STATISTICAL ANALYSIS PLAN ADDENDUM

BlackThorn Therapeutics, Inc.

K2-MDD-201

Protocol Title:	A Phase 2a, Randomized, Double-blind, Placebo-controlled Proof of Concept Study to Evaluate the Effects of Oral BTRX-335140 Versus Placebo in Subjects With Major Depressive Disorder
Protocol Version and Date:	Amendment 7 Version 8.0; 24 January 2022
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1 STATISTICAL ANALYSIS PLAN ADDENDUM APPROVAL

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3 LIST OF ABBREVIATIONS

Table 1 List of Abbreviations

Abbreviation	Definition
ANCOVA	analysis of covariance
CGI-I	Clinical Global Impression of Improvement
CGI-S	Clinical Global Impression of Severity
ET	end of treatment
eCRF	electronic case report form
HADS	Hospital Anxiety and Depression Scale
HAM-A	Hamilton Anxiety Rating Scale
[redacted]	[redacted]
HAMD-17	Hamilton Depression Rating Scale - 17-item Version
LOCF	last observation carried forward
MDD	major depressive disorder
MMRM	mixed-models repeated measures
[redacted]	[redacted]
SAP	statistical analysis plan
SDS	Sheehan Disability Scale
SHAPS	Snaith-Hamilton Pleasure Scale
[redacted]	[redacted]

4 INTRODUCTION

Detailed descriptions of the methods and presentation of data analyses proposed for BlackThorn Therapeutics, Inc. Protocol K2-MDD-201 (A Phase 2a, Randomized, Double-blind, Placebo-controlled Proof of Concept Study to Evaluate the Effects of Oral BTRX-335140 Versus Placebo in Subjects With Major Depressive Disorder) Amendment 7 Version 8.0 (24 January 2022) are described in the original statistical analysis plan (SAP), version 2.0, 03 June 2022 (hereafter referred to as "the main SAP").

The purpose of this addendum is to provide clarifications to the definitions and methodology related to the analyses described in the main SAP.

5 STATISTICAL METHODS

5.1 General Methodology

5.1.1 Analysis Visit Window

For efficacy and safety analyses by visit, assessments at scheduled and unscheduled visits will be mapped to the appropriate analysis window as detailed in Table 3 and Table 4 of the main SAP. The analysis visit windows for the standard ophthalmologic examination at Visit 3 (Week 2) and Visit 4 (Week 4) from Table 4 of the main SAP will instead be mapped as shown in Table 2.

Table 2 Analysis Windows for Standard Ophthalmologic Examination by Visit

Period	Screening	Baseline	Study Drug Treatment				Safety Follow-up	
Visit	1	2	3	4	5	6		
	Days –7 to –28	Day 1	Day 15 (±2 d)	Day 29 (±2 d)	Day 43 (±2 d)	Day 57 (±2 d)	Day 71	Day 85
Analysis Visit Name		**Baseline**	**Week 2**	**Week 4**	**Week 6**	**Week 8**	**Week 10**	**Week 12**
Standard ophthalmologic examination	NA	≤ 1 Pre-dose	[2, 14]	NA	NA	≥ 15	NA	NA

5.1.2 Baseline Definition

For efficacy and safety analyses presented by study visit, the main SAP defines the baseline value as "the last measurement prior to the start of study drug administration". The baseline value will differ for summaries of Hamilton Depression Rating Scale - 17-item Version (HAMD-17), Clinical Global Impression of Improvement (CGI-I), Snaith-Hamilton Pleasure Scale (SHAPS), Hospital Anxiety and Depression Scale (HADS), Hamilton Anxiety Rating Scale (HAM-A), Sheehan Disability Scale (SDS), Clinical Global Impression of Severity (CGI-S), [redacted] and [redacted]

[redacted] presented by study visit, in which case the baseline value will be defined as the last measurement prior to or within 1 hour of the first administration of study drug. For all other efficacy and safety analyses presented by study visit, the baseline value will be defined as stated in the main SAP.

5.2 Efficacy Evaluation

5.2.1 Measurements of Treatment Compliance

As stated in the main SAP, percentage compliance to the study treatment regimen will be determined as the total dose actually taken divided by the expected dose received, multiplied by 100. The expected dose received will be calculated as (the date of last dose of study drug – the date of first dose of study drug + 1 day) x 1 capsule/day. If the date of last dose is missing or unknown, the date of last dose will instead be equal to the date of last available visit prior to Visit 7 or the date of study completion/termination, whichever is earlier. The date of the last available visit prior to Visit 7 is defined as the date of the last available visit between Visit 2 (Baseline) and Visit 6 (Week 8)/ET (end of treatment). Any bottles dispensed on or after the date of last dose will be excluded from the calculation of total dose actually taken.

In the case of missing values, the following calculations will differ from the main SAP. If the number of capsules taken is missing, but the corresponding number of capsules dispensed and returned are not missing, then the number of capsules taken will be calculated as the number of capsules dispensed minus the number of capsules returned. If both the number of capsules returned and the number of capsules taken are missing, then it will be assumed that 0 capsules were returned and all capsules dispensed were taken. In addition, if the number of capsules dispensed is 0, then the corresponding number of capsules taken and returned will be set to 0.

Dosing compliance will be summarized using descriptive statistics, by treatment group, based on the Efficacy Population. As an exception rule, the compliance rate for subjects with $\leq$ 14 days exposure duration will not be calculated, as the compliance may not be calculated reliably for such short duration.

5.2.2 Sensitivity Analyses of the Primary Efficacy Endpoint

The main SAP describes the sensitivity analyses that will be performed. The Last Observation Carried Forward (LOCF) Imputation technique will be performed as follows: the last observed non-missing post-baseline value will be used to fill in missing values at Week 8, regardless of when the missing value occurred. The imputed dataset generated with the LOCF technique will be used as input data in the analysis of covariance (ANCOVA) analysis.

5.2.3 Subgroup Analyses of the Primary Efficacy Endpoint

The primary efficacy endpoint (change from baseline to Week 8 in HAMD-17 total score) will be summarized using the subgroups described in the main SAP. Additionally,

the mixed-models repeated measures (MMRM) analysis described for the primary efficacy endpoint in the main SAP will be repeated for all subgroups.

5.2.4 Subgroup Analyses of the Secondary Efficacy Endpoints

The secondary efficacy endpoint SHAPS will be summarized using the subgroups described in the main SAP. HAM-A total score will instead be presented by baseline SHAPS Total Score (14 to 56) (< median and ≥ median) and SHAPS Original Total Score (0 to 14) (< median and ≥ median).

5.3 Safety Evaluation

5.3.1 Vital Signs, Physical Findings, and Other Observations Related to Safety

5.3.1.1 Ophthalmology Examination

The main SAP states "Standard ophthalmologic examination and corneal specular microscopy examination results will be summarized by presenting shift from baseline to end of study results as normal, abnormal not clinically significant, and abnormal clinically significant." This summary will be presented by visit.

Additionally, the subjects who have endothelial dystrophy medical history prior to treatment used for the subgroup analysis will be identified by having a preferred term of "Corneal Dystrophy" collected on the Medical and Psychiatric History Other Than MDD (major depressive disorder) electronic case report form (eCRF) prior to date of first dose of study drug.

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STATISTICAL ANALYSIS PLAN

BlackThorn Therapeutics, Inc.

K2-MDD-201

Protocol Title:	A Phase 2a, Randomized, Double-blind, Placebo-controlled Proof of Concept Study to Evaluate the Effects of Oral BTRX-335140 Versus Placebo in Subjects With Major Depressive Disorder
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3 LIST OF ABBREVIATIONS

Table 1 List of Abbreviations

Abbreviation	Definition
Ab	antibody
AE	adverse event
AESI	adverse event of special interest
ALT/SGPT	alanine aminotransferase
ANC	absolute neutrophil count
ANCOVA	analysis of covariance
AST/SGOT	aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical
BMI	body mass index
BP	blood pressure
BUN	blood urea nitrogen
C-SSRS	Columbia Suicide Severity Rating Scale
CGI-I	Clinical Global Impression of Improvement
CGI-S	Clinical Global Impression of Severity
CI	confidence interval
CK	creatine kinase
CPK	creatine phosphokinase
COWS	Clinical Opiate Withdrawal Scale
COVID-19	coronavirus disease 2019
CSM	Corneal Specular Microscopy
CSR	clinical study report
CV	coefficient of variation
DSM-5	Diagnostic and Statistical Manual of Mental Disorders Fifth Edition
ECG	electrocardiogram
eCRF	electronic case report form
GGT	gamma-glutamyltransferase
GLMM	generalized linear mixed model
HADS	Hospital Anxiety and Depression Scale
HADS-A	Hospital Anxiety and Depression Scale - Anxiety Subscale
HADS-D	Hospital Anxiety and Depression Scale - Depression Subscale
HAM-A	Hamilton Anxiety Rating Scale
HAMD-17	Hamilton Depression Rating Scale - 17-item Version
HAV-Ab	hepatitis A virus antibody
HBc Ab	hepatitis B core antibody

Abbreviation	Definition
HBs Ag	hepatitis B surface antigen
HC Ab	hepatitis C antibody
HIV	human immunodeficiency virus
HPMC	hydroxypropyl methylcellulose
ICF	informed consent form
ICH	International Council for Harmonisation
IgM	immunoglobulin M
INR	International normalized ratio
IWRS	interactive web-response system
KORA	kappa opioid receptor antagonist
[redacted]	
LOCF	last observation carried forward
LS	least-square
MCV	mean cell volume
MCHC	mean cell hemoglobin concentration
MDD	major depressive disorder
MedDRA	Medical Dictionary for Regulatory Activities
MI	multiple imputation
MMRM	mixed-models repeated measures
OD	oculus dextrus
[redacted]	
OS	oculus sinister
PC	pre COVID
PCS	potentially clinically significant
[redacted]	
[redacted]	
POC	proof of concept
PP	Per-Protocol
[redacted]	
QD	once daily
QTcB	corrected QT interval using Bazett's correction
QTcF	corrected QT interval using Fridericia's correction
RBC	red blood cells
[redacted]	
SAE	serious adverse event
SAP	statistical analysis plan
SD	standard deviation
SDS	Sheehan Disability Scale

