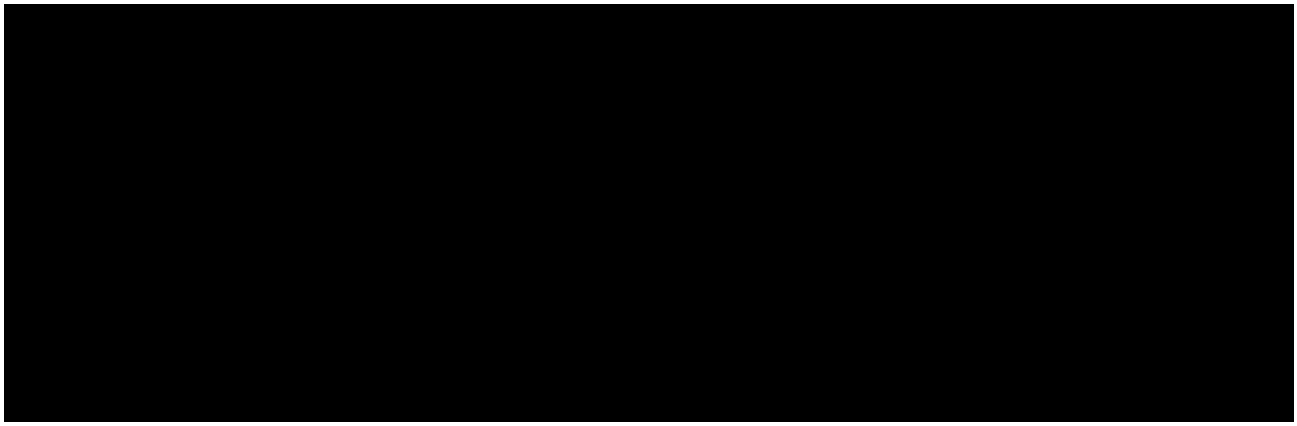
- the month of AE start < the month of first dose of study drug, then the day will be set to the last day of the month.
- the month of AE start > the month of first dose of study drug, then the day will be set to the 1st day of the month.
- If the year of AE start < the year of first dose of study drug, then the day will be set to the last day of the month.
- If the year of AE start > the year of first dose of study drug, then the day will be set to the 1st day of the month.
- If the imputed AE start date is after the AE stop date, then the AE start date will be set to the AE stop date.

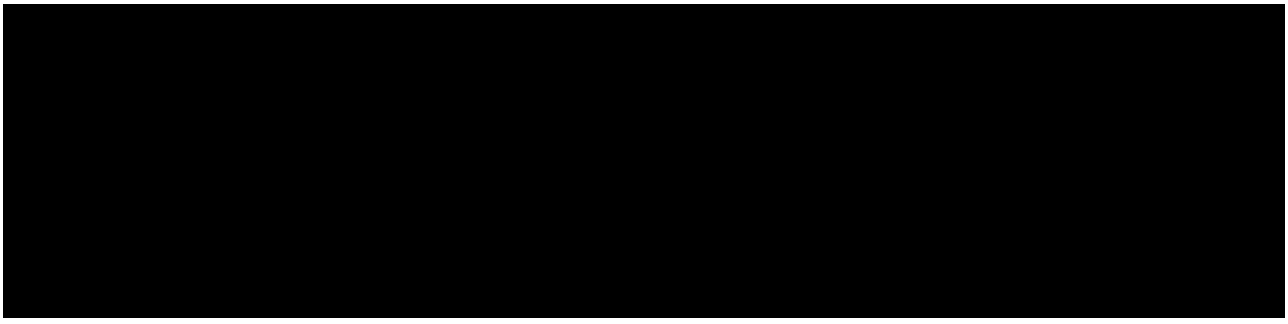
For each AE, a treatment emergent flag will be calculated using the recorded AE start date (or imputed start date, where applicable).

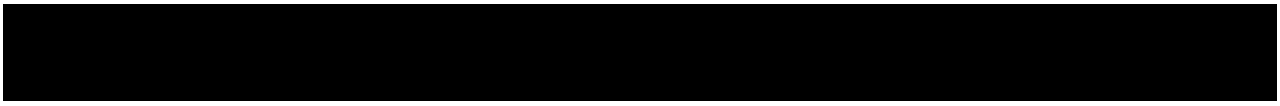
- IF the date of first dose of study drug ≤ AE start date, THEN set the treatment emergent flag equal to "Y".

In addition, a post-treatment flag will be calculated using the recorded AE start date (or imputed start date, where applicable).

- IF the AE start date > date of last dose of study drug, THEN set the post-treatment flag equal to "Y".







[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[a] Collected at Screening only.
[b] Collected at Screening for post-menopausal women only.
[c] Collected at Screening and Day 15 only, for adjunctive treatment participants only.

4.13.4 Vital Sign Results

The following vital sign data will be recorded in the eCRF: assessment date, height, weight, BMI, oral temperature, temperature method (oral or tympanic), pulse rate, respiratory rate, systolic blood pressure (BP), and diastolic BP.

Change from baseline values to each post-baseline assessment will be calculated for pulse rate, respiratory rate, systolic BP, and diastolic BP. In addition, potentially clinically important flags will be derived for systolic BP, diastolic BP, pulse rate, and respiratory rate (see Table 5).

Table 5: Potentially Clinically Important Criteria for Vital Sign Results

Vital Sign	Lower PCI criterion	Upper PCI criterion
Pulse rate	≤50 bpm	≥130 bpm
Systolic BP	<90 mmHg	>160 mmHg
Diastolic BP	<50 mmHg	>100 mmHg
Change in systolic BP	decrease ≥30 mmHg	increase ≥30 mmHg
Change in diastolic BP	decrease ≥20 mmHg	increase ≥20 mmHg

BP = blood pressure; PCI = potentially clinically important

4.13.5 Electrocardiogram Results

Standard 12-lead ECGs will be performed using the equipment supplied by the central ECG vendor. For all ECGs performed, the following data will be recorded in the eCRF: assessment date, if the ECG was performed in triplicate ("Yes" or "No"), overall interpretation ("Normal" or "Abnormal") and if the ECG was clinically significant ("Yes" or "No").

