PROTOCOL NEP-MDD-201

A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, PARALLEL-GROUP EFFICACY AND SAFETY STUDY OF BTRX-246040 ADMINISTERED ONCE DAILY IN PATIENTS WITH MAJOR DEPRESSIVE DISORDER WITH OR WITHOUT ANHEDONIA

Sponsor:	BlackThorn Therapeutics, Inc.
	780 Brannan Street
	San Francisco, CA 94103
Date:	November 20, 2018

1 SYNOPSIS

Name of Investigational Study Product: BTRX-246040	
Title of Study: A Randomized, Double-blind, Placebo-Controlled, Parallel-group Efficacy and Safety Study of BTRX-246040 Administered Once Daily in Patients with Major Depressive Disorder with or without Anhedonia	
Approximate Number of Planned Patients: 100 patients randomized with treatment	**Phase of Development:** 2a
Length of Study: Approximately 17 months	
Primary Objective: To evaluate the effects of BTRX-246040 on symptoms of depression in adult patients with Major Depressive Disorder (MDD) after 8 weeks of double-blind treatment	
Secondary Objectives: 1) To evaluate the effects of BTRX-246040 on anhedonia in adult patients with MDD 2) To evaluate the safety and tolerability of BTRX-246040 in adult patients with MDD 3) To evaluate the effects of BTRX-246040 on the performance of tasks as measured by the Facial Expression Recognition Task (FERT), Probabilistic Reward Task (PRT), and Effort Expenditure for Reward Task (EEfRT) in adult patients with MDD	
Exploratory Objectives: 1) To evaluate the effects of BTRX-246040 on anxiety-related symptoms in adult patients with MDD 2) To evaluate the effects of BTRX-246040 on pain-related symptoms in adult patients with MDD 3) To evaluate the effects of BTRX-246040 as measured by biomarkers in adult patients with MDD	
Study Design: Study NEP-MDD-201 is an 8-week, randomized, double-blind, placebo-controlled, parallel-group, multicenter study to evaluate the effects of BTRX-246040 on symptoms of depression in adult patients with MDD after 8 weeks of double-blind treatment. There will be a 28-day screening period, 8-week active treatment period (during which patients will receive either BTRX-246040 or placebo), and an approximate 1- to 2-week off-drug safety follow-up period. Patients will be consented and screened (Visit 1) for the study until approximately 100 patients are randomized. Randomization will be performed in a 1:1 ratio to one of 2 treatment arms (placebo or BTRX-246040 QD) after meeting DSM-5 diagnostic criteria for MDD and study inclusion (see section 8). Randomization will be stratified by anhedonia symptom severity, as indicated by a SHAPS total score ≤ 4 and > 4. The BTRX-246040 starting dose for all eligible	

patients will be 40 mg QD, initiated at Visit 2 (baseline). A dose escalation to 80 mg QD will occur at Visit 3 (week 1) for all patients. Patients who tolerate the dose increase will remain at a dose of 80 mg QD for the subsequent 7 weeks. The dose may be decreased back to 40 mg QD anytime through Visit 4 (week 2), if required, for tolerability. Patients who cannot tolerate 40 mg daily will be discontinued from participation in the study. Subsequent visits include Visit 5 (week 4), Visit 6 (week 6), and the end of the treatment period, Visit 7 (week 8). All patients will return to the study site for an off-drug safety follow-up visit approximately 1 to 2 weeks after discontinuation of study drug. Overall duration in the study for each patient is expected to be approximately 14 weeks.
Diagnosis and Main Criteria for Inclusion and Exclusions (see section 8 of this protocol for complete list of criteria): The study population will consist of men and women aged 18 to 65 years (inclusive) with a diagnosis of MDD as defined by DSM-5 criteria and have had at least 1 prior major depressive episode in the past 10 years. Patients must present with a new current episode of MDD and the duration of the current episode must be at least 4 weeks but not longer than 18 months. At Visit 1 (screening) and Visit 2 (baseline), patients must have clinically significant depressive symptoms defined by tandem (investigator- and computer-administered) Montgomery-Asberg Depression Rating Scale® (MADRS) total scores ≥ 26 with a difference of ≤ 7 points between the Investigator- and computer-administered MADRS total scores, a CGI-S score ≥ 4. Patients will be excluded from the study if they present with any current DSM-5 disorder other than MDD which is the focus of treatment. Are homicidal in the opinion of the Investigator or are at suicidal risk (any suicide attempts within 12 months prior to Visit 1 [screening] or any suicidal intent, including a plan, within 3 months prior to Visit 1 [screening]; C-SSRS answer of "YES" on item 4 or 5 [suicidal ideation]; Investigator- or computer-administered MADRS score of ≥ 5 on item 10 [suicidal thoughts]; by Investigator clinical evaluation). Patients cannot have any history of substance or alcohol use disorder within 12 months prior to Visit 1 (screening) per DSM-5 criteria or clinically significant comorbid disease.
Investigational Study Product, Dose and Mode of Administration: A starting dose of BTRX-246040 40 mg (1 capsule) QD at Visit 2 (baseline), orally for the first 7 days. A dose escalation to 80 mg QD (2 capsules) will occur at Visit 3 (week 1) and this dose will be maintained for the subsequent 7 weeks. The dose may be decreased back to 40 mg QD anytime through Visit 4 (week 2), if required, for tolerability. Patients who cannot tolerate 40 mg daily will be discontinued from participation in the study.
Reference Therapy, Dose and Mode of Administration: Placebo will be assigned as 1 capsule QD at Visit 2 (baseline), orally for the first 7 days. Two placebo capsules will be given at Visit 3 (week 1) for the subsequent 7 weeks. Placebo capsules will consist of inactive ingredients and look identical to BTRX-246040. Placebo may be decreased back to 1 capsule QD anytime through Visit 4 (week 2), if required, for tolerability. Patients who cannot tolerate 1 capsule daily will be discontinued from participation in the study.
Planned Duration of Treatment: Double-blind Treatment period: 8 weeks

Criteria for Evaluation:

Primary Efficacy: Primary efficacy will be determined using change from baseline to Week 8 on the Investigator-administered MADRS total score in the Full Analysis Set of patients.

Secondary Efficacy: Secondary efficacy will be assessed using change from baseline to 8 in the following endpoints:

- Investigator-administered MADRS-6 subscale
- Investigator-administered MADRS individual items
- Hospital Anxiety and Depression Scale (HADS) subscales: anxiety subscale (HADS-A) and depression subscale (HADS-D)
- Dimensional Anhedonia Rating Scale (DARS)
- Snaith-Hamilton Pleasure Scale (SHAPS)

In addition, outcome measures from the following performance tasks will be analyzed.

- FERT
- PRT
- EEfRT

Exploratory Efficacy & Tolerability:

- Change in HAM-A total score from baseline to Week 4 and from baseline to Week 8
- Change from baseline to Week 8 on the average pain in the last 24 hours
- Changes in plasma levels of nociceptin, and changes in serum levels of interleukin 1 beta, 2, 6 and 10 (IL-1ß, IL-2, IL-6, IL-10), tumor necrosis factor alpha (TNFα), interferon gamma (IFNγ) and C-reactive protein (CRP) from baseline to Week 8, correlated to treatment outcome and exposure

All efficacy endpoints will be summarized by visit, including change from baseline information.

Digital Assessments: Vocal and behavioral digital assessment data are collected in this study. These data will be in a separate report.

Safety: Safety and tolerability will be assessed by clinically significant events. Safety assessments include AEs, SAEs, vital signs, weight and BMI, laboratory values, neurological exams, C-SSRS, SHSF, SHFU, and ECGs.

Bioanalytical: nonpharmacogenetic biomarkers: changes in plasma levels of nociceptin and serum levels of IL-1ß, IL-2, IL-6, IL-10, TNFα, IFNγ, and CRP.

Pharmacokinetics: Pharmacokinetic (PK) data collected in this study will be listed. Population PK analysis of the plasma concentrations in this study will be reported separately and the results of multiple clinical trials may be combined for this analysis.

Statistical Methods:

The sample size determination was based on the primary endpoint, change from baseline in the Investigator-administered MADRS total score, at Visit 7 (week 8). Approximately 100 patients will be randomized to either BTRX-246040 or placebo in a 1:1 allocation ratio. A sample size of 44 patients per treatment group will provide 75% power to detect a relative effect size of 0.5 on the MADRS total score, based on a two-sample t-test and a significance level of 0.10. All tests are two-sided. To account for patients who may discontinue early and be excluded from the analysis population, a sample size of approximately 50 patients per treatment group (total of 100 patients) will be randomized.

All statistical tests will be 2-sided. In this Phase 2 study, statistical tests that are significant at the level of 10% will be considered as demonstrating a trend. Statistical tests that are significant at the 5% level will be considered as demonstrating a strong trend. Where appropriate, analyses of the secondary efficacy parameters and the exploratory endpoints will report nominal p-values to aid the interpretation of results. No adjustments for multiplicity will be made for secondary and exploratory analyses.

The primary efficacy analysis will be on the Investigator-administered MADRS total score and the key comparison will be the contrast between BTRX-246040 and placebo at the last visit of the 8-week double-blind treatment period (Visit 7/Week 8) from a mixed-model repeated measures (MMRM) analysis on the change from baseline in the Investigator-administered MADRS. This primary efficacy analysis will be performed on the Full Analysis Set of patients that includes all randomized patients who received at least one dose of study drug and have at least one post-dose efficacy assessment.

The following secondary efficacy endpoints will be analyzed by the MMRM model:

1. Change from baseline to Week 8 on the Investigator-administered MADRS-6 subscale
2. Change from baseline to Week 8 on the Investigator-administered MADRS individual items
3. Change from baseline to Week 8 on the total scores of the HADS subscales, the anxiety subscale (HADS-A) and the depression subscale (HADS-D)
4. Change from baseline to Week 8 on the DARS
5. Change from baseline to Week 8 on the SHAPS (using the original 0 – 14 scoring method and also using an alternative scoring method ranging from 14 – 56)

The following secondary endpoints will be analyzed by logistic regression with fixed effects for treatment and investigative site with the addition of the baseline Investigator-administered MADRS as a covariate.

Responder rates defined by:

6. Proportion of patients who demonstrate clinical response, where **response** was defined by a reduction of at least 50% in the baseline Investigator-administered MADRS total

score at Week 8.

7. Proportion of patients with MDD who achieved remission, where **remission** was defined by a total score of ≤10 on the Investigator-administered MADRS at Week 8.
8. Proportion of patients who demonstrate significant clinical improvement, where **improvement** is defined by a score of 1 (very much improved) or 2 (much improved) on the CGI-I at Week 8.

The following secondary efficacy endpoints based on the performance tasks will be analyzed and presented in summary and graphical displays.

9. Outcome measures from the FERT
10. Outcome measures from the PRT
11. Outcome measures from the EEfRT

The exploratory endpoints will include:

1. Change in HAM-A total score from baseline to Week 4 and from baseline to Week 8
2. Change from baseline to Week 8 on the average pain in the last 24 hours
3. Changes in plasma levels of nociceptin, and changes in serum levels of IL-1ß, IL-2, IL-6, IL-10, TNFα, IFNγ, and CRP from baseline to Week 8, correlated to treatment outcome and exposure

Exploratory endpoints will be summarized and, where appropriate, represented graphically.

All safety data will be listed and summarized for the Safety Analysis Set of patients who received at least one dose of study drug.

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3 PROTOCOL SIGNATURE PAGE

This protocol has been approved by:

19 NOV 2018

Atul R. Mahableshwarkar, M.D. **Date**
VP Clinical Development & Responsible Medical Officer
BlackThorn Therapeutics, Inc.

Principal Investigator Declaration:

I have read and understood the requirements and conditions of the study protocol. I am aware of my responsibilities as an Investigator under the guidelines of the Internal Conference on Harmonization Good Clinical Practice (ICH GCP) standards, the Declaration of Helsinki, local regulations (as applicable) and the study protocol. I agree to conduct the study according to these guidelines and to appropriately direct and assist the study team assigned to me who will be involved in the study.

I agree to use the study material, including investigational treatment medication, only as specified in the protocol.

I understand that the protocol must be approved by the Ethics Committee and Regulatory Authorities prior to its implementation. I confirm that if I or any of my staff are members of the ethical review board, we will abstain from deliberation and voting on this protocol.

I understand that non-compliance with the study protocol may lead to early termination of the study.

Principal Investigator Signature **Date**

Principal Investigator Name (printed)

4 ABBREVIATIONS

Ab	Antibody
AE	Adverse Event
ALT	Alanine Transaminase
AST	Aspartate Transaminase
ANCOVA	Analysis of the Covariance
ANOVA	Analysis of the Variance
AUC	Area Under the Curve
AUD	Alcohol Use Disorder
BMI	Body Mass Index
BUN	Blood Urea Nitrogen
CGI-I	Clinical Global Impression of Improvement
CGI-S	Clinical Global Impression of Change
CI	Confidence Interval
CIOMS	Council for International Organizations of Medical Sciences
C_{max}	Maximum Plasma Drug Concentration
CNS	Central Nervous System
CRF	Case Report Form
CRP	C-reactive protein
C-SSRS	Columbia Suicidality Severity Rating Scale
DARS	Dimensional Anhedonia Rating Scale
DSM-5	Diagnostic and Statistical Manual of Mental Disorders Fifth Edition
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EEfRT	Effort Expenditure for Reward Task
EPD	Edinburgh Postnatal Depression Scale
ETB	P1vital® Oxford Emotional Test Battery
FAS	Full Analysis Set
FERT	Facial Expression Recognition Task

FOIA	Freedom of Information Act
GCP	Good Clinical Practice
GEE	Generalized Estimating Equation
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
HAV	Hepatitis A Virus
HAV-Ab [IgM]	Hepatitis A Virus Antibody
HBc Ab	Hepatitis B Core Antibody
HBs Ag	Hepatitis B Surface Antigen
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use
IFNγ	interferon gamma
IL-1ß, IL-2, IL-6, IL-10	interleukin 1 beta, 2, 6 and 10
IgM	Immunoglobulin M
IND	US Investigational New Drug Application
INR	International Normalized Ratio
IRB	Investigational Review Board
IWRS	Interactive Web-response System
LC-MS/MS	Liquid Chromatography-tandem Mass Spectrometry
LICQ	Lifetime Illness Characteristics Questionnaire
LS Means	Least-square means
MADRS	Montgomery-Asberg Depression Rating Scale®
MDD	Major Depressive Disorder
MMRM	Mixed-model Repeated Measures

Noc/OFQ	Nociceptin/Orphanin FQ
MOS	Margin of Safety
NOAEL	No-Observed- Adverse-Effect-Level
NONREM	Nonlinear Mixed Effect Modeling
NOPR	Nociceptin Receptor
PET	Positron Emission Tomography
PK	Pharmacokinetic
POC	Proof-of-concept
PRF	Pre-Randomization Form
PRT	Probabilistic Reward Task
QD	Once Daily
QTcF	Fridericia's correction method for QT Interval
RO	Receptor Occupancy
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SCID-CT	Structured Clinical Interview for DSM Disorders-Clinical Trials
SHAPS	Snaith-Hamilton Pleasure Scale
SHFU	Self-Harm Follow-up Form
SHSF	Self-Harm Supplement Form
SNRI	Serotonin Norepinephrine Reuptake Inhibitor
SSRI	Selective Serotonin Reuptake Inhibitor
TEAE	Treatment-emergent Adverse Event
TNFα	Tumor necrosis factor alpha
TPO	Third-party Organization
TSH	Thyroid Stimulating Hormone
ULN	Upper Limit of Normal
US	United States
WHODD	World Health Organization Drug Dictionary

5 INTRODUCTION

Major depressive disorder (MDD) is the most common mood disorder and imposes considerable economic and humanitarian suffering, such as decreased quality of life, functional impairment, and increased mortality rate. By 2020, depressive disorders are expected to be the second highest cause of morbidity in the world (Murray et al., 2006) and has been predicted to become the leading cause of disease burden by the year 2030 (WHO, 2008). The lifetime prevalence of MDD is approximately 16.6% in the United States (US) (Kessler et al., 2003). Less than one quarter of patients with MDD are correctly identified and appropriately treated (Olfson et al., 2005). Patients who receive appropriate medications with available drugs are often under-treated, which is attributed to poor compliance and tolerability, and lack of efficacy. Episodes of MDD are often chronic and recurrent, with a relapse chance rate of 55% to 90% for individuals who experienced one or two prior depressions. More than 80% of the individuals who experience a second episode and who are not treated will experience a third episode within 3 years (Thase and Sullivan, 1995).