Abbreviation	Definition
SE	standard error
SHAPS	Snaith-Hamilton Pleasure Scale
SI	Système International
SIGH-A	Structured Interview Guide for the Hamilton Anxiety Rating Scale
SIGH-D	Structured Interview Guide for the Hamilton Depression Rating Scale
SOC	system organ class
[redacted]	
TEAE	treatment-emergent adverse event
TESAE	treatment-emergent serious adverse event
THC	delta-9-tetrahydrocannabinol
TSH	thyroid stimulating hormone
ULN	upper limit of normal
WBC	white blood cell
WHODrug	World Health Organization Drug classification

4 INTRODUCTION

The purpose of this statistical analysis plan (SAP) is to provide comprehensive and detailed descriptions of the methods and presentation of data analyses proposed for BlackThorn Therapeutics, Inc. Protocol K2-MDD-201 (A Phase 2a, Randomized, Double-blind, Placebo-controlled Proof of Concept Study to Evaluate the Effects of Oral BTRX-335140 Versus Placebo in Subjects With Major Depressive Disorder) Amendment 7 Version 8.0 (24 January 2022). The statistical methods applied in the design and planned analyses of this study are consistent with the International Council for Harmonisation (ICH) guideline *Statistical Principles for Clinical Trials* (E9) (1998).

This SAP will be finalized before treatment unblinding and final database lock. There are no changes to the planned analyses described in the study protocol in this SAP; any deviations from this SAP will be documented in the clinical study report (CSR).

5 STUDY OBJECTIVES

5.1 Primary Study Objective

The primary objective of this study is to establish Proof of Concept (POC) for BTRX 335140, a kappa opioid receptor antagonist (KORA), by evaluating the impact of BTRX-335140 relative to placebo on symptoms of major depressive disorder (MDD) in adult subjects with MDD and symptoms of anhedonia and anxiety following 8 weeks of double-blind treatment as assessed by the Hamilton Depression Rating Scale (HAMD-17).

5.2 Secondary Study Objectives

The secondary objectives of this study are:

- To evaluate the effects of BTRX-335140 on self-reported anhedonia in adult subjects with MDD
- To evaluate the effects of BTRX-335140 on anxiety-related symptoms in adult subjects with MDD
- To evaluate the effects of BTRX-335140 on functional impairment in adult subjects with MDD
- To evaluate the safety and tolerability of BTRX-335140 in adult subjects with MDD

5.3 [redacted]

[redacted]

6 INVESTIGATIONAL PLAN

6.1 Overall Study Design

Study K2-MDD-201 is a US-based, randomized, double-blind, placebo-controlled, parallel-group, multicenter study to evaluate the effects of BTRX-335140 on symptoms of depression in approximately 180 adult subjects with MDD and symptoms of anhedonia and anxiety after 8 weeks of double-blind treatment. The study consists of a 7- to 28-day screening period, an 8-week active treatment period (during which subjects will receive either BTRX-335140 or placebo), and a 4-week safety follow-up period.

Subjects sign an informed consent form (ICF) and then enter the screening period to ensure they meet the required inclusion and exclusion criteria, including diagnosis and stability of depressive symptoms and absence of psychiatric and medical conditions that would preclude study participation. The 28-day screening period may be extended to 35 days (after consultation with and approval by the medical monitor), if necessary, for reasons including, but not limited to, discontinuation of a subject's current inefficacious medications or to obtain an ophthalmologic examination.

Subjects who meet the Diagnostic and Statistical Manual of Mental Disorders Fifth Edition (DSM5) diagnostic criteria for MDD with symptoms of anhedonia and anxiety and all other eligibility criteria for the study are randomized in a 1:1 ratio to 1 of 2 treatment arms (placebo or BTRX-335140 once daily [QD]). Beginning at Visit 2 (Baseline), subjects receive treatment with BTRX-335140 80 mg QD or placebo for 8 weeks. Scheduled visits take place every two weeks (Week 2, 4, 6, and 8). During the Safety Follow-up, all subjects receive a telephone call from study site personnel for safety follow-up approximately 2 weeks after discontinuation of study drug for assessment of AEs, concomitant medication usage, and Columbia Suicide Severity Rating Scale (C-SSRS). The overall duration in the study for each subject is approximately 14 to 16 weeks.

Efficacy and safety endpoints are described in Section 6.4 and are measured according to the Schedule of Assessments described in Appendix A: Schedule of Procedures and Assessments.

6.2 Schedule of Assessments

For the complete schedule of assessments, refer to Appendix A: Schedule of Procedures and Assessments.

6.3 Treatments

6.3.1 Treatments Administered

Treatment is initiated at 1 capsule of study drug (80mg BTRX-335140 or placebo) QD and this dose is maintained for all 8 weeks of double-blind treatment. Subjects are instructed to swallow capsules whole and not crush or chew and to store the capsules in their original packaging at the temperature listed on the package label. Subjects are instructed to take their first dose of study drug under the supervision of clinical study personnel during the baseline visit and to try to take their study medication at approximately the same time each day, preferably in the morning with food.

6.3.2 Method of Assigning Subjects to Treatment Groups

Subjects who meet all inclusion criteria and are not excluded by the exclusion criteria are randomized to double-blind treatment at Visit 2 (Baseline) using an interactive web-response system (IWRS). Subjects are randomized in a 1:1 ratio to 1 of 2 treatment arms (placebo or BTRX-335140 QD).

The IWRS is used to assign bottles containing double-blind study drug or placebo to each subject. Further details are provided in the Pharmacy manual.

6.4 Efficacy and Safety Variables

6.4.1 Efficacy Variables

6.4.1.1 Primary Efficacy Variable

The primary efficacy endpoint is the change from baseline to Week 8 on the HAMD-17 score in the Efficacy population.

This instrument is completed based on a semi-structured interview (Structured Interview Guide for the Hamilton Depression Rating Scale [SIGH-D], Williams 1988). The 17-item HAM-D comprises individual ratings related to the following symptoms: depressed mood (sadness, hopeless, helpless, worthless), feelings of guilt, suicide, insomnia (early, middle, late), work and activities, retardation (slowness of thought and speech; impaired ability to concentrate; decreased motor activity), agitation, anxiety (psychic and somatic), somatic symptoms (gastrointestinal and general), genital symptoms, hypochondriasis, loss of weight, and insight. The HAMD-17 scoring ranges from 0 to 52 with individual items ranging from 0-4 points and 0-2 points where higher scores corresponding to higher levels of symptom severity.

6.4.1.2 Secondary Efficacy Variables

Secondary efficacy endpoints include the following:

- HAMD-17 response rate at Week 4 and Week 8. The response rate is the percentage of subjects with ≥ 50% decrease from baseline in HAMD-17 score.

- CGI-I. CGI-I score assessed at each postbaseline timepoint compared to baseline. The CGI-I scale (Guy 1976) is a clinician-rated instrument that measures the improvement of the subject's symptoms. It is a 7-point scale where a score of 1 indicates that the subject is "very much improved," a score of 4 indicates that the subject has experienced "no change," and a score of 7 indicates that the subject is "very much worse." For analysis purposes, a CGI-I response indicating symptom improvement will be defined as "much improved" or "very much improved".

- SHAPS. Change from baseline to each timepoint assessed in SHAPS scores. The SHAPS is a 14-item self-report instrument which measures anhedonia (Snaith et al 1995). Each of the 14 items has a set of 4 responses, 2 of which endorse agreement (Definitely/Strongly Agree, Agree) and 2 of which endorse disagreement (Disagree, Strongly Disagree). There are two scoring methods.

    - SHAPS Original Total Score (0 to 14): The original method assigned a score of 1 to either of the disagreement responses (Disagree, Strongly Disagree) and a score of 0 to either of the agreement responses (Definitely/Strongly Agree, Agree). Therefore, original scores on the SHAPS can range from 0-14 with higher scores corresponding to higher levels of anhedonia.

    - SHAPS Total Score (14 to 56): The second method derives a total score by summing the item responses where Definitely/Strongly Agree responses score 1 point, Agree responses score 2 points, Disagree responses score 3 points, and Strongly Disagree responses score 4 points. Therefore, scores on the SHAPS can range from 14 to 56 by the second method, with higher scores corresponding to higher levels of anhedonia. Both methods will be used for analyses.

- HADS. Change from baseline to each timepoint assessed in HADS total score and HADS subscales (ie, anxiety subscale [HADS-A] and depression subscale [HADS-D] scores). The HADS measures levels of anxiety and depression without regards to somatic symptoms (Zigmond and Snaith 1983). This self-report scale consists of 14 items. Seven of the items are used to evaluate anxiety and 7 evaluate depression. Each item on the questionnaire is scored from 0 to 3. Therefore, the anxiety subscale (HADS-A) and the depression subscale (HADS-D) each range from 0 to 21.

- HAM-A. Change from baseline to each timepoint assessed in HAM-A total score. This instrument will be completed based on a semi-structured interview

(Structured Interview Guide for the Hamilton Anxiety Rating Scale [SIGH-A], Williams 1988). The scale consists of 14 items. Each item is rated on a scale of 0 (feeling not present) to 4 (very severe prevalence of the feeling). The HAM-A total score is the sum of the 14 items and the score ranges from 0 to 56 where higher scores indicate more severe anxiety.