Electronic ECG tracings will be analyzed by the central ECG vendor for the calculation of reported values for the following ECG tests: heart rate, RR interval, PR interval, QRS duration, QT interval, corrected QT interval using the Fridericia formula (QTcF), and evaluator interpretation. ECG vendor data will include ECG test name, reported result/finding, reported unit, standard result/finding, standard

unit, lead location, method of ECG Test ("12 Lead Standard") position of the subject during the ECG ("Supine"), and ECG category ("Measurement", "Finding", "Interpretation", "Technical"). The evaluator interpretation result will be recorded as "NORMAL", "ABNORMAL", or "UNABLE TO EVALUATE". Data from the ECG vendor will be combined with the ECG eCRF data prior to analysis.

Change from baseline values to each post-baseline assessment will be calculated for heart rate, RR interval, PR interval, QRS duration, QT interval and QTcF interval. In addition, potentially clinically important flags will be calculated for heart rate, PR interval, QRS duration, QT interval, QTcF interval, change from baseline in heart rate, and change in QT, QTcB, and QTcF intervals (see Table 6).

Table 6: Potentially Clinically Important Criteria for ECG Results

ECG measure	Lower PCI criterion	Upper PCI criterion
Heart rate	≤50 bpm	≥130 bpm
PR interval		≥300 msec
QRS duration		≥140 msec
QT interval		≥500 msec
QTcF interval		≥500 msec
Change in QTcF		increase ≥40 msec

ECG = electrocardiogram; PCI = potentially clinically important

4.13.6 Columbia–Suicide Severity Rating Scale

The Columbia–Suicide Severity Rating Scale (C-SSRS; available at www.cssrs.columbia.edu) is a semi-structured interview that captures the occurrence, severity, and frequency of suicide-related thoughts and behaviors during the assessment period. The interview includes definitions and suggested questions to solicit the type of information needed to determine if a suicide-related thought or behavior has occurred. The C-SSRS has a "Screening/Baseline" version that will be completed at the Screening Visit (Visit 1) and a "Since Last Visit" version that will be completed at all other visits. Both versions contain all 11 categories of suicidal ideation and behavior defined in the FDA guidance document on suicidal behavior and ideation (FDA, 2012). Each category is assessed using a single question requiring a "Yes" or "No" response with follow-up questions for any "Yes" responses. The assessment date and each C-SSRS response will be recorded in the eCRF.

In addition, three C-SSRS composite indicator variables will be calculated as follows:

4.13.6.1 C-SSRS Suicidal Ideation Indicator

A composite indicator of C-SSRS suicidal ideation will be calculated as:

- IF the response to any one of the five suicidal ideation questions is "Yes", THEN set the suicidal ideation indicator equal to 1.
- OTHERWISE, set the suicidal ideation indicator equal to 0.

4.13.6.2 C-SSRS Suicidal Behavior Indicator

A composite indicator of C-SSRS behavior ideation will be calculated as:

- IF the response to any one of the five suicidal behavior questions is "Yes", THEN set the suicidal behavior indicator equal to 1.
- OTHERWISE, set the suicidal behavior indicator equal to 0.

4.13.6.3 C-SSRS Suicidal Ideation or Behavior Indicator

A composite indicator of C-SSRS suicidal ideation or behavior will be calculated as:

- IF the response to any one of the 5 suicidal ideation questions or any of the 5 suicidal behavior questions is "Yes", THEN set the suicidal ideation or behavior indicator equal to 1.
- OTHERWISE, set the suicidal ideation or behavior indicator equal to 0.

4.14 Pharmacokinetic Variables

For all patients, blood samples will be collected by site staff for PK testing. For all PK samples collected, the following data will be recorded in the eCRF: sample collection date and sample collection time. A validated bioanalytical method will be utilized for the determination of plasma and urine concentrations of PRAX-114 and its metabolites.

Additionally, a sub-study to investigate the PK of PRAX-114 and its metabolites dosed in the evening in participants with MDD will be conducted in a subset of participants at selected research sites with serial PK sampling capabilities. All study participants at the designated research sites will be offered participation in the PK sub-study, which will be conducted within 3 days of completing the Visit 7/Day 29 visit assessments (Table 3). To qualify for entrance into the PK sub-study, participants must have completed the treatment period of the parent study through Visit 7/Day 29. Participants will be admitted to the clinical research in-patient unit and remain in the unit until the last PK sample is collected. Sub-study participants will receive the same treatment to which they were assigned during the parent study. Sub-study participants will return for follow-up study procedures as defined in the main study schedule of assessments (as per the Appendix in Section 9 of this SAP). It is anticipated that approximately 20 participants will participate in the PK sub-study.

Analysis of plasma samples for concentrations of PRAX-114 and its metabolites will be conducted by Syneos Health (Princeton, New Jersey, USA) using a validated bioanalytical assay. The following data will be transferred from Syneos Health: study ID ("PRAX-114-214"), site ID, subject ID, visit name, laboratory name ("Syneos Health"), specimen reference ID, specimen collection date, planned timepoint (where applicable), category for lab test ("ANALYTE"), specimen material type ("plasma"), method of test or examination ("LC0MS/MS"), lower limit of quantitation, pharmacokinetic test name, test status along with reason not done, if applicable, and results or finding in original units, and any comments.