A clinical diagnosis of MDD is made based on the continuous presence of at least 5 of 9 symptoms over at least two weeks. One of these symptoms must be either depressed mood or anhedonia which is defined as diminished interest or pleasure in response to rewarding stimuli (American Psychiatric Association, 2013: Diagnostic and Statistical Manual of Mental Disorders, 5th Edition). This diagnostic classification assigns equal importance to depressed mood and anhedonia thus highlighting anhedonia as a core feature of the disorder. The classification system also acknowledges that anhedonia may be present in a subset of individuals with MDD. Estimates of significant anhedonia among patients with MDD are rare, one report describes an anhedonia rate of approximately 37% among a sample of 65 individuals diagnosed with MDD (Pelizza and Ferrari, 2009). Moreover, published data indicates that the presence of anhedonia may represent a distinct physiologically based subset of patients with MDD. Evidence from functional neuroimaging assessments indicates that anhedonia is linked to disruptions in central reward system functioning and is an important aspect of depression pathophysiology (Keedwell et al., 2005).

The current mainstays of pharmacological treatments for depression include the use of selective serotonin reuptake inhibitors (SSRIs) (Fava et al., 2007; Zupancic et al., 2006) and serotonin-norepinephrine reuptake inhibitors (SNRIs) (Stahl et al., 2005), which provide reasonable treatment options; however, these therapies are not effective in all patients, and are often associated with undesirable side effects, such as weight gain and sexual dysfunction. The lack of efficacy and the adverse events (AEs) associated with the use of these antidepressants lead to high levels of treatment discontinuation (Zajecka, 2000). Even with multiple consecutive treatments, only a small proportion of patients remain asymptomatic (Rush, 2007). Thus, there continues to be a substantial unmet medical need for new antidepressants with greater response rates and improved tolerability.

The presence of anhedonia is associated with difficulty in treating MDD. Research findings indicate that available therapies such as SSRIs do not target depression-related motivational and reward-processing deficits sufficiently (APA, 2000; Dunlop and Nemeroff, 2007; McCabe et al., 2009; Nutt et al., 2007; Price et al., 2009; Shelton and Tomarken, 2001) and that anhedonic symptoms predict inadequate treatment response (Spijker et al., 2001).

Nociceptin/Orphanin FQ (Noc/OFQ) is a neuropeptide that acts as a natural ligand for a G protein-coupled receptor (Meunier et al., 1995; Reinscheid et al., 1995) encoded by the gene OPRL1. OPRL1 is expressed in brain regions associated with mood disorders and obesity (Florin et al., 2000; Berthele et al., 2003) and regulates neuronal activity in regions underlying hedonic processing and motivation (Norton et al., 2002). In non-clinical animal models, nociceptin modulates several physiological functions and behaviors including depression, stress and anxiety, feeding, locomotor activity, body temperature, substance abuse, memory, and pain (Lambert, 2008; Zaveri, 2016).

BTRX-246040 is a potent and selective antagonist of human nociceptin receptors (NOPR). Preclinical evidence indicates that it has anxiolytic, ethanol-consumption reducing/relapse preventing, antidepressant-like, body weight-reducing and analgesic effects (Witkin et al., 2014; Rorick-Kehn et al., 2016; Statnick et al., 2016). Open-label studies have documented increased plasma levels of nociceptin in different subpopulations of depressive disorder patients, including bipolar depressed and postpartum patients, as compared to non-depressed controls, which were found to be correlated with severity of the depressed symptoms as assessed by Hamilton Depression Rating Scale (HAM-D) and Edinburgh Postnatal Depression (EPD) Scale score (Gu et al. 2003; Wang et al. 2009).

Completed Phase 1 studies showed that BTRX-246040 was well-tolerated in healthy males after single administrations of doses ranging from 2 to 800 mg and after multiple daily doses of 40 to 200 mg (n = 78). There were no deaths, serious adverse events (SAEs), or severe AEs, and the overall incidence of treatment-emergent adverse events (TEAEs) in the entire Phase 1 program was relatively low. There were no findings noted in the clinical laboratory, electrocardiogram (ECG), and physical examination, including neurological examination. No suicidal ideation or behavior was detected following BTRX-246040 administration, as assessed by the Columbia Suicide Severity Rating Scale® (C-SSRS). All vital signs were within normal ranges, with no notable dose- or treatment-related changes noted following single and multiple doses of BTRX-246040 at any dose tested. BTRX-246040 showed a predictable pharmacokinetic (PK) profile with low- moderate variability. The terminal half-life of BTRX-246040 was estimated to be 66 to 96 hours.

Completed Phase 2 studies in male and female patients with MDD (Study I5J-MC-NOAC [NOAC]) and male and female patients with Alcohol Use Disorder (AD) (Study I5J-MC-NOAD [NOAD]) showed BTRX-246040 was well tolerated at doses of 40 mg QD over an 8-week period. The dose of 40 mg QD was chosen because results from a single-dose study assessing brain Noc/OFQ receptor occupancy (Study I5J-MC-NOAB [NOAB]) predicted that this dose

would result in receptor occupancy (RO) above 80% for 24-hours in at least 75% of patients at steady state. There were no deaths or SAEs with BTRX-246040 administration in either study. In study NOAC, the most common TEAEs that occurred more frequently with BTRX-246040 than placebo were nausea (7/69 [10.1%] vs. 1/65 [1.5%] patients), insomnia (7/69 [10.1%] vs. 0/65 patients) and dizziness (5/69 [7.2%] vs. 0/65 patients). In study NOAD, the frequency of TEAEs reported was similar between the BTRX-246040 and placebo treatment groups. In both studies, most TEAEs were mild or moderate in severity. There were no notable treatment-related clinical laboratory findings or abnormal ECGs. Vital signs were generally within normal range, with no notable treatment-related changes reported following 40 mg QD BTRX-246040. In Study NOAC, no increase in suicidal ideation or behavior relative to placebo was detected following BTRX-246040 administration, as assessed by the C-SSRS. In Study NOAD, there was no suicidal ideation or behavior following BTRX-246040 administration, as assessed by the C-SSRS. In general, the observed BTRX-246040 concentration time data in patients with MDD were in a similar range to the concentrations observed in the healthy subject study (Study I5J-MC-NOAA [NOAA]) when the data were compared after normalizing for administered dose.

Based on data from rat and rabbit reproduction toxicity studies, BTRX-246040 does not pose a significant safety hazard to a developing embryo or fetus. In rats, BTRX-246040 did not cause major malformations or effects on embryo-fetal survival. Slightly reduced fetal growth and an increase in minor fetal anomalies occurred at the maternally toxic dose of 200 mg/kg/day which corresponded to an AUC of 204485 ng.h/mL on Gestation Day 17. The Margin of Safety (MOS) for the AUC at the no-observed- adverse-effect-level (NOAEL) of 75 mg/kg/day relative to the AUC at the 40 mg in study NOAA is 69-fold and 57-fold above the expected AUC at the 80 mg dose. In rabbits, BTRX-246040 did not cause major or minor fetal anomalies or adversely affect embryo-fetal survival. A slight reduction in fetal weights occurred concurrently with significant maternal toxicity (body weight loss and reduced food consumption) at 100 mg/kg/day (AUC – 11381 ng.h/mL). The MOS for the AUC at the NOAEL of 30 mg/kg/day relative to the AUC at the 40 mg dose in study NOAA is 2.5-fold and 0.9-fold at the expected AUC at the 80 mg dose. In general toxicity studies, dose-limiting toxicity was mainly related to dose- and exposure-dependent increases in severity of central nervous system (CNS) effects in rats and dogs including ataxia, tremors (dogs only), and convulsions (dogs only). The lowest maximum drug plasma concentration (Cmax) that produced convulsions in dogs (the most sensitive preclinical species) was 2400 ng/mL (approximately 19-fold above the mean Cmax anticipated with 40 mg QD and 7-fold above the mean Cmax anticipated with 80 mg QD).

The current study will use a starting dose of 40 mg QD for which observed Cmax estimates after dosing for 14 days to healthy male patients in Study NOAA ranged from 87.9 to 183 ng/mL (mean=127 ng/mL). The exposure multiple to the lowest convulsive dose in dogs is 19-fold and the exposure multiple to $1/10^{th}$ the convulsive threshold in dogs is approximately 2-fold based on the mean Cmax at 40 mg observed in Study NOAA. Since this dose was well-tolerated in two separate Phase 2 studies (n = 113 male and female patients treated with BTRX-246040) in which

a trend towards efficacy was observed, dose escalation to 80 mg QD based on individual patient tolerability will be employed in the current study to further explore the effective dose range. PK data are available for the dose regimens of 40, 120, and 200 mg QD, but not for 80 mg QD. The mean Cmax on Day 14 of dosing with 120 mg QD to healthy male patients in Study I5J-MC-NOAA (NOAA) was 570 ng/mL. Thus, the mean Cmax and AUC_{0-24} with steady-state dosing with 80 mg QD is anticipated to be approximately 350 ng/mL and 3550 ng.h/mL, respectively (intermediate between that of 40 mg QD and 120 mg QD).

More information about the known and expected benefits, risks and reasonably anticipated AEs may be found in the Investigator's Brochure (IB).

6 OBJECTIVES

6.1 PRIMARY OBJECTIVE

To evaluate the effects of BTRX-246040 on symptoms of depression in adult patients with Major Depressive Disorder after 8 weeks of double-blind treatment.

6.2 SECONDARY OBJECTIVES

- To evaluate the effects of BTRX-246040 on anhedonia in adult patients with MDD
- To evaluate the safety and tolerability of BTRX-246040 in adult patients with MDD
- To evaluate the effects of BTRX-246040 on the performance of tasks as measured by the Facial Expression Recognition Task (FERT), Probabilistic Reward Task (PRT), and Effort Expenditure for Reward Task (EEfRT) in adult patients with MDD

6.3 EXPLORATORY OBJECTIVES

- To evaluate the effects of BTRX-246040 on anxiety-related symptoms in adult patients with MDD
- To evaluate the effects of BTRX-246040 on pain-related symptoms in adult patients with MDD
- To evaluate the effects of BTRX-246040 as measured by biomarkers in adult patients with MDD

7 INVESTIGATIONAL PLAN

7.1 SUMMARY OF STUDY DESIGN

Study NEP-MDD-201 is an 8-week, randomized, double-blind, placebo-controlled, parallel-group, multicenter study. There will be a 28-day screening period, 8-week active treatment period (during which patients will receive either BTRX-246040 or placebo), and an approximate 1- to 2-week off-drug safety follow-up period (Figure 1).

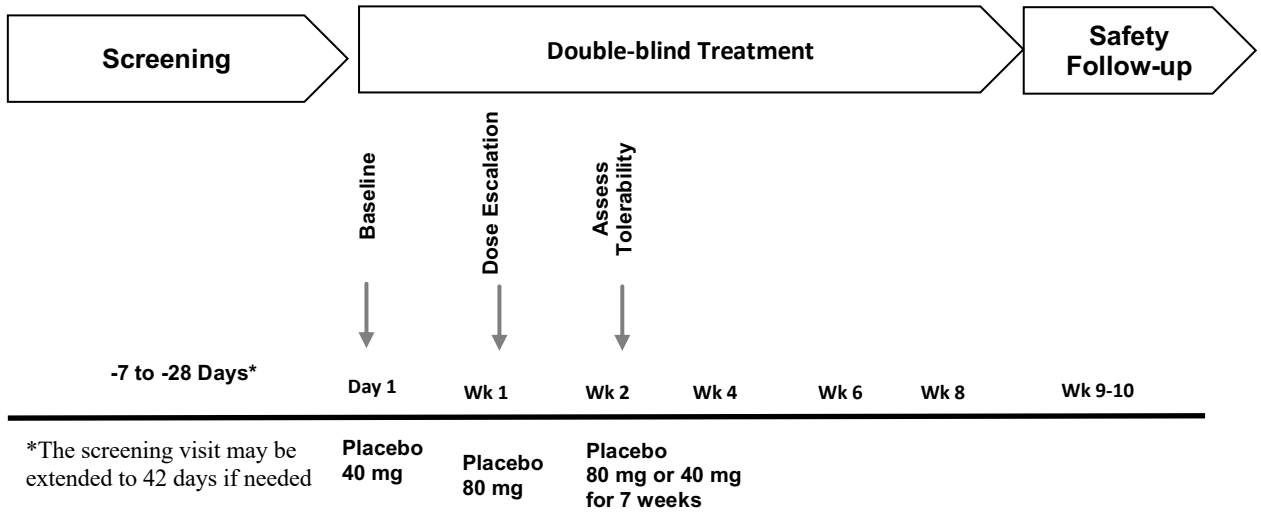

Figure 1 Study Design

Patients will be consented and screened for the study until approximately 100 patients are randomized. Randomization will be performed at Visit 2 (baseline) in a 1:1 ratio to one of two treatment arms (placebo or BTRX-246040 QD) after meeting disease diagnostic criteria for MDD and study inclusion (see section 8). Randomization will be stratified by anhedonia symptom severity, as indicated by a SHAPS total score ≤ 4 and > 4. The BTRX-246040 starting dose for all eligible patients will be 40 mg QD. A dose escalation to 80 mg QD will occur at Visit 3 (week 1) visit for all patients. Patients who tolerate the dose increase will remain at a dose of 80 mg QD for the subsequent 7 weeks. The dose may be decreased back to 40 mg QD anytime through Visit 4 (week 2), if required, for tolerability. Patients who cannot tolerate 40 mg daily will be discontinued from participation in the study. Subsequent visits include Visit 5 (week 4), Visit 6 (week 6), and the end of the treatment period, Visit 7 (week 8). All patients will return to the study site for a safety follow-up visit approximately 1 to 2 weeks after discontinuation of study drug. Overall duration in the study for each patient is expected to be approximately 14 weeks.

7.2 Study Design and Control

The initial screening period is designed to ensure patients 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 randomization, investigational product blinding, and placebo controls will serve to minimize the chance of bias in the implementation and execution of the study. Randomization to treatment assignment reduces the likelihood that results will reflect selection bias. Having both Investigators and patients blinded to treatment assignment (double-blind) controls for expectation bias relative to any particular treatment condition. A placebo arm is included to control the patient and Investigator expectations of improvement during the study that could confound interpretation of pharmacological effects of treatment.

Double-blind, placebo-controlled studies are the standard in assessment of efficacy in randomized clinical trials evaluating patients with conditions such as MDD. These patients can be significantly responsive to placebo and it is important to use a placebo comparator to discern this effect despite the risk of a possible worsening of symptoms. There have been estimates of placebo response rates in MDD clinical trials between 30% and 50%. Certain factors are associated with improvement in placebo-treated patients. These include therapeutic effects secondary to interaction with a medical professional, misdiagnosis of the psychiatric disorder, clinicians overestimating baseline symptom severity or symptom change, and regression to the mean in patients who began the study with more severe symptoms of illness (Fava et al. 2003). In this study, we take several measures to mitigate these influences.

Patients will complete a computer-administered diagnostic validation assessment, the Lifetime Illness Characteristics Questionnaire (LICQ), at Visit 1 (screening) using a secure computer device provided to the site. The patient's diagnostic information, based on the responses to the computerized interview, will be reviewed by the Sponsor-designated independent clinical reviewer. Any uncertainty raised by the patient's responses on the diagnostic interview will be discussed with the Investigator.

A tandem assessment process will be used to administer and rate the MADRS. Two MADRS administrations will be conducted at each study visit using a secure computer device provided to the site. One will be administered by a qualified site Investigator. The second will be computer-administered and will consist of a series of probe and follow-up questions with multiple-choice response options to be completed by the patient.

The Investigator-administered MADRS scores will be compared with the computer-administered MADRS scores. This will ensure that the MADRS is administered in a reliable, consistent manner throughout the study and avoids potential introduction of bias.

8 STUDY POPULATION

Patients who meet all Inclusion Criteria and are subsequently not excluded by the Exclusion Criteria will be randomized to double-blind treatment at Visit 2.

8.1 INCLUSION CRITERIA

Patients are eligible to be included in the study only if they meet **all** the following criteria:

1. Are adult men or women 18 to 65 years of age (inclusive) at informed consent, who provide informed consent by signing the appropriate informed consent forms (ICFs).
2. Have a diagnosis at screening of Major Depressive Disorder as assessed by the Investigator using the Structured Clinical Interview for DSM Disorders-Clinical Trials (SCID-CT).
    a. Must have had at least 1 other major depressive episode previously diagnosed and/or treated in the past 10 years Note: Patients who did not receive treatment will require adequate explanation for discussion with the Medical Monitor.
    b. Must present with a new episode of MDD and the duration of the current episode must be at least 4 weeks but not longer than 18 months
3. Total scores of ≥ 26 on both the Investigator-administered and computer-administered Montgomery-Asberg Depression Rating Scale® (MADRS) at Visit 1 (screening) and Visit 2 (baseline).
4. A difference of ≤ 7 points between rater and computer MADRS total scores at Visit 1 (screening) and Visit 2 (baseline).
5. Clinical Global Impression - Severity score of ≥ 4 at Visit 1 (screening) and Visit 2 (baseline).
6. Agree to the following birth control:
    a. Male patients agree to use a reliable method of birth control during the study and for at least 90 days following the last dose of BTRX-246040 or placebo.
    b. Female patients of child-bearing potential (women not surgically sterilized and between menarche and 2 years postmenopausal) who test negative for pregnancy at the time of enrollment based on a serum pregnancy test and agree to use a reliable method of birth control (e.g., oral contraceptives or Norplant®; a reliable double barrier method of birth control [diaphragms with contraceptive jelly; cervical caps with contraceptive jelly; condoms with contraceptive foam]; intrauterine devices; partner with vasectomy; or abstinence) during the study and for 8 weeks following the last dose of the investigational product BTRX-246040 or placebo.
7. Willing and able to give written informed consent to participate.
8. Patient must have a personal smartphone (iOS or Android operating system).
9. Able to understand and comply with instructions in English.
10. Are judged to be reliable and agree to keep all appointments for clinic visits, tests, and

procedures, including venipuncture, and examinations required by the protocol.

11. Body mass index (BMI) between 18-40 kg/m^2.

8.2 Exclusion Criteria

Patients will be excluded from the study if they meet **any** of the following criteria:

1. Are homicidal in the opinion of the Investigator or are at suicidal risk by any of the following criteria:
    a. any suicide attempts within 12 months prior to Visit 1 (screening) or any suicidal intent, including a plan, within 3 months prior to Visit 1 (screening)
    b. C-SSRS answer of "YES" on item 4 or 5 (suicidal ideation) within 3 months prior to Visit 1 (Screening)
    c. Investigator- or computer-administered MADRS score of ≥ 5 on item 10 (suicidal thoughts)
    d. by Investigator clinical evaluation
2. Have a current or previous diagnosis of OCD, PTSD, eating disorder, bipolar I or II disorder, psychotic depression, schizophrenia or other psychotic disorder.
3. Have any other current Axis I DSM-5 disorder other than MDD, which is the focus of treatment.
4. Have any other clinically significant medical condition or circumstance prior to randomization that, in the opinion of the Investigator, could affect patient safety, preclude evaluation of response, interfere with the ability to comply with study procedures, or prohibit completion of the study, such as uncontrolled diabetes mellitus, renal or hepatic impairment, coronary artery disease, evidence of significant active cardiac, respiratory, or hematologic disease, cancer with < 5 year remission (basal cell carcinoma is not excluded), chronic pain, fibromyalgia or gastric bypass.
5. Have had prior seizures (other than remote history of childhood febrile seizure) or other condition that would place the patient at increased risk of seizures or is taking anticonvulsants for seizure control.
6. Have a history of serious head injury (e.g., skull fracture, cerebral contusion, or trauma resulting in prolonged unconsciousness), intracranial neoplasm or hemorrhage.
7. Have had electroconvulsive treatment, transcranial magnetic stimulation or vagal stimulation in the 6 months prior to Visit 2 (baseline).
8. Have initiated psychotherapy or have had a change in psychotherapy (such as Cognitive Behavioral Therapy) or other non-pharmacological therapies (such as acupuncture or hypnosis) within 6 weeks prior to Visit 2 (baseline) or at any time during the acute phase of the study.
9. Have a visual or physical motor impairment that could interfere with the study tasks.
10. Have alanine aminotransferase (ALT) or aspartate aminotransferase (AST) levels ≥ 2x upper limit of normal (ULN) or glomerular filtration rate ≤ 60 mL/min at Visit 1 (screening).

11. Positive HCV antibody (Ab), hepatitis B surface antigen (HBs Ag), HAV IgM antibody (HAV-Ab [IgM]) or HIV test at Visit 1 (screening).
12. Have nephritic syndrome, end-stage renal disease (and using renal replacement therapy such as hemodialysis or peritoneal dialysis), or a serum creatinine ≥ 2 mg/dL (≥ 172 µmol/L) at Visit 1 (screening).
13. Have a thyroid-stimulating hormone (TSH), free T4, or total T3 level outside of the established reference range that is deemed clinically significant by the Investigator at Visit 1 (screening). Patients previously diagnosed with hyperthyroidism or hypothyroidism who have been treated with a stable dose of thyroid supplement for at least 3 months prior to Visit 1 (screening) and are clinically and chemically (normal free T4) euthyroid will be allowed to participate in the study.
14. Have any other clinically significant abnormalities (significant would include laboratory deviations requiring acute medical intervention or further medical evaluation) in laboratory results at screening, including clinical chemistries, hematology, and urinalysis, and any clinical information that, in the judgment of the Investigator, should preclude a patient's participation at study entry.
15. Have ECG abnormalities obtained at Visit 1 (screening) or Visit 2 (baseline) that, in the judgment of the Investigator, are clinically significant regarding the patient's participation e.g. QTcF > 450 msec in males, > 470 msec in females, Left Bundle Brunch Block, Myocardial Infarction findings with current coronary artery disease.
16. Have a history of substance or alcohol use disorder within 12 months prior to Visit 1 (screening) as defined by DSM-5. Note: Nicotine and caffeine use disorder are not excluded.
17. Have a positive urine drug screen at Visit 1 (screening) or Visit 2 (baseline) that has not been deemed acceptable by the Medical Monitor (see section 11.4.2).
18. Use of the following concomitant medications (contact the Sponsor-designated medical monitor to determine eligibility when in doubt):
    a. psychoactive medication including stimulants, benzodiazepines and anxiolytics, oral antipsychotics, mood stabilizers (carbamazepine, lamotrigine, etc.), lithium, antidepressants and hypnotics/sedatives within 5 half-lives of Visit 2 (baseline).
    b. proton pump inhibitors within 5 half-lives of Visit 2 (baseline)
    c. depot antipsychotics, fluoxetine and irreversible monoamine oxidase inhibitors within 4 weeks of Visit 2 (baseline)
19. Are currently taking or have taken within 5 half-lives of Visit 2 (baseline) any medications or supplements that are strong inhibitors or inducers of CYP3A4 (see Appendix 1).
20. Have a known hypersensitivity to gelatin capsules.
21. Are women who are either pregnant or breastfeeding.
22. Are Investigator site personnel directly affiliated with this study, and/or their immediate families. Immediate family is defined as a spouse, parent, child, or sibling, whether

biological or legally adopted.

23. Are employees of the Sponsor or are employees of any third-party organizations (TPOs) involved in study who require exclusion of their employees.
24. Have participated in a clinical trial or any other type of medical research judged by the Investigator to be scientifically or medically incompatible with this study within 30 days prior to Visit 1 (screening). Contact the Sponsor-designated medical monitor to determine eligibility when in doubt.
25. Have previously completed or withdrawn from this study or any other study investigating BTRX-246040 (previously called LY2940094).
26. Not willing to install and maintain smartphone applications for the digital assessments throughout participation in the study

8.2.1 Rationale for Exclusion of Certain Patients

The exclusion criteria in section 8.2 are intended to ensure that patients with conditions that could be exacerbated by participation in the study are excluded, and that patients with conditions that could confound the interpretation of efficacy or safety results are excluded. No patient is to be excluded on the basis of race, ethnicity, or gender.

8.3 Discontinuations

8.3.1 Early Discontinuation

Patients who discontinue from the study early will have end of treatment assessments performed as shown in the Schedule of Assessments, Table 1. Every effort will be made to encourage them to complete all Visit 7 (week 8) assessments and follow-up visit assessments.

If early discontinuation occurs, the primary reason will be determined and reported on the study completion CRF. Reasons for early discontinuation listed in the CRF are: Investigator/Physician decision, patient decision (withdrawal of consent), Sponsor decision, AE(s), unsatisfactory therapeutic effect, and loss to follow-up.

1. Investigator/Physician Decision

    a. The Investigator/physician decides that the patient should be withdrawn from the study for any reason. For example, the patient is significantly noncompliant with the study drug regimen (see section 10.8; Patients with investigational product compliance ≤ 70% over any 2-week visit interval)
    b. After Visit 2 (baseline) the patient is found to have met an exclusionary criterion or not met an entry criterion required for study participation.
    c. The patient is enrolled in any other clinical trial involving an investigational product or is enrolled in any other type of medical research judged not to be scientifically or medically compatible with this study.

2. Patient Decision
    a. The patient requests to be withdrawn from the study for any reason. If the reason to request withdrawal is an adverse event this should be documented as the primary reason for discontinuation.
3. Sponsor Decision
    a. An Investigator, site personnel performing assessments or patient is unblinded (see section 10.6).
4. The Sponsor stops the study or stops the patient's participation in the study for medical, safety, regulatory or other reasons consistent with applicable laws, regulations and good clinical practice (GCP).
5. Adverse Event
    a. The patient experiences an AE that would necessitate discontinuation of investigational product. If this decision is made because of an SAE or a clinically significant laboratory value, the investigational product is to be discontinued and appropriate measures are to be taken (see item 5. d. below). The Sponsor or its Medical Monitor designee is to be alerted immediately (see section 11.3).
    b. Discontinuation should be considered by the Investigator after consultation with the Sponsor or its Medical Monitor designee when a patient has the following abnormal liver tests:
        i. ALT or AST > 8 x ULN
        ii. ALT or AST > 5 x ULN for more than 2 consecutive weeks
        iii. ALT or AST > 3 x ULN and total bilirubin level > 2 x ULN
        iv. ALT or AST > 3 x ULN and prothrombin time > 1.5 x ULN
        v. ALT or AST > 3 x ULN with the appearance of fatigue, nausea, vomiting, right upper-quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%).
    c. If the patient is thought to be at risk of harm to him/herself or others at any time during the study, a further psychiatric assessment by the Investigator must be conducted. Patients must be discontinued from study participation if they:
        i. are assessed as homicidal
        ii. are actively suicidal (any suicidal ideation with intent or specific plan or any suicide attempt)
        iii. are at serious suicidal risk as assessed by the C-SSRS (score of "YES" on item 4 or 5)
        iv. score ≥ 5 on item 10 of either the computer- or Investigator-administered MADRS item 10.

    The Investigator must assure that these patients receive the appropriate psychiatric care and follow-up.

d. Any patient who is discontinued from investigational product due to AEs or clinically significant laboratory abnormalities will be followed until resolution or clinical stabilization.

e. The Investigator remains responsible for following, through an appropriate healthcare option, AEs that caused the patient to discontinue study drug.

6. Unsatisfactory Therapeutic Effect

   a. If the patient requires treatment with another therapeutic agent for MDD, discontinuation from the study will occur prior to introduction of the new agent.

7. Loss to Follow-Up

   a. For patients who are lost to follow-up, all steps performed to contact them (dates of telephone calls, registered letters, etc.) will be reported in the source documents. The Investigators are required to make at least two attempts to contact the patient for a follow-up safety visit.

8. Pregnancy

   a. Study drug treatment will be discontinued for female patients who become pregnant during study. See section 11.3.3 for additional information.

8.3.2 Discontinuation of the Study

The study will be discontinued if the Sponsor judges it necessary for medical, safety, regulatory, or other reasons consistent with applicable laws, regulations, and GCP.

9 STUDY PROCEDURES

A visit window of +/- 1 day is acceptable for all visits with the exception of Visit 2 (randomization) and Visit 8 (safety follow-up). Every effort should be made to have the same qualified rater perform scales at each visit for a given patient. As much as possible, schedule the patient to complete the PRT/EEfRT tasks at approximately the same time at all visits where they are conducted. Only outcome measures or assessment tools provided by the Sponsor for this study are allowed to be used. Study drug is administered in clinic at Visits 2, 3, 4, 5, and 6 and patients should be reminded to withhold home-dosing on these days.

9.1 VISIT 1: SCREENING

Before any screening procedure is performed, patient informed consent must be obtained. After obtaining the patient's informed consent, these procedures will be conducted. The following order of procedures is recommended to prioritize eligibility and safety assessments:

1. The Investigator will conduct/administer the following assessments (*or his/her designee where asterisked):
    a. Enter patient information in IWRS*
    b. Height*
    c. Weight*
    d. Allow patient to complete the LICQ*
    e. Computer-administered MADRS followed by the Investigator-administered MADRS
    f. Psychiatric history to complete the SCID-CT including personal history of traumatic events
    g. C-SSRS
    h. CGI-S
    i. Previous and current MDD psychiatric therapy / pharmacologic / non-pharmacologic
    j. Self-Harm Supplement Form (SHSF) and if applicable, Self-Harm Follow-up Form (SHFU)
    k. Medical history review
    l. Identify substance or alcohol abuse or dependence
    m. Concomitant therapies review*
    n. Physical examination and neurological examination
    o. Vital signs (BP and pulse both supine and standing, respiratory rate, temperature)*
    p. 12-lead ECG*

q. Blood and urine collection for central laboratory analysis (including urine drug screen and serum pregnancy test for women of child-bearing potential)*
r. Assist patient to download, register and launch digital applications on their smartphone device*
s. Collect and/or monitor digital assessments, as applicable*
t. Complete the Pre-Randomization Form (PRF) if the PI determines that the patient is eligible for randomization. There is no need to submit the PRF if the patient is a known screen failure.*

The screening visit may be completed over two days for scheduling convenience and must be completed between 7 and approximately 28 days prior to Visit 2 (baseline). The screening visit may be extended to 42 days if needed for washout of concomitant medications, to receive lab or ECG reports, or to accommodate scheduling of assessments. Any additional extension requires prior approval from the Sponsor-designated Medical Monitor.

The Investigator will review the results of the central laboratory tests for inclusion/exclusion criteria and submit the PRF to the Sponsor-designated Medical Monitor. The PRF will be reviewed and the Investigator will be notified of the Medical Monitor's eligibility decision prior to Visit 2 (baseline).

9.2 VISIT 2: BASELINE (DAY 1)

The following order of procedures is recommended to prioritize eligibility and safety and assessments:

1. The Investigator will conduct/administer the following assessments (*or his/her designee where asterisked):
    a. Weight*
    b. Investigator-administered MADRS followed by the computer-administered MADRS
    c. Allow patient to complete Hospital Anxiety and Depression Scale (HADS), Snaith-Hamilton Pleasure Scale (SHAPS), Dimensional Anhedonia Rating Scale (DARS), and pain question; reviewed by site personnel qualified to oversee completeness*
    d. Hamilton Anxiety Rating Scale (HAM-A)
    e. C-SSRS
    f. CGI-S and CGI-I
    g. SHSF and if applicable, SHFU
    h. Concomitant therapies review*
    i. Adverse events review
    j. Neurological examination
    k. Vital signs (BP and pulse both supine and standing, respiratory rate, temperature)*

l. 12-lead ECG*
m. Urine drug screen and urine pregnancy test for women of child-bearing potential*

The Investigator will confirm the patient's eligibility prior to completing the remaining randomization visit procedures. The following order of these procedures is recommended:

2. Complete randomization via IWRS
3. The rater will administer the FERT (Note the FERT must not immediately precede or follow the EEfRT or PRT)
4. Blood and urine collection for central laboratory analysis. Note: blood and urine may be collected earlier in the visit as long as it precedes administration of the FERT by > 1 hour.
5. The rater will administer the Probabilistic Reward Task (PRT) and Effort Expenditure for Reward Task (EEfRT) in the order assigned by IWRS
6. Collect and/or monitor digital assessments, as applicable
7. After completing all study procedures, the patient will take their first dose of investigational study drug **(one capsule)** with a light snack at the site from the bottle dispensed at this visit
8. Collect two blood samples for pharmacokinetic analysis (one between 15 min and 2 hours after dosing and another between 4 and 8 hours after dosing)
9. Dispense allocated study drug and instruct patient on the importance of compliance and returning the dispensed bottle with any unused study drug at each visit.
10. Instruct all patients to **take one capsule of their assigned study drug daily and not take study drug the day of their next visit**.

The Investigator will review the results of the central laboratory tests for safety prior to the next visit.

9.3 VISIT 3: WEEK 1 (DAY 8) DOSE ESCALATION

The following order of procedures is recommended to prioritize safety and efficacy assessments:

1. The Investigator will conduct/administer the following assessments (*or his/her designee where asterisked):
    a. Weight*
    b. Investigator-administered MADRS followed by the computer-administered MADRS
    c. Allow patient to complete SHAPS; reviewed by site personnel qualified to oversee completeness*
    d. C-SSRS
    e. CGI-I
    f. SHSF and if applicable, SHFU
    g. Concomitant therapies review*
    h. Adverse events review

   i. Neurological examination
   j. Vital signs (BP and pulse both supine and standing, respiratory rate, temperature)*
2. The rater will administer the FERT
3. Collect and/or monitor digital assessments, as applicable
4. Blood and urine collection for central laboratory analysis (including urine pregnancy test for women of child-bearing potential). Note: blood and urine may be collected earlier in the visit as long as it precedes administration of the FERT by > 1 hour.
5. The patient-returned investigational study drug will be accounted for and patient will be re-instructed if found to be out of compliance
6. Complete IWRS and dispense allocated study drug
7. Collect three blood samples for pharmacokinetic analysis at the following time points: (1) prior to dosing, (2) between 15 min and 2 hours after dosing, and (3) between 4 and 8 hours after dosing
8. After completing the pre-dose pharmacokinetic blood sample, **the patient will take the increased dose of investigational study drug** (i.e., two capsules) with a light snack at the site from the bottle dispensed at this visit
9. Dispense allocated study drug and instruct patient on the importance of compliance and returning the dispensed bottle with any unused study drug at each visit.
10. Instruct all patients to **take two capsules of their assigned study drug daily, and not take study drug the day of their next clinic visit**.

The Investigator will review the results of the central laboratory tests for safety prior to the next visit.

9.4 VISIT 4: WEEK 2 (DAY 15)

The following order of procedures is recommended to prioritize safety and efficacy assessments:

1. The Investigator will conduct/administer the following assessments (*or by his/her designee where asterisked):
   a. Weight*
   b. Investigator-administered MADRS followed by the computer-administered MADRS
   c. Allow patient to complete SHAPS and pain question; reviewed by site personnel qualified to oversee completeness*
   d. C-SSRS
   e. CGI-I
   f. SHSF and if applicable, SHFU
   g. Concomitant therapies review*
   h. Adverse events review
   i. Neurological examination

   j. Vital signs (BP and pulse both supine and standing, respiratory rate, temperature)*
2. The rater will administer the FERT
3. Collect and/or monitor digital assessments, as applicable
4. The patient-returned investigational study drug will be accounted for and patient will be re-instructed if found to be out of compliance
5. The Investigator will **assess tolerability and decrease the investigational study drug dose** via IWRS if deemed appropriate
6. Complete IWRS
7. Blood and urine collection for central laboratory analysis (including urine pregnancy test for women of child-bearing potential). Note: blood and urine may be collected earlier in the visit as long as it precedes administration of the FERT by > 1 hour.
8. After completing all study procedures, the patient will take their investigational study drug at the tolerated dose (i.e., either two capsules or one capsule) with a light snack at the site from the bottle dispensed at this visit
9. Dispense allocated study drug and instruct patient on the importance of compliance and returning the dispensed bottle with any unused study drug at each visit.
10. Instruct all patients to take their assigned study drug dose daily, **and not take study drug the day of their next clinic visit**.

The Investigator will review the results of the central laboratory tests for safety prior to the next visit.

9.5 VISIT 5, WEEK 4 (DAY 29)

The following order of procedures is recommended to prioritize safety and efficacy assessments:

1. The Investigator will conduct/administer the following assessments (*or by his/her designee where asterisked):
   a. Weight*
   b. Investigator-administered MADRS followed by the computer-administered MADRS
   c. Allow patient to complete HADS, SHAPS, DARS, and pain question; reviewed by site personnel qualified to oversee completeness*
   d. HAM-A
   e. C-SSRS
   f. CGI-I
   g. SHSF and if applicable, SHFU
   h. Concomitant therapies review*
   i. Adverse events review
   j. Neurological examination
   k. Vital signs (BP and pulse both supine and standing, respiratory rate,

temperature)*
   l. 12-lead ECG*
2. Blood and urine collection for central laboratory analysis (including urine pregnancy test for women of child-bearing potential)
3. The patient-returned investigational study drug will be accounted for and patient will be re-instructed if found to be out of compliance
4. Complete IWRS
5. The rater will administer the PRT and EEfRT in the order assigned by IWRS
6. Collect and/or monitor digital assessments, as applicable
7. Collect three blood samples for pharmacokinetic analysis at the following time points: (1) prior to dosing, (2) between 15 min and 2 hours after dosing, and (3) between 4 and 8 hours after dosing
8. After completing all study procedures including the pre-dose pharmacokinetic blood sample, the patient will take their dose of investigational study drug from the bottle dispensed at this visit with a light snack at the site
9. Dispense allocated study drug and instruct patient on the importance of compliance and returning the dispensed bottle(s) with any unused study drug at each visit.
10. Instruct all patients to take their assigned study drug dose daily, **and not take study drug the day of their next clinic visit**.

The Investigator will review the results of the central laboratory tests for safety prior to the next visit.

9.6 VISIT 6, WEEK 6 (DAY 43)

The following order of procedures is recommended to prioritize safety and efficacy assessments:

1. The Investigator will conduct/administer the following assessments (*or his/her designee where asterisked):
   a. Weight*
   b. Investigator-administered MADRS followed by the computer-administered MADRS
   c. C-SSRS
   d. Allow patient to complete pain question; reviewed by site personnel qualified to oversee completeness*
   e. CGI-I
   f. SHSF and if applicable, SHFU
   g. Concomitant therapies review*
   h. Adverse events review
   i. Neurological examination
   j. Vital signs (BP and pulse both supine and standing, respiratory rate,

temperature)*

2. Blood and urine collection for central laboratory analysis (including urine pregnancy test for women of child-bearing potential)
3. Collect and/or monitor digital assessments, as applicable
4. The patient-returned investigational study drug will be accounted for and patient will be re-instructed if found to be out of compliance
5. Complete IWRS and dispense allocated study drug
6. After completing all study procedures, the patient will take their investigational study drug with a light snack at the site from the bottle dispensed at this visit
7. Instruct patient on the importance of compliance and returning the dispensed bottle(s) with any unused study drug at each visit.
8. Instruct all patients to take their assigned study drug dose daily **and not take study drug the day of their next clinic visit**.

The Investigator will review the results of the central laboratory tests for safety prior to the next visit.

9.7 Visit 7, Week 8 (Day 57) End of Treatment

Special effort will be made by the site to encourage patients who discontinue from the study early to complete all Visit 7 (week 8) assessments and safety follow-up visit assessments. If the patient does not attend the discontinuation visit, the C-SSRS should be completed if the site has become aware of a potential suicide-related thought of behavior by other communications. The following order of procedures is recommended to prioritize safety and efficacy assessments:

1. The Investigator will conduct/administer the following assessments (*or his/her designee where asterisked):
    a. Weight*
    b. Investigator-administered MADRS followed by the computer-administered MADRS
    c. Allow patient to complete HADS, SHAPS, DARS, and pain question; reviewed by site personnel qualified to oversee completeness*
    d. HAM-A
    e. C-SSRS
    f. CGI-I
    g. SHSF and if applicable, SHFU
    h. Concomitant therapies review*
    i. Adverse events review
    j. Neurological examination
    k. Vital signs (BP and pulse both supine and standing, respiratory rate, temperature)*
    l. 12-lead ECG *

2. Blood, pharmacokinetic, and urine collection (including urine drug screen and local urine pregnancy test for women of child-bearing potential)*
3. Collect and/or monitor digital assessments, as applicable
4. The patient-returned investigational study drug will be accounted for
5. Complete IWRS
6. The rater will administer the PRT and EEfRT in the order assigned by IWRS.

The Investigator will review the results of the central laboratory tests for safety prior to the next visit.

9.8 VISIT 8, SAFETY FOLLOW-UP

This visit will be conducted approximately 7-14 days after patients complete their study drug. The following order of procedures is recommended to prioritize safety and efficacy assessments:

1. The Investigator will conduct/administer the following assessments (*or his/her designee where asterisked):
    a. Weight*
    b. Investigator-administered MADRS followed by the computer-administered MADRS
    c. C-SSRS
    d. CGI-I
    e. SHSF and if applicable, SHFU
    f. Concomitant therapies review*
    g. Adverse events review
    h. Neurological examination
    i. Vital signs (BP and pulse both supine and standing, respiratory rate, temperature)*
2. Collect and/or monitor digital assessments, as applicable
3. Blood and urine collection (including local urine pregnancy test for women of child-bearing potential)
4. Any remaining patient-returned investigational study drug will be accounted for
5. Complete IWRS
6. After all study assessments are complete, assist patient to remove digital applications from their smartphone device, as applicable.

Table 1 Schedule of Assessments

Schedule of Assessments	Screening Period	Baseline	Study Drug Treatment				End of Treatment [l]	Safety Follow-Up
Visit	**1[a]**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Day	**D (-7 to -28)**	**D1**	**D8**	**D15**	**D29**	**D43**	**D57**	
Week		**W0**	**W1**	**W2**	**W4**	**W6**	**W8**	**W9 - 10**
Clinician-administered Clinical Assessments								
SCID-CT	X							
CGI-S	X	X						
CGI-I		X	X	X	X	X	X	X
HAM-A		X			X		X	
C-SSRS	X	X	X	X	X	X	X	X
Investigator- and computer-administered MADRS	X	X	X	X	X	X	X	X
Inclusion/exclusion criteria	X	X [b]						
Neurological examination	X	X	X	X	X	X	X	X
Medical/psychiatric history	X							
Previous and current MDD psychiatric therapy/pharmacologic and non-pharmacologic	X							
Personal history: traumatic events	X							
Demographics & baseline habits	X							
Physical examination	X							
Study Assessments								
Informed consent (before procedures/tests)	X							
Height	X							
Weight	X	X	X	X	X	X	X	X
Complete IWRS	X	X	X	X	X	X	X	X
Study Medication Activities								
Dispense and administer /study drug [c]		X[c1]	X[c2]	X[c3]	X	X		
Study drug compliance		X	X	X	X	X	X	
Safety Assessments								
Adverse events review		X	X	X	X	X	X	X
Vital signs [d]	X	X	X	X	X	X	X	X
12-lead ECG	X	X			X		X	
Concomitant therapies review	X	X	X	X	X	X	X	X
SHSF, and SHFU as appropriate	X	X	X	X	X	X	X	X
Urine drug and alcohol screen	X	X					X	
Urinalysis	X	X	X	X	X	X	X	X
Pregnancy Test [e]	X	X	X	X	X	X	X	X
Hematology	X	X	X	X	X	X	X	X
Clinical chemistry, including Amylase and Lipase	X	X	X	X	X	X	X	X
Thyroid function tests	X							
Hepatitis screen	X							
Hepatic monitoring [f]	X							

Schedule of Assessments	Screening Period	Baseline	Study Drug Treatment				End of Treatment [l]	Safety Follow-Up
Visit	**1[a]**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Day	**D (-7 to -28)**	**D1**	**D8**	**D15**	**D29**	**D43**	**D57**	
Week		**W0**	**W1**	**W2**	**W4**	**W6**	**W8**	**W9 - 10**
Other Blood Collections								
PK sample		X	X		X		X	
Nonpharmacogenetic biomarker samples		X					X	
Pharmacogenetic sample		X						
ePatient Reported Outcome Assessments								
LICQ	X [g]							
Pain question		X		X	X	X	X	
HADS		X			X		X	
DARS		X			X		X	
SHAPS		X	X	X	X		X	
Emotional Tasks								
FERT [h]		X	X	X				
Behavioral Tasks [i]								
PRT		X			X		X	
EEfRT		X			X		X	
Digital Assessments [j]								
Collect/monitor digital assessments [k]	X	X	X	X	X	X	X	X

a Visit 1 procedures may be completed over 2 days, if necessary and extended for up to 42 days if needed for washout.

b Eligibility must be re-confirmed prior to randomization.

c Patients will take their study drug in the clinic from the bottle dispensed that day with a light snack. If a patient discontinues before completing the study drug treatment, an effort should be made to obtain a blood sample at the time of discontinuation

c1 All patients initiate dosing with 40 mg (one capsule) through week 1

c2 All patients increase dosing to 80 mg (two capsules)

c3 The dose may be decreased back to 40 mg (one capsule) anytime through Visit 4 (week 2), if required, for tolerability

d Collect BP and pulse in supine and standing; collect RR and temperature in any position

e Serum pregnancy test at Visit 1 (screening) performed by a Sponsor-designated laboratory; urine pregnancy tests will be performed at subsequent visits by the site for all women of childbearing potential (women not surgically sterilized and between menarche and 2 years postmenopausal).

f Based on laboratory safety values, unscheduled hepatic monitoring testing may be performed as part of patient follow-up, in consultation with the Sponsor-designated Medical Monitor.

g Patient completes the LICQ before clinician SCID-CT interview.

h The rater administers the FERT prior to blood draw collections or at least 1 hour after blood draw collection. The FERT should not immediately precede or follow the PRT/EEfRT.

i As much as possible, schedule the patient to complete the PRT/EEfRT tasks at approximately the same time at all visits where they are conducted. The patient will complete the tasks in the order assigned by IWRS.

j At the screening visit, assist patient to download, register and launch digital applications on their smartphone device, as applicable.

k Schedule assignments for patients who have downloaded the app to complete "in clinic" and "out of clinic" vocal samples. Out of clinic assignments should be assigned on a weekday approximately 2-3 days after a clinic visit.

l Patients who discontinue early from the study should be encouraged to complete the End of Treatment visit assessments. If the patient does not attend the discontinuation visit, the C-SSRS should be completed if the site has become aware of a potential suicide-related thought of behavior by other communications.

10 TREATMENT

10.1 TREATMENTS ADMINISTERED

This study involves a comparison of BTRX-246040 versus placebo taken orally once daily for 8 weeks. Treatment will be initiated at 40 mg (1 capsule) once daily for approximately one week then will be increased to 80 mg (2 capsules) once daily at Visit 3 (week 1). One dose reduction back to 40 mg (1 capsule) once daily will be allowed through Visit 4 (week 2) for patients who cannot tolerate the 80 mg (2 capsule) dose. Refer to section 10.5 for timing of dose administration. Table 2 shows the treatment regimens.

Table 2 Treatment Regimens

Treatment Group	Regimen*
BTRX-246040	40 mg (1 capsule BTRX-246040) orally QD x 1 week, then 80 mg (2 capsules BTRX-246040) orally QD x 7 weeks
Placebo	1 capsule orally QD x 1 week, then 2 capsules orally QD x 7 weeks

* dose reduction back to 40 mg (1 capsule) once daily will be allowed through Visit 4 (week 2)

The Investigator or his/her designee is responsible for explaining the correct use of the investigational product to the patient and site personnel, verifying that instructions are followed properly, maintaining accurate records of investigational product dispensing and collection, and returning all unused medication to the Sponsor or its designee at the end of the study.

10.2 MATERIALS AND SUPPLIES

BTRX-246040 and placebo capsules and packaging will be identical to maintain Investigator and patient blinding. Patients should be instructed to swallow capsules whole and not crush or chew and store the capsules in their original packaging at the temperature listed on the package label. Clinical trial materials will be labeled according to the regulatory requirements.

10.3 METHOD OF ASSIGNMENT TO TREATMENT

Patients who meet all inclusion criteria and are subsequently not excluded by the exclusion criteria will be randomized to double-blind treatment at Visit 2 (baseline) using an interactive web-response system (IWRS). Patients will be randomized in a 1:1 ratio to one of two treatment arms (placebo or BTRX-246040 QD). Randomization will be stratified by anhedonia symptom severity, as indicated by SHAPS total score ≤ 4 and total score > 4. The order in which the PRT and EEfRT administrations are conducted will be assigned via IWRS.

The IWRS will be used to assign bottles containing double-blind investigational product or placebo to each patient. Site personnel may be required to confirm that they have located the correct bottles by entering a confirmation number found on the package label into the IWRS prior to dispensing the bottle(s) to the patient (see section 10.5).

10.4 Rationale for Selection of Doses in the Study

The starting dose for this study will be 40 mg QD taken for 1 week. A dose of 40 mg BTRX-246040 given QD has been tested and was well-tolerated in two efficacy studies, in male and female patients with MDD (Study NOAC) and male and female patients with AD (Study NOAD). There were no deaths or SAEs with BTRX-246040 administration in either study. In study NOAC, the most common TEAEs that occurred more frequently with BTRX-246040 than placebo were nausea (7/69 [10.1%] vs. 1/65 [1.5%] patients), insomnia (7/69 [10.1%] vs. 0/65 patients) and dizziness (5/69 [7.2%] vs. 0/65 patients). In study NOAD, the frequency of TEAEs reported was similar between the BTRX-246040 and placebo treatment groups. In both studies, most TEAEs were mild or moderate in severity. There were no notable treatment-related clinical laboratory findings or abnormal ECGs. Vital signs were generally within normal ranges, with no notable treatment-related changes reported following 40 mg QD BTRX-246040. In both studies, no increase in suicidal ideation or behavior relative to placebo was detected following BTRX-246040 administration, as assessed by the C-SSRS.

In male healthy volunteer study NOAA, BTRX-246040 was shown to be safe and well-tolerated up to 800 mg given as a single dose and up to 200 mg QD after multiple dosing for 14 days. In general, the observed BTRX-246040 concentration time data in patients with MDD were in a similar range to the concentrations observed in this healthy subject study when the data were compared after normalizing for administered dose. In this study, observed Cmax estimates after dosing 40 mg QD for 14 days ranged from 87.9 to 183 ng/mL (mean=127 ng/mL). The exposure multiple to the lowest convulsive dose in the nonclinical study in dogs is 19X and the exposure multiple to 1/10th the convulsive threshold in dogs is approximately 2X based on the mean Cmax at 40 mg.

The 40 mg dose was well-tolerated in two separate Phase 2 studies (n = 113 male and female patients treated with BTRX-246040) and a trend towards efficacy in MDD was observed although the MDD study did not meet the success criteria of the primary endpoint. For this reason, dose titration from 40 mg to 80 mg QD based on individual patient tolerability will be employed in the current study to further explore the effective dose range. A dose decrease to 40 mg QD will be allowed if the 80 mg QD dose is not tolerated.

10.5 Selection and Timing of Doses

BTRX-246040 has only been dosed with a light breakfast in clinical pharmacology studies to date; there are currently no PK data available for BTRX-246040 when dosed in the fasted state. Therefore, in the current study, patients will take the investigational study drug orally at approximately the same time every morning with a light breakfast.

All patients will be instructed to take their first dose of BTRX-246040 or placebo on the day of Visit 2 (baseline) after completing all baseline study procedures. Patients will take their study drug in the clinic with a light snack.

Patients will be instructed to refrain from taking their investigational study drug at home on the morning of Visits 3, 4, 5, and 6. Study drug will be administered with a light snack at the site from the bottle dispensed at the visit.

10.6 BLINDING

This is a double-blind study. Patients, site personnel, and the Sponsor will be blinded to treatment.

Emergency unblinding for AEs must be performed through the IWRS. This option may be used ONLY if the patient's well-being requires knowledge of the patient's treatment assignment. All requests resulting in an unblinding event are recorded and reported by the IWRS.

The Investigator should make every effort to contact the medical monitor prior to unblinding a patient's treatment assignment. If an Investigator, site personnel performing the assessments, or patient is unblinded, the patient must be discontinued from the study. In cases where there are ethical reasons to have the patient remain in the study, the Investigator must obtain specific approval from the Sponsor-designated Medical Monitor for the patient to continue in the study.

10.7 CONCOMITANT THERAPIES

In general, concomitant medications with primarily CNS activity are not allowed in the study. The Investigator should instruct the patient to notify the study site about any new medications he/she takes and about any significant non-pharmacological therapies administered after the start of the study drug (e.g. acupuncture, hypnosis etc.). All medications, including over the counter medications and supplements, and also significant non-pharmacological therapies, administered after the patient starts treatment with study drug must be listed on the corresponding CRFs.

10.8 TREATMENT COMPLIANCE WITH INVESTIGATIONAL PRODUCT

Each patient will be instructed to return all investigational product packaging and unused material to the study site at each visit. The study site will keep a record of all drug dispensed to and returned by the patients throughout the study.

Patient compliance with BTRX-246040 or placebo will be assessed by direct questioning and counting returned capsules.

For patients who demonstrate noncompliance (patient has taken < 80% or ≥120% of the prescribed dosage for a visit interval), investigative sites must counsel patients on the importance of investigational product compliance. Any missed investigational product or extra dose of investigational product will be recorded as a protocol deviation.

Patients with investigational product (BTRX-246040 or placebo) compliance ≤ 70% over any 2-week visit interval (beginning with the Visit 2 [baseline] to Visit 4 [week 2] interval) will require early discontinuation.

11 Safety, Efficacy Evaluations, Sample Collection and Testing, and Appropriateness of Measurements

Study procedures and their timing are summarized in the Schedule of Assessments (Table 1). All scales and tasks will be completed and/or administered by qualified and trained site raters who meet the training requirements and qualifications of the scale or task standards set by the Sponsor and training vendors.

11.1 Efficacy Measures

11.1.1 Montgomery-Asberg Depression Rating Scale (MADRS)

The MADRS is a semi-structured, Investigator-administered interview to assess the severity of depression among patients with a diagnosis of depression. It is designed to be sensitive to change resulting from antidepressant therapy. (Montgomery, 1979).

The MADRS includes 10 items assessing the following symptoms: apparent sadness, reported sadness, inner tension, reduced sleep, reduced appetite, concentration difficulties, lassitude, inability to feel, pessimistic thoughts, and suicidal thoughts. Each item is scored from 0 (absence of symptom) to 6 (severe symptom); the overall score ranges from 0 to 60. MADRS total scores from 0 to 6 indicate normal/symptom absent, from 7 to 19 indicate mild depression, from 20 to 34 indicate moderate depression, and from 35 to 60 indicate severe depression.

The MADRS-6 subscale focuses on the core symptoms of depression and is the sum of items about the following symptoms: apparent sadness, reported sadness, inner tension, lassitude, inability to feel, and pessimistic thoughts. Overall score ranges from 0 to 36.

The MADRS will be administered by qualified and trained Investigator site raters. Every effort should be made to have the same rater perform this scale at each visit for a given patient.

A tandem assessment process will be used to administer and rate the MADRS. Two MADRS administrations will be conducted using a secure computer device. One will be administered by a qualified site Investigator. The second will be computer-administered and will consist of a series of probe and follow-up questions with multiple-choice response options to be completed by the patient.

The Investigator-administered MADRS scores will be compared the computer-administered MADRS scores. This will ensure that the MADRS is administered in a reliable, consistent manner throughout the study and avoids potential introduction of bias.

At Visit 1 (screening), the computer-administered MADRS will be conducted prior to the Investigator-administered MADRS. At all other visits, the Investigator-administered MADRS will be conducted first.

The Investigator-administered MADRS data will be collected and maintained independently from the computer-administered MADRS data. Investigator-administered MADRS scores will never be altered or adjusted.

11.1.2 Snaith Hamilton Pleasure Scale (SHAPS)

The SHAPS is a 14-item self-report instrument which measures anhedonia. It has been shown to be valid and reliable in normal and clinical samples. The scale will be completed by the patient and reviewed by site personnel qualified to oversee completeness. Each of the 14 items has a set of four responses, two of which endorse agreement (Definitely Agree, Agree) and two of which endorse disagreement (Disagree, Strongly Disagree). Under the original scoring method, responses endorsing agreement receives a score of 0 and responses endorsing disagreement receive a score of 1. The total score ranges from 0 to 14. A higher total score indicates higher levels of state anhedonia (Snaith 1995). In this study, in additional to the traditional scoring method, an alternative scoring method will assign 1 = Strongly Agree, 2 = Agree, 3 = Disagree, 4 = Strongly Disagree. Under this scoring method, total score ranges from 14 to 56, with higher scores indicating higher levels of anhedonia.

11.1.3 Clinical Global Impression Scale – Severity (CGI-S)

The Clinical Global Impression Scale – Severity (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 seven days. The score should reflect the average severity level across the seven days. The CGI-S is scored on a 7-point scale where a score of 1 indicates that the patient is “normal, not at all ill” a score of 4 indicates that the patient is "moderately ill" and a score of 7 indicates that the patient is “among the most extremely ill patients.” (Guy 1976).

This scale will be completed by qualified and trained Investigator site raters. Every effort should be made to have the same rater perform this scale at each visit for a given patient.

11.1.4 Clinical Global Impression Scale – Improvement (CGI-I)

The CGI-I scale (Guy 1976) is a clinician-rated instrument that measures the improvement of the patient’s symptoms. It is a 7-point scale where a score of 1 indicates that the patient is “very much improved,” a score of 4 indicates that the patient has experienced “no change,” and a score of 7 indicates that the patient is “very much worse.”

This scale will be completed by qualified and trained Investigator site raters. Every effort should be made to have the same rater perform this scale at each visit for a given patient.

11.1.5 Pain Question

The patient will rate a single item to assess their average pain in the last 24 hours. The item will be reviewed by site personnel qualified to oversee completeness. The pain question is scored from 0 (no pain) to 10 (pain as severe as you can imagine).

11.1.6 Hamilton Anxiety Rating Scale (HAM-A)

The HAM-A is administered to assess severity of anxiety, its improvement during the course of treatment, and the timing of such improvement (Hamilton 1960). This instrument will be completed by qualified and trained Investigator site raters based on his/her assessment of the patient. 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.

11.1.7 Dimensional Anhedonia Rating Scale (DARS)

The DARS is a dynamic self-report scale that measures desire, motivation, effort and consummatory pleasure across hedonic domains. The patient will complete the scale and it will be reviewed by site personnel qualified to oversee completeness. It is a 17-item self-report measure that consists of 4-component structure mapping onto the domains of anhedonia: hobbies, food/drink, social activities, and sensory experience (Rizvi, et al. 2015).

11.1.8 Probabilistic Reward Task (PRT)

The PRT is a measure of reward learning consisting of trials in which patients are asked to respond to two hardly distinguishable cues, of which one is more frequently rewarded. The task will be administered to the patient and reviewed by site personnel qualified to oversee completeness. Response bias for the rewarded category is the main outcome of interest (Pizzagalli, et al, 2008). Other outcomes include discriminability (subject's ability to differentiate between the two cues), accuracy (hit rates), and reaction time. This task will be completed by the patient using the Sponsor-provided study equipment. The order of administration of the PRT will be assigned by IWRS.

11.1.9 Effort Expenditure for Reward Task (EEfRT)

The EEfRT was developed in an effort to dissociate reward components in anhedonia. The task will be administered to the patient and reviewed by site personnel qualified to oversee completeness. The EEfRT task is a multi-trial game in which participants are given an opportunity on each trial to choose between two different task difficulty levels in order to obtain monetary rewards. Patients are required to complete the task with a specific number of button responses within a constrained period of time in order to obtain a monetary reward. This task isolates patient willingness to exert effort relative to size and likelihood of reward that measures reward "wanting", in contrast to commonly used anhedonia questionnaires, which focus on reward "liking". (Treadway et al, 2009). This task will be completed by the patient using the Sponsor-provided study equipment. The order of administration of the EEfRT will be assigned by IWRS.

11.1.10 The Facial Expression Recognition Task (FERT)

The FERT is a computer test used to assess attention to, and perception of, social cues and affective information. The task will be administered to the patient and reviewed by site personnel qualified to oversee completeness. Faces with 6 different basic emotions (happiness, fear, anger, disgust, sadness, surprise) are displayed on the screen and the patient is required to indicate the expression of the face via a button-press. Different intensity levels of each emotion are presented, which increases the ambiguity of the facial expression and the sensitivity of the task (Harmer et al, 2009). This task will be completed by the patient using the Sponsor-provided study equipment. The FERT will be completed prior to blood draw collections and should not immediately precede or follow the PRT/EEfRT.

11.1.11 Digital Assessments

Vocal and/or behavioral digital assessments will be conducted with HIPAA compliant digital applications that have been download onto the patient's smartphone with an iOS or Android operating system. The applications will be used for active collection of brief vocal samples for subsequent analysis of acoustic features that are associated with affective states and for passive acquisition of metadata to assess behavioral patterns that are associated with affective or cognitive states. Baseline values will be collected during the screening period prior to the first dose of study drug. Post-baseline values will be collected at periodic intervals during the study to be analyzed for potential treatment effects (analysis to be performed in a separate report). Digital samples will be reviewed by site personnel qualified to oversee completeness.

11.1.12 Hospital Anxiety and Depression Scale (HADS)

The HADS measures levels of anxiety and depression without regards to somatic symptoms (Zigmond and Snaith, 1983). The scale consists of 14 items. Seven of the items are used to evaluate anxiety and seven 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. Higher scores indicate higher levels of anxiety and depression, respectively.

11.2 Assessments Only at Visit 1 (Screening)

11.2.1 The Structured Clinical Interview for DSM-5, Clinical Trials Version (SCID-CT)

The SCID-CT will be completed by qualified and trained Investigator site raters. The interview begins with an overview section, which is abridged to entail questions relevant to the current clinical trial. In this version of the SCID, only essential elements for MDD incorporating typical inclusion and exclusion criteria are included. Also, the subtypes and specifiers have been eliminated, and criteria ratings have been streamlined into a "-" and "+" format. Current and lifetime history will be collected.

11.2.2 Lifetime Illness Characteristics Questionnaire (LICQ)

Patients will complete a computer-administered diagnostic validation assessment, ideally prior to the Investigator-administered SCID-CT, on a secure computer device provided to the site. The LICQ will collect data about the patient's history relative to a lifelong history of MDD and the current major depressive episode. The patient's diagnostic information, based on the responses to the computerized interview, will be reviewed by the Sponsor-designated independent clinical reviewer. Any uncertainty raised by the patient's responses on the diagnostic interview will be discussed with the Investigator/site clinician.

11.3 Safety Measures

Investigators are responsible for monitoring the safety of patients who have entered this study and for alerting the Sponsor or its designee to any event that seems unusual, even if this event may be considered an unanticipated benefit to the patient.

The Investigator is responsible for the appropriate medical care of patients during the study.

The Investigator remains responsible for following, through an appropriate health care option, AEs that are serious or that caused the patient to discontinue before completing the study. The patient should be followed until the event is resolved or explained. Frequency of follow-up evaluation is left to the discretion of the Investigator.

11.3.1 Adverse Events

An AE is any sign, symptom, illness, clinically significant abnormal laboratory value or ECG finding, or other untoward medical occurrence associated with the use of an investigational product that appears or worsens in a subject during a clinical study. This definition does not imply a causal relationship between the AE and the investigational product.

Adverse events beginning with the initial dose of investigational product or during the subsequent duration of their enrollment in the study will be considered treatment-emergent AEs (TEAE). TEAEs will be recorded on the AE CRF 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 (e.g., discontinue study drug, begin concomitant medication, begin non-pharmacological treatment, etc.), outcome, and relationship to the investigational product. All TEAEs related to study drug will be followed through resolution or 30 days after the patient terminates from the study, whichever occurs first.

Medical conditions present or AEs occurring prior to the first dose of investigational product will be captured as medical history in the CRF. 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 investigational product will be recorded as a TEAE.

Lack of drug effect is not an AE in clinical studies because the purpose of the clinical study is to establish drug effect.

11.3.2 Overdose

In the event of an overdose of study drug, the Investigator should use clinical judgment in treating the overdose and contact the Sponsor-designated Medical Monitor, or designee. The Investigator should refer to the relevant document(s) for detailed information regarding warnings, precautions, contraindications, AEs, and other significant data pertaining to the study drug. Such documentation may include, but not be limited to the Investigator's Brochure.

11.3.3 Pregnancy

Any pregnancy that occurs from baseline after study drug exposure until study completion must be reported to the Sponsor-designated Medical Monitor on a Pregnancy Notification Form within 24 hours of learning of its occurrence.

Each pregnancy must be reported as well to the Sponsor-designated Medical Monitor within 24 hours of learning of its occurrence. The pregnancy must be followed up to determine outcome (including premature termination) and status of mother and child.

11.3.4 Serious Adverse Events

A serious adverse event (SAE) is any AE that results in one of the following outcomes, regardless of the Investigator's opinion of causation:

1. death
2. initial or prolonged inpatient hospitalization
    a. 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.
3. a life-threatening experience (that is, immediate risk of dying)
4. persistent or significant disability/incapacity
5. congenital anomaly/birth defect
6. other medically important serious event as determined by the Investigator (for example, an AE that jeopardizes the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition).

If a patient experiences an SAE after signing informed consent, but prior to receiving investigational product, the event will be captured as medical history in the CRF and not submitted as reportable-event unless the Investigator feels the event may have been caused by a protocol procedure.

SAEs reported after a patient has taken the last dose of investigational product will be collected in the pharmacovigilance system for 30 days after the last dose of investigational product.

Thereafter, only SAEs that the Investigator feels were related to the investigational product or a protocol procedure must be reported.

All SAEs must be captured on a study-specific SAE form in addition to the AE CRF. Study site personnel must alert the Sponsor-designated Medical Monitor of the SAE and must submit the completed study-specific SAE form to the Sponsor-designated Medical Monitor within 24 hours of the site's awareness of the SAE.

The Investigator and supporting personnel responsible for patient care should discuss with the Sponsor-designated Medical Monitor or designee any need for supplemental investigations of SAEs. The results of these additional assessments conducted must be reported to the Sponsor-designated Medical Monitor. If a patient death occurs during participation in the study and a post-mortem examination is performed and a copy of the autopsy report is available, it should be submitted to the Sponsor-designated Medical Monitor.

The Investigator is responsible for safety reporting in compliance with their Institutional Review Board (IRB).

11.3.5 Suspected Unexpected Serious Adverse Reactions

Suspected unexpected serious adverse reactions (SUSARs) are serious events that are not listed in the IB and that the Investigator identifies as related to investigational product or procedure.

The Sponsor is responsible for IND safety reporting as per 21 CFR 312.32.

11.4 Sample Collection and Testing

Standard clinical laboratory tests will be performed at times specified in the Schedule of Assessments. Chemistry laboratory tests should be conducted after a minimum of fasting 8 hours to avoid the need of repeat testing. Laboratory analyte results that could unblind the study will not be reported to investigative sites or other blinded personnel until the study has been unblinded.

Samples collected for safety laboratory tests will be destroyed within 60 days of receipt of confirmed test results. Tests are run and confirmed promptly whenever scientifically appropriate. When scientific circumstances warrant, however, it is acceptable to retain samples to batch the tests run, or to retain the samples until the end of the study to confirm that the results are valid. Certain samples may be retained for a longer period, if necessary, to comply with applicable laws, regulations, or laboratory certification standards.

Table 1 lists the schedule for sample collections in this study. Table 3 lists the specific laboratory analyses that will be performed for this study.

Based on laboratory safety values, unscheduled hepatic monitoring testing may be performed as part of patient follow-up as described in Table 4.

Detailed instructions on sample requirements and preparation are described in the central laboratory manual.

Investigators must document their review of each safety laboratory report.

Table 3 Clinical Laboratory Tests[a]

Hematology: 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	**Clinical Chemistry:** Serum concentrations of: Sodium Potassium Sodium 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)[b] Magnesium
Urinalysis: Specific gravity pH Protein Glucose Ketones Blood Urine leukocyte esterase	
Urine Drug Screen[c] Amphetamines Barbiturates Benzodiazepine Cannabinoids Cocaine Ethyl alcohol Opiates Phencyclidine Propoxyphene Methadone	**Viral Serology:** Hepatitis B Surface Antigen (HBs Ag) Hepatitis B Core Antibody (HBc Ab) Hepatitis C Antibody (HC Ab) Hepatitis A Antibody (HAV-Ab [IgM]) HIV
Thyroid function: Thyroid stimulating hormone (TSH)	

Total triiodothyronine (T3) Free thyroxine (T4)	
Pregnancy Test (females of child bearing potential only)[d] **Pancreas:** Amylase, total Lipase	**Nonpharmacogenetic biomarkers:** Nociceptin IL-1ß IL-2 IL-6 IL-10 TNFα IFNγ CRP **Pharmacogenetic sample** **Pharmacokinetic (PK) concentration of BTRX-246040**

[a] Assayed by a Sponsor-designated laboratory, unless specified otherwise.
[b] Creatine kinase muscle-brain isoenzyme (CK-MB) is to be assayed if CK results >1000 IU/L.
[c] Performed at screening and baseline and at the Investigator's discretion throughout the study (see below).
[d] Serum pregnancy test at Visit 1 (screening) performed by a Sponsor-designated laboratory; urine pregnancy tests will be performed at subsequent visits by the site.
[e] Performed at entry.

11.4.1 Hepatic Safety

If a study patient experiences elevated ALT or AST > 3X ULN or elevated total bilirubin > 2X ULN, clinical and laboratory monitoring should be initiated by the Investigator. Details for hepatic monitoring depend upon the severity and persistence of observed laboratory test abnormalities. To ensure patient safety and comply with regulatory guidance, the Investigator is to consult with the Sponsor-designated medical monitor regarding collection of specific recommended clinical information and follow-up laboratory tests as guided in Table 4.

These results will be reviewed in a timely fashion by the Sponsor-designated medical monitor, along with the patient's clinical case, to determine possible cause. Patients may be discontinued from the investigational drug or the study based on hepatotoxicity criteria outlined in section 8.3.

Table 4 Hepatic Monitoring Tests

Hepatic Hematology[a] Hemoglobin Hematocrit RBC WBC Neutrophils, segmented Lymphocytes Monocytes Eosinophils Basophils Platelets	**Haptoglobin**[a] **Anti-smooth muscle antibody**[a] **Anti-nuclear antibody**[a] **Hepatic Coagulation**[a] Prothrombin Time Prothrombin Time, INR
Hepatic Chemistry[a] Total bilirubin Direct bilirubin Alkaline phosphatase ALT AST GGT CPK	**Hepatic Serologies**[a,b] Hepatitis A antibody Total HAV-Ab [IgM] HBs Ag Hepatitis B surface antibody (anti-HBs) HBc Ab HC Ab Hepatitis E antibody, IgG Hepatitis E antibody, IgM

[a] Assayed by a Sponsor-designated or local laboratory.
[b] Reflex/confirmation dependent on regulatory requirements and/or testing availability.

11.4.2 URINE DRUG SCREEN

A urine drug screen for drug abuse will include: amphetamines, barbiturates, benzodiazepine, cannabinoids, cocaine, ethyl alcohol, opiates, phencyclidine, propoxyphene, and methadone. No retest is allowed for a positive result with a drug of abuse (e.g., cocaine, cannabinoids, phencyclidine, and methadone).

The site must contact the Sponsor-designated Medical Monitor or designee for pre-approval before a retest of other drugs that presented as positive as long as the patient has a valid prescription and there is no evidence of a Substance Use Disorder. In the case of Alcohol (Ethyl Alcohol) as long as there is clear justification for the finding and no history nor lab pattern consistent with Alcohol Use Disorder. If the results from an allowed repeat urine drug screen are negative, the patient may be included. If the repeat results are positive, the patient must be excluded from the study. A second retest is not admissible.

11.4.3 Samples for Drug Concentration Measurements Pharmacokinetics

Two blood samples will be collected on the day of randomization and three blood samples will be collected at the Week 1 and Week 4 visits, at sporadic time points. Population pharmacokinetic analysis will be conducted at a later time. An additional blood sample will be collected at Visit 7 (end of treatment) to monitor compliance with study medication.

Approximately 2 mL of blood will be drawn at each collection time point at the visits specified in the Schedule of Assessments for determination of plasma concentrations of BTRX-246040. Samples will be analyzed for BTRX-246040 at a laboratory approved by the Sponsor using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. It is intended that the blood samples collected from patients receiving placebo will not be analyzed.

If a patient discontinues before study completion, an effort should be made to obtain a blood sample at the time of discontinuation.

The approximate time of the dose administration should be recorded on the day of the blood sampling and for the 2 days preceding the day of blood sampling. Blood samples should be drawn at or within the time period specified and the actual date and time of sample collection will be recorded on the laboratory requisition.

Bioanalytical samples collected to measure study drug concentrations will be retained for a maximum of 1 year following the last patient visit for the study. During this time, samples remaining after the bioanalyses may be pooled and used for exploratory analyses such as metabolic profiling and/or protein binding work.

Drug concentration information that would unblind the study will not be reported to investigative sites or blinded personnel until the study has been unblinded.

11.4.4 Pharmacogenetic Samples

There is growing evidence that genetic variation may impact a patient's response to therapy. Variable response to therapy may be due to genetic determinants that impact drug absorption, distribution, metabolism, and excretion, the mechanism of action of the drug, the disease etiology and/or the molecular subtype of the disease being treated. Therefore, where local regulations allow, a blood sample will be collected for pharmacogenetic analysis. It is a one-time collection, as noted in the Schedule of Assessments.

Samples will be stored and analysis may be performed on genetic variants thought to play a role in the nociceptin signaling pathway including, but not limited to OPRL1, to evaluate their association with observed clinical outcomes to BTRX-246040.

In the event of an unexpected AE or the observation of unusual response, the samples may be genotyped and analysis may be performed to evaluate a genetic association with response to BTRX-246040. These investigations may be limited to a focused candidate gene study as described above or, if appropriate, genome-wide association studies may be performed to

identify regions of the genome associated with the variability observed in drug response. Samples will only be used for investigations related to the disease and the drug or class of drugs under study in the context of this clinical program. They will not be used for broad exploratory unspecified disease or population genetic analysis.

Samples will be identified by the patient number (coded) and stored for up to a maximum of 15 years after the last patient visit for the study at a facility selected by the Sponsor. The sample and any data generated from it can only be linked back to the patient by Investigator site personnel. The duration allows the Sponsor to respond to regulatory requests related to the study drug.

11.4.5 Nonpharmacogenetic Biomarker Samples

Collection of samples for nonpharmacogenetic biomarker research is a required part of this study. Blood samples, including sampling for nociceptin plasma levels assessments will be collected at the times specified in the Schedule of Assessments. The study will assess changes in plasma levels of nociceptin, and changes in serum levels of IL-1ß, IL-2, IL-6, IL-10, TNFα, IFNγ, and CRP from Visit 2 (baseline) to Visit 7 (week 8) in patients treated with BTRX-246040 versus placebo to better understand the relationships between nociceptin levels, depressive symptoms, and the relationship to clinical features and treatment response.

Stored samples may be used to conduct biomarker research related to better understanding the drug target, the mechanism of BTRX-246040 drug action and the disease process. Stored specimens might also be used to develop or evaluate a potential biomarker method.

Samples will be identified by patient number (coded) and may be stored for a maximum of 15 years after the last patient visit for the study at a facility selected by the Sponsor.

11.5 Other Safety Measures

11.5.1 Vital Sign Measurements

Vital signs (respiratory rate, oral temperature, blood pressure, and pulse) will be measured at each visit throughout the screening and treatment periods of the study after resting for 10 minutes. Blood pressure and pulse will be measured in standing and supine position; respiratory rate and oral temperature will be measured in any position at each visit throughout the study (see Table 1).

11.5.2 Height and Weight

Height and weight will be measured during the study as indicated in the Schedule of Assessments. Height should be measured only once at screening with no shoes for correct measurement. The BMI will be calculated based on height measured at screening and weight measured at each visit.

11.5.3 Physical Examination

Examination of general appearance, skin, neck (including thyroid), eyes, ears, nose, throat, lungs, heart, abdomen, back, lymph nodes, extremities, and vascular status will be conducted at the screening visit.

11.5.4 Neurological Examination

Neurological examination of cranial nerves, motor system, sensory, and reflexes will be conducted at screening and all subsequent study visits.

11.5.5 Collection of Electrocardiograms

For each patient, single 12-lead digital ECGs will be collected as indicated in the Schedule of Assessments after resting for 5 min.

The ECGs will initially be interpreted by a qualified physician (the Investigator or qualified designee) at the site as soon after the time of ECG collection as possible, and ideally while the patient is still present, to determine whether the patient meets entry criteria at the relevant visit(s) and for immediate patient management, should any clinically relevant findings be identified.

After enrollment, if a clinically significant increase in the Fridericia's correction method for the QT (QTcF) interval from baseline (e.g. QTcF ≥500 msec and/or QTcF increased ≥ 60 msec) or other clinically significant quantitative or qualitative change from baseline is identified, the patient will be assessed by the Investigator for symptoms (e.g., palpitations, near syncope, syncope) and to determine whether the patient can continue investigational product. The Investigator or qualified designee is responsible for determining if any change in patient management is needed and must document his/her review of the ECG printed at the time of evaluation for each time point.

All digital ECGs will be electronically transmitted to a designated central ECG laboratory. A cardiologist at the central ECG laboratory will then conduct a full over-read. A report based on data from this over-read will be issued to the investigative site. All data from the over-reads will be placed in the Sponsor database for analytical and study report purposes. Any clinically significant finding that was present on the fully over-read ECG will be reported to the Investigator and to the Sponsor.

When there are differences in ECG interpretation between the Investigator (or qualified designee) and the cardiologist at the central ECG laboratory, the Investigator's (or qualified designee's) interpretation will be used for study entry and immediate patient management. Interpretations from the cardiologist at the central ECG laboratory will be used for data analysis and report writing purposes.

The Investigator (or qualified designee) must document his/her review of ECGs printed at the time of collection, the final over-read ECG report issued by the central ECG laboratory, and any alert reports.

11.5.6 Suicidal Ideation/Suicidality

Suicide-related events (behavior and/or ideation) will be assessed and evaluated at every scheduled and unscheduled visit with the administration of the C-SSRS (Posner 2009). The C-SSRS captures the occurrence, severity, and frequency of suicide-related thoughts and behaviors during the assessment period. The scale includes suggested questions to solicit the type of information needed to determine if a suicide-related thought or behavior occurred. The C-SSRS was developed by the Columbia group (Posner 2009) to prospectively categorize suicide-related events. The 'Baseline' version will be used at Visit 1 (screening) and 'Since Last Visit' will be used for all subsequent visits.

If a suicide-related event is identified at any time during the study, a thorough evaluation should be performed by a study physician and appropriate medical care should be provided. In some patients taking antidepressants, worsening of depression, suicidal events (suicidal thinking and/or behavior), or unusual changes in behavior have been reported, especially, at the beginning of the drug therapy, at the time of dose changes, or early after treatment discontinuation. It is important that patients are instructed to notify their doctor immediately if they have any distressing thoughts or feelings at any time related to harm to either self or others.

The Self-Harm Supplement Form (SHSF) captures the number of discrete events of suicidal behavior, possible suicidal behavior, and non-suicidal self-injurious behavior. This scale must be administered by the Investigator at every visit.

The Self-Harm Follow-up Form (SHFU) captures the details of each episode counted on the SHSF. These details include episode description, start and end dates, associated adverse events, severity, relationship to study drug, method, intentions, cognition, actions taken, plan, stressors, and follow-up hospital visits. The SHFU is completed at any time that the SHSF number of discrete events is ≥1.

11.5.7 Safety Monitoring

The Sponsor-designated Medical Monitor and the study team will monitor blinded safety data throughout the course of the study. This will include review of AEs, SAEs, any AEs of special interest, ECG findings, neurological exams, laboratory results, vital signs, and other safety data.

In the event that safety monitoring uncovers an issue that needs to be addressed by unblinding at the group level, a data monitoring committee will be formed to protect the integrity of data and to evaluate additional analyses of the safety data.

11.5.7.1 Complaint Handling

The Sponsor collects product complaints on investigational products and drug delivery systems used in clinical studies in order to ensure the safety of study participants, to monitor quality, and to facilitate process and product improvements.

Patients will be instructed to contact the Investigator as soon as possible if he or she has a complaint or problem with the investigational product so that the situation can be assessed.

For blinded studies, all product complaints associated with material packaged, labeled, and released by the Sponsor or its delegate will be reported.

The Investigator or his/her designee is responsible for handling the following aspects of the product complaint process in accordance with the instructions provided for this study:

1. recording a complete description of the product complaint reported and any associated AEs using the study-specific complaint forms provided for this purpose
2. submitting the completed product complaint form within 24 hours to the Sponsor or its designee.

11.6 Pharmacokinetic Analysis

Plasma concentrations of BTRX-246040 will be listed by patient, visit, date, time, and when available, time lapse between the last dose and collection of the pharmacokinetic sample. Population PK analysis of the plasma concentrations in this study will be reported separately and the results of multiple clinical trials may be combined for that analysis.

11.7 Appropriateness of Measurements

Efficacy and safety assessments used in the study are generally regarded as reliable, accurate, and relevant in this patient population. The change from Visit 2 (baseline) to Visit 7 (week 8) in the Investigator-administered MADRS total score is the primary efficacy measure. Its psychometric properties have been established in various study samples. This study will include secondary and exploratory outcome measures.

12 DATA QUALITY ASSURANCE

To ensure accurate, complete, and reliable data, the Sponsor or its representatives will do the following:

1. provide instructional material to the study sites, as appropriate
2. provide start-up training to instruct the Investigators and study site personnel. This training will give instruction on the protocol, the completion of the eCRFs, study procedures, and on handling and administering the study drug
3. make periodic visits to the study site
4. be available for consultation and stay in contact with the study site personnel by email and telephone
5. review and evaluate eCRF data and standard computer edits to detect errors in data collection
6. conduct a quality review of the database.

In addition, the Sponsor or its representatives may periodically check a sample of the patient data recorded against source documents at the study site. The study may be audited by the Sponsor, its representatives, and/or applicable regulatory agencies at any time. Investigators will be given notice before an audit occurs.

To ensure the safety of participants in the study, and to ensure accurate, complete, and reliable data, the Investigator will keep records of laboratory tests, clinical notes, and patient medical records in the patient files as original source documents for the study. If requested, the Investigator will provide the Sponsor, applicable regulatory agencies, and applicable IRBs with direct access to original source documents.

12.1 DATA CAPTURE SYSTEM

The Sponsor will select an electronic data capture system (EDC) and provide appropriately trained site personnel user permissions to enter information on the electronic CRFs (Case Report Forms). The site maintains a separate source for the data entered onto CRFs.

Electronic data collected on other devices will be transferred to the Sponsor's selected EDC, as applicable.

An identification code assigned by IWRS to each patient will be used in lieu of the patient's name to protect the patient's identity when reporting study-related data.

13 SAMPLE SIZE AND STATISTICAL METHODS

13.1 DETERMINATION OF SAMPLE SIZE

The sample size determination was based on the primary endpoint, change from baseline in the Investigator-administered MADRS total score, at Visit 7 (week 8). Approximately 100 patients will be randomized to either BTRX-246040 or placebo in a 1:1 allocation ratio. A sample size of 44 patients per treatment group will provide 75% power to detect a relative effect size of 0.5 on the MADRS total score, based on a two-sample t-test and a significance level of 0.10. All tests are two-sided. To account for patients who may discontinue early and be excluded from the analysis population, a sample size of approximately 50 patients per treatment group (total of 100 patients) will be randomized.

13.2 STATISTICAL AND ANALYTICAL PLANS

13.2.1 GENERAL CONSIDERATIONS

Data collected from eCRFs, IWRS, ECG, performance tasks, and clinical laboratory evaluations will be listed by treatment group and patient identification.

Generally, if not specified, continuous data will be summarized by descriptive statistics, including number of patients with data, mean, standard deviation, median, and range. Categorical data will be summarized by the number and percentage of patients for each category or classification.

All statistical tests will be 2-sided. In this Phase 2 study, statistical tests that are significant at the level of 10% will be considered as demonstrating a trend. Statistical tests that are significant at the 5% level will be considered as demonstrating a strong trend. Where appropriate, analyses of the secondary efficacy parameters and the exploratory endpoints will report nominal p-values to aid the interpretation of results. No adjustments for multiplicity will be made for secondary and exploratory analyses. Data analysis will be performed using SAS® version 9.2 or greater.

Any change to the data analysis methods described in the protocol will require an amendment ONLY if it changes a principal feature of the protocol. Any other change to the data analysis methods described in the protocol, and the justification for making the change, will be described in the clinical study report. Additional exploratory analyses of the data will be conducted as deemed appropriate.

All total and subscale scores are derived from individual items. Unless otherwise specified, if any individual item is missing, the corresponding total and subscale scores may be considered missing.

A detailed statistical analysis plan (SAP) describing the statistical methodologies will be developed by the Sponsor or its designee and will be finalized before locking the study database.

13.2.2 Analysis Sets

- All Patients Enrolled Set: includes all patients who provided informed consent for this study.
- Full Analysis Set (FAS): includes all randomized patients who received at least one dose of study drug and have at least one post-dose efficacy assessment.
- Safety Analysis Set: includes all patients who received at least one dose of study drug.
- Pharmacokinetic Analysis Set: includes all randomized patients with any PK data.
- Per-Protocol Analysis Set: The Per-Protocol analysis set will include only those patients who completed the study with no major protocol deviations.
- Completers Analysis Set: All patients in the Full Analysis Set who completed the treatment period.
- MADRS Non-responders at Week 2 Analysis Set: All patients in the Full Analysis Set who have a Week 2 Investigator-administered MADRS total score that is >50% of the baseline MADRS total score. This analysis set excludes patients who exhibited an early response at Week 2 on the Investigator-administered MADRS.

Unless otherwise specified, all efficacy analyses will be conducted on the Full Analysis Set. Analyses will be performed according to the treatment the patient received. Efficacy analyses will be repeated on a Per-Protocol Analysis Set, the Completers Analysis Set, and the MADRS Non-responders at Week 2 Analysis Set for the primary efficacy endpoint and other selected secondary efficacy endpoints of interest. Safety analyses will be conducted on the Safety Analysis Set. The Pharmacokinetic listing will include the Pharmacokinetic Analysis Set.

13.2.3 Patient Disposition

The number of randomized patients will be summarized by investigative site and treatment group. Frequency counts and percentages of all patients who are randomized and complete the treatment period or discontinue early will be presented for each treatment group. Reasons for discontinuation from the treatment period will be summarized. The proportion of subjects who discontinue early will be compared between treatment groups using Fisher's exact test. The number of subjects who complete and reasons for failing to complete the follow-up visit will be presented for each treatment group. These analyses will be conducted on all patients enrolled, the Full Analysis Set, and the Safety Analysis Set.

13.2.4 Baseline Patient Characteristics

Baseline characteristics including age, sex, and ethnic origin will be summarized for all randomized patients by treatment group and for patients overall. Comparisons between treatment groups will be performed using Fisher's exact test for categorical data and an ANOVA with treatment and investigative site in the model for continuous data. These analyses will be conducted on the Full Analysis Set, the Safety Analysis Set, the Per-Protocol Analysis Set, the Completers Analysis Set, and the MADRS Non-responders at Week 2 Analysis Set. Baseline

habits, including alcohol consumption and smoking status, will also be compared between treatment groups. This analysis will be conducted on the Safety Analysis Set.

13.2.5 Concomitant Therapies

Previous and concomitant drug therapy will be coded to Anatomical, Therapeutic, or Chemical (ATC) level and preferred drug name according to the World Health Organization Drug Dictionary (WHODD). These medications will be summarized by treatment group, ATC classification, and preferred drug name. This analysis will be conducted on the Safety Analysis Set.

All Non-pharmacological therapies will be listed. Use of Cognitive Behavioral Therapy will be summarized. Changes in therapy (e.g. start, stop) during the study will be summarized. This analysis will be conducted on the Safety Analysis Set.

13.2.6 Treatment Compliance with Investigational Product

A patient 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 proportion of compliant patients will be summarized by treatment group. This analysis will be conducted on the Full Analysis Set, the Safety Analysis Set, and the MADRS Non-responders at Week 2 Analysis Set.

13.2.7 Multiplicity Issue

No adjustments will be made for multiple comparisons.

13.2.8 Efficacy Analysis

All efficacy endpoints will be summarized by visit, including change from baseline information.

13.2.8.1 Primary Efficacy Analysis

The primary efficacy analysis will be on the change in Investigator-administered MADRS total score from Visit 2 (baseline) to the last visit of the 8-week double-blind treatment period Visit 7 (week 8). The key comparison will be the contrast between BTRX-246040 and placebo at Visit 7 (week 8) from a mixed-model repeated measures (MMRM) analysis. The primary analysis will use the Full Analysis Set.

A repeated measures analysis refers to a restricted-maximum-likelihood- (REML-) based, MMRM analysis using all the longitudinal observations at each post-baseline visit. The model for this analysis will include the fixed class effects of treatment, investigative site, visit, and treatment-by-visit interaction, as well as the continuous, fixed covariates of baseline Investigator-administered MADRS total score. An unstructured covariance structure will be used to model the within-patient errors. If the unstructured covariance matrix results in a lack of convergence, the heterogeneous autoregressive variance-covariance structure will be used. Least-square means (LS Means) for the difference to placebo at Week 8, with corresponding 2-sided 95% confidence intervals will be presented in tabular and graphical displays.

13.2.8.2 SENSITIVITY ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT

The following sensitivity analyses will be conducted on the primary efficacy endpoint to ensure robustness of the primary analysis.

The first sensitivity analysis will use the same mixed model repeated measures (MMRM) analysis used for the primary efficacy endpoint. The change from baseline in Investigator-administered MADRS total score at each post-baseline visit will be analyzed to provide information on the onset and the time course of efficacy. No imputation will be applied to missing data.

The second sensitivity analysis will utilize an ANCOVA method. This ANCOVA model will be used to analyze the change in Investigator-administered MADRS total score from baseline to Visit 7 (week 8). This ANCOVA model will include treatment group and investigative site as fixed effects and baseline MADRS total score as a covariate. The ANCOVA will be applied utilizing two methods of imputation for missing values.

- Multiple imputation for missing values: The results from the ANCOVA on multiple imputed datasets will be combined using the usual Rubin's rules for multiple imputation.
- Single imputation for missing values: The last observation carried forward (LOCF) imputation will be used.

The primary endpoint will be also analyzed based on the Per-Protocol Analysis Set, the Completer Analysis Set, and the MADRS Non-responders at Week 2 Analysis Set using MMRM.

An analysis of change from baseline in the computer-administered MADRS will also be performed using MMRM.

13.2.8.3 SECONDARY EFFICACY ANALYSES

The analysis of the secondary and exploratory endpoints will be based on the Full Analysis Set. All statistical tests will be 2-sided with a significance level of 10%. Where appropriate, secondary efficacy analyses will report nominal p-values to aid the interpretation of results. No adjustments for multiplicity will be made for these secondary analyses.

Analysis of each of the secondary efficacy variables will be based on patients with available data at respective time points used in defining the specific secondary efficacy variable. Missing data for secondary endpoints will not be imputed.

The MMRM models used for the primary efficacy endpoint will be used to analyze continuous secondary efficacy endpoints collected longitudinally similar to the primary endpoint (#1-6 listed below). The MMRM models will contain fixed effects for treatment, investigative site, visit, and treatment-by-visit interaction as well as the continuous fixed covariates of baseline measurement. Change from baseline will be the response parameter of interest.

The following secondary efficacy endpoints will be analyzed by the MMRM model:

1. Change from baseline to Week 8 on the Investigator-administered MADRS-6 subscale
2. Change from baseline to Week 8 on the Investigator-administered MADRS individual items
3. Change from baseline to Week 8 on the total scores of the HADS subscales, the anxiety subscale (HADS-A) and the depression subscale (HADS-D).
4. Change from baseline to Week 8 on the DARS
5. Change from baseline to Week 8 on the SHAPS (using the original 0 – 14 scoring method and also using an alternative scoring method ranging from 14 – 56)

The following secondary endpoints will be used to assess the proportion of responders. They will be analyzed by logistic regression with fixed effects for treatment and investigative site with the addition of the baseline Investigator-administered MADRS as a covariate.

Responder rates defined by:

6. Proportion of patients who demonstrate clinical response, where **response** was defined by a reduction of at least 50% in the baseline Investigator-administered MADRS total score at Week 8.
7. Proportion of patients with MDD who achieved remission, where **remission** was defined by a total score of ≤10 on the Investigator-administered MADRS at Week 8.
8. Proportion of patients who demonstrate significant clinical improvement, where **improvement** is defined by a score of 1 (very much improved) or 2 (much improved) on the CGI-I at Week 8.

The following secondary efficacy endpoints based on the performance tasks will be analyzed and presented in summary and graphical displays.

Data for the FERT will be summarized (mean, standard deviation, range) for each treatment group at each visit. All treatment differences will be estimated with 95% CI. No adjustments will be made for multiple comparisons. The analysis will employ ANOVA models without adjusting for covariates. However, if significant confounds are identified, a secondary analysis will be performed to examine the relationship between FERT outcome measures and other covariates. A full description of the statistical analysis methods for FERT outcome measures will be provided in in the SAP.

9. Outcome measures from the FERT
    a) Accuracy
    b) Misclassification
    c) Average reaction time
    d) Target sensitivity
    e) Response bias

Data for all PRT outcome measures will be summarized (mean, standard deviation, range) for each treatment group at each visit. The ANOVA models will be used to compare between treatment groups. For "Response bias" and "Discriminability," treatment group, block, and treatment-by-block interactions will be considered fixed effects. For "Reaction time" and "Hit rates," the stimulus type will be included as an additional fixed effect. The details of the analysis methods for these outcome measures will be described in the SAP.

10. Outcome measures from the PRT
    a) Response bias
    b) Discriminability
    c) Reaction time
    d) Hit rates

Data for all EEfRT outcome measures will be summarized (mean, standard deviation, range) for each treatment group at each visit. Statistical analyses of the Preference during the EEfRT trial will be performed using Generalized Estimating Equation (GEE) models. A binary logistic distribution will be used to model the probability of the Preference. The relationship and interactions between Preference and independent variables of reward magnitude, reward probability, expected value, age, sex, and efficacy parameters will be explored. The ANOVA model will be used to compare the completion rates between treatment groups. The details of the analysis method for these outcome measures will be described in the SAP.

11. Outcome measures from the EEfRT
    a) Preference (hard or easy task choice)
    b) Completion rates

13.2.8.4 Exploratory Efficacy and Health Outcomes Analyses

The exploratory endpoints will include:

1. Change in HAM-A total score from baseline to Week 4 and from baseline to Week 8
2. Change from baseline to Week 8 on the average pain in the last 24 hours.
3. Changes in plasma levels of nociceptin, and changes in serum levels of IL-1ß, IL-2, IL-6, IL-10, TNFα, IFNγ, and CRP from baseline to Week 8, correlated to treatment outcome and exposure

Exploratory endpoints will be summarized and, where appropriate, represented graphically. The analysis of the continuous exploratory endpoints will depend on the frequency of their measurements. If they are measured at baseline and multiple post-baseline visits, then an MMRM approach will be used. If they are measured at baseline and Week 8, an ANCOVA will be used. If the endpoint is only measured after treatment, an ANOVA will be used.

Further details on the analyses will be provided in the SAP.

13.2.8.5 SENSITIVITY ANALYSIS

Analyses will be conducted to assess the validity of assumptions inherent to the primary analysis and to assess the sensitivity of results to untestable assumptions. The primary analysis will also be repeated using the Per-Protocol Analysis Set, the Completer Analysis Set, and the MADRS Non-responders at Week 2 Analysis Set. In addition, analyses will be conducted to assess the impact of missing data. Departures from the assumed missing at random mechanism will be assessed by implementing inclusive models for dropout within the multiple imputation frameworks. Full details of these analyses will be provided in the SAP.

13.2.9 DIGITAL ASSESSMENTS

Vocal and behavioral digital assessment data are collected in this study. These data will be in a separate report.

13.2.10 PHARMACOKINETIC/PHARMACODYNAMIC ANALYSES

Pharmacokinetic data collected in this study will be listed for the Pharmacokinetic Analysis Set.

Population PK analysis of the plasma concentrations in this study will be reported separately. Pharmacokinetic data in this study may be combined with data from other studies to obtain improved model estimates and have adequate data for analysis.

Other exploratory analyses, such as the relationship between BTRX-246040 exposure and efficacy measures or AEs or other safety endpoints, may be undertaken as deemed appropriate.

13.2.11 SAFETY ANALYSES

Safety assessments include AEs, SAEs, vital signs, weight and BMI, laboratory values, neurological exams, C-SSRS, SHSF, SHFU, and ECGs. All safety analyses will be performed on the Safety Analysis Set unless otherwise specified.

The safety/tolerability of BTRX-246040 and placebo will be compared for rates of spontaneously reported TEAEs, SAEs, AEs associated with self-harm episodes, early study discontinuations, discontinuations from BTRX-246040 or placebo, and AEs leading to discontinuation from the study or from BTRX-246040 or placebo.

Data will be pooled from all investigative sites for the analyses.

Safety results will be summarized by treatment. For categorical variables, frequencies and percentages will be reported and for continuous endpoints by visit and change from baseline summaries will be presented.

13.2.12 SUBGROUP ANALYSES

Subgroup analysis based on gender, baseline severity of anhedonia symptoms (SHAPS total score ≤ 4 and > 4; also SHAPS total score ≤ 7 and > 7) and baseline severity of anxiety (HAM-A total score < 20 and ≥ 20) will be performed. Subgroups based on other characteristics may be considered. All subgroup analyses will be exploratory in nature.

13.2.13 Interim Analysis

No interim analyses are planned for this study.

14 INFORMED CONSENT, ETHICAL REVIEW, AND REGULATORY CONSIDERATIONS

14.1 INFORMED CONSENT

The Investigator is responsible for ensuring that the patient understands the potential risks and benefits of participating in the study, including answering any questions the patient may have throughout the study and sharing in a timely manner any new information that may be relevant to the patient's willingness to continue his or her participation in the trial.

The ICF will be used to explain the potential risks and benefits of study participation to the patient in simple terms before the patient is entered into the study, and to document that the patient is satisfied with his or her understanding of the risks and benefits of participating in the study and desires to participate in the study.

The Investigator is responsible for ensuring that informed consent is given by each patient. This includes obtaining the appropriate signatures and dates on the ICF prior to the performance of any protocol procedures and prior to the administration of investigational product.

14.2 ETHICAL REVIEW

The Sponsor or its representatives must approve all ICFs before they are submitted to the IRB and are used at investigative sites(s). All ICFs must be compliant with the ICH guideline on GCP.

Documentation of IRB approval of the protocol and the ICF must be provided to the Sponsor before the study may begin at the investigative site(s). The IRB(s) will review the protocol as required.

Any member of the IRB who is directly affiliated with this study as an Investigator or as site personnel must abstain from the IRB's vote on the approval of the protocol.

14.3 REGULATORY CONSIDERATIONS

This study will be conducted in accordance with:

1. consensus ethics principles derived from international ethics guidelines, including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
2. the ICH GCP Guideline [E6]
3. applicable laws and regulations.

The Sponsor certifies that this study is being conducted under an active US Investigational New Drug (IND) application.

Some of the obligations of the Sponsor will be assigned to a TPO.

14.3.1 Investigator Information

Physicians with a specialty in psychiatry or physicians with experience or with medical site staff with experience in treating MDD will participate as Investigators in this clinical trial.

14.3.2 Protocol Signatures

The Sponsor's responsible medical officer will approve the protocol, confirming that to the best of his or her knowledge, the protocol accurately describes the planned design and conduct of the study.

After reading the protocol, each principal Investigator will sign the protocol signature page and provide a copy of the signed page to a Sponsor representative.

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Appendix 1 Examples of Inhibitors and Inducers of CYP3A4

Per exclusion criterion 19: **Are currently taking or have taken within 5 half-lives of Visit 2 (baseline) any medications or supplements that are strong inhibitors or inducers of CYP3A4. The listed moderate inhibitors/inducers below are not exhaustive. See also:**

http://www.fda.gov/drugs/developmentapprovalprocess/developmentresources/druginteractionslabeling/ucm093664.htm.

Table 1.a Inhibitors of CYP3A4

Strong inhibitors [a]	Moderate inhibitors [b]
boceprevir	amprenavir
clarithromycin	aprepitant
conivaptan	atazanavir
grapefruit juice	ciprofloxacin
indinavir	darunavir
itraconazole	diltiazem
ketoconazole	erythromycin
lopinavir	fluconazole
mibefradil	fosamprenavir
nefazodone	imatinib
nelfinavir	verapamil
posaconazole	
ritonavir	
saquinavir	
telaprevir	
telithromycin	
voriconazole	

[a] Increases the area under the curve (AUC) of the substrate by ≥ 5-fold.
[b] Increases the AUC of the substrate by 2- to 5-fold.

Table 1.b Inducers of CYP3A4

Strong inducers [a]	Moderate inducers [b]
avasimibe	bosentan
carbamazepine	efavirenz
enzalutamide	
phenytoin	etravirine
rifampin	modafinil
St John's wort	nafcillin

[a] Decreases the AUC of the substrate by ≥ 80%.

[b] Decreases the AUC of the substrate by 50–80%.