- SDS. Change from baseline to each timepoint assessed in SDS scores. SDS is a brief self-rated tool where the subject rates how symptoms of depression have disrupted the 3 domains (related to work/school, social, and family life) on an anchored 10-point visual analogue scale (Sheehan 1983). The SDS total score will be the sum of the 3 individual item scores where higher scores indicate more severe disability related to MDD symptoms.

- CGI-S. Change in Clinical Global Impression of Severity (CGI-S) from baseline to Week 8. The CGI-S is a clinician-rated instrument that measures the severity of depression at the time of assessment. This rating is based upon observed and reported symptoms, behavior, and function in the past 7 days. The score should reflect the average severity level across the 7 days. The CGI-S is scored on a 7-point scale where a score of 1 indicates that the subject is "normal, not at all ill" a score of 4 indicates that the subject is "moderately ill," and a score of 7 indicates that the subject is "among the most extremely ill subjects" (Guy 1976).

6.4.1.3 Exploratory Efficacy Variables

[redacted]

6.4.2 *Safety Variables*

6.4.2.1 Adverse Events

An AE is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

An AE may be any unfavorable and unintended sign, symptom, illness, clinically significant abnormal laboratory value or ECG finding, or other untoward medical occurrence that appears or worsens in a subject during a clinical study. This definition does not imply a causal relationship between the AE and the study drug.

Adverse events beginning with the initial dose of study drug or during the subsequent duration of their enrollment in the study will be considered treatment-emergent adverse events (TEAEs). TEAEs will be recorded on the AE electronic case report form (eCRF) to include the event, date of onset, whether the AE is associated with an episode of self-harm, severity, frequency, seriousness, date of resolution, action taken with respect to the AE (eg, discontinue study drug, begin concomitant medication, begin non-pharmacological treatment, etc.), outcome, and relationship to the study drug. All TEAEs related to study drug will be followed to a satisfactory resolution or until the event

becomes stable or can be explained by another known cause (ie, concurrent condition or medication).

Medical conditions present or AEs occurring prior to the first dose of study drug will be captured as medical history in the eCRF. Any AE occurring during the study that is related to a pre-existing condition or event that worsens in intensity or frequency after the first dose of study drug will be recorded as a TEAE.

The investigator will assess the severity of each AE based on his/her clinical judgment using one of the following categories:

Mild: Usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.

Moderate: Usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the subject.

Severe: Interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.

The investigator will assess the relationship (ie, causality) of each AE to study drug based on his/her clinical judgment. Relationship to study drug will be assessed according to the following guidelines:

Possibly related: The AE is known to occur with the study drug, there is a reasonable possibility that the study drug caused the AE, or there is a temporal relationship between the study drug and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study drug and the AE.

Unlikely related: There is not a reasonable possibility that the administration of the study drug caused the event, there is no temporal relationship between the study drug and event onset, or an alternate etiology has been established.

6.4.2.2 Serious Adverse Events

An SAE is any AE that results in 1 of the following outcomes, regardless of the investigator's opinion of causation:

- Death
- Initial or prolonged inpatient hospitalization
    - Surgeries planned prior to signing the ICF will not be considered SAEs. However, worsening of the underlying medical condition during the study will be considered an AE and must be captured as serious if any SAE-defining outcomes occur as a result.

- A life-threatening experience (that is, immediate risk of dying)
- Persistent or significant disability/incapacity
- Congenital anomaly/birth defect
- Other medically important serious event as determined by the investigator (for example, an AE that jeopardizes the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition)

The following events do not meet the definition of an SAE:

- Hospitalization for elective treatment of a pre-existing condition that does not worsen from baseline
- Hospitalizations for a standard procedure for study drug administration and routine monitoring of the studied indication not associated with any deterioration in condition
- Social or convenience admission to a hospital
- Prolongation of a hospitalization for social or convenience reasons not associated with the occurrence of an AE
- Hospitalization or an emergency room visit that lasts less than 24 hours that does not meet the criteria of an important medical or a life-threatening event

6.4.2.3 Adverse Events of Special Interest

AESIs (serious or nonserious) are TEAEs of particular safety importance as identified by the Sponsor. Reported TEAEs that are classified in the system organ classes (SOCs) listed below will be considered AESIs:

- Skin and subcutaneous tissue disorders
- Eye disorders

Nonserious AEs in the above SOCs that occur prior to study drug dosing will be recorded in medical history.

6.4.2.4 Laboratory Parameters

A central laboratory will be used for safety laboratory tests. Standard clinical laboratory tests are performed at times specified in the Schedule of Procedures and Assessments section of the clinical study protocol. Repeat testing of samples may be permitted during screening, after review and approval by the medical monitor.

Abnormal laboratory values are graded by the investigator as "clinically significant" or "not clinically significant," where applicable. Clinically significant abnormal laboratory values will be reported as AEs. Investigators may repeat laboratory tests for any parameter that is abnormal and/or clinically significant.

Table 2 lists the specific laboratory analyses that will be performed for this study.

Table 2 Clinical Laboratory Tests

Hematology[a]: Hemoglobin Hematocrit Erythrocyte count (red blood cell [RBC]) Mean cell volume (MCV) Mean cell hemoglobin concentration (MCHC) Leukocytes (white blood cell [WBC]) Neutrophils, segmented Absolute Neutrophil Count (ANC) Lymphocytes Monocytes Eosinophils Basophils Platelets Prothrombin time[b]	**Chemistry[a]:** Serum concentrations of: Sodium Potassium Bicarbonate Chloride Total bilirubin Direct bilirubin Alkaline phosphatase Alanine aminotransferase/serum glutamic pyruvic transaminase (ALT/SGPT) Aspartate aminotransferase/serum glutamic oxaloacetic transaminase (AST/SGOT) Gamma-glutamyl transferase (GGT) Blood urea nitrogen (BUN) Creatinine Uric acid Phosphorous Calcium Glucose (random) Albumin Total cholesterol Creatine kinase (CK)[c] Magnesium Amylase Lipase
Urinalysis[a]: Specific gravity pH Protein Glucose Ketones Blood Urine leukocyte esterase Urobilinogen	
Urine Drug Screen and Breathalyzer Alcohol Screen[a] Amphetamines Barbiturates Benzodiazepines Delta-9-tetrahydrocannabinol (THC) Cocaine Ethyl alcohol (via breathalyzer) Opioids	**Viral Serology[d]** Hepatitis B Surface Antigen (HBs Ag) Hepatitis B Core Antibody (HBc Ab) Hepatitis C Antibody (HC Ab) Hepatitis A Antibody (HAV-Ab [IgM]) Human immunodeficiency virus (HIV)

Phencyclidine Propoxyphene Methadone	
Thyroid function[d]: Thyroid-stimulating hormone (TSH); if abnormal perform Free T3 and Free T4 reflex testing	
Pregnancy Test (females of child bearing potential only)[e]	[redacted]

[a] Performed at each visit.

[b] To be conducted at baseline only, unless indicated for hepatic monitoring testing

[c] Creatine kinase is to be fractionated if CK results >1000 IU/L.

[d] Screening only.

[e] Serum pregnancy test at Visit 1 (Screening) is to be performed by a Sponsor-designated laboratory; urine pregnancy tests will be performed at all subsequent visits by the site.

[f] [redacted].

Note: All safety laboratory tests will be analyzed by a Sponsor-designated laboratory, unless otherwise specified.

6.4.2.5 Physical Examinations

A full physical examination is performed at Screening and includes an evaluation of cardiovascular, respiratory, gastrointestinal, neurological (examination of cranial nerves, motor system, sensory, and reflexes), dermatological, and musculoskeletal systems and include general appearance, skin, head, neck, eyes, ears, nose, throat, lungs, heart, abdomen, back, lymph nodes, extremities, and vascular status. Additional physical examinations are performed as needed.

Height and weight are measured during the study as indicated in the Schedule of Procedures and Assessments. Height should be measured only once at screening with no shoes for correct measurement. The body mass index (BMI) is calculated based on height measured at screening and weight measured at Screening, Baseline (Week 0), Week 4, and Week 8.

6.4.2.6 Vital Signs

Vital signs (respiratory rate, blood pressure [BP], and pulse) are measured at each visit throughout the screening and treatment periods of the study in supine and standing positions. Subjects should be supine for 10 minutes before vital signs (respiratory rate first, then BP and pulse) are measured (respiratory rate and pulse should be assessed for 30 seconds, then doubled to record breaths/minute and beats/minute, respectively). Subjects are then asked to stand for 3 minutes prior to measurement of standing respiratory rate, BP, and pulse. Oral temperature can be measured in any position at each visit throughout the study.

6.4.2.7 Electrocardiography

ECG is performed at Screening, Baseline (Week 0), Week 4, and Week 8. For each subject, single 12-lead digital ECGs are collected after the subject has rested for 5 minutes. All digital ECGs are electronically transmitted to a designated central ECG laboratory. Any clinically significant finding that was present on the fully over-read ECG is reported to the investigator and to the Sponsor.

The results include ventricular rate, PR interval, RR interval, QRS duration, QT interval, QTcB interval (corrected QT interval using Bazett's correction), QTcF interval (corrected QT interval using Fridericia's correction), interpretation (normal, abnormal) and evaluation.

The corrected QT interval will be corrected for respiratory rate using Fridericia's correction: $QTcF = QT/RR^{0.33}$ and Bazett's correction: $QTcF = QT/RR^{0.5}$.

6.4.2.8 Ophthalmologic Examination

The following ocular assessments are performed, using these standard clinical operating procedures, at specified visits, on both eyes of each subject:

1. Standard Ophthalmologic Examinations: performed at Screening/Baseline, between post-dose days 7-15, and at end of treatment/Week 8.
2. Corneal Specular Microscopy (CSM): noncontact or contact specular microscopy for qualitative and quantitative examination of the central corneal endothelium (cell count, cell shape, density, and morphology), with image capture at Screening/Baseline and 12 weeks following first dose.

Local investigators assess and record the results of items 1 and 2 on the examination worksheet. For both the standard ophthalmologic examination and CSM, an assessment of normal/abnormal is captured as well as whether the abnormal result is clinically significant or not. CSM results are sent to [redacted] where the designated ophthalmology expert will review. Additional ophthalmologic examinations (standard ophthalmologic exams and/or corneal specular microscopies) may be conducted at any time during the study to assess subject safety.

The final ocular examination for corneal specular microscopy is conducted approximately 12 weeks (+2 days) after the first dose of study drug (including both subjects who complete the study and subjects who discontinue early from the study).

6.4.2.9 Suicidal Ideation/Suicidality

Suicide-related events (behavior and/or ideation) are assessed and evaluated at every scheduled and unscheduled visit with the administration of the C-SSRS (Posner 2007). The C-SSRS captures the occurrence, severity, and frequency of suicide-related thoughts and behaviors during the assessment period. The 2009 "Baseline" version is used at Visit

1 (Screening), evaluating past year and past 3 months, and the 2009 "Since Last Visit" version is used for all subsequent visits.

6.4.2.10 Opiate Withdrawal

Opiate withdrawal is assessed at screening and at all subsequent treatment visits through Week 8 using the COWS. The COWS is a clinician administered instrument used to rate 11 common opiate withdrawal signs or symptoms (Wesson and Ling 2003). The total score of the 11 items can then be used to assess a subject's level of opiate withdrawal and to determine their level of physical dependence on opioids. This assessment provides an additional safeguard to ensure no subjects are enrolled with opiate withdrawal or for whom withdrawal may begin during the course of the study. Scores range from 0 to 48 with higher scores indicating greater severity of opioid withdrawal symptoms.

6.4.3 [redacted]

[redacted]

6.5 Data Quality Assurance

Report summaries will be generated using validated Base SAS® software, version 9.4 or higher, on a PC or server-based platform. Additional validated software may be used to generate analyses, as needed.

All SAS programs that create outputs or supporting analysis datasets will be validated by a second statistical programmer or biostatistician. At a minimum, validation of programs will consist of a review of the program log, review of output or dataset format and structure, and independent confirmatory programming to verify output results or dataset content. Additionally, all outputs will undergo a review by a senior level team member before finalization.

The content of the source data will be reviewed on an ongoing basis by project statistical programmers and statisticians. Data will be checked for missing values, invalid records, and extreme outliers through defensive programming applications, analysis-based edit checks, and other programmatic testing procedures. All findings will be forwarded to the project data manager for appropriate action and resolution.

7 STATISTICAL METHODS

7.1 General Methodology

Data will be analyzed by Precision for Medicine biostatistics personnel. Statistical analyses will be reported with tables, figures, and listings, presented in rich text format, and using recommended ICH numbering. Output specifications for all tables, figures, and listings will be in conformance with guidelines specified by the ICH in Appendix 7 of the *Electronic Common Technical Document Specification* (Apr 2003).

7.1.1 Reporting Conventions

Tables and figures will be summarized by treatment group. Tables summarizing demographics and other baseline characteristics will also include a column for all subjects combined. In general, all data collected and any derived data will be presented in subject data listings for all randomized subjects except for the screening failures listing which will be presented for only screen failures and the informed consent and eligibility criteria listing which will be presented for all screened subjects. Listings will be ordered by treatment group, subject number, and assessment or event date. The treatment groups will be displayed in the same order as they appeared in the summary tables. The treatment group presented in safety listings will be based on the actual assignment. The treatment group presented in all other listings will be based on the planned assignment, unless otherwise noted.

In general, continuous variables will be summarized to indicate the study population sample size (N), number of subjects with available data (n), mean, SD, median, 25^{th} (Q1) and 75^{th} (Q3) quartiles, minimum, and maximum values. Categorical variables will be summarized by the population size (N), number of subjects with available data (n), number of subjects in each category, and the percentage of subjects in each category. Unless otherwise noted, the denominator to determine the percentage of subjects in each category will be based on the number of subjects with available data. The denominator for percentages for incidence data (such as adverse events) will be based on the number of subjects in the analysis population. Select ordinal data may be summarized using both descriptive statistics and counts and percentages of subjects in each category, as appropriate.

7.1.2 Analysis Visit Window

For efficacy analyses and safety analysis by visit, assessments at scheduled and unscheduled visits will be mapped to the appropriate analysis window as detailed in Table 3 and Table 4. In the event where more than one visit falls in the same analysis window, the following rules will be used in sequence to determine the record that will be analyzed:

- If there is a scheduled visit in the analysis window, then the scheduled visit's data will be used.
- If there is no scheduled visit in the analysis visit window, the data closest to the scheduled day/time visit will be used.
- If there is no scheduled visit in the analysis visit window and there is a tie between the visits with regards to the number of days/hours before and after the scheduled day, the later data will be used.

Visit windows will not apply to listings. All data will be included in subject listings.

Windowing will be applied to the data prior to any missing data calculations.

Table 3 Analysis Windows for Efficacy Analyses

Period	Screening	Baseline	Study Drug Treatment				Safety Follow-up	
Visit	1	2	3	4	5	6		
	Days –7 to –28	Day 1	Day 15 (±2 d)	Day 29 (±2 d)	Day 43 (±2 d)	Day 57 (±2 d)	Day 71	Day 85
Analysis Visit Name		**Baseline ***	**Week 2**	**Week 4**	**Week 6**	**Week 8**	**Week 10**	**Week 12**
CGI-I HADS	NA	≤ 1 Pre-dose	[2, 22]	[23, 36]	[37, 50]	[51, 71]	NA	NA
HAMD-17 HAM-A SHAPS [redacted]	NA	≤ 1 Pre-dose	NA	[16, 43]	NA	[44, 71]	NA	NA
CGI-S [redacted]	NA	≤ 1 Pre-dose	NA	NA	NA	[44, 71]	NA	NA
SDS	NA	≤ 1 Pre-dose	NA	[23, 36]	[37, 50]	[51, 71]	NA	NA

* Baseline for analysis visit refers to baseline value for efficacy analysis defined in Section 7.1.5

Table 4 Analysis Windows for Safety Analyses by Visit

Period	Screening	Baseline	Study Drug Treatment				Safety Follow-up	
Visit	1	2	3	4	5	6		
	Days –7 to –28	Day 1	Day 15 (±2 d)	Day 29 (±2 d)	Day 43 (±2 d)	Day 57 (±2 d)	Day 71	Day 85
Analysis Visit Name		**Baseline**	**Week 2**	**Week 4**	**Week 6**	**Week 8**	**Week 10**	**Week 12**
Vital signs Clinical laboratory tests COWS	NA	≤ 1 Pre-dose	[2, 22]	[23, 36]	[37, 50]	[51, last dose +30]	NA	NA
Body weight ECG	NA	≤ 1 Pre-dose	NA	[2, 43]	NA	[44, last dose +30]	NA	NA
Standard Ophthalmologic examination	NA	≤ 1 Pre-dose	NA	[2, 14]	NA	≥ 15	NA	NA
Corneal specular microscopy	NA	≤ 1 Pre-dose	NA	NA	NA	NA	NA	≥2
C-SSRS	Nominal	≤ 1 Pre-dose	[2, 22]	[23, 36]	[37, 50]	[51, 64]	[65, last dose +30]	NA

7.1.3 Data Handling Rules

Unless otherwise noted, values reported as greater than or less than some quantifiable limit (e.g., "< 1.0") will be summarized with the sign suppressed in summary tables and figures, using the numeric value reported. Data will display on subject listings to include the sign.

7.1.4 Standard Calculations

Where appropriate, the calculated study day of each assessment or event will be presented with the assessment or event date on subject data listings, where study day will be determined as:

- The assessment/event date minus the date of first dose of study drug, if the assessment/event date is prior to the date of first dose; and
- The assessment/event date minus the date of first dose of study drug, plus one, if the assessment/event date is on or after the date of first dose.

Start and stop dates will be imputed when partial dates are present as needed to determine treatment emergent events and concomitant medications/procedures. No imputation will

be done for a completely missing start/stop date or for subjects who did not receive study treatment.

Start dates with a missing day but which have month and year provided will be imputed such that:

- If the provided month and year match the month and year for that subject's first dose date, then the Day 1 date will be used
- In all other cases the 1st of the month will be used with the provided month and year

Start dates with a missing day and month but which have year populated will be imputed such that:

- If the provided year matches the year for that subject's first dose date, then the first dose date will be used
- In all other cases the 1st of January will be used with the provided year

Stop dates will be imputed as follows:

- Missing day with a provided year and month will use the last day of the month
- Missing day and month with provided year will use December 31

If the imputed stop date is greater than the last study date for the subject, then the imputed date will be replaced with the last known subject date.

The date of the start of the current depressive episode and date of diagnosis of MDD will be imputed when the month or day are missing as follows:

- Missing day is set to 1 if the same year and month as the date of assessment. Otherwise it is set to 15

- Missing month and day are set to Jan 1 if the same year as the date of assessment. Otherwise it is set to July 1.

Other variables requiring calculations will be derived using the following formulas:

- **Days:** A duration between two dates expressed in days will be calculated using the following conventions:

    - Later date – earlier date + 1, if the earlier date is on or after the date of first dose of study drug; or

    - Later date – earlier date, if the earlier date is prior to the date of first dose of study drug.

- **Months:** A duration expressed in months will be calculated by dividing the duration in days by (365.25 / 12);

- **Years:** A duration expressed in years will be calculated by dividing the duration in days by 365.25;

- **Change from Baseline:** Change from baseline will be calculated as the post-baseline value minus the baseline value;

- **Percentage Change from Baseline:** Percentage change from baseline will be calculated as the change from baseline divided by the baseline value, multiplied by 100.

7.1.5 Baseline Definition

For efficacy and safety analyses presented by study visit, the baseline value will be defined as the last measurement prior to the start of study drug administration.

7.1.6 Multiple Comparisons/Multiplicity

The prespecified primary efficacy analysis will be conducted using a two-sided test at the α=0.05 level of significance. All other analyses will be also conducted with no adjustments for multiple comparisons.

7.2 Analysis Populations

The analysis populations are defined as follows:

- The Safety population is defined as all subjects who received study drug and will be used for summaries of safety parameters. Subjects will be analyzed according to treatment received.

- The Efficacy population includes all randomized subjects who received at least 1 dose of study drug, have a baseline HAMD-17 assessment total score, and have at least 1 post-baseline HAMD-17 assessment total score. Subjects will be analyzed according to randomized treatment assignment. This population will be used for the primary analysis and for all other efficacy analyses.

- The Per-Protocol (PP) population includes subjects in the efficacy population except those who experienced an important protocol deviation that is determined to substantially impact efficacy analysis, or did not have a HAMD-17 score at Week 8 analysis visit, or whose compliance at the end of the study was less than 70%. Subjects will be analyzed according to randomized treatment assignment. This population will be used for supportive efficacy analyses. The list of important protocol deviations for PP population will be classified by the sponsor before the final database lock and the unblinding of the study.

7.3 Study Subjects

7.3.1 Disposition of Subjects

Subject disposition will be summarized for all subjects by treatment group and over all subjects combined. Summaries will include the number and percentage of subjects screened, randomized, in each analysis population, completing the treatment, completing the study, and discontinuing the study early by the primary reason for discontinuation. Subject disposition will also be summarized separately for each study site.

Subject completion status, date of study completion/discontinuation, study day of discontinuation, and reason for discontinuation will be listed. Inclusion and exclusion eligibility will be listed separately.

7.3.2 Important Protocol Deviations

Important protocol deviations will be summarized by treatment group and over all subjects combined for the Efficacy Population. Important protocol deviations are protocol deviations captured on-study that are deemed by the Sponsor to potentially impact the efficacy or safety conclusions of the study.

Important protocol deviation rules will be determined and appropriately categorized prior to database lock and prior to breaking the blind of the treatment group assignments. The number and percentage of subjects with any important protocol deviations as well as the number and percentage of subjects with deviations within each category will presented.

The important protocol deviations will be listed by treatment group and subject.

7.4 Efficacy Evaluation

7.4.1 Datasets Analyzed

All efficacy summaries will be produced on the Efficacy Population; select efficacy summaries (including HAMD-17, response rate of HAMD-17, subgroup analysis of HAMD-17 (<22 vs ≥ 22), SHAPS, CGI-I) will also be produced on the PP Population. A data listing of subjects excluded from the Efficacy Population or PP Population, to include the reason for exclusion, will be presented. All data listings will be listed for all randomized or treated subjects.

7.4.2 Demographic and Other Baseline Characteristics

Demographic variables including age, sex, ethnicity and race will be summarized by treatment group and over all subjects combined for the Safety, Efficacy, and PP Populations. Age at the time of informed consent, as collected on the eCRF, will be summarized using descriptive statistics. Sex, ethnicity, and race will be summarized with the number and percentage of subjects in each parameter category (including the number missing).

Baseline characteristics include height, weight, BMI, baseline CGI-S score, HAMD-17 score, SHAPS scores by both scoring methods, HAM-A score, [redacted] HADS subscale and total scores, [redacted] SDS total score, duration of current depressive episode, time since first diagnosis of depression, MDD medication use for the current episode of MDD prior to the start of study drug administration, non-pharmacological procedures for the current episode of MDD, and medical and psychiatric history other than MDD. Height, weight, BMI, baseline CGI-S score, HAMD-17, SHAPS scores by both scoring methods, HAM-A score, [redacted] HADS total and subscale scores, [redacted] SDS total score, duration of current depressive episode, and time since first diagnosis of depression will be summarized using descriptive statistics by treatment group and over all subjects combined for the Safety, Efficacy, and PP Populations.

Medications for the current episode of MDD prior to the start of study drug administration will be coded using World Health Organization Drug Classification (WHODrug) for Drug Statistics Methodology (B3 Global) September 2019 version. For the MDD medication summary, the number and percentage of subjects receiving any medication will be summarized by treatment group, as will the number and percentage receiving any medication by Anatomical Therapeutic Chemical (ATC) drug class and generic drug name. Subjects reporting use of more than one medication at each level of summarization (any medication received, ATC class, and generic drug name) will be counted only once. ATC class terms will be displayed by descending order of incidence, as will generic drug names within each ATC class.

Medical and psychiatric history other than MDD will be presented only for the Safety Population. Medical and psychiatric history conditions will be coded using the Medical Dictionary for Regulatory Activities (MedDRA, version 22.1). Medical and psychiatric history will be summarized using frequency counts and percentages by treatment group, system organ class and preferred term.

All other baseline characteristics except MDD non-pharmacologic procedures will be summarized by treatment group and over all subjects combined for the Safety, Efficacy, and PP Populations. Demographic and baseline characteristic information will be listed for all randomized or treated subjects.

7.4.3 Measurements of Treatment Compliance

Percentage compliance to the study treatment regimen will be determined as the total dose actually taken divided by the expected dose received, multiplied by 100. The expected dose received will be calculated as (the date of last dose of study drug – the date of first dose of study drug + 1) x 1 capsule/day. If the date of last dose is not collected or missing, the date of last dose will be equal to the date of Visit 6/EOT or date of study completion/termination, whichever is earlier.

The total dose actually taken will be determined as the difference between the sum of all capsules dispensed before the date of last dose and the sum of all capsules returned. If number of capsules taken is not missing, the number of capsules returned will be

calculated as the number of capsules dispensed minus the number of capsules taken. If both the number of capsules returned and the number of capsules taken are missing, then it will be assumed that 0 capsules were returned. In addition, if the number of capsules dispensed is 0, then the corresponding number of capsules returned will be set to 0, if applicable.

Dosing compliance will be summarized using descriptive statistics, by treatment group, based on the Efficacy Population. As an exception rule, the compliance rate for subjects with < 14 days exposure duration will not be calculated, as the compliance may not be calculated reliably for such short duration. A subject is defined to be compliant if he/she has taken ≥80% and <120% of the number of doses expected to be taken during the study overall. The number and percentages of subjects who are compliant and non-compliant within each treatment group will be summarized.

7.4.4 Primary Efficacy Endpoint Analysis Methods

The primary efficacy endpoint is the change from baseline to Week 8 on the HAMD-17 score. The estimand for the primary efficacy endpoint is the difference in means between treatment groups in the change of HAMD-17 scores from baseline to Week 8 for all subjects in the Efficacy population. For efficacy analyses, baseline is defined as the latest measurement prior to the first administration of study drug. The null hypothesis to be tested is that there is no difference between BTRX-335140 and placebo:

$$H_0: \ \mu_A = \mu_B;$$

Where μ_A and μ_B represent the mean values for BTRX-335140 and placebo, respectively. The alternate hypothesis to be tested is that the treatment group means differ:

$$H_1: \ \mu_A \neq \mu_B;$$

The analysis will be conducted using a mixed-models repeated measures (MMRM) with change from baseline in HAMD-17 score as the dependent variable and will include treatment group, week, and week by treatment group interaction as factors, and baseline HAMD-17 score as covariate. Variance estimation will be based on an unstructured covariance matrix. If the unstructured covariance matrix results in a lack of convergence, the heterogeneous autoregressive variance-covariance structure will be used.

The primary analysis will compare the groups at Week 8. The primary results obtained from the model will be the estimated treatment difference at Week 8. Significance will be assessed based on a two-sided test at the α=0.05 level of significance. In addition, the estimated treatment difference at Week 4 will be provided. The model-based least square (LS) means, standard errors, 95% CIs, and 2-sided p-values will be reported.

In addition, descriptive summary of HAMD-17 and each individual item will be generated by treatment group and by visit.

7.4.5 Sensitivity Analyses of the Primary Efficacy Endpoint

Sensitivity analyses will be used to investigate the impact of important protocol deviations or missing data for the primary efficacy endpoint assessment (ie, HAMD-17 total score). The primary efficacy analysis will be repeated using

- The per-protocol population.
- If ≥10% of subjects have missing data for the primary efficacy endpoint assessment (i.e., HAMD-17 total score), the following will be conducted:
    - Rubin's rules for multiple imputation (MI) with SAS Proc MI and MIANALYSIS for the efficacy population. See Appendix B : Steps for Multiple Imputation for further details.
    - Last Observation Carried Forward (LOCF) Imputation technique, ie, the last observed non-missing value will be used to fill in missing values at a later point in the study, regardless of when the missing value occurred. The imputed dataset generated with the LOCF technique will be used as input data in the ANCOVA. The efficacy population will be used.

7.4.6 Subgroup Analyses of the Primary Efficacy Endpoint

The primary efficacy endpoint (change from baseline to Week 8 in HAMD-17 total score) will be summarized using the following subgroups:

- Gender (male and female)
- Baseline HAMD-17 scores: < 22 vs ≥ 22
- by baseline SHAPS Total Score (14 to 56) (< median and ≥ median) and SHAPS Original Total Score (0 to 14) (< median and ≥ median).

[redacted]

Summaries by subgroup will only be produced if there are at least 20 subjects in the category of interest. Additional subgroup analyses may be performed post-hoc, as appropriate.

The primary endpoint (HAMD-17 total score) will also be summarized in subgroups of the Efficacy Population that include all subjects screened on or before March 16, 2020 (labeled as PreCOVID) versus the subjects screened after March 16, 2020 (labeled as PostCOVID).

7.4.7 Secondary Endpoint Analysis Methods

Secondary endpoints including the change from baseline to each timepoint assessed for comparisons between BTRX-335140 and placebo for the measures listed below, will be analyzed using an MMRM model for treatment group, week, and week by treatment group interaction as fixed factors and a covariate adjustment for baseline score. For CGI-I, the baseline CGI-I score will not be included as a covariate, as the baseline assessment of CGI-I refers to the comparison with screening visit. For all other measures the baseline score included as a covariate will be the baseline score for that measure. Variance estimation will be based on an unstructured covariance matrix unless there is a lack of convergence in which case a heterogeneous autoregressive variance-covariance structure will be used.

- CGI-I at each post-baseline visit
- Change in SHAPS from baseline at each post-baseline visit
- Change in HADS total score and HADS subscales: HADS-A and HADS-D from baseline at each post-baseline visit
- Change in HAM-A total score from baseline at each post-baseline visit
- Change in SDS from baseline at each post-baseline visit

The response rates (the percentage of subjects with ≥ 50% decrease in HAMD-17 from baseline to Week 4 and Week 8) will be compared between BTRX-335140 and placebo-treated groups. The response rates, response rate difference and corresponding 95% Wald CI, and Cochran-Mantel-Haenszel p-value will be reported.

The change at Week 8 from baseline in CGI-S will be estimated using an analysis of the covariance (ANCOVA) model with main effects for treatment group and a covariate adjustment for baseline CGI-S score. The model-based LS means, standard errors, 95% CIs, and 2-sided p-values will be reported.

For SDS, analysis will be performed for the three SDS items separately and the SDS total score as the sum of the individual domains. Note that the work/school domain may be not applicable for all subjects and therefore the analysis of SDS total score will be performed for the subjects with all 3 domain scores and the checkbox of “No work or school” is NOT marked. In addition, a sensitivity analysis of SDS total score will be conducted for all subjects, where the SDS total score will be calculated as (sum of social and family life)*30/20 for the subjects for whom the checkbox of “No work or school” is marked.

For CGI-I, an additional analysis will be performed to compare the symptom improvement rate, i.e. the percentage of subjects with responses at each post-baseline visit of ‘much improved’ or ‘very much improved’ (i.e. CGI-I score ≤2) between BTRX-335140 and placebo. The symptom improvement rate, improvement rate difference and corresponding 95% Wald CI, and Cochran-Mantel-Haenszel p-value will be reported.

For SHAPS, the analyses will be performed for the SHAPS total scores based on both methods of scoring (SHAPS Total Score [14 to 56] and SHAPS Original Total Score [0 to 14]).

In addition, descriptive summaries of each secondary efficacy variable will be generated by treatment group and by visit.

7.4.8 Subgroup Analyses of the Secondary Efficacy Endpoints

The secondary efficacy endpoints HAM-A and SHAPS will be summarized using the following subgroups:

- HAM-A total score will be presented by baseline SHAPS score (< median and ≥ median).
- SHAPS score (both SHAPS Total Score [14 to 56] and SHAPS Original Total Score [0 to 14]) will be presented by baseline [redacted]

Summaries by subgroup will only be produced if there are at least 20 subjects in the category of interest. Additional subgroup analyses may be performed post-hoc, as appropriate.

[redacted]

[redacted]

[redacted]

7.4.10 Interim Analyses and Data Monitoring

There are no interim analyses planned.

7.4.11 Additional supportive analyses to assess impact of COVID-19

Efficacy Endpoints Ascertainment

In the event that a subject cannot come to the clinic after the baseline visit due to self-quarantine, local restrictions, illness, or other reasons as described in the protocol, the site personnel may conduct a virtual (by telephone contact) visit. A table summarizing the mode of ascertainment of efficacy endpoints (in-clinic vs. phone) will be presented by treatment assignment, type of efficacy assessment and visit.

A listing will also be provided. The impact of the efficacy endpoints ascertainment (in-person vs. phone) will be explored in additional analyses if a large imbalance in ascertainment method between treated and placebo is present at study visits.

Study Discontinuation

Reasons for study discontinuation identified by the investigator as related to COVID-19 will be listed.

7.4.12 [redacted]

[redacted]

7.5 Safety Evaluation

Safety analysis will be carried out for the Safety Population, to include all subjects who receive at least one dose of study drug. Safety analyses will include data for each subject during the treatment-emergent period (from first dose of study drug to 30 days after last dose) except for the summary of AESIs. Subjects who do not complete the study, for whatever reason, will have all available data up until the time of termination included in the analysis.

7.5.1 Adverse Events

TEAEs are defined as those AEs with onset after the first dose of study drug or existing events that worsened after the first dose during the study and that occur prior to 30 days after last dose of treatment. TEAEs will be summarized by treatment group. Events reported with a partial onset date (e.g., month and year are reported but the day is missing) will be considered to be treatment-emergent unless a partial date clearly indicates that it occurred prior to first dose of study treatment or more than 30 days after last dose of treatment.

Verbatim terms on case report forms will be mapped to preferred terms and system organ classes using MedDRA version 22.1.

Summaries that are displayed by system organ class and preferred terms will be ordered by descending incidence of system organ class and preferred term within each system organ class. Summaries displayed by preferred term only will be ordered by descending incidence of preferred term. Summaries of the following types will be presented:

- Overall summary of number of unique TEAEs and treatment-emergent serious adverse events (TESAEs) and subject incidence of TEAEs meeting various criteria;
- Subject incidence of TEAEs by MedDRA system organ class and preferred term;
- Subject incidence of TEAEs by MedDRA preferred term;
- Subject incidence of TEAEs by severity grade, MedDRA system organ class, and preferred term;
- Subject incidence of TEAEs related to study drug, MedDRA system organ class, and preferred term;
- Subject incidence of TEAEs related to study drug by MedDRA preferred term;
- Subject incidence of TEAEs leading to discontinuation of study drug by MedDRA system organ class and preferred term;
- Subject incidence of severe TEAEs related to study drug by MedDRA system organ class and preferred term;
- Subject incidence of SAEs by MedDRA system organ class and preferred term;
- Subject incidence of AESIs by MedDRA system organ class and preferred term.
- Subject incidence of AESIs by severity grade, MedDRA system organ class, and preferred term; and

- Subject incidence of AESIs related to study drug, MedDRA system organ class, and preferred term

At each level of summarization (e.g., any AE, system organ class, and preferred term), subjects experiencing more than one TEAE will be counted only once. In the summary of TEAEs by severity grade, subjects will be counted once at the highest severity reported at each level of summarization.

When summarizing the related adverse events, the adverse events with relationship of unlikely related, possibly related, and missing are counted.

All adverse events will be presented in data listings by treatment group, subject, and event date. Serious AEs, AEs leading to discontinuation of the study drug, and AEs leading to death will be presented in separate data listings.

7.5.2 Extent of Exposure

Extent of exposure to study treatment will be summarized for the Safety Population by treatment group. The duration of exposure will be presented in days and calculated as the date of last dose of study drug minus the date of first dose of study drug, plus one. If the date of last dose is not collected, the date of last dose will be equal to the date of Visit 6/EOT or date of study completion/termination, whichever is earlier. The total dose received is calculated as (sum of all capsules dispensed before date of last dose – sum of all capsules returned) x 80mg. If the number of capsules taken is not missing, the number of capsules returned will be calculated as the number of capsules dispensed minus the number of capsules taken. If both the number of capsules returned and the number of capsules taken are missing, then it will be assumed that 0 capsules were returned. In addition, if the number of capsules dispensed is 0, then the corresponding number of capsules returned will be set to 0, if applicable. Duration of exposure and total dose received (mg) will be summarized using descriptive statistics.

7.5.3 Deaths, Other Serious Adverse Events, and Other Significant Adverse Events

All deaths during the study, including the post treatment follow-up period, will be listed by treatment group and subject, to include the primary cause of death. Treatment-emergent serious AEs and other significant AEs, including those that led to discontinuation of the study drug, will be provided in separate subject data listings.

7.5.4 Clinical Laboratory Evaluation

All descriptive summaries of laboratory results will be based on data analyzed by the central laboratory and presented in Système International (SI) units, as suggested by the Center for Biologics Evaluation and Research and the Center for Drug Evaluation and Research *Position on Use of SI Units for Lab Tests* (Oct 2013). All data will be included in by-subject data listings. Laboratory measurements identified as abnormal (ie, outside the normal range) will also be listed separately.

Clinical laboratory measurements, including serum chemistry and hematology, will be summarized by treatment group. Descriptive statistics will be presented for observed values and changes from baseline at each visit where parameters were scheduled to be collected per the clinical study protocol.

Where applicable, laboratory results will be classified as "low," "normal," or "high" with respect to the parameter-specific reference ranges (ie, below the lower limit of the normal range, within the normal range, or above the upper limit of the normal range). Three-by-three contingency tables will be presented for each laboratory parameter to summarize the shift from the baseline category to the worst post-baseline measurement, defined as the value numerically farthest outside of the normal range across all post-baseline visits through the end of the study. Summary results will include the count and percentage of subjects within each shift category and treatment group.

The number and percentage of subjects with a PCS (potentially clinically significant) laboratory result for the select analytes listed below in [redacted] will be presented by treatment group and study visit. PCS laboratory results will also be listed.

Table 5 Potentially Clinically Significant Laboratory Results

Analyte	PCS Cutoffs
Hemoglobin	**Low**:< 10.0 g/dL; < 6.2 mmol/L; <100 g/L **High:** > 2 g/dL (20 g/L) above ULN or above baseline if baseline is above ULN
Platelets count	<75,000/mm3; <75.0 x 10e9 /L
WBC count	<3000/mm3; <3.0 x 10e9 /L
Neutrophils	<1500/mm3; <1.5 x 10e9 /L
Lymphocytes	**Low:** < 800/mm3; < 0.8 x 10e9 /L **High:** >4000/mm3 - 20,000/mm3; > 4 x 10e9 /L - 20 x 10e9 /L
Total bilirubin	> 1.5 x ULN
AST	> 3.0 x ULN
ALT	> 3.0 x ULN
Alkaline phosphatase	> 3.0 x ULN
Creatinine	> 1.5 x above baseline; > 1.5 x ULN
Sodium	**Low:** < 130 mmol/L **High:** > 150 mmol/L
Potassium	**Low:** < 3.0 mmol/L **High:** > 5.5 mmol/L
CK	> 5 x ULN
GGT	>2.5 x ULN
BUN	> 30 mg/dL; > 10.71 mmol/L

7.5.5 Vital Signs, Physical Findings, and Other Observations Related to Safety

7.5.5.1 Vital Signs

Vital signs will include pulse (supine and standing), systolic/diastolic blood pressure (supine and standing), respiratory rate, body temperature, height, weight, and BMI will be listed by treatment group, subject, and visit date. Vital sign parameter measurements will be summarized by treatment group. Descriptive statistics will be presented for results and change from baseline at each visit where parameters were scheduled to be collected.

The number and percentage of subjects with a PCS measurement in supine will be presented by treatment group and study visit. PCS measurements include systolic blood pressure ≤ 90 mmHg, systolic blood pressure ≥ 180 mmHg, diastolic blood pressure ≤ 50 mmHg, diastolic blood pressure ≥ 105 mmHg, heart rate ≤ 40 bpm, and heart rate ≥ 120 bpm. PCS measurements in supine will also be listed.

Additionally, the number and percentage of subjects with an orthostatic systolic BP decrease > 20 mmHg or diastolic BP decrease > 10 mmHg from supine to standing position at any post-baseline measurement during the study will be summarized and listed.

7.5.5.2 12-Lead Electrocardiogram

Twelve-Lead ECG interval parameters including heart rate, PR, RR, QRS, QT, QTc interval calculated using the Fridericia method (QTcF), and QTc interval calculated using the Bazett method (QTcB) will be summarized by treatment group. Descriptive statistics will be presented for observed values and changes from baseline at each visit where parameters were scheduled to be collected.

The number and percentage of subjects with a PCS QTcF result will be presented by treatment group and study visit. PCS results at post-baseline visits include QTcF ≥ 500 msec, QTcF change from baseline ≥ 60 msec, QTcF change from baseline ≥ 30 msec. PCS results at post-baseline visits will also be listed.

7.5.5.3 Ophthalmology Examination

Standard ophthalmologic examination and corneal specular microscopy examination results will be summarized by presenting shift from baseline to end of study results as normal, abnormal not clinically significant, and abnormal clinically significant. Clinically significant abnormalities on ophthalmologic exam will be listed by treatment group, subject, and visit date.

The baseline, post-baseline and percent (%) change from baseline of cell density and coefficient of variation (CV) results from the central reading group for CSM will be summarized descriptively for each eye (oculus dextrus [OD] and oculus sinister [OS]). The number and percentage of subjects with percent decrease from baseline in cell density >= 5%, 10%, and 15% will be summarized for each eye and for both eyes. Results from the central reading group for CSM will also be presented in a data listing.

Additional subgroup summaries will be performed by three device types (Konan, Nidek and Tomey) and by age groups (18-29, 30-39, 40-49, 50-59, 60-65). All the above summaries will be repeated based on the set of subjects excluding the subjects who had endothelial dystrophy medical history prior to treatment.

7.5.5.4 Physical Examination

Abnormal results of the physical examination will be presented in subject data listings by treatment group, subject, and visit date.

7.5.5.5 Prior and Concomitant Medications

Medications will be coded using WHODrug (B3 Global) September 2019 version. Medications entered on the eCRF will be mapped to ATC drug class (level 4) and drug name. If ATC drug class level 4 is not available, then ATC drug class level 3 will be utilized. If ATC drug class levels 4 and 3 are not available, then ATC drug class level 2 will be utilized. If ATC drug class levels 4, 3, and 2 are not available, then ATC drug class level 1 will be utilized.

Prior and concomitant medications will be summarized separately and the study phase of each medication will be determined programmatically based on medication start and end dates. A prior medication is defined as any medication administered and stopped prior to the date of the first dose of study drug. A concomitant medication is defined as any medication administered on or after the date of the first dose of study drug. If it cannot be determined whether a medication was received after the start of study drug dosing, it will be considered concomitant.

For both prior and concomitant medications summaries, the number and percentage of subjects receiving any medication will be summarized by treatment group, as will the number and percentage receiving any medication by ATC drug class and generic drug name. Prior medications will also be summarized over all subjects combined. Subjects reporting use of more than one medication at each level of summarization (any medication received, ATC class, and generic drug name) will be counted only once. ATC class terms will be displayed by descending order of incidence, as will generic drug names within each ATC class. Prior and concomitant medications will be listed separately.

7.5.5.6 Prior and Concomitant Non-Pharmacological Procedures

Non-pharmacological procedures will be listed by treatment group, subject, and date.

7.5.5.7 Columbia Suicide Severity Rating Scale

Suicidality data collected on the C-SSRS at screening, baseline, and by visit during the treatment period will be listed for all subjects. Listings will include all data collected on the C-SSRS.

At screening visit, the number and percentage of subjects with a response of ‘Yes’ to:

- Any Suicidal Ideation from questions #1-3 (#1: Wish to be dead, #2: Nonspecific active suicidal thoughts, #3: Active suicidal ideation with any methods (not plan) without intent to act) for the past 3 months and each individual question #1-3 will be presented by treatment group
- The Preparatory Acts or Behavior question for the past 12 months will be presented by treatment group
- Any suicide attempts (Actual Attempt, Interrupted Attempt, or Aborted Attempt) in past 12 months will be presented by treatment group

At each of the scheduled visits following screening visit, the number and percentage of subjects with a response of 'Yes' to

- Any Suicidal Ideation from questions #1-5 (#1: Wish to be dead, #2: Nonspecific active suicidal thoughts, #3: Active suicidal ideation with any methods (not plan) without intent to act, #4: Active suicidal ideation with some intent to act, without specific plan, #5: Active suicidal ideation with specific plan and intent) or any Suicidal Behavior from questions for Actual Attempt, Interrupted Attempt, Aborted Attempt, or Preparatory Acts or Behavior will be presented by treatment group.
- Any Suicidal Ideation from questions #1-5 (#1: Wish to be dead, #2: Nonspecific active suicidal thoughts, #3: Active suicidal ideation with any methods (not plan) without intent to act, #4: Active suicidal ideation with some intent to act, without specific plan, #5: Active suicidal ideation with specific plan and intent) and each individual question #1-5 will be presented by treatment group.
- Any Suicidal Behavior from questions for Actual Attempt, Interrupted Attempt, Aborted Attempt, or Preparatory Acts or Behavior and each individual question will be presented by treatment group.

7.5.5.8 Clinical Opiate Withdrawal Scale

The COWS will provide an ongoing assessment of subjects for any symptoms suggestive of opiate withdrawal. COWS scale items and total scores, along with the numbers of subjects with a total score of ≥ 5, will be summarized by treatment group. Descriptive statistics for total scores and the number and percentage of subjects with a total score of ≥ 5 will be presented for each visit that the COWS was scheduled to be administered.

[redacted]

[redacted]

7.7 Changes in the Conduct of the Study or Planned Analyses

There are no changes to the planned analyses described in the study protocol in this SAP.

8 REFERENCE LIST

[redacted]

Guy W. *ECDEU assessment manual for psychopharmacology-revised*. Rockville, MD: National Institute of Mental Health, Psychopharmacology Research Branch, Division of Extramural Research Programs. 1976. 217–22.

[redacted]

Posner K. State of the science: measurement of suicidal adverse events and the Columbia Suicide Severity Rating Scale. Poster presented at: *47th Annual Meeting of the New Clinical Drug Evaluation Unit (National Institute of Mental Health);* June, 2007; Boca Raton, FL.

Rubin, DB. and Schenker, N.. Interval estimation from multiply-imputed data: a case study using agriculture industry codes. *Journal of the American Statistical Association.* 1987; 81: 366–374.

Sheehan DV. The Sheehan Disability Scales. In: *The Anxiety Disease and How to Overcome It*. Sheehan DV, ed. New York, NY: Charles Scribner and Sons; 1983:151

Snaith RP, Hamilton M, Morley S, et al. A scale for the assessment of hedonic tone. The Snaith-Hamilton Pleasure Scale. *Br J Psychiatry*. 1995;167(1):99–103.

Wesson DR, Ling, W. The Clinical Opiate Withdrawal Scale (COWS). *J Psychoactive Drugs*. 2003;35(2):253–9.

Williams JBW. A Structured Interview Guide for the Hamilton Depression Rating Scale. *Arch Gen Psychiatry*. 1988;45(8):742–747. doi:10.1001/archpsyc.1988.01800320058007

Zigmond AS, Snaith RP. The hospital anxiety and depression scale. *Acta Psychiatr Scand*. 1983;67(6):361–70.

APPENDIX A: SCHEDULE OF PROCEDURES AND ASSESSMENTS

Period	Screening	Baseline	Study Drug Treatment				Safety Follow-up	
Visit	1[b]	2	3	4	5	6		
Procedure and Assessments	Days –7 to –28 NA	Day 1 Week 0	Day 15 (±2 d) Week 2	Day 29 (±2 d) Week 4	Day 43 (±2 d) Week 6	Day 57/ EOT[c] (±2 d) Week 8	TC[a] Week 10	Ocular Exam Week 12
Informed consent	X							
Inclusion/exclusion criteria	X	X[d]						
Medical/psychiatric history	X							
Demographics	X							
Vital signs[e]	X	X	X	X	X	X		
Physical examination[f]	X							
Body weight[g]	X	X		X		X		
12-Lead ECG	X	X		X		X		
Standard ophthalmologic examination[h]		X[i, j]				X		
Corneal specular microscopy[h]		X[i]						X[t]
Prior/concomitant medication/therapy	X	X	X	X	X	X	X	
Randomization		X						
Clinical laboratory tests (hematology, chemistry, urinalysis)	X	X	X	X	X	X		
Serum/urine pregnancy test[k]	X	X	X	X	X	X		
UDS (onsite and central laboratory) and breathalyzer alcohol screen	X	X	X	X	X	X		
[redacted]				[redacted]		[redacted]		
[redacted]		[redacted]						
Thyroid function tests	X							
Viral serology	X							
Hepatic safety testing[n]	←							→
SCID-5-CT	X							
CGI-S	X	X				X		
CGI-I		X	X	X	X	X		
C-SSRS[o]	X	X	X	X	X	X	X	
HAMD-17	X	X		X		X		
HAM-A	X	X		X		X		
[redacted]		[redacted]				[redacted]		
SDS		X		X	X	X		
HADS		X	X	X	X	X		

Period	Screening	Baseline	Study Drug Treatment				Safety Follow-up	
Visit	1[b]	2	3	4	5	6		
Procedure and Assessments	Days –7 to –28 NA	Day 1 Week 0	Day 15 (±2 d) Week 2	Day 29 (±2 d) Week 4	Day 43 (±2 d) Week 6	Day 57/ EOT[c] (±2 d) Week 8	TC[a] Week 10	Ocular Exam Week 12
SHAPS	X	X		X		X		
[redacted]		[redacted]				[redacted]		
[redacted]	[redacted]	[redacted]		[redacted]		[redacted]		
[redacted]	[redacted]					[redacted]		
COWS	X	X	X	X	X	X		
Adverse events[p]		X	X	X	X	X	X	
Complete IWRS	X	X	X	X	X	X		
Dispense study drug		X[q]	X[q]	X[q]	X[q]			
Study drug administration		X[r]		X[s]				
Study drug compliance			X	X	X	X		

BMI = body mass index; CRF=case report form; CGI-I = Clinical Global Impression of Improvement; CGI-S = Clinical Global Impression of Severity; COWS = Clinical Opiate Withdrawal Scale; CSSRS = Columbia Suicide Severity Rating Scale; d = day; DSM = Diagnostic and Statistical Manual of Mental Disorders; ECG = electrocardiogram; eCRF=electronic case report form; EOT = End of Treatment; HADS = Hospital Anxiety and Depression Scale; HAM-A = Hamilton Anxiety Rating Scale; HAMD-17 = Hamilton Rating Scale for Depression – 17Item Version; IWRS = Interactive Web-response System; [redacted] NA = not applicable; [redacted]; [redacted]; [redacted]; SDS = Sheehan Disability Scale; SCID5CT = Structured Clinical Interview for DSM-5 Disorders, Clinical Trials Version; SHAPS = SnaithHamilton Pleasure Scale; [redacted]; TC = telephone call; UDS=urine drug screen.

[a] Study site personnel will contact subjects by telephone for Safety Follow-up assessments (adverse events, concomitant medication usage, and C-SSRS) approximately 2 weeks after discontinuation of study drug.

[b] Visit 1 procedures may be completed over 2 days, if necessary.

[c] Subjects who discontinue early will complete an EOT visit <2 days after their last dose of study drug; efficacy assessments are to be performed only if the subject has remained on study drug. For subjects who do not return, the site should complete a C-SSRS if they are aware of a potential suicide-related thought or behavior by other communications. The 12-week corneal specular microscopy examination should still be conducted approximately 12 weeks after the first dose of study drug (Section 7.7.2 of the protocol).

[d] Inclusion/exclusion criteria must be reconfirmed for eligibility prior to randomization.

[e] Collect vital signs measurements (respiratory rate first, then blood pressure and pulse) after subject has been supine for 10 minutes; subjects should then stand for 3 minutes before obtaining standing vital signs measurements [see Section 6.3.4 of the protocol]. Oral temperature can be assessed in any position.

[f] A full physical examination involving an evaluation of cardiovascular, respiratory, gastrointestinal, neurological (examination of cranial nerves, motor system, sensory, and reflexes), dermatological, and musculoskeletal systems, and include general appearance, skin, head, neck, eyes, ears, nose, throat, lungs, heart, abdomen, back, lymph nodes, extremities, and vascular status will be conducted at the screening visit. Further physical examination will be conducted as needed. (Section 6.3.3 of the protocol).

[g] At Screening, collect height for BMI calculation.

[h] Ophthalmologic examinations will be performed by a site-affiliated ophthalmologist. Details for the standard ophthalmologic examination and corneal specular microscopy will be described in a separate ocular monitoring manual. Additional standard ophthalmologic exams and / or corneal specular microscopies may be conducted to assess subject safety.

[i] Baseline ophthalmologic examinations (standard and corneal specular microscopy) must be performed before randomization.

[j] Prior to Visit 3 (Week 2) (between Days 7 and 14 [+2 days]), a standard ophthalmologic examination will be performed.

[k] Serum pregnancy test is to be performed at screening visit only and urine pregnancy tests will be performed by the site at all subsequent visits.

[l] [redacted]

[m] [redacted]

[n] Unscheduled hepatic monitoring testing may be performed at any time on the basis of clinical laboratory testing results, after consultation with the Sponsor-designated medical monitor (see Section 6.3.2.2 of the protocol).

[o] The C-SSRS Baseline version will be used at Screening and "Since Last Visit" version will be used for all subsequent visits.

[p] Adverse events reported before first dose of study drug to be recorded as medical history. Serious AEs will be recorded on the AE eCRF from the time of informed consent.

[q] Subjects will be instructed to take their study drug daily in the morning and preferably with food.

[r] Subjects will take their first dose of study drug (BTRX-335140 or placebo) under the supervision of clinical study personnel during the baseline visit.

[s] [redacted]

[t] The Week 12 corneal specular microscopy should be completed for all subjects (ie, completers and early termination) unless consent is withdrawn. The corneal specular microscopy should be conducted approximately 12 weeks after the first dose of study drug; exceptions to be discussed with the medical monitor.

APPENDIX B: STEPS FOR MULTIPLE IMPUTATION

For the MI to be applied to the MMRM for the sensitivity analysis of the primary efficacy endpoint, the following steps will be followed:

Imputation distribution

The imputation distribution for the missing absolute change from baseline **at visit *t*** (eg, Week 4, Week 8) will be a normal distribution.

Imputation algorithm

We will use the following algorithm that relates the mean of the missing absolute change from baseline **at visit *t***. The algorithm will be implemented within each treatment group separately by each visit as follows:

- randomly draw a sample from the normal distribution (μ, σ^2), where μ is the mean of the non-missing absolute changes from baseline **at visit *t*** and σ^2 is the sample variance estimated using the non-missing absolute changes **at visit *t***.

Analysis model

The complete MI method is described below:

- Form an "imputed dataset" by imputing missing change from baseline values at each visit for those subjects who have a missing value at the visit. The appropriate normal distribution specified in the algorithm above will be used for each such subject.
- Repeat this process *K* (K=20) times to form *K* imputed datasets.

- Fit the same MMRM model as the one used for primary analysis to each imputed dataset to estimate the absolute change.
- Combine the results from the analyses of K imputed datasets using the SAS procedure MIANALYZE to derive the MI estimator.

Let θ be the true treatment difference. Denote by $\tilde{\theta}_k$ the estimate of θ from the k^{th} imputed dataset, and the corresponding estimate of the variance is denoted by V_k. The MI estimator of θ, $\tilde{\theta}_{\mathbf{MI}}$, is the average of the K individual estimates.

The estimated variance of $\tilde{\theta}_{\mathbf{MI}}$ is a combination of the between- and within-imputation variability as follows: $V_{\mathbf{MI}} = W + \left(1 + \frac{1}{K}\right) B$, where $W = \frac{1}{K}\sum_{k=1}^{K} V_k$ is the within-imputation variability and is $B = \frac{1}{K-1}\sum_{k=1}^{K}(\tilde{\theta}_k - \tilde{\theta}_{\mathbf{MI}})^2$ is the between-imputation variance. The statistic $T=\frac{\tilde{\theta}_{\mathbf{MI}}-\theta}{\sqrt{V_{\mathbf{MI}}}}$ has an approximate t_V distribution Rubin (1987), where $V = (K-1)(1+\frac{W}{B})^2$.

[redacted]