4.15 Biomarker Variables

Biomarker data will be collected by the central laboratory and will include levels of high sensitivity C-reactive protein (hs-CRP), proinflammatory panel: interferon gamma (IFN-γ), interleukin 10 (IL-10,)

[redacted]

5 ANALYSIS METHODS

All personnel involved in the conduct and analysis of the study will remain blinded to individual participant treatment assignments until the study has been completed, the data have been checked for accuracy with any errors corrected, and all analysis sets have been identified (where applicable). Analysis will be carried out using SAS® (version 9.4 or newer, SAS Institute Inc, Cary, NC). Templates for the summary tables, figures, and participant data listings will be available separately. Data listings will include all data recorded for all enrolled participants; if captured, data entered for screen failure participants will be flagged in the listings.

Descriptive statistics for continuous data will include number of participants (n), mean, standard deviation (SD), median, minimum, and maximum, unless otherwise noted. Summaries of change from baseline variables will include only participants who have both a baseline value and corresponding value at the timepoint of interest. Descriptive statistics for categorical data will include frequency and percentage. Where appropriate, 90 or 95% confidence intervals (CIs) may be reported.

The minimum and maximum will be reported with the same degree of precision (ie, the same number of decimal places) as the recorded data. Measures of location (eg, mean and median) will be reported to 1 degree of precision more than the recorded data, and measures of spread (eg, standard deviation) will be reported to 2 degrees of precision more than the recorded data.

Because there is a stratification variable (pharmacotherapy status), summary tables and analyses will be repeated for each of the two stratification levels (monotherapy and adjunctive therapy). Listings will include an indicator of pharmacotherapy status.

In general, all collected data on randomized participants and any derived efficacy parameters from the questionnaire data will be presented in subject data listings. Listings will be ordered by treatment, subject number, and assessment visit or assessment date. The treatment group presented in listings will be based on the planned assignment for efficacy endpoints and actual assignment for safety endpoints.

Any changes to the analyses that are not included in this SAP will be documented in the CSR.

5.1 Participant Disposition

All participants who provide informed consent will be included in disposition summaries, including the number of patients who participated in the optional PK sub-study. The number of participants screened, screen failed, randomized, completed and discontinued study treatment (with reason for treatment discontinuation), completed and discontinued from the study, as well as reason for discontinuation, will be summarized by treatment group and overall. The number of participants in each analysis set will also be summarized. All disposition data will be listed by participant.

5.2 Demographics and Other Baseline Characteristics

Descriptive summaries of the demographic and other baseline characteristics will be provided by treatment group and overall using the Randomized analysis set. These tabulations will include the following variables:

- Age
- Sex
- Child bearing potential
- Ethnicity
- Race
- Height
- Weight
- BMI
- DSM-5 MDD Anxious Distress Specifier severity category and dichotomous representation
- HAM-D17 total score, anxiety/somatisation factor, dichotomized anxiety/somatisation factor, and sleep factor
- CGI-S score
- ATRQ inadequate treatment response category

All demographics and other baseline characteristics data will be listed by participant.

5.3 Medical History

Descriptive summaries of medical history will be provided by treatment group and overall using the safety analysis set. Medical history will be displayed by MedDRA system organ class (SOC) and preferred term (PT). All medical history data will be listed by participant.

5.4 Psychiatric History

Descriptive summaries of psychiatric history will be provided by treatment group and overall using the safety analysis set. All psychiatric history data will be listed by participant.

5.5 Prior and Concomitant Medications and Procedures

Descriptive summaries of prior medications will be provided by treatment group and overall using the Randomized analysis set. Descriptive summaries of concomitant medications will be provided by treatment group using the safety analysis set. Both prior and concomitant medications will be displayed by Anatomical Therapeutic Chemical (ATC) classification level 3 and preferred term. All prior and concomitant medication data will be listed by participant.

All prior and concomitant procedure data will be listed by participant.

5.6 Protocol Deviations

Major protocol deviations will be summarized by treatment group and overall using the Randomized analysis set. All inclusion/exclusion criteria and protocol deviation data will be listed by participant.

5.7 Primary Efficacy Analysis

Estimand attributes for the primary efficacy analysis are defined according to the five attributes specified in ICH E9(R1) Addendum:

- Treatment – Daily administration of PRAX-114 as compared to the reference treatment of placebo
- Population – Participants diagnosed with recurrent MDD and who are currently experiencing a moderate to severe MDE and who may be treated with an antidepressant at a stable dose as described in the study inclusion criteria
- Variable – Change from baseline in the HAM-D17 total score at Day 15
- Intercurrent event (IE) – Any discontinuation of study drug during the intervention period
- Population level summary – The mean change from baseline to Day 15 in HAM-D17 total score will be estimated for each dosing group (including placebo). The presence of a dose-response relationship at Day 15 will be assessed. Additionally, PRAX-114 will be compared to placebo using the difference in group means

The hypothetical strategy will be used to account for intercurrent events in the primary analysis. Under the hypothetical strategy, any efficacy data collected after the occurrence of an intercurrent event (ie, any discontinuation of study drug) will be set to missing. Because participants are asked to complete an early termination visit upon treatment discontinuation, efficacy data collected within two study days of the last confirmed dose will not be set to missing. Efficacy data collected more than two study days after treatment discontinuation will be set to missing. This strategy assumes that any participants discontinuing study drug would have had values similar to those participants continuing to receive the same randomized treatment at the timepoint of interest.

To demonstrate the dose-response relationship between PRAX-114 treatment (including placebo) and the primary endpoint, the primary analysis will be based on a Multiple Comparisons and Modeling (MCP-Mod) analysis. This analysis tests for a dose-response relationship, allowing for uncertainty in the dose-response relationship through inclusion of contrasts from a candidate set of pre-specified dose-response models. If a dose-response relationship exists, fitted estimates for the change from baseline in HAM-D17 total scores will be calculated for each treatment group using the candidate dose-response model associated with the largest test statistic.

Six candidate models will be tested using the MCP-Mod methodology. All 6 candidate dose response models assume a monotonic relationship between efficacy and PRAX-114 dose. The candidate models considered are shown in Table 7 below.

Table 7**: Candidate Models**

Model number	**Candidate model specification**
1	Logistic (ED50=30, δ=0.5)
2	Logistic (ED50=20, δ=0.01)
3	Logistic (ED50=20, δ=5.0)
4	Linear

5	E_{max} (ED50=30)
6	E_{max} (ED50=10)

For each candidate model, the dose response will be tested using the optimal contrast vector for the model. The additional covariates of pharmacotherapy strata and baseline HAM-D17 score will be included in each model. The primary comparison of interest will be a test of the dose response at Day 15. A multiplicity-adjusted p-value will be compared to the overall one-sided alpha level of 0.05. If any of the candidate dose-response models are associated with an adjusted p-value less than 0.05, a dose-response relationship will be demonstrated. If more than one candidate dose-response model meets this threshold, the candidate dose-response model associated with the largest test statistic will be selected. If none of the six candidate dose-response models meet this threshold, then the primary analysis will end indicating that a dose-response relationship cannot be established. The test statistic and adjusted p-value will be reported for each of the six candidate models.

Model-based point estimates and standard errors will be reported for the selected dose-response model and the two covariates (pharmacotherapy strata and baseline HAM-D17 score). Using the mean baseline HAM-D17 scores for each pharmacotherapy strata, the mean (standard error) for each treatment group will be reported and plotted (ie, one plot for monotherapy and one plot for adjunctive therapy). Template code based on the DoseFinding package in R can be found in Appendix B.

5.8 Sensitivity Analysis

5.8.1 Intent-to-Treat Approach

Estimand attributes for the intent-to-treat sensitivity analysis are similar to those described for the primary efficacy analysis in Section 5.7.

The treatment policy strategy will be used to account for intercurrent events in this sensitivity analysis. Under the treatment policy strategy, an intercurrent event is considered to be irrelevant to the participant's outcome and any efficacy data collected after the occurrence of an IE will be included as is (ie, the value for the variable of interest is used regardless of whether or not the intercurrent event occurs). This strategy assumes that any participants discontinuing study drug would have had values similar to those observed despite no longer receiving randomized treatment.

The MCP-Mod procedure will be performed with the full analysis set using similar methods to those described for the primary efficacy analysis.

5.9 Secondary Efficacy Analysis

All secondary efficacy analyses will use a similar estimand as the primary endpoint analysis (ie, using the hypothetical strategy), but will replace the population level summary with the appropriate endpoint-specific summary.

Additional hypothesis testing will be conducted comparing the 60mg dose level to placebo. The overall Type I error rate will be controlled using a hierarchical testing procedure. Provided the primary analysis declares a dose-response relationship at the 5% significance level, the order of testing will be as follows:

- Day 15, PRAX-114 60mg versus placebo
- Day 29, PRAX-114 60mg versus placebo
- Day 8, PRAX-114 60mg versus placebo
- Day 4, PRAX-114 60mg versus placebo
- Day 43, PRAX-114 60mg versus placebo

Note that statistical significance at the two-sided level of 0.05 must be achieved for each test in order to continue testing to the next timepoint in the hierarchy.

The presence of a dose-response signal at the Day 29 visit will be tested using the same MCP-Mod methods described for the primary efficacy analysis in Section 5.7. In addition to using the hypothetical strategy, the dose-response signal at the Day 29 visit will also be tested using the treatment policy strategy (similar to the sensitivity analysis).

The efficacy for each dose of PRAX-114 compared to placebo, as measured by changes from baseline in HAM-D17 total scores, will be analyzed using mixed model repeated measures (MMRM) methods and the full analysis set. The model will include visit, treatment group, and the treatment-by-visit interaction as fixed factors and pharmacotherapy strata and baseline HAM-D17 total score as covariates. Robust variance estimates for the fixed effects will be used for testing treatment differences. Within-subject variability will be modeled using an unstructured covariance pattern. In case of convergence problems, a banded Toeplitz (ie, TYPE = TOP(6)) structure for the covariance pattern will be used. The Kenward-Roger approximation will be used to estimate the denominator degrees of freedom. The comparisons of interest will be the difference between each dosing level of PRAX-114 and the placebo treatment group at each visit. Model-based point estimated (ie, least square [LS] means for each treatment group and the treatment difference), 95% CI for the difference, and p-value will be reported for each windowed visit.

Similar MMRM methods will be used for other secondary change from baseline efficacy variables (ie, HAM-D17 anxiety/somatisation and sleep factors, CGI-S, CGI-I, SDQ total and subscales, and WSAS total and domain scores). Since the PGI-I is a comparative scale and does not have a baseline value, total scores will be analyzed using an MMRM without the baseline covariate.

For the analysis of dichotomous efficacy variables (HAM-D17 response and remission, dichotomized HAM-D17 anxiety/somatisation factor, CGI-I response), each variable will be analyzed using generalized estimating equation (GEE) methods using the logit link function and binomial distribution. The GEE models will include visit, treatment group, and treatment-by-visit interaction as fixed factors, pharmacotherapy strata and baseline score (ie, HAM-D17 total score or CGI-S score) as a covariate. Within subject variability will be modeled using an unstructured (ie, TYPE = UN) covariance pattern. In case of convergence problems, an exchangeable (ie, TYPE = EXCH) structure for the covariance pattern will be used. The comparison of interest will be the difference between each dosing level of PRAX-114 and the placebo treatment group at each visit. Model based point estimates (ie, odds ratios), 95% confidence interval for the odds ratio, and p-value will be reported for each comparison within each windowed visit.

Descriptive statistics for efficacy scores, change from baseline scores, and response/remission indicators will be summarized by treatment group and windowed visit (see Section 4.12). All efficacy data will be listed by participant and visit.

5.10 Subgroup Analyses

Additional subgroup analyses will be performed for the MMRM-based comparisons of 40mg or 60mg versus placebo and 60mg versus placebo. Similar MMRM methods will be used as described for the secondary efficacy MMRM analyses. The following list details the pre-specified subgroup analyses:

- Combined 40mg and 60mg versus placebo MMRM analysis
- 60mg MMRM subgroup analyses:
    - Age (< median, >= median)
    - Sex (Female, Male)
    - Age-by-sex (Female < median, >= median; Male < median, >= median)
    - Race (Black or African American, Other, White)
    - BMI (< median, >= median)
    - Baseline HAM-D17 score (< median, >= median)
    - HAM-D17 sleep factor (< median, >= median)
    - AE of interest (somnolence, sedation, or dizziness)
    - One week (Day 8) decrease in HAM-D17 sleep factor of 4 or more points or having experienced an AE of interest

Median refers to the baseline median value of the given grouping variable (eg, baseline median BMI for the BMI grouping variable). Should the sample size within a subgroup be insufficient to achieve model convergence with either the unstructured or banded Toeplitz covariance structures, the subgroup analysis will not be performed.

5.11 Exploratory Efficacy Analyses

5.11.1 Perceived Stress Scale

Change from baseline in the Perceived Stress Scale (PSS) at Day 15, Day 29, and Day 43 will be analyzed using similar MMRM models as described above in Section 5.9.

To investigate the baseline PSS score and its relationship to primary and key secondary endpoints, baseline PSS score for each participant will be categorized according to tertiles. The change from baseline in HAM-D17 total score will be presented using descriptive statistics for each baseline PSS tertile category by treatment group and windowed visit.

Descriptive statistics for PSS scores and change from baseline scores will be summarized by treatment group and windowed visit (see Section 4.12). All PSS data will be listed by participant and visit.

5.11.2 Changes in Sexual Functioning Questionnaire

Change from baseline in the Changes in Sexual Functioning Questionnaire (CSFQ-14) total score and subscale scores at Day 15, Day 29, Day 36, and Day 43 will be analyzed using similar MMRM models as described above in Section 5.9. Additionally, change from baseline in the CSFQ-14 total score will be analyzed separately for females and males again using the MMRM models as described above in Section

[redacted]

[redacted]

[redacted]

5.12 Pharmacokinetic Analyses

5.12.1 Plasma Concentrations

Plasma concentrations of PRAX-114 and its metabolites (M14, M15, and M16) will be summarized by nominal sampling time point (ie, nominal visit for main portion of the study and nominal timepoint for the PK sub-study) and treatment group, using the appropriate PK analysis set (see SAP sections 3.6 and 3.7). The concentration data as reported by the bioanalytical laboratory will be used without rounding for all analyses.

Descriptive statistics for plasma concentrations will include n, number of participants with concentrations below the limit of quantification (BLQ), mean, SD, coefficient of variation (CV%), geometric mean, geometric CV%, median, minimum, and maximum. For descriptive summaries, plasma concentrations reported as BLQ will be set to zero. For each analyte, if the mean concentration value is BLQ, then the mean concentration value will be presented as 0.

For the PK sub-study only, plots of individual plasma concentrations versus actual time will be produced by treatment (PRAX-114 dose group), analyte and timepoint, overlaid by participant on a linear scale. In addition, individual plasma concentration plots will be produced by participant, with analytes overlaid, on both linear and semi-log scales. Plots of mean plasma concentrations versus nominal timepoint will also be produced by treatment and analyte on both linear and semi-log scales; mean plots will also include error bars for +SD.

Plasma concentration data will be listed by treatment, participant, analyte, and nominal visit or timepoint. Deviations from scheduled times will be presented in listings.

5.12.2 Pharmacokinetic Parameters

For plasma concentrations from the PK sub-study, parent drug and metabolite PK parameters will be estimated from concentration-time data using non-compartmental methods with Phoenix® Win-Nonlin® software Version 8.0 or higher or SAS® software Version 9.4 or higher. The actual dose administered (mg) and actual postdose blood sampling times will be used in the calculation of the PK parameters if possible. If an actual sampling time is missing, the sample concentration result will be treated as missing unless there is scientific justification to include the result using the nominal time.

For the non-compartmental analysis, the following conventions will be utilized to handle data below the limit of quantification (BLQ):

Plasma BLQ concentrations will be assigned a value of 0 before the first measurable concentration and, thereafter, BLQ concentrations will be treated as missing. The following rules apply to the specific situations defined below:

- If an entire concentration-time profile is BLQ, it will be excluded from PK analysis.
- Where 2 or more consecutive concentrations are BLQ at the end of a profile, the profile will be deemed to have terminated and any further quantifiable concentrations will be set to missing for the calculation of the PK parameters, unless they are considered to be a true characteristic of the profile of the drug.
- If the predose plasma concentration is missing prior to the initial treatment with PRAX-114, it will be set to 0 by default within Phoenix WinNonliN.

If the actual time of sample collection deviates from the nominal time by more than ±10%, the plasma concentration will be flagged and excluded from the summary statistics. Any quantifiable predose concentration value prior to the initial treatment with PRAX-114 will be considered anomalous and set to missing for the PK analysis. This will be set to 0 by default in Phoenix WinNonlin.

The following PK parameters shown in Table 8 below will be determined where possible from the plasma concentrations of PRAX-114 and its metabolites M14, M15, and M16.

Table 8: PK Parameters PRAX-114 and metabolites M14, M15, and M16

Parameter	Units[a]	Definition
AUC_{0-t}	h*ng/mL	area under the concentration-time curve from time 0 to the time of the last quantifiable concentration (t_{last})[b]
AUC_{inf}	h*ng/mL	area under the concentration-time curve from time 0 extrapolated to infinity[c]
$\%AUC_{extrap}$	%	percentage of area under the concentration-time curve due to extrapolation from the last quantifiable concentration to infinity
C_{max}	ng/mL	maximum observed concentration
t_{max}	h	time of the maximum observed concentration
$t_{1/2}$	h	apparent terminal elimination half-life
CL/F	L/h	apparent total clearance (PRAX-114 only)
V_z/F	L	apparent volume of distribution during the terminal phase (PRAX-114 only)
MR_{AUC}	NA	metabolite:parent ratio based on AUC_{inf}

[a] Units are based on concentration units (provided by the bioanalytical lab or preferred units for presentation of PK parameters) and dose units used in the study.
[b] The AUC will be calculated using the linear trapezoidal rule for increasing concentrations and the logarithmic rule for decreasing concentrations (linear up/log down rule).
[c] Based on the last observed quantifiable concentration

PK parameters will be summarized by PRAX-114 dose group using descriptive statistics The parameter t_{max} will be summarized using n, median, minimum, and maximum only. All other PK parameters will be summarized using n, mean, SD, CV%, geometric mean, geometric CV%, median, minimum, and maximum. The non-compartmental parameters will not be reported to any greater accuracy than that of the concentration data as reported by the bioanalytical group.

Any parameter values that cannot be calculated will be presented as not reportable (NR). If r^2 is less than 0.8, the parameter $t_{1/2}$ will be listed as "NR". The number and percentage of NR parameter values will also be displayed on the summary tables.

Collected plasma concentration data may also contribute to population PK analyses. Any population PK analyses will be presented separately from the main clinical study report (CSR).

PK parameter data will be listed by treatment group, analyte and participant.

[redacted] and change from baseline values will be included.

5.14 Safety Analyses

For all safety variables, descriptive statistics will be presented by treatment group and visit (where applicable) using the safety analysis set. In summaries of change from baseline safety variables, only participants with both baseline and change from baseline values will be included. No formal hypothesis testing will be performed for any safety variable.

5.14.1 AEs

The overall incidence of participants having at least one AE in each of the following categories will be summarized by treatment group:

- Any TEAE
- Any serious adverse event (SAE)
- Any AE leading to death
- Any TEAE leading to study drug discontinuation
- Any related TEAE

All AE data will be listed by participant. Separate listings by participant will be presented for all SAEs, AEs leading to death, and TEAEs leading to study drug withdrawal.

5.14.1.1 TEAEs

The incidence of TEAEs will be summarized by treatment group, SOC, and preferred term. Each participant will be counted only once per SOC and preferred term. An additional display may be produced by treatment group and preferred term.

5.14.1.2 SAEs

The incidence of SAEs will be summarized by treatment group, SOC, and preferred term. Each participant will be counted only once per SOC and preferred term.

5.14.1.3 TEAEs Leading to Study Drug Discontinuation

The incidence of TEAEs leading to study drug discontinuation will be summarized by treatment group, SOC, and preferred term. Each participant will be counted only once per SOC and preferred term.

5.14.1.4 TEAEs by Maximum Relationship to Study Drug

The incidence of TEAEs will be summarized by maximum relationship to study drug, treatment group, SOC, and preferred term. Each participant is counted only once per preferred term and most related category reported.

5.14.1.5 TEAEs by Maximum Severity

The incidence of TEAEs will be summarized by maximum severity to study drug, treatment group, SOC, and preferred term. Each participant is counted only once per preferred term and most severe category reported.

5.14.1.6 Post-treatment TEAEs

The incidence of post-treatment TEAEs will be summarized by treatment group, SOC, and preferred term. Each participant is counted only once per SOC and preferred term.

5.15 Laboratory Test Results

Descriptive statistics for all reported values and change from baseline values will be summarized by test category, treatment group, and nominal visit. Only the original assessment for each visit will contribute to descriptive statistics; repeat or unscheduled assessments will not be included in the calculation of descriptive statistics by visit. All laboratory test results data (including pregnancy test and urine drug screen data and any repeat or unscheduled assessments) will be listed by participant and visit.

5.16 Vital Sign Results

Descriptive statistics for all reported values and change from baseline values will be summarized by treatment group and nominal visit. Only the original assessment for each visit will contribute to these summaries; repeat or unscheduled assessments will not be included in the calculation of descriptive statistics by visit. The number and percentage of participants with PCI values at any time will be tabulated by treatment group. Summaries of PCI values will include all assessments regardless of whether an original, repeat, or unscheduled assessment. All vital sign results data will be listed by participant and visit.

[redacted]

5.18 C-SSRS

The incidence of suicidal ideation/behavior at any time will be summarized by treatment group for each C-SSRS ideation, behavior, and ideation/behavior indicator. All C-SSRS data will be listed by participant and visit.

[redacted]

6 INTERIM ANALYSES

No formal interim analysis is planned for this clinical trial.

7 CHANGES TO PLANNED ANALYSES

7.1 Changes to Analysis as Described in Protocol

The following analysis sets were not defined in the protocol but added for presentation and analysis:

- Randomized Analysis Set
- PK Sub-study Analysis Set
- Per Protocol Analysis Set

Subgroup analyses are pre-specified for the 60mg dose group versus placebo (Section 5.10).

7.2 Changes to Approved Version of the SAP

Not applicable.

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9 APPENDIX A

Schedule of Activities

Source: Table 2 from Protocol Version 3.0, 22 November 2021

Table 2: Schedule of Activities

Period	**Screening** [a]	**Intervention**							**Follow-Up**	
Visit Day (Visit window in days)	**D-28 to D-1**	**D1 (Baseline)**	**D4** [b] **(±1)**	**D8 (±1)**	**D15 (±2)**	**D22 (±2)**	**D29 (±2)**	**Optional PK Sub-study (Table 3)**	**D36 (±2)**	**D43 (±2)**
Visit	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7/ET** [c]		**V8**	**V9**
CLINCIAL TRIAL ENTRY AND GENERAL ASSESSMENTS										
Informed Consent	X [d]									
Admission to Optional PK Sub-Study							X [e]			
Clinical Trial Registry	X									
Inclusion/Exclusion Criteria	X	X [f]								
Psychiatric and Medical History	X									
History of Alcohol, Tobacco and Drug Use	X									
Demographic Data	X									
Body Weight	X						X			
Height	X									
hCG Test [g]	X *(serum)*	X [f] *(urine)*					X *(urine)*			
Hepatitis Screen	X									
Drug Screen	X [h]						X			X

Note: Table and footnotes continue onto next page.

Schedule of Activities (continued)

Table 2: Schedule of Activities

Period	Screening [a]	Intervention							Follow-Up	
Visit Day (Visit window in days)	D-28 to D-1	D1 (Baseline)	D4 [b] (±1)	D8 (±1)	D15 (±2)	D22 (±2)	D29 (±2)	Optional PK Sub-study (Table 3)	D36 (±2)	D43 (±2)
Visit	V1	V2	V3	V4	V5	V6	V7/ET [c]		V8	V9
MINI [a]	X									
ATRQ [a]	X									
Independent Eligibility Verification	X									
DSM-5 Anxious Distress Specifier [a]	X									
PCRS	X	X	X	X	X	X	X		X	X
HAM-D17 [a]	X	X [f]	X	X	X	X	X		X	X
CGI-S [a]	X	X [f]	X	X	X	X	X		X	X
CGI-I [a]			X	X	X	X	X		X	X
PGI-I			X	X	X	X	X		X	X
In-person Administered SDQ		X		X	X	X	X		X	X
Remotely Administered SDQ [i]					X [i]				X [i]	
CSFQ-14		X			X		X		X	X
WSAS		X			X		X			X
[redacted]										

Note: Table and footnotes continue onto next page

Schedule of Activities (continued)

Table 2: Schedule of Activities

Period	Screening [a]	Intervention							Follow-Up	
Visit Day (Visit window in days)	**D-28 to D-1**	**D1 (Baseline)**	**D4 [b] (±1)**	**D8 (±1)**	**D15 (±2)**	**D22 (±2)**	**D29 (±2)**	**Optional PK Sub-study (Table 3)**	**D36 (±2)**	**D43 (±2)**
Visit	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7/ET [c]**		**V8**	**V9**
SAFETY ASSESSMENTS										
Clinic Visit	X	X		X	X	X	X		X	X
Vital Signs [j]	X	X [f]		X	X	X	X		X	X
Physical Examination	X						X			
Clinical Laboratory Evaluations [k]	X	X					X		X	
12-lead ECG	X	X [l]					X			
C-SSRS (Baseline/Screening) [a]	X									
C-SSRS (Since Last Visit) [a]		X [f]	X	X	X	X	X		X	X
[redacted]										
AE monitoring [m]	X								X	
Concomitant Meds/ Procedures [m]	X								X	
PHARMACOKINETICS/PHARMACODYNAMICS										
Blood Collection for Study Drug and Metabolite Concentration [n]				X	X	X	X		X	X

Note: Table and footnotes continue onto next page

Schedule of Activities (continued)

Table 2: Schedule of Activities

Period	Screening [a]	Intervention							Follow-Up	
Visit Day (Visit window in days)	**D-28 to D-1**	**D1 (Baseline)**	**D4 [b] (±1)**	**D8 (±1)**	**D15 (±2)**	**D22 (±2)**	**D29 (±2)**	**Optional PK Sub-study (Table 3)**	**D36 (±2)**	**D43 (±2)**
Visit	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7/ET [c]**		**V8**	**V9**
[redacted]										
Blood Sample for Antidepressant Treatment (adjunctive treatment participants only)	X				X					
STUDY DRUG										
Randomization [o]		X								
Dispense Study Drug		X		X	X	X				
Study Drug Administration [p]		X [q]								

AE=adverse event; ATRQ=Antidepressant Treatment Response Questionnaire; CGI-S=Clinical Global Impression-Severity; CGI-I=Clinical Global Impression-Improvement ; CSFQ-14=Changes in Sexual Functioning Questionnaire; C-SSRS=Columbia-Suicide Severity Rating Scale; D=Day; d=days; DESS= Discontinuation-Emergent Signs and Symptoms Scale; DSM-5=Diagnostic and Statistical Manual of Mental Disorders 5th Edition; ECG=electrocardiogram; ET=early termination; HAM-D17=17-item Hamilton Depression Rating Scale; hCG=human chorionic gonadotropin; MINI=Mini International Neuropsychiatric Interview; PCRS= Placebo-Control Reminder Script; PK=pharmacokinetic; PSS=Perceived Stress Scale; PGI-I=Patient Global Impression-Improvement; SDQ=Symptoms of Depression Questionnaire; V=Visit; WSAS= Work and Social Adjustment Scale.

a The recommended order of assessments at Screening: MINI, DSM-5 Anxious Distress Specifier, HAM-D17, C-SSRS, then all other assessments with the CGI being administered after all other rater-administered assessments. Starting at the Day 1 visit and for all subsequent visits, the HAM-D17 should be the first rater-administered assessment, and the CGI should be administered after all other rater-administered assessments (which may be administered at any point during the visit).

b Visit will be performed remotely via telehealth procedures.

c All participants who discontinue study drug early should attend an ET visit as soon as possible, as well as return for the post-treatment follow-up visits (unless they withdraw consent for study participation).

Note: Table and footnotes continue onto next page

Schedule of Activities (continued)

Table 2: Schedule of Activities

Period	Screening [a]	Intervention							Follow-Up	
Visit Day (Visit window in days)	D-28 to D-1	D1 (Baseline)	D4 [b] (±1)	D8 (±1)	D15 (±2)	D22 (±2)	D29 (±2)	Optional PK Sub-study (Table 3)	D36 (±2)	D43 (±2)
Visit	V1	V2	V3	V4	V5	V6	V7/ET [c]		V8	V9

d Date of informed consent can be prior to screening procedures to allow for appropriate washout of excluded medications.

e All study participants at the designated research sites will be offered participation in the PK sub-study, which will be conducted within 3 days of the Visit 7/Day 29 visit assessments. Participants who enroll in the optional PK sub-study should also follow the procedures in Table 3.

f To be performed prior to randomization to confirm eligibility. HAM-D17 and CGI-S scores should be re-confirmed at Baseline (Day 1) to ensure participants continue to meet eligibility criteria.

g hCG (serum) is required for all women at Screening, and for women of childbearing potential only, post-screening.

h See Section 8.2.7 for exception for positive cannabis test at Screening.

i Remotely administered SDQ will be Days 4, 11 (+1d), 18 (+1d), 25 (+1d), 32 (+1d), 39 (+1d).

j Including oral/tympanic temperature, supine blood pressure, respiratory rate, and pulse rate. Vitals should be taken prior to blood draws when applicable.

k Including hematology, clinical chemistry, coagulation (Screening only), TSH (Screening only), FSH (Screening for post-menopausal women only), and urinalysis.

l Baseline (Day 1) ECG should be performed in triplicate and individual ECG tracings should be obtained as closely as possible in succession, but no more than 2 minutes apart.

m Will be collected throughout the clinical trial from time of signing informed consent until end of trial. Reference Section 12, Appendix 2 for procedures for collecting adverse events that are ongoing at the last scheduled visit.

n Collected at any time during the visit.

o Only to be completed after independent verification of eligibility and all Baseline (Day 1) assessments have been performed and continued eligibility has been confirmed.

p Dosed every night at bedtime (QHS).

q Final day of study drug administration in the parent study will be Day 28 unless the visit window is used; in that case, the final day of PRAX-114 dosing will be the day prior to the final Intervention Period clinical trial visit (ie, the D29 visit).

Schedule of Activities: Optional PK Sub-study

Source: Table 3 from Protocol Version 3.0, 22 November 2021

Table 3: Schedule of Activities (Optional PK Sub-study)

Day	**Admission**	**Sub-study Day 1**											**Sub-study Day 2**			**Sub-study Day 3** [a]
Visit		**S1**											**S2**			**S3/ET** [b]
Hours Post Dose		-4	0 [c]	0.25	0.5	0.75	1	1.5	2	2.5	3	4	12	16	24	36
Review Informed Consent	X [d]															
Admission to Clinic	X															
Physical Examination [e]	X															
Urine Drug Screen	X															
Vital Signs [f]			X								X		X			X
C-SSRS (Since Last Visit)	X															X [k]
AE Monitoring [g]	X															
Concomitant Meds/ Procedures [g]	X															
Pre-Dosing Meal [h]		X														
Study Drug Administration			X [i]													
Blood Collection for Study Drug and			X	X	X	X	X	X	X	X	X	X	X	X	X	X

Note: Table and footnotes continue onto next page

Schedule of Activities: Optional PK Sub-study (continued)

Day	Admission	Sub-study Day 1											Sub-study Day 2			Sub-study Day 3 [a]
Visit		S1											S2			S3/ET [b]
Metabolite Concentration j																
Discharge from Clinic																X

AE=adverse event; C-SSRS=Columbia-Suicide Severity Rating Scale; ET=early termination; S=sub-study.

a After Sub-study Day 3, participants should return for the post-treatment follow-up visits of the parent study (see Table 2) beginning at Day 36.

b If the participant discontinues early from the Sub-study, the S3/ET procedures should be completed prior to discharge. In addition, participants who ET from the Sub-study should return for the post-treatment follow-up visits of the parent study (unless they withdraw consent for study participation).

c Protocol activities are prior to study treatment administration.

d ICF will have been previously reviewed and signed; the objective of this review is to refresh participants' knowledge of sub-study procedures.

e Abbreviated physical examination at Investigator's discretion if there were findings during previous exam or new/open AEs if appropriate.

f Including oral/tympanic temperature, supine blood pressure, respiratory rate, and pulse rate. Vitals should be taken prior to blood draws when applicable (within a 20-minute window).

g Will be collected throughout the clinical trial from time of signing informed consent until end of trial.

h Participants should be provided dinner at approximately 16:00 (4 PM).

i Dosing at approximately 20:00 (8 PM).

j Assessment windows are as follows: ±2 minutes for <1 hour postdose, ±5 minutes for 1 to 3 hours postdose, ±10 minutes for 4 to 8 hours postdose, and ±20 minutes for all other timepoints.

k The C-SSRS can be performed before or after the blood draw within a 4-hour window.

10 APPENDIX B

There are several statistical software packages capable of performing the MCP-Mod procedure. The template code below uses the DoseFinding package available in R. In the template code, the HAMD17_DAY15 dataset contains the changes from baseline to Day 15 in HAM-D17 total scores (variable name: resp), the dose (0 = placebo and 10, 20, 40, and 60 for PRAX-114 treated participants), each participants baseline HAM-D17 total score (variable name: BASELINE) and whether the participant was part of the monotherapy or adjunctive pharmacotherapy strata (variable name: ADJFLN, 0 = monotherapy, 1 = adjunctive). The example below assumes that a logistic model was chosen and that the mean baseline HAM-D17 score in the monotherapy strata was 26.

```
# Load the DoseFinding package and other useful packages
library(tidyverse)
library(ggplot2)
library(DoseFinding)

# Pre-specified set of candidate models
dose_seq <- c(0, 10, 20, 40, 60)
MODS <- Mods(linear = NULL,
        emax = c(10, 30),
        logistic = matrix(c(20, 20, 30, 0.01, 5, 0.5), byrow = FALSE, nrow = 3),
        doses = dose_seq)

# Multiple comparison testing procedure (Choose model with largest test statistic)
MCPMOD_01 <- DoseFinding::MCTtest(dose       = dose,
                        resp       = resp,
                        data       = HAMD17_DAY15,
                        models     = MODS,
                        addCovars  = ~ BASELINE + ADJFLN,
                        alpha      = 0.05,
                        alternative = "one.sided")
MCPMOD_01

# Modeling procedure given that logistic model is selected
MCPMOD_02 <- fitMod(dose     = dose,
                resp     = resp,
                data     = HAMD17_DAY15,
                model    = "logistic",
```