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CLINICAL STUDY PROTOCOL: K2-MDD-201

Protocol Title:	A Phase 2a, Randomized, Double-blind, Placebo-controlled Proof of Concept Study to Evaluate the Effects of Oral BTRX-335140 Versus Placebo in Subjects With Major Depressive Disorder
Brief Title:	Proof of Concept Phase 2a, Double-blind Study in Major Depressive Disorder with BTRX-335140 vs Placebo
Investigational Product:	BTRX-335140
Phase of Development:	Phase 2a
Sponsor:	BlackThorn Therapeutics, Inc. 1700 Owens Street, Suite 535 San Francisco, CA 940158 Telephone: 866-258-5277
Medical Monitor:	[redacted], [redacted] Executive Medical Director Medical Affairs Worldwide Clinical Trials Telephone: [redacted]
Medical Project Leader:	[redacted], [redacted] VP, Clinical Development BlackThorn Therapeutics, Inc. Telephone: [redacted]
Amendment 7 (Version/Effective Date):	8.0/24 January 2022

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SPONSOR SIGNATURE

Protocol Title:	A Phase 2a, Randomized, Double-blind, Placebo-controlled Proof of Concept Study to Evaluate the Effects of Oral BTRX-335140 Versus Placebo in Subjects With Major Depressive Disorder
Protocol Number:	K2-MDD-201

This Protocol Amendment 7, Version 8.0 has been reviewed and approved by the Sponsor.

DocuSigned by:
[redacted]
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Jan 27, 2022

Company/Sponsor signatory
[redacted], [redacted]
VP Clinical Development
BlackThorn Therapeutics, Inc.
Tel: [redacted]
e-mail: [redacted]

Date

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INVESTIGATOR AGREEMENT

Protocol Title:	A Phase 2a, Randomized, Double-blind, Placebo-controlled Proof of Concept Study to Evaluate the Effects of Oral BTRX-335140 Versus Placebo in Subjects With Major Depressive Disorder
Protocol Number:	K2-MDD-201

This Protocol Amendment 7, Version 8.0 has been reviewed and approved by the Sponsor.

The signature below constitutes agreement with this protocol and attachments, and provides assurance that this study will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to national and local legal and regulatory requirements and applicable regulations and guidelines.

Principal Investigator: _____

Address: _____

Signature: _____

Date: _____

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SUMMARY OF CHANGES

This section describes significant changes made in Version 8.0 of the protocol. Changes to punctuation, formatting (ie, changes in numbering, word order, location, etc.), the table of contents, and the list of abbreviations and definitions of terms are not listed in the summary of changes table (Table 1).

Table 1: Summary of Changes

Section Number and Title	Description of Change	Rationale
Synopsis – Statistical Methods, Section 9 Statistical Considerations, Efficacy Analyses	Changed alpha, power, and confidence interval (CI) for efficacy analyses to reflect 2-sided alpha=0.05; power of 84% and 95% CIs	To decrease the chance of a Type I error.
Synopsis – Study Design, Schedule of Procedures and Assessments (footnotes c, h, and t), Section 3.1, Section 6.3.6, Section 7.6, Section 7.7.2, Section 7.8	Clarified wording around the Week 12 corneal specular microscopy, which should be completed for all subjects (ie, completers and early termination) unless consent is withdrawn. Also added clarification that additional ophthalmologic examinations (standard ophthalmologic exams and/or corneal specular microscopies) may be conducted at any time during the study to assess subject safety.	In the event of early termination, every attempt should be made to get a subject to come back for corneal specular microscopy at 12 weeks after the first dose of study drug. Added text to reflect potential of unscheduled ophthalmologic and/or corneal specular microscopies to assess safety
Section 3.2.2, Section 3.2.3, Section 9.3.3.2, Section 9.3.5	Moved CGI-S from an exploratory to a secondary endpoint.	Supports the secondary objectives; consistency with the SAP
Synopsis – Statistical Methods, Section 9.3.3.2	Added description for analysis of CGI-S using generalized estimating equations (GEE)	Consistency with the SAP
[redacted]		
Synopsis – Statistical Methods, Section 9.3.3.2	Revised statistical text for secondary efficacy analyses to allow for ANCOVA as appropriate.	Clarity and consistency with the SAP
Synopsis – Study Endpoints, Section 3.2.2, Section 9.3.3.2, Section 9.3.5	Moved HAMD-17 response rates from an exploratory endpoint to a secondary endpoint	Consistency with the SAP
Section 9.3.3.2	Added bullet for CGI-I	Inadvertently missed
Section 9.3.6	Clarified text describing subgroup analyses	Consistency with the SAP

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SYNOPSIS

Name of Finished Product: BTRX-335140	
Protocol Number: K2-MDD-201	**Phase of Development:** 2a
Protocol Title: A Phase 2a, Randomized, Double-blind, Placebo-controlled Proof of Concept Study to Evaluate the Effects of Oral BTRX-335140 Versus Placebo in Subjects With Major Depressive Disorder	
Number of Planned Subjects: Approximately 180 subjects randomized with treatment	
Study Sites: Approximately 40 study centers in the United States (US)	
Duration of Study: Approximately 24 months	
Study Objectives: **Primary Objectives:** To establish Proof of Concept (POC) for BTRX-335140, a kappa opioid receptor antagonist, by evaluating the impact of BTRX-335140 relative to placebo on symptoms of major depressive disorder (MDD) in adult subjects with MDD and symptoms of anhedonia and anxiety following 8 weeks of double-blind treatment as assessed by the Hamilton Depression Rating Scale (HAMD-17)	
Secondary Objectives: 1. To evaluate the effects of BTRX-335140 on self-reported anhedonia in adult subjects with MDD 2. To evaluate the effects of BTRX-335140 on anxiety-related symptoms in adult subjects with MDD 3. To evaluate the effects of BTRX-335140 on functional impairment in adult subjects with MDD 4. To evaluate the safety and tolerability of BTRX-335140 in adult subjects with MDD	
[redacted]	
Study Design: Study K2-MDD-201 is an 8-week, randomized, double-blind, placebo-controlled, parallel-group, multicenter study to evaluate the effects of BTRX-335140 on symptoms of depression in adult subjects with MDD and symptoms of anhedonia and anxiety after 8 weeks of double-blind treatment. The study will consist of a 7- to 28-day screening period, an 8-week active treatment period (during which subjects will receive either BTRX-335140 or placebo), and a 4-week Safety Follow-up period. Subjects will sign an informed consent form (ICF) and will then enter the screening period to ensure they meet the required inclusion and exclusion criteria, including diagnosis and stability of depressive symptoms and absence of psychiatric and medical conditions that would preclude study participation. The 28-day screening period may be extended to 35 days (after consultation with and approval by the medical monitor), if necessary, for reasons including, but are not limited to, discontinuation of a subject's current inefficacious medications or to obtain an ophthalmologic examination. In the event that an investigator requests to re-screen a subject that previously screen failed Study K2-MDD-201, the medical monitor and Sponsor will consider allowing re-screening on a case-by-case basis. After eligibility is confirmed by the medical monitor, and before randomization, baseline ophthalmologic examinations (standard and corneal specular microscopy) must be performed. Subjects who meet the Diagnostic and Statistical Manual of Mental Disorders Fifth Edition (DSM5) diagnostic criteria for MDD and all other eligibility criteria for the study will be randomized in a 1:1 ratio to 1 of 2 treatment arms	

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(placebo or BTRX 335140 once daily [QD]) until approximately 180 subjects are randomized. Beginning at Visit 2 (Baseline), subjects will receive treatment with BTRX-335140 80 mg QD or placebo for 8 weeks. During the Safety Follow-up, all subjects will receive a telephone call from study site personnel approximately 2 weeks after discontinuation of study drug for assessment of AEs, concomitant medication usage, and CSSRS; and at Week 12, subjects will have corneal specular microscopy performed by a study site affiliated ophthalmologist. The overall duration in the study for each subject is expected to be approximately 14 to 16 weeks. Additional ophthalmologic examinations (standard ophthalmologic exams and/or corneal specular microscopies) may be conducted to monitor subject safety.

Possible Changes in Conduct of Study: Study sites will maintain, at a minimum, coronavirus disease 2019 (COVID-19) risk mitigation procedures as per regulatory and local guidelines and/or laws. Allowed modifications to the conduct of the study, in the event of site staff and/or patient restrictions due to COVID-19, are outlined in Appendix 1 to the protocol.

Duration of Treatment: 8 weeks

Study Population:

Inclusion criteria:

Subjects 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
2. Have a primary DSM-5 diagnosis of MDD, with prominent symptoms of anhedonia confirmed by Structured Clinical Interview for DSM-5 Disorders, Clinical Trials Version (SCID-5-CT)
   a. The current episode must have started at least 3 weeks prior to screening but no more than 12 months before the screening visit.
   b. Have not failed 2 or more courses of antidepressant treatment in the current episode
   c. No more than a 3-point change in HAMD-17 between screening and baseline
   d. Have sufficient history or an independent report to confirm that symptoms are causing functional impairment or clinically significant distress
3. Meet the blinded-rule list based on clinical scale criteria
4. Body mass index (BMI) of 18 to 40 kg/m^2 (inclusive)
5. Medically stable (in the opinion of the investigator and Sponsor/Sponsors delegate) based on medical history, vital signs, clinical laboratory tests, and 12-lead electrocardiogram (ECG) performed at screening and baseline
6. Agree to the following birth control:
   a. Nonvasectomized men must agree to use a condom with spermicide, if sexually active during the study, until 90 days after the last dose of study drug administration. No restrictions are required for a vasectomized man, provided his vasectomy was performed 4 months or more prior to the first dose of study drug. A man who has been vasectomized less than 4 months prior to the first dose of study drug must follow the same restrictions as a nonvasectomized man. Additionally, men must refrain from sperm donation during study treatment and for at least 90 days following the last dose of study drug.
   b. Women of child-bearing potential (women not surgically sterilized and between menarche and 2 years postmenopausal) must have a negative serum pregnancy test at screening and a negative urine pregnancy test at enrollment and agree to use reliable birth control (eg, 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 10 days following the last dose of the study drug (BTRX-335140 or placebo). Women will be considered surgically sterile, if they have had tubal ligation, bilateral salpingo oophorectomy, or a hysterectomy.

      Note: Abstinence will be allowed if, in the investigator's judgement, it is determined that the subject is reliable, that abstinence is the preferred and usual lifestyle of the subject, and that abstinence will

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be continued for the duration of the study including the 10 days (women) or 90-day period (men) following last dose of study drug as noted above.

c. Or engaged exclusively in a non-heterosexual relationship

7. Willing and able to give written informed consent to participate
8. Able to understand and comply with instructions in English
9. Are judged by the investigator to be reliable and agree to keep all appointments for clinic visits, tests, and procedures, including venipuncture, and examinations required by the protocol

Exclusion Criteria:

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

1. Have a history of any of the following DSM-5 disorders within the specified timeframe:
   a. Currently or in the past year: diagnosis of personality disorder, attention deficit disorder/attention deficit hyperactivity disorder, anorexia nervosa, or bulimia nervosa. Subjects with comorbid generalized anxiety disorder, social anxiety disorder, or panic disorder for whom MDD is considered the primary diagnosis are not excluded.
   b. Lifetime: diagnosis of bipolar 1 or 2, schizophrenia, obsessive compulsive disorder, or post-traumatic stress disorder
2. Have a history of substance or alcohol use disorder (AUD), per DSM-5 criteria, within the past year
3. Are actively suicidal (eg, any suicide attempts within the past 12 months) or are at serious suicidal risk as indicated by any current suicidal intent, including a plan, as assessed by the Columbia Suicide Severity Rating Scale (C-SSRS) (score of "YES" on suicidal ideations item 4 or 5 within 3 months prior to Visit 1 [Screening]) and/or based on clinical evaluation by the investigator; or are homicidal, in the opinion of the investigator
4. Have a history or signs of Cushing's disease, Addison's Disease, primary amenorrhea or other evidence of significant disorders of the hypothalamus-pituitary-adrenal axis
5. Have any other clinically significant medical or psychiatric condition or circumstance prior to randomization that, in the opinion of the investigator, or Sponsor could affect subject safety, preclude evaluation of response, interfere with the ability to comply with study procedures, or prohibit completion of the study, such as acute stress disorder, adjustment disorder, impulse control disorder, 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, gastric bypass, lap band placement, or any other significant gastrointestinal condition
6. Have had prior seizures (other than remote history of childhood febrile seizure) or other condition that would place the subject at increased risk of seizures or is taking anticonvulsants for seizure control
7. Have a history of serious head injury (eg, skull fracture, cerebral contusion, or trauma resulting in prolonged unconsciousness), intracranial neoplasm, or hemorrhage
8. Have ever had electroconvulsive treatment, vagal nerve stimulation, or treatment with ketamine or esketamine for MDD
9. Have initiated transcranial magnetic stimulation, psychotherapy (such as Cognitive Behavioral Therapy) or have had a change in psychotherapy, or other non-drug therapies (such as acupuncture or hypnosis) within 4 weeks prior to Visit 1 (Screening) or at any time during the acute phase of the study
10. Have a visual or physical motor impairment that could interfere with subject's ability to perform study assessments, as assessed by the investigator
11. Have alanine aminotransferase (ALT) or aspartate aminotransferase (AST) levels ≥2 x upper limit of normal (ULN) or a bilirubin level 1.5 x ULN unless due to a documented history of Gilbert's syndrome
12. Estimated glomerular filtration rate (eGFR) ≤60 mL/min/1.73m^2 as calculated by the Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] 2009 creatinine equation at Visit 1 (Screening)
13. Positive hepatitis C virus (HCV) antibody (Ab), hepatitis B surface antigen (HBs Ag), hepatitis A virus (HAV) IgM antibody (HAV-Ab [IgM]) or human immunodeficiency virus (HIV) test at Visit 1 (Screening)

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14. Have a thyroid-stimulating hormone (TSH) level of <0.9 x lower limit of normal (LLN) or >1.2 x ULN on or off stable treatment for hyperthyroidism or hypothyroidism; if TSH is abnormal, evaluate reflex Free T3 and Free T4. If reflex testing is normal, the assessment of normal thyroid function will be determined based on the judgement of the investigator, following discussion with the medical monitor.
15. 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 or Sponsor, should preclude a subject's participation at study entry
16. Exclusionary ECG abnormalities obtained at Visit 1 (Screening) or Visit 2 (Baseline) are QT interval corrected using Fridericia's formula (QTcF) >450 msec in males or >470 msec in females, complete bundle branch block, evidence of myocardial infarction or ischemia, and predominantly nonsinus conducted rhythms. Other abnormalities can be exclusionary at the discretion of the principal investigator or medical monitor. See Section 6.3.5 of protocol for guidance on ECG interpretations.
17. Have a positive urine drug screen for amphetamines, barbiturates, cocaine, methadone, opioids, propoxyphene, tetrahydrocannabinol (THC), phencyclidine, or a positive blood alcohol level assessed by breathalyzer at Visit 1 (Screening) and Visit 2 (Baseline). For occasional (1 to 2 times per month maximum) cannabis users only, 1 retest is allowed and subject must agree to abstain from use for the duration of the study; a positive second test is exclusionary.
18. Have any use, by history, of Salvinorin A
19. 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/anticonvulsants (carbamazepine, lamotrigine, etc.), lithium, antidepressants, S-adenosylmethionine, melatonin, agomelatine, and hypnotics/sedatives within 5 half-lives or 14 days (whichever is longer) of Visit 2 (Baseline)
    b. Fluoxetine and irreversible monoamine oxidase inhibitors within 4 weeks of Visit 2 (Baseline) depot antipsychotics within 2 months of Visit 2 (Baseline)
    c. Opioid agonists and antagonists
20. Are currently taking or have taken within 5 half-lives of Visit 2 (Baseline) any medications or supplements [redacted]
21. Are women who are either pregnant or breastfeeding
22. Have participated (received study treatment) in a clinical study or any other type of medical research judged by the investigator or Sponsor 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.
23. Have participated in multiple interventional clinical studies, such that, in the opinion of the investigator, the subject is not a suitable candidate for participation
24. Have previously completed or withdrawn from this study or any other study investigating BTRX-335140
25. 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.
26. Are employees of the Sponsor or are employees of any third-party organizations (TPOs) (eg, laboratory staff, study vendors and transportation providers) involved in study who require exclusion of their employees
27. Has any of the following: 1) useful vision in only 1 eye from a pre-existing ophthalmic disease or amblyopia; 2) a corneal transplant in either eye; 3) corneal dystrophy or family history of corneal dystrophy; 4) severe dry eye syndrome [keratitis sicca]; 5) will not or cannot cooperate with ophthalmic examination requiring pupillary dilation (includes history of severe adverse reaction to mydriatic agents or untreated narrow angle glaucoma). Note: The following ocular disorders are allowed: cataracts, prior

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cataract surgery, glaucoma (narrow angle glaucoma is allowed if definitively treated with laser peripheral iridectomy), macular degeneration, or ocular changes associated with diabetes mellitus or multiple sclerosis.
Investigational Product, Dose, and Mode of Administration: A starting dose of BTRX-335140 80 mg (1 capsule) QD at Visit 2 (Baseline), orally and this dose will be maintained for the subsequent 8 weeks.
Reference Therapy, Dose, and Mode of Administration: Placebo will be assigned as 1 capsule QD at Visit 2 (Baseline), orally and will be maintained for the subsequent 8 weeks. Placebo capsules will consist of inactive ingredients and look identical to BTRX-335140.
Study Assessments: **Efficacy:** • HAMD-17 • Clinical Global Impression Scale – Severity (CGI-S) • Clinical Global Impression of Improvement (CGI-I) • Snaith-Hamilton Pleasure Scale (SHAPS) • Hamilton Anxiety Rating Scale (HAM-A) • Hospital Anxiety and Depression Scale (HADS) subscales (ie, anxiety subscale [HADS-A] and depression subscale [HADS-D]) • Sheehan Disability Scale (SDS) • [redacted] **Safety:** Safety assessments include adverse events (AEs), serious adverse events (SAEs), adverse events of special interest (AESIs), vital signs, weight and BMI, clinical laboratory tests, physical, ECG, and ophthalmologic examinations (standard and corneal specular microscopy), C-SSRS, and Clinical Opiate Withdrawal Scale (COWS). [redacted] will also be obtained; all assessments are optional [redacted]
Study Endpoints: **Primary Efficacy:** The primary efficacy endpoint is the change from baseline to Week 8 on the HAMD-17 score in the Efficacy population. **Secondary:** Secondary endpoints will include the following: • CGI-I score assessed at each postbaseline timepoint • Change from baseline to each timepoint assessed in SHAPS score • Change from baseline to each timepoint assessed in HADS subscales (ie, HADS-A and HADS-D) scores • Change from baseline to each timepoint assessed in HAM-A total score • Change from baseline to each timepoint assessed in SDS score • Change from baseline to end of study in CGI-S scores • HAMD-17 response rate • Assessment of safety through the occurrence of treatment-emergent AEs (TEAEs), SAEs, and AESIs; change from baseline in vital signs measurements, body weights, BMI, clinical laboratory test results, C-SSRS scores, COWS scores; physical examination findings, ECG results, and ophthalmologic examination findings

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[redacted]

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

Statistical Methods: All statistical comparisons will be made at a 2-sided alpha of 0.05. No adjustment for multiplicity is planned. SAS Version 9.4 or higher will be used to analyze data.

Subjects will be analyzed according to randomized treatment assignment for efficacy assessments and according to treatment received for safety assessments.

Primary Efficacy Analysis: The primary efficacy analysis is a comparison of mean reduction in HAMD-17 scores between BTRX-335140 and placebo at Week 8. The analysis will be conducted using a Mixed-Models Repeated Measures (MMRM) with change from baseline HAMD-17 score as the dependent variable and will include treatment group, Week, and Week by treatment group interaction as factors, and baseline HAMD-17 score as covariate. Variance estimation will be based on an unstructured covariance matrix. The model-based Week 8 least square (LS) means, standard errors, 95% confidence intervals (CIs), and *p*-values will be reported.

Secondary Efficacy Analysis: Comparisons between BTRX-335140 versus placebo for the change from baseline in score at each timepoint assessed for the secondary endpoints will be conducted using MMRM analyses as specified above or analysis of covariance (ANCOVA), as appropriate. For CGI-I an additional analysis comparing the percentage of subjects with responses of 'much improved' or 'very much improved' at each timepoint between BTRX-335140 and placebo will be performed using generalized estimating equations (GEE). Response rates for decrease in HAMD-17 (e.g., the percentage of subjects with ≥ 50% decrease in HAMD-17 from baseline to Week 4 and Week 8) will be compared between BTRX-335140 and placebo using Cochran-Mantel-Haenszel tests. [redacted]

Safety Analyses: Safety data, including AEs, safety laboratory results, physical examination results, vital signs, suicidality, COWS scores, ECGs, and ophthalmologic examination findings will be summarized by treatment group and/or listed.

[redacted]

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SCHEDULE OF PROCEDURES AND ASSESSMENTS

Possible Changes in Conduct of Study: Study sites will maintain, at a minimum coronavirus disease 2019 (COVID-19) risk mitigation procedures as per regulatory and local guidelines and/or laws. Allowed modifications to the conduct of the study, if needed, are outlined in Appendix 1 to the protocol.

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

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Period	Screening	Baseline	Study Drug Treatment				Safety Follow-up	
Visit	1[b]	2	3	4	5	6		
Procedure and Assessments	Days –7 to –28 NA	Day 1 Week 0	Day 15 (±2 d) Week 2	Day 29 (±2 d) Week 4	Day 43 (±2 d) Week 6	Day 57/ EOT[c] (±2 d) Week 8	TC[a] Week 10	Ocular Exam Week 12
HAMD-17	X	X		X		X		
HAM-A	X	X		X		X		
[redacted]								
SDS		X		X	X	X		
HADS		X	X	X	X	X		
SHAPS	X	X		X		X		
[redacted]								
COWS	X	X	X	X	X	X		
Adverse events[p]		X	X	X	X	X	X	
Complete IWRS	X	X	X	X	X	X		
Dispense study drug		X[q]	X[q]	X[q]	X[q]			
Study drug administration		X[r]		X[s]				
Study drug compliance			X	X	X	X		

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

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

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

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

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

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

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

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

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

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

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

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

[l] [redacted]

[m] Optional assessment; subject refusal to perform will not prohibit study participation.

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

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

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

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

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

[s] [redacted]

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

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LIST OF ABBREVIATIONS

Abbreviation or Term	Definition
Ab	antibody
ADD	attention deficit disorder
ADHD	attention deficit hyperactivity disorder
AE	adverse event
ALT	alanine aminotransferase
APA	American Psychiatric Association
AESI(s)	adverse event(s) of special interest
AST	aspartate aminotransferase
ANCOVA	analysis of the covariance
AUC	area under the curve
AUD	alcohol use disorder
BMI	body mass index
BP	blood pressure
CAT	Clinical Assessment Technology
CFR	Code of Federal Regulations
cGCP	current Good Clinical Practice
CGI-I	Clinical Global Impression of Improvement
CGI-S	Clinical Global Impression of Severity
CI	confidence interval
CIOMS	Council for International Organizations of Medical Sciences
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
C_{max}	maximum plasma drug concentration
CNS	central nervous system
COVID-19	coronavirus disease 2019
COWS	Clinical Opiate Withdrawal Scale
CREB	cAMP response binding protein
CRF	case report form
CRO	Contract Research Organization
C-SSRS	Columbia Suicide Severity Rating Scale
[redacted]	
DSM-5	Diagnostic and Statistical Manual of Mental Disorders Fifth Edition
DSM-IV-TR	Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision
EC	Ethics Committee
ECG	electrocardiogram, electrocardiographic
eCRF	electronic case report form
EDC	electronic data capture
eGFR	estimated glomerular filtration rate

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Abbreviation or Term	Definition
FDA	Food and Drug Administration
GAD	generalized anxiety disorder
GEE	generalized estimating equations
HADS	Hospital Anxiety and Depression Scale
HADS-A	Hospital Anxiety and Depression Scale – Anxiety Subscale
HADS-D	Hospital Anxiety and Depression Scale – Depression Subscale
HAM-A	Hamilton Anxiety Rating Scale
HAMD-17	Hamilton Depression Rating Scale – 17-item Version
HAV	hepatitis A virus
HAV-Ab (IgM)	hepatitis A virus antibody
HBs Ag	hepatitis B surface antigen
HCV	hepatitis C virus
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
HPMC	hydroxypropyl methylcellulose
IB	investigator's brochure
IC_{50}	half-maximal inhibitory concentration
ICC	intraclass correlation coefficient
ICF	informed consent form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IEC	Independent Ethics Committee
IgM	immunoglobulin M
IND	US Investigational New Drug Application
IRB	Institutional Review Board
IWRS	interactive web-response system
KOR	kappa opioid receptor
KORA	kappa opioid receptor antagonist
[redacted]	
LC-MS/MS	liquid chromatography-tandem mass spectrometry
LLN	lower limit of normal
LS Means	least-square means
MedDRA	Medical Dictionary for Regulatory Activities
MDD	major depressive disorder
MMRM	mixed-models repeated measures
NOAEL	no-observed-adverse-effect-level
[redacted]	
PDF	portable document format
PET	positron emission tomography
[redacted]	

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Abbreviation or Term	Definition
[redacted]	[redacted]
[redacted]	[redacted]
POC	proof of concept
PP	per-protocol
[redacted]	
PTSD	post-traumatic stress disorder
QD	once daily
QTcF	Fridericia's correction method for QT Interval
REB	Research Ethics Board
RO	receptor occupancy
[redacted]	
SAE	serious adverse event
SAP	statistical analysis plan
SCID-5-CT	Structured Clinical Interview for DSM-5 Disorders, Clinical Trials Version
SD	standard deviation
SDS	Sheehan Disability Scale
SHAPS	Snaith-Hamilton Pleasure Scale
SIGH-A	Structured Interview Guide for the Hamilton Anxiety Rating Scale
SIGH-D	Structured Interview Guide for the Hamilton Depression Rating Scale
SNRI	serotonin norepinephrine reuptake inhibitor
SOP	Standard Operating Procedure
SSRI	selective serotonin reuptake inhibitor
[redacted]	[redacted]
SUSAR	serious unexpected suspected adverse reaction
[redacted]	[redacted]
TC	telephone call
TEAE	treatment-emergent adverse event
THC	tetrahydrocannabinol
TPO	third-party organization
TSH	thyroid-stimulating hormone
UDS	urine drug screen
ULN	upper limit of normal
US	United States
WHO	World Health Organization
WHODrug	World Health Organization Drug classification

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1 INTRODUCTION

1.1 Background Information

1.1.1 Major Depressive Disorder

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. Currently, MDD is the leading cause of disability and afflicts approximately 322 million people worldwide (4.4% of the global population), with prevalence increasing 18% between 2005 and 2015 (World Health Organization [WHO] 2017). By 2020, depressive disorders are expected to be the second highest cause of morbidity in the world (Murray and Lopez 2006) and have been predicted to become the leading cause of disease burden by the year 2030 (WHO 2004). The lifetime prevalence of MDD is approximately 16.6% in the United States (US) (Kessler et al 2003). 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 2 weeks. One of these symptoms, as defined in the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5), must be either depressed mood or anhedonia, which is defined as diminished interest or pleasure in response to rewarding stimuli (APA 2013). 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; 1 report describes an anhedonia rate of approximately 37% among a sample of 65 individuals diagnosed with MDD (Pelizza and Ferrari 2009). Moreover, published data indicate that the presence of anhedonia may represent a distinct physiologically based subset of patients with MDD, as 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). This is an important finding from the standpoint of therapeutic interventions because the presence of anhedonic symptoms has been found to be associated with poorer longitudinal symptom progression among patients with depression (Morris et al 2009) and is a major predictor of suicide (Fawcett et al 1990; Winer et al 2014).

1.1.2 Treatments

The current mainstays of pharmacological treatments for depression include the use of selective serotonin reuptake inhibitors (SSRIs) (Fava et al 2007; Zupancic and Guilleminault 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

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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 2010; Nutt et al 2007; Price et al 2009; Shelton and Tomarken 2001) and that anhedonic symptoms predict inadequate treatment response (Spijker et al 2001).

The presence of anhedonia is associated with inadequate treatment response to antidepressant drugs (McMakin et al 2012; Uher et al 2012) and potentially also to psychological treatments (Craske et al 2016). Unfortunately, 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 2010; Nutt et al 2007; Price et al 2009; Shelton and Tomarken 2001). For these reasons, recent efforts have been directed towards developing therapies that effectively target anhedonia, such as ketamine and Positive Affect Treatment (Craske et al 2016; Lally et al 2014).

1.1.3 BTRX-335140

BTRX-335140 is a selective kappa opioid receptor (KOR) antagonist that is being evaluated for the treatment of neurobehavioral disorders. Research studies carried out in animals and a proof of mechanism study carried out in humans suggest that medications which block KORs have the potential for being effective new treatments for patients with mood and anxiety spectrum disorders (Krystal et al 2020). The KOR system is an important mediator of the negative effects of stress-induced alterations on mood, emotional regulation, and motivation. This system has been a target for drug development in neurobehavioral disorders where dysregulation of stress signaling is associated with increased vulnerabilities. It is thought that KOR antagonists may improve anhedonia and reduce the vulnerabilities associated with stress.

In laboratory animals, stress or repeated exposure to drugs of abuse triggers intracellular events involving cAMP response binding protein (CREB) in the nucleus accumbens – an area of the mesolimbic dopamine system known to be involved in reward and motivation (Carlezon and Krystal 2016). This increased CREB activity leads to elevated expression of the opioid peptide dynorphin (Bruchas et al 2009), which in preclinical models causes core symptoms of depression and anxiety. In preclinical models of depression and anxiety such as the forced swim test or tail suspension test, KOR antagonism blocks the biochemical and behavioral response to stress, leading to antidepressant and anxiolytic behavioral effects (Carlezon and Krystal 2016). In preclinical studies of addiction behavior, KOR antagonists prevent stress-induced drug-seeking behavior (Beardsley et al 2005).

In humans, KOR agonists, including the plant-derived Salvinorin A, have been reported to trigger symptoms of dysphoria and anxiety (Pfeiffer et al 1986; Walsh et al 2001) suggesting

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that antagonists may play a beneficial role in the treatment of anxiety disorders and anhedonic symptoms in depression.

In the recently completed NIMH FAST-MAS Phase 2 Proof of concept (POC) study, patients with mood and anxiety disorders (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision [DSM-IV-TR] diagnostic criteria for: MDD, Bipolar 1 or 2 Depressed, Generalized Anxiety Disorder [GAD], Social Phobia, Panic Disorder, or Post-Traumatic Stress Disorder [PTSD]) who had high levels of anhedonia (Snaith-Hamilton Pleasure Scale [SHAPS] >20) were treated with a KOR antagonist (KORA) or placebo for 8 weeks. In that study, the group receiving the KORA treatment showed greater ventral striatal activation (a neural target related to hedonic response) during anticipation of both gain and loss during an incentivized test of working memory as well as a significant improvement in their anhedonia score as evaluated by the SHAPS compared to those patients who received placebo (Krystal et al 2020).

Nonclinical studies indicate that the risk for cardiovascular, respiratory, and central nervous system (CNS) AEs in the BTRX-335140 clinical program is low. Body weight loss or decreased body weight gain associated with decreased food intake was observed across species tested. BTRX-335140 was nonmutagenic in in vitro Ames and chromosomal mutation assays.

[redacted] Ocular phototoxicity (corneal edema), with confirming histopathologic findings was observed at the 100 mg/kg/day dose. [redacted] No phototoxicity was noted in the retina or any other ocular structure at any dose level.

BTRX-335140 is orally bioavailable across animal species studied. BTRX-335140 is a [redacted] Based on nonclinical studies, BTRX-335140 is not anticipated to significantly inhibit or induce [redacted] at concentrations relevant in vivo. [redacted]

The first-in-human Phase 1 study in healthy adult subjects evaluated single and multiple ascending doses of BTRX335140. Treatment with BTRX-335140 exhibited a favorable pharmacokinetic (PK) profile, and BTRX-335140 was well tolerated. There were no deaths, serious adverse events (SAEs), or subject discontinuations due to AEs in this study. Overall, AEs were infrequent, and the majority of events were Grade 1 in intensity. In the multiple-dose phase, 13 (54%) subjects experienced a total of 42 treatment-emergent adverse

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events (TEAEs) following BTRX-335140 administration, and all reported TEAEs in the multiple-dose phase were Grade 1 in severity. The most common events reported were pruritus and fatigue. Grade 1 pruritus was reported 4 times among 3 (13%) subjects overall, with 1 subject receiving 20 mg BTRX-335140 and 2 subjects receiving 160 mg BTRX-335140 daily for 10 days. A total of 3 (13%) subjects each experienced a single TEAE of Grade 1 fatigue, including 2 subjects who received 80 mg BTRX-335140 and 1 subject who received 160 mg BTRX-335140 daily for 10 days. All remaining AEs were reported by 2 or fewer subjects each in both the single- and multiple-dose phases. Following multiple-dose administration of BTRX-335140, the following AEs were considered at least possibly related to the study treatment: abnormal dreams (40 mg and 80 mg), fatigue (80 mg and 160 mg), headache (80 mg and 160 mg), sleep disorder (40 mg), asthenia, chills, dizziness, nausea, somnolence (80 mg), generalized pruritus, macular rash, tremor (160 mg), and papule (placebo). Overall, there were no remarkable findings in the remaining safety assessments in the study for clinical laboratory parameters, vital sign measurements, electrocardiogram (ECG) findings, and Columbia Suicide Severity Rating Scale (C-SSRS) scores. Overall, safety findings were comparable for BTRX-335140 and placebo and there were no trends observed in safety data with respect to ascending single and multiple BTRX-335140 doses.

[redacted] After 10 days of once-daily oral dosing, some accumulation (up to 3.9-fold for AUC and up to 4.6-fold for C_{max} exposures) of BTRX-335140 in plasma is observed and steady state is reached by Day 10. The administration of a high-fat diet with BTRX-335140 resulted in an increase in exposure by 17% (AUC) and 12% (C_{max}) compared to fasted conditions.

The second Phase 1 study used positron emission tomography (PET) imaging study in healthy adult males to assess KOR Occupancy in the human brain using the radiotracer [^{11C}]LY2795050 following single ascending oral doses of BTRX-335140 showed tracer uptake was reduced by treatment with BTRX-335140, and the relationship between KOR Occupancy and BTRX-335140 concentration was IC_{50} = 9.85 ±1.40 ng/mL.

1.2 Rationale for Study Design, Doses, and Control Groups

The current study is the first Phase 2 study designed to establish POC for BTRX-335140 by evaluating the impact of treatment with BTRX-335140 relative to treatment with matching placebo on symptoms of MDD in adult patients.

The randomization, study drug 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 subjects blinded to treatment assignment (double-blind) controls for expectation bias relative to any particular treatment condition. A placebo group is included to control the subject and investigator expectations of improvement during the study that could confound interpretation of pharmacological effects of treatment.

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Double-blind, placebo-controlled studies are the standard in assessment of efficacy in randomized clinical studies evaluating subjects with conditions such as MDD. There have been estimates of placebo response rates in MDD clinical studies between 30% and 50% therefore it is important to use a placebo comparator despite the risk of a possible worsening of symptoms. Certain factors are associated with improvement in placebo-treated subjects. 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 subjects who began the study with more severe symptoms of illness (Fava et al 2003).

A starting dose of 80 mg of BTRX-335140 was selected for this study on the basis of pharmacokinetic/pharmacodynamic modeling from the PK and PET receptor occupancy (RO) studies that indicates that this dose should provide approximately 80–90% RO at steady state. The study dose of 80 mg BTRX 335140 as well as a higher dose of 160 mg have been given once daily (QD) for 10 consecutive days and was well-tolerated in healthy subjects (Study K2-ADS-101). Pharmacokinetics of a single 80 mg dose were similar under fed and fasted conditions. The brain KOR occupancy of a single 160 mg dose of BTRX-335140 is approximately 90% (Study K2-HV-102). Human PK modeling based on drug exposure levels in Study K2-ADS-101 indicates that this same level of occupancy will be achieved with an 80-mg daily dose at steady state. This occupancy level is projected to be sufficient to produce a KORA mechanism-based effect in an MDD population.

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1.3 Potential Risks and Benefits

Information about the known and expected benefits, risks, and reasonably anticipated AEs with BTRX-335140 treatment may be found in the Investigator's Brochure (IB). No data are available on the long-term effect of BTRX-335140 on fertility; therefore, the possibility of a negative impact on fertility cannot be excluded.

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2 OBJECTIVES

2.1 Primary Objective

To establish POC for BTRX-335140, a KORA, by evaluating the impact of BTRX-335140 relative to placebo on symptoms of MDD in adult subjects with MDD and symptoms of anhedonia and anxiety following 8 weeks of double-blind treatment as assessed by the Hamilton Depression Rating Scale (HAMD-17).

2.2 Secondary Objectives

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

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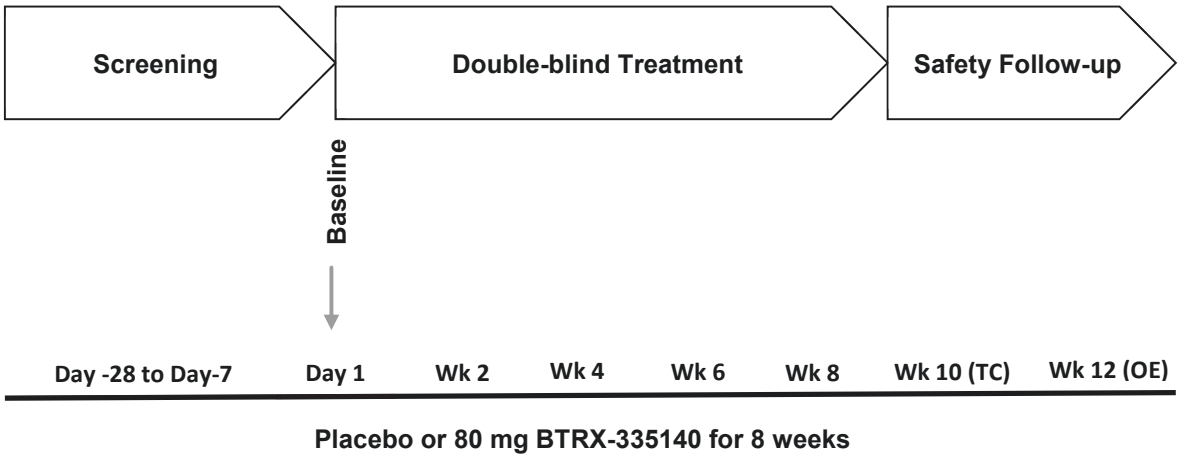
3 INVESTIGATIONAL PLAN AND ENDPOINTS

3.1 Description of Study Design

Study K2-MDD-201 is an 8-week, randomized, double-blind, placebo-controlled, parallel-group, multicenter study to evaluate the effects of BTRX-335140 on symptoms of depression in adult subjects with MDD and symptoms of anhedonia and anxiety after 8 weeks of double-blind treatment. The study will consist of a 7- to 28-day screening period, an 8-week active treatment period (during which subjects will receive either BTRX-335140 or placebo), and a 4-week Safety Follow-up period (Figure 1).

Figure 1: Study Design



OE = ophthalmologic examinations; TC = telephone contact; Wk = Week.
Note: To reduce subject burden, subjects may be allowed to perform 1 virtual visit at Week 2 (Visit 3), 4 (Visit 4), or 6 (Visit 5). Virtual visits will be performed according to procedures specified in Appendix 1.

Subjects will sign an informed consent form (ICF) and will then enter the screening period to ensure they meet the required inclusion and exclusion criteria, including diagnosis and stability of depressive symptoms and absence of psychiatric and medical conditions that would preclude study participation. The 28-day screening period may be extended up to a maximum of 35 days (after consultation with and approval by the medical monitor), if necessary, for reasons including, but are not limited to, discontinuation of a subject's current inefficacious medications or to obtain an ophthalmologic examination. Some of the enrollment criteria in this study are based on analyses of depression data by BlackThorn using explainable artificial intelligence to inform about predictors of response to a KORA. Sites will be provided with a tablet computer preprogramed with an algorithm to confirm subject eligibility on the clinical assessment scales. In addition, for those subjects who are deemed to meet eligibility criteria, data will be requested to allow review by the medical monitor and the Clinical Assessment Technology (CAT) team to monitor data quality, and the ophthalmologic examinations (standard ophthalmologic and corneal specular microscopy) will be performed prior to randomization.

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In the event that an investigator requests to re-screen a subject that previously screen failed Study K2-MDD-201, the medical monitor and Sponsor will consider allowing re-screening on a case-by-case basis.

Subjects who meet the DSM-5 diagnostic criteria for MDD and all other eligibility criteria for the study will be randomized in a 1:1 ratio to 1 of 2 treatment arms (placebo or BTRX-335140 QD) until approximately 180 subjects are randomized. Beginning at Visit 2 (Baseline), subjects will receive treatment with BTRX-335140 80 mg QD or placebo for 8 weeks. During the Safety Follow-up, all subjects will receive a telephone call (TC) from study site personnel approximately 2 weeks after discontinuation of study drug for assessment of AEs, concomitant medication usage, and C-SSRS; and at Week 12, subjects will have corneal specular microscopy performed by a study site-affiliated ophthalmologist. The overall duration in the study for each subject is expected to be approximately 14 to 16 weeks.

The Week 12 corneal specular microscopy should be completed for all subjects (ie, completers and early termination) unless consent is withdrawn. The corneal specular microscopy should be conducted approximately 12 weeks after the first dose of study drug; subjects who terminate the study early would need to plan for the corneal specular microscopy at 12 weeks after the first dose of study drug. Any exceptions are to be discussed with the medical monitor.

Additional ophthalmologic examinations (standard ophthalmologic exams and/or corneal specular microscopies) may be conducted to assess subject safety.

Details of study procedures and assessments are provided in Section 6; for details on the timings of study assessments refer to Section 7 and the Schedule of Procedures and Assessments.

Study sites will maintain, at a minimum, coronavirus disease 2019 (COVID-19) risk mitigation procedures as per regulatory and local guidelines and/or laws. Allowed modifications to the conduct of the study, in the event of site staff and/or patient restrictions due to COVID-19, are outlined in Appendix 1.

3.2 Study Endpoints

3.2.1 Primary

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

3.2.2 Secondary

- Clinical Global Impression of Improvement (CGI-I) score assessed at each postbaseline timepoint
- Change from baseline to each timepoint assessed in SHAPS scores

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- Change from baseline to each timepoint assessed in Hospital Anxiety and Depression Scale (HADS) subscales (ie, anxiety subscale [HADS-A] and depression subscale [HADS-D] scores)
- Change from baseline to each timepoint assessed in Hamilton Anxiety Rating Scale (HAM-A) total score
- Change from baseline to each timepoint assessed in Sheehan Disability Scale (SDS) scores
- Change from baseline to end of study in Clinical Global Impression Scale – Severity (CGI-S) scores
- HAMD-17 response rate
- Assessment of safety through the occurrence of TEAEs, SAEs, and adverse events of special interest (AESIs); change from baseline in vital signs measurements, body weights, body mass index (BMI), clinical laboratory test results, C-SSRS scores, Clinical Opiate Withdrawal Scale (COWS) scores; physical examination findings, ECG results, and ophthalmologic examination findings.

3.3 Study Duration

The overall duration of the study is expected to be up to approximately 24 months.

The expected participation period for a subject is approximately 14 to 16 weeks, including up to 28 days screening, approximately 8 weeks of double-blind treatment, and 4 weeks for safety follow–up.

The end of the study is defined as the date when the last scheduled study procedure, as outlined in the Schedule of Procedures and Assessments, is performed for the last subject.

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4 STUDY ENROLLMENT AND WITHDRAWAL

4.1 Study Population

Approximately 180 subjects will be enrolled in the study at approximately 40 study centers in the US.

4.1.1 Inclusion Criteria

Subjects must fulfill all of the following inclusion criteria to be eligible for participation in the study:

1. Are adult men or women 18 to 65 years of age (inclusive) at informed consent
2. Have a primary DSM-5 diagnosis of MDD, with prominent symptoms of anhedonia confirmed by Structured Clinical Interview for DSM-5 Disorders, Clinical Trials Version (SCID-5-CT)
    a. The current episode must have started at least 3 weeks prior to screening but no more than 12 months before the screening visit.
    b. Have not failed 2 or more courses of antidepressant treatment in the current episode
    c. No more than a 3-point change in HAMD-17 between screening and baseline
    d. Have sufficient history or an independent report to confirm that symptoms are causing functional impairment or clinically significant distress
3. Meet the blinded-rule list based on clinical scale criteria
4. BMI of 18 to 40 kg/m^2 (inclusive)
5. Medically stable (in the opinion of the investigator and Sponsor/Sponsors delegate) based on medical history, vital signs, clinical laboratory tests, and 12-lead ECG performed at screening and baseline
6. Agree to the following birth control:
    a. Nonvasectomized men must agree to use a condom with spermicide, if sexually active during the study, until 90 days after the last dose of study drug administration. No restrictions are required for a vasectomized man, provided his vasectomy was performed 4 months or more prior to the first dose of study drug. A man who has been vasectomized less than 4 months prior to the first dose of study drug must follow the same restrictions as a nonvasectomized man. Additionally, men must refrain from sperm donation during study treatment and for at least 90 days following the last dose of study drug.
    b. Women of child-bearing potential (women not surgically sterilized and between menarche and 2 years postmenopausal) must have a negative serum pregnancy test at screening and a negative urine pregnancy test at enrollment and agree to use reliable birth control (eg, 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

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vasectomy; or abstinence) during the study and for 10 days following the last dose of the study drug (BTRX-335140 or placebo). Women will be considered surgically sterile, if they have had tubal ligation, bilateral salpingo oophorectomy, or a hysterectomy.

Note: Abstinence will be allowed if, in the investigator's judgement, it is determined that the subject is reliable, that abstinence is the preferred and usual lifestyle of the subject, and that abstinence will be continued for the duration of the study including the 10 days (women) or 90-day period (men) following the last dose of study drug as noted above.

c. Or engaged exclusively in a non-heterosexual relationship

7. Willing and able to give written informed consent to participate
8. Able to understand and comply with instructions in English
9. Are judged by the investigator to be reliable and agree to keep all appointments for clinic visits, tests, and procedures, including venipuncture, and examinations required by the protocol

4.1.2 Exclusion Criteria

Subjects will be excluded from participating in this study if they meet **any** of the following criteria:

1. Have a history of any of the following DSM-5 disorders within the specified timeframe:
   a. Currently or in the past year: diagnosis of personality disorder, attention deficit disorder/attention deficit hyperactivity disorder, anorexia nervosa, or bulimia nervosa. Subjects with comorbid GAD, social anxiety disorder, or panic disorder for whom MDD is considered the primary diagnosis are not excluded
   b. Lifetime diagnosis of bipolar 1 or 2, schizophrenia, obsessive compulsive disorder, or post-traumatic stress disorder
2. Have a history of substance or alcohol use disorder (AUD), per DSM-5 criteria, within the past year
3. Are actively suicidal (eg, any suicide attempts within the past 12 months) or are at serious suicidal risk as indicated by any current suicidal intent, including a plan, at screening or baseline as assessed by the C-SSRS (score of "YES" on suicidal ideations item 4 or 5 within 3 months prior to Visit 1 [Screening]) and/or based on clinical evaluation by the investigator; or are homicidal, in the opinion of the investigator
4. Have a history or signs of Cushing's disease, Addison's Disease, primary amenorrhea or other evidence of significant disorders of the hypothalamus-pituitary-adrenal axis
5. Have any other clinically significant medical or psychiatric condition or circumstance prior to randomization that, in the opinion of the investigator, or Sponsor could affect subject safety, preclude evaluation of response, interfere with the ability to comply with study procedures, or prohibit completion of the study, such as acute stress disorder, adjustment disorder, impulse control disorder, uncontrolled diabetes mellitus, renal or

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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, gastric bypass, lap band placement, or any other significant gastrointestinal condition

6. Have had prior seizures (other than remote history of childhood febrile seizure) or other condition that would place the subject at increased risk of seizures or is taking anticonvulsants for seizure control
7. Have a history of serious head injury (eg, skull fracture, cerebral contusion, or trauma resulting in prolonged unconsciousness), intracranial neoplasm, or hemorrhage
8. Have ever had electroconvulsive treatment, vagal nerve stimulation, or treatment with ketamine or esketamine for MDD
9. Have initiated transcranial magnetic stimulation, psychotherapy (such as Cognitive Behavioral Therapy) or have had a change in psychotherapy, or other non-drug therapies (such as acupuncture or hypnosis) within 4 weeks prior to Visit 1 (Screening) or at any time during the acute phase of the study
10. Have a visual or physical motor impairment that could interfere with the subject's ability to perform study assessments, as assessed by the investigator
11. Have alanine aminotransferase (ALT) or aspartate aminotransferase (AST) levels ≥2 x upper limit of normal (ULN) or a bilirubin level 1.5 x ULN unless due to a documented history of Gilbert's syndrome
12. Estimated glomerular filtration rate (eGFR) ≤60 mL/min/1.73m^2 as calculated by the Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] 2009 creatinine equation (Levey et al 2009) at Visit 1 (Screening)
13. Positive hepatitis C virus (HCV) antibody (Ab), hepatitis B surface antigen (HBs Ag), hepatitis A virus (HAV) IgM antibody (HAV-Ab [IgM]) or human immunodeficiency virus (HIV) test at Visit 1 (Screening)
14. Have a thyroid-stimulating hormone (TSH) level of <0.9 x lower limit of normal (LLN) or >1.2 x ULN on or off stable treatment for hyperthyroidism or hypothyroidism; if TSH is abnormal, evaluate reflex Free T3 and Free T4. If reflex testing is normal, the assessment of normal thyroid function will be determined based on the judgement of the investigator, following discussion with the medical monitor.
15. 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 or Sponsor, should preclude a subject's participation at study entry
16. Exclusionary ECG abnormalities obtained at Visit 1 (Screening) or Visit 2 (Baseline) are QT interval corrected using Fridericia's formula (QTcF) >450 msec in males or >470 msec in females, complete bundle branch block, evidence of myocardial infarction or ischemia, and predominantly nonsinus conducted rhythms. Other abnormalities can be exclusionary at the discretion of the principal investigator or medical monitor. See Section 6.3.5 for guidance on ECG interpretations.

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17. Have a positive urine drug screen for amphetamines, barbiturates, cocaine, methadone, opioids, propoxyphene, tetrahydrocannabinol (THC), phencyclidine, or positive blood alcohol level assessed by breathalyzer at Visit 1 (Screening) and Visit 2 (Baseline). For occasional (1 to 2 times per month maximum) cannabis users only, 1 retest is allowed and subject must agree to abstain from use for the duration of the study; a positive second test is exclusionary.
18. Have any use, by history, of Salvinorin A
19. 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/anticonvulsants (carbamazepine, lamotrigine, etc.), lithium, antidepressants, S-adenosylmethionine, melatonin, agomelatine, and hypnotics/sedatives within 5 half-lives or 14 days (whichever is longer) of Visit 2 (Baseline)
    b. Fluoxetine and irreversible monoamine oxidase inhibitors within 4 weeks of Visit 2 (Baseline); depot antipsychotics within 2 months of Visit 2 (Baseline)
    c. Opioid agonists and antagonists
20. Are currently taking or have taken within 5 half-lives of Visit 2 (Baseline) any medications or supplements that are [redacted]
21. Are women who are either pregnant or breastfeeding
22. Have participated (received study treatment) in a clinical study or any other type of medical research judged by the investigator or Sponsor 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.
23. Have participated in multiple interventional clinical studies, such that, in the opinion of the investigator, the subject is not a suitable candidate for participation
24. Have previously completed or withdrawn from this study or any other study investigating BTRX-335140
25. 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.
26. Are employees of the Sponsor or are employees of any third-party organizations (TPOs) (eg, laboratory staff, study vendors and transportation providers) involved in study who require exclusion of their employees
27. Has any of the following: 1) useful vision in only 1 eye from a pre-existing ophthalmic disease or amblyopia; 2) a corneal transplant in either eye; 3) corneal dystrophy or family history of corneal dystrophy; 4) severe dry eye syndrome [keratitis sicca]; 5) will not or cannot cooperate with ophthalmic examination requiring pupillary dilation (includes

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history of severe adverse reaction to mydriatic agents or untreated narrow angle glaucoma). Note: The following ocular disorders are allowed: cataracts, prior cataract surgery, glaucoma (narrow angle glaucoma is allowed if definitively treated with laser peripheral iridectomy), macular degeneration, or ocular changes associated with diabetes mellitus or multiple sclerosis.

4.2 Method of Assigning Subjects to Treatment

Subjects 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). Subjects will be randomized in a 1:1 ratio to 1 of 2 treatment arms (placebo or BTRX-335140 QD).

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

4.3 Blinding

This is a double-blind study. Subjects, site personnel, and the Sponsor will be blinded to treatment. In the event there is a safety emergency, the investigator is requested, where possible, to contact the medical monitor or the Sponsor before unblinding; however, if there is insufficient time to do so, the investigator may perform emergency unblinding for SAEs through the IWRS as described in Section 8.1.2.

4.4 Subject Withdrawal

A subject's participation in this study is voluntary, and the subject may refuse to participate or withdraw from the study at any time for any reason. The investigator also has the right to withdraw a subject from the study for any reason. The investigator should make a reasonable effort to ascertain the primary reason for withdrawal and document that reason in the electronic case report form (eCRF).

Possible reasons for early discontinuation are as follows:

- Investigator/physician decision
  - The investigator/physician decides that the subject should be withdrawn from the study for any reason, eg, the subject is significantly noncompliant with the study drug regimen (see Section 5.5; subjects with study drug compliance ≤70% over any 2-week visit interval).
  - After Visit 2 (Baseline), the subject is found to have met an exclusionary criterion or not met an entry criterion required for study participation.
  - The subject is enrolled in any other clinical study 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.

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- Subject Decision
  - The subject requests to be withdrawn from the study for any reason. If the reason to request withdrawal is an AE this should be documented as the primary reason for discontinuation.
- Sponsor Decision
  - An investigator, site personnel performing assessments, or subject is unblinded (see Section 4.3).
- Adverse Event
  - The subject experiences an AE that would necessitate discontinuation of study drug. Any subject who is discontinued from study drug due to AEs or clinically significant laboratory abnormalities will be followed until resolution or clinical stabilization. If this decision is made because of an SAE or a clinically significant laboratory value, the study drug is to be discontinued and appropriate measures are to be taken. The Sponsor or its medical monitor designee is to be alerted immediately (see Section 8.5.1).
  - Discontinuation should be considered by the investigator after consultation with the Sponsor or its medical monitor designee when a subject has the following clinically significant abnormal laboratory findings:
    - ALT or AST >8 x ULN
    - ALT or AST >5 x ULN for more than 2 consecutive weeks
    - ALT or AST >3 x ULN and total bilirubin level >2 x ULN
    - ALT or AST >3 x ULN and prothrombin time (or INR) >1.5 x ULN
    - 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 %).
  - If the subject 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. This includes subjects who score 3 or above on item 11 of the HAMD-17. Subjects must be discontinued from study participation and offered appropriate treatment and care if they:
    - are assessed as homicidal
    - are actively suicidal (any suicidal ideation with intent or specific plan or any suicide attempt) or at serious suicidal risk
    - if suicidal gestures are present
  - Any subject who is found to be dependent on, or abusing opioids, should be withdrawn from the study and provided with appropriate medical care. If at any time during the study a score of 5 or more is obtained on the COWS, the investigator (or designee) must determine if the subject is experiencing opioid withdrawal (see Section 6.3.8).
  - The investigator remains responsible for following, through an appropriate healthcare option, AEs that caused the subject to discontinue study drug.
- Unsatisfactory Therapeutic Effect

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- If the investigator determines that a subject requires treatment with another therapeutic agent for MDD and that this alternative treatment cannot be safely delayed until completion of the current study, discontinuation from the study will occur prior to introduction of the new agent.

- Loss to Follow-Up
  - For subjects who are lost to follow-up, all steps performed to contact them (dates of telephone calls, registered letters, etc.) will be documented in the source documents. The investigators are required to make at least 3 attempts to contact the subject for a follow-up safety visit.

- Pregnancy
  - Study drug treatment will be discontinued for female subjects who become pregnant during study. See Section 8.5.4 for additional information.

- Other Reasons
  - If a subject misses, or is expected to miss, more than 1 sequential clinic visit, the investigator must contact the medical monitor to discuss continuation in the study. If it is determined that the subject must be discontinued the reason for termination, eg, termination related to COVID-19, must be documented as such in EDC (see Appendix 1).

4.5 Early Termination of Subjects From the Study

For subjects who discontinue study drug, every attempt should be made to complete the End-of-Treatment Visit assessments within 2 days after their last dose of study drug (Section 7.8). The 12-week corneal specular microscopy examination should be completed for subjects who discontinue participation in the study unless consent for this safety procedure is withdrawn. The corneal specular microscopy should be conducted approximately 12 weeks after the first dose of study drug; exceptions are to be discussed with the medical monitor.

Additional ophthalmologic examinations (standard ophthalmologic exams and/or corneal specular microscopies) may be conducted to monitor subject safety.

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5 STUDY TREATMENTS

5.1 Description of Treatments

The drug product BTRX-335140 is supplied for clinical study use as capsules and [redacted]

The placebo capsule product consists of a capsule size and color that matches the active drug product contains [redacted] exclusively.

5.2 Manufacturing, Packaging, and Labeling

BTRX-335140 and placebo capsules and packaging will be identical to maintain investigator and subject blinding. Clinical study materials will be labeled according to the regulatory requirements. Each bottle will contain 16 capsules of study drug with a unique 5-digit identification number.

5.3 Storage

[redacted] consistent with the product label. The drug product is stable when stored according to the labeling instructions.

5.4 Study Drug Administration

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

Subjects will also be instructed to refrain from taking their investigational study drug at home on the morning of Visit 4 (to allow PK sample to be withdrawn predose). Study drug will be administered at the site from the bottle dispensed at Visit 4. [redacted]

Subjects who cannot tolerate 1 capsule daily will be discontinued from participation in the study.

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5.5 Study Drug Compliance

Each subject will be instructed to return all study drug 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 subjects throughout the study.

Subject compliance with BTRX-335140 or placebo will be assessed by direct questioning and counting of returned capsules.

For subjects who demonstrate noncompliance (subject has taken <80% of the prescribed dosage for a visit interval), investigative sites must counsel subjects on the importance of study drug compliance. Any missed study drug or extra dose of study drug will be recorded as a protocol deviation.

Subjects with study drug (BTRX-335140 or placebo) compliance ≤70% over any 2-week visit interval (beginning with the Visit 2 [Baseline] to Visit 3 [Week 2] interval) will require early discontinuation.

5.6 Study Drug Accountability

The study drug provided for this study will be used only as directed in the study protocol. In accordance with current Good Clinical Practice (cGCP), investigators are required to maintain accurate and up-to-date records of all study drugs to permit reconciliation. The investigator or designee must maintain adequate records of distribution, including the date received, number and units received, manufacturer, lot numbers, dispensing, and return or destruction of all study drug (ie, accountability or dispensing logs).

All study drug records must be up to date and accurate for inspection by the site's clinical monitor and/or auditor. Instructions for returns, disposal, or destruction will be provided in the study reference manual and/or pharmacy manual.

If there are any issues during the course of the study related to the quality of the study drug, the investigator, clinical site pharmacist or pharmacy designee should contact the Sponsor or designated Contract Research Organization (CRO).

5.7 Prior and Concomitant Therapy

All nonpsychiatric medications, including over-the-counter medications and supplements, and also significant nonpharmacological therapies, taken by the subjects from the time of screening (ie, signing of informed consent) through the end of the Safety Follow-up period (ie, last contact) will be recorded in the subject's eCRF.

Previous and current MDD psychiatric therapy history (pharmacologic and nonpharmacologic) during the current episode will be recorded separately. Concomitant therapies will be reviewed for disallowed medications.

BTRX-335140 is a [redacted]. This

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restriction includes [redacted] (Appendix 2). However, nonsystemic (eg, topical creams, eye drops, mouthwashes) applications are permissible.

In vitro, BTRX-335140 is an [redacted]

In general, concomitant medications with primary CNS activity are not allowed in the study. The investigator should instruct the subject to notify the study site about any new medications he/she takes and about any significant nonpharmacological therapies administered after the start of the study drug (eg, acupuncture, hypnosis etc.).

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6 STUDY PROCEDURES AND ASSESSMENTS

Study procedures and their timing are summarized in the Schedule of Procedures and Assessments. Investigator/clinician-rated 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. Subject-rated assessments will be recorded using a tablet computer. The SCID and COWS are not included on the tablet; they are available only in paper form.

Rater consistency is critical in assessing the HAM-D, HAM-A, CGI-S, and CGI-I. Therefore, every attempt should be made, where possible, to have the same rater assess the subject at each visit to provide consistency in these ratings. It is important that the site strives to maintain rater consistency for the primary endpoint, the HAM-D. The CGI-S and CGI-I rater should also be the same rater and should take into account all available information in the assessment, particularly when the CGI rater is not rating the HAM-D and/or HAM-A.

6.1 Medical History and Demographics

Medical history and demographics will be collected at Screening and will be reviewed at the baseline visit.

6.1.1 Medical History

A complete medical history will be obtained at screening and will be reviewed prior to randomization to ensure subjects qualify for the study. Confirmation of the subject's MDD will also be obtained.

Psychiatric history (including a lifetime history of substance or alcohol abuse or dependence) will be assessed using the SCID-5-CT. The SCID-5-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 study.

Ophthalmologic history will be obtained at screening to assess general ocular disorders and specifically whether the subject has any of the following exclusionary conditions: 1) useful vision in only 1 eye from a pre-existing ophthalmic disease or amblyopia; 2) a corneal transplant in either eye; 3) corneal dystrophy or family history of corneal dystrophy; 4) severe dry eye syndrome [keratitis sicca]; or 5) history of severe adverse reaction to mydriatic agents. If a subject has narrow angle glaucoma, then there must be confirmation of definitive treatment with laser iridectomy as verified by the subject's ophthalmologist in order to be enrolled.

Nonserious AEs reported before the first dose of study drug will be recorded as medical history.

6.1.2 Demographics and Baseline Characteristics

Demographic information and baseline characteristics collected at Screening will include age, sex, race, ethnicity, height, weight, and BMI.

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6.2 Efficacy Assessments

6.2.1 Hamilton Rating Scale for Depression – 17-item Version

The HAM-D 17-item version (Hamilton 1967) will be the primary endpoint to evaluate changes in depression severity with treatment. This interviewer-administered semi-structured interview (Structured Interview Guide for the Hamilton Depression Rating Scale [SIGH-D], Williams 1988) is one of the most widely used instruments in depression treatment studies and its reliability and validity has been extensively studied. The HAMD-17 scoring ranges from 0 to 52 with individual items ranging from 0-4 points and 0-2 points to capture symptom severity. The HAMD-17 is validated for videoconference and telephone assessment (Kobak et al 2008).

6.2.2 Snaith Hamilton Pleasure Scale

The SHAPS is a 14-item self-report instrument which measures anhedonia (Snaith et al 1995). It has been shown to be valid and reliable in normal and clinical samples, with adequate construct validity, satisfactory test-retest reliability (intraclass correlation coefficient [ICC] = 0.70), and high internal consistency (Cronbach's alpha of 0.94) (Franken et al 2007). The scale will be completed by the subject and reviewed by site personnel qualified to oversee completeness. Each of the 14 items has a set of 4 responses, 2 of which endorse agreement (Definitely Agree, Agree) and 2 of which endorse disagreement (Disagree, Strongly Disagree). A total score can be derived by summing the responses items answered with strongly agree are coded as 1 while a strongly disagree response will be coded as 4. Therefore, scores on the SHAPS can range from 14 to 56, with higher scores corresponding to higher levels of anhedonia.

6.2.3 Clinical Global Impression Scale – Severity

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

6.2.4 Clinical Global Impression Scale – Improvement

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

6.2.5 Hamilton Anxiety Rating Scale

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 a semi-structured

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interview (Structured Interview Guide for the Hamilton Anxiety Rating Scale [SIGH-A], Williams 1988) his/her assessment of the subject. 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.

6.2.7 Hospital Anxiety and Depression Scale

The HADS measures levels of anxiety and depression without regards to somatic symptoms (Zigmond and Snaith 1983). This self-report scale consists of 14 items. Seven of the items are used to evaluate anxiety and 7 evaluate depression. Each item on the questionnaire is scored from 0 to 3. Therefore, the anxiety subscale (HADS-A) and the depression subscale (HADS-D) each range from 0 to 21. Higher scores indicate higher levels of anxiety and depression, respectively.

6.2.9 Sheehan Disability Scale

The Sheehan Disability Scale (SDS) was developed to assess functional impairment in 3 related domains of work/school, social, and family life. It is a brief self-rated tool where the subject rates the 3 items on an anchored 10-point visual analogue scale (Sheehan 1983). Following the subject's self-assessment, the rater should review the Work/School and Days Lost items to confirm appropriateness of the selected rating by evaluating the impact of MDD symptoms across those sectors. SDS may be administered via telephone assessment; however, the subject must be provided with a portable document format (PDF) copy of the assessment for reference of the visual analogue scale when selecting their ratings.

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6.3 Safety Assessments

6.3.1 Adverse Events

All AEs will be evaluated from the time the subject provides written informed consent through the last Safety Follow-up TC. Nonserious AEs reported before the first dose of study drug will be recorded as medical history (see Section 6.1.1). Serious AEs will be recorded on the AE eCRF from the time of informed consent. Additional safety information, including the definition of an AE/SAE/AESI and reporting requirements is provided in Section 8. Clinically significant findings for laboratory results, vital signs, ECGs, ophthalmologic examinations, and abnormal physical examination should be recorded as AEs; a clinical diagnosis, rather than the changes in laboratory analyte or other assessment should be recorded.

6.3.2 Clinical Laboratory Tests

6.3.2.1 Sample Collections

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

Abnormal laboratory values will be graded by the investigator as “clinically significant” or “not clinically significant,” where applicable. Clinically significant abnormal laboratory

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values will be reported as AEs as per Section 6.3.1 above). Investigators may repeat laboratory tests for any parameter that is abnormal and/or clinically significant.

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

Table 2: Clinical Laboratory Tests

Hematology[a]: Hemoglobin Hematocrit Erythrocyte count (red blood cell [RBC]) Mean cell volume (MCV) Mean cell hemoglobin concentration (MCHC) Leukocytes (white blood cell [WBC]) Neutrophils, segmented Absolute Neutrophil Count (ANC) Lymphocytes Monocytes Eosinophils Basophils Platelets Prothrombin time[b]	**Chemistry[a]:** Serum concentrations of: Sodium Potassium Bicarbonate Chloride Total bilirubin Direct bilirubin Alkaline phosphatase Alanine aminotransferase/serum glutamic pyruvic transaminase (ALT/SGPT) Aspartate aminotransferase/serum glutamic oxaloacetic transaminase (AST/SGOT) Gamma-glutamyl transferase (GGT) Blood urea nitrogen (BUN) Creatinine Uric acid Phosphorous Calcium Glucose (random) Albumin Total cholesterol Creatine kinase (CK)[c] Magnesium Amylase Lipase
Urinalysis[a]: Specific gravity pH Protein Glucose Ketones Blood Urine leukocyte esterase Urobilinogen	
Urine Drug Screen and Breathalyzer Alcohol Screen[a] Amphetamines Barbiturates Benzodiazepines Delta-9-tetrahydrocannabinol (THC) Cocaine Ethyl alcohol (via breathalyzer) Opioids Phencyclidine Propoxyphene Methadone	**Viral Serology[d]** Hepatitis B Surface Antigen (HBs Ag) Hepatitis B Core Antibody (HBc Ab) Hepatitis C Antibody (HC Ab) Hepatitis A Antibody (HAV-Ab [IgM]) Human immunodeficiency virus (HIV)
Thyroid function[d]: Thyroid-stimulating hormone (TSH); if abnormal perform Free T3 and Free T4 reflex testing	

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Pregnancy Test (females of child bearing potential only)[e]	[redacted]

[a] Performed at each visit.
[b] To be conducted at baseline only, unless indicated for hepatic monitoring testing (see Table 3)
[c] Creatine kinase is to be fractionated if CK results >1000 IU/L.
[d] Screening only.
[e] Serum pregnancy test at Visit 1 (Screening) is to be performed by a Sponsor-designated laboratory; urine pregnancy tests will be performed at all subsequent visits by the site.
[f] Baseline only; optional collection.
Note: All safety laboratory tests will be analyzed by a Sponsor-designated laboratory, unless otherwise specified.

6.3.2.2 Hepatic Safety and Enhanced Hepatic Testing

Bilirubin, ALT, and AST will be measured at each scheduled visit (as part of the scheduled clinical laboratory tests). If a subject has elevated ALT or AST (>3 x ULN) or elevated total bilirubin (>2 x ULN), the following procedures must be performed:

1. Contact the medical monitor/Sponsor to assess whether the subject should be discontinued from study drug (see Section 4.4).
2. Perform clinical laboratory testing according to the list of hepatic monitoring tests in Table 3 within 48 to 72 hours. The frequency of repeat testing to be performed will be determined in consultation with the medical monitor/Sponsor. In general, repeat laboratory testing should be performed 2 to 3 times per week; frequency of retesting can decrease to once a week or less if, the laboratory abnormality(ies) stabilizes and/or subject is asymptomatic.
3. Provide additional history from the subject including:
   - history of concomitant medication (prescription, nonprescription, over-the-counter, herbal supplements, dietary supplements)
   - alcohol use, recreational drug use, and special diets
   - recent illnesses, exposures, and exposures to environmental chemical agents

These results will be reviewed in a timely fashion by the Sponsor-designated medical monitor, along with the subject's clinical case, to determine possible cause.

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Table 3: Hepatic Monitoring Tests

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

Ab = antibody; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CPK = creatine phosphokinase; GGT = Gamma-glutamyltransferase; HAV-Ab [IgM] = hepatitis A virus antibody; HBc Ab = hepatitis B core antibody; HBs Ag = hepatitis B surface antigen; HCV Ab = hepatitis C virus antibody; Ig = immunoglobulin; INR = international normalized ratio; RBC = red blood cells; WBC = white blood cells.

[a] Assayed by a Sponsor-designated or local laboratory.

[b] Reflex/confirmation dependent on regulatory requirements and/or testing availability.

[c] Autoimmune hepatic antibodies and hepatic serologies need only be obtained at the time of the first hepatic monitoring test. Additional testing will be directed by the medical monitor/Sponsor.

6.3.2.3 Urine Drug Screen

The urine drug screen for drugs of abuse will include amphetamines, barbiturates, benzodiazepines, THC, cocaine, opioids, phencyclidine, propoxyphene, and methadone. This test will be done at the site using the dip sticks provided as well as at the central laboratory.

For occasional (1 to 2 times per month maximum) cannabis users only, 1 retest is allowed; a positive second test is exclusionary.

The site must contact the Sponsor-designated medical monitor or designee for preapproval before a retest of drugs other than THC that presented as positive; a retest will be permitted if the positive results can be explained by a valid prescription, and if there is no evidence of a substance use disorder. If the results from an allowed repeat urine drug screen prior to randomization are negative, the subject may be randomized. The investigator must be satisfied that the subject has been appropriately counseled regarding the restrictions of study participation and that the subject intends to comply. If the repeat results are positive, the subject must be excluded from the study. A second retest is not admissible.

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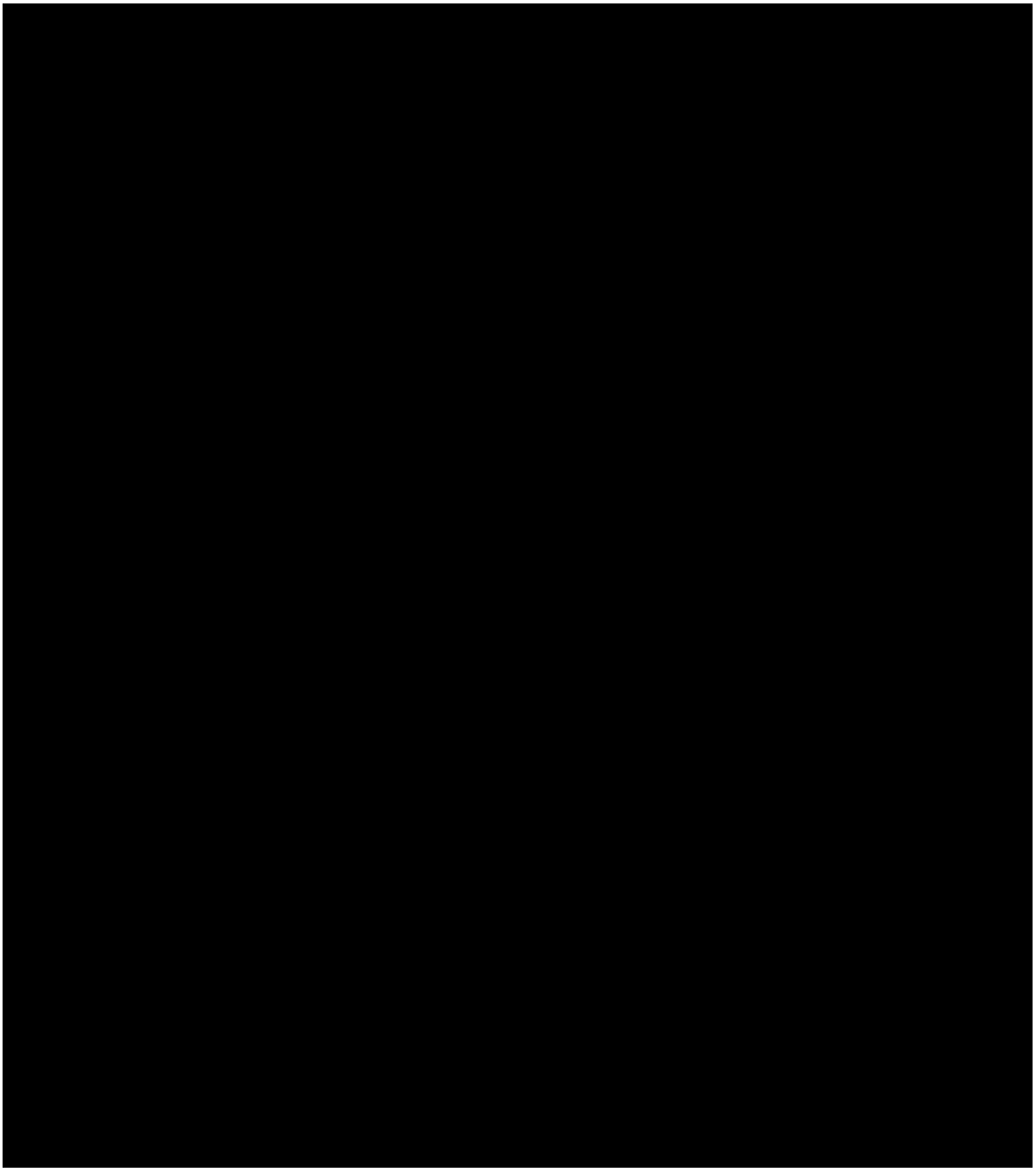
If a subject tests positive following randomization, the site must contact the Sponsor-designated medical monitor or designee, to discuss the subject's continued participation in the study. In the event that the subject is allowed to continue in the study, the investigator will document the discussion with the medical monitor and counsel the subject regarding study restrictions.

Blood alcohol level will be assessed by breathalyzer testing.



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6.3.3 Physical Examinations

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

Height and weight will be measured during the study as indicated in the Schedule of Procedures and Assessments. Height should be measured only once at screening with no shoes for correct measurement. The BMI will be calculated based on height measured at screening and weight measured at Visits 1, 2, 4, and 6.

6.3.4 Vital Signs

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

6.3.5 Electrocardiography

For each subject, single 12-lead digital ECGs will be collected after the subject has rested for 5 minutes. 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 subject is still present, to determine whether the subject meets entry criteria at the relevant visit(s) and for immediate subject management, should any clinically relevant findings be identified.

After enrollment, clinically significant increases in the QTcF interval from baseline (eg, QTcF ≥500 msec and/or QTcF increased ≥60 msec) or other clinically significant quantitative or qualitative changes from baseline should be reported as AEs. The subject will be assessed by the investigator for symptoms (eg, palpitations, near syncope, syncope) and to determine whether the subject can continue study drug. The investigator or qualified designee is responsible for determining if any change in subject 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.

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The cardiologist's ECG interpretation will be used for inclusion/exclusion determination for the screening ECG; the baseline ECG will be based on the investigator review and interpretation of the machine read for inclusion/exclusion determination at Visit 2 (Baseline). Exclusionary QT interval criteria, corrected using Fridericia's formula (QTcF), at Visit 1 (Screening) or Visit 2 (Baseline), are QTcF >450 msec in males or >470 msec in females. In the event that the QTcF is greater than the specified cutoffs and the investigator (in consultation with the medical monitor) determines that additional ECGs may aid in the further evaluation to determine enrollment at either screening or baseline visits, triplicate ECGs may be obtained. The average of the triplicate QTcF values may be used to evaluate eligibility at screening or baseline. If the average exceeds the protocol-specified cutoff, then the subject is not eligible for enrollment. At baseline, if central review subsequently determines the subject is not eligible by the cardiologist's interpretation, the subject must be bought in to discontinue.

When there are differences in ECG interpretation between the investigator (or qualified designee) and the cardiologist at the central ECG laboratory, the investigator (or qualified designee) should consult with the cardiologist at the central ECG laboratory. The investigator (or qualified designee) will be responsible for the medical management of the subject. 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.

6.3.6 Ophthalmologic Examinations

Ophthalmologic examinations will be conducted in this study as follows (further details will be described in an ophthalmologic examination manual):

1. Standard ophthalmologic examinations: to include best corrected visual acuity assessment, slit-lamp examination of both eyes including assessment of intraocular pressure, as well as dilation of the pupils to examine the optic nerve and retina
2. Corneal specular microscopy: noncontact or contact specular microscopy for qualitative and quantitative examination of the central corneal endothelium (cell count, cell shape, density, and morphology), with image capture

After confirmation that the subject has met all eligibility criteria, and is approved for randomization, the 2 examinations will be performed and will serve as baseline ophthalmologic examinations. The study site will refer the subject to an affiliated ophthalmologist. Ophthalmologic examinations will be conducted as per the Schedule of Procedures and Assessments and Section 7. Any ocular abnormalities at baseline should be recorded in medical history. Corneal specular microscopy results will be sent to a designated expert ophthalmology center for review and analysis. Additional examinations may be required in the event a subject has an ocular AESI (see Section 8.4).

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Any subject who discontinues from the study early must return to the clinic for the follow-up ophthalmologic examinations 12 weeks from the start of study drug.

6.3.7 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 2007). 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 to prospectively categorize suicide-related events. The 2009 "Baseline" version will be used at Visit 1 (Screening), evaluating past year and past 3 months, and the 2009 "Since Last Visit" version will be used for all subsequent visits.

Subjects who score 3 or above on Item 11 of the HAMD-17 must be further evaluated to determine is they are at risk or harm to themselves or others (see Section 4.4). If a suicide-related event is identified at any time during the study, a thorough evaluation should be performed by the investigator or suitably qualified study physician and appropriate medical care 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 subjects 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.

6.3.8 Opiate Withdrawal

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

The COWS will provide an ongoing assessment of subjects for any symptoms suggestive of opiate withdrawal. If at any time during the study, a score of 5 or more is obtained on the COWS, the investigator (or designee) must determine if the subject is experiencing opioid withdrawal. Any subject who is found to be dependent on opioids, abusing opioids, or experiencing opioid withdrawal should be withdrawn from the study and provided with appropriate medical care.

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7 TIMING OF PROCEDURES AND ASSESSMENTS

Information on study procedures and assessments is provided in Section 6. The timing for all assessments is provided in the Schedule of Procedures and Assessments.

Possible COVID-19 related changes in conduct of study: Study sites will maintain, at a minimum, COVID-19 risk mitigation procedures as per regulatory and local guidelines and/or laws. Allowed modifications to the conduct of the study, in the event of site staff and/or patient restrictions due to COVID-19, are outlined in Appendix 1 of the protocol. In addition, to reduce subject burden, subjects may take Visit 3 (Week 2), 4 (Week 4), or 5 (Week 6) as a virtual visit according to procedures outlined in Appendix 1.

7.1 Screening Period (Day –28 to Day–7)

Before any screening procedure is performed, subject informed consent must be obtained. Reinforce the need for the subject to cover skin, and to wear sunscreen and sunglasses when exposed to the sun, to minimize the potential risk of sun damage. After obtaining the subject's informed consent, the investigator will conduct/administer the screening assessments. Administration of the assessments in the order listed is strongly recommended. If any given criterion is not met, the screening should be stopped without completing any of the subsequent assessments.

1. Have subject sign ICF
2. Enter subject information in IWRS
3. Demographic details, height and weight
4. Breathalyzer for blood alcohol level
5. Subject and investigator will complete the following study eligibility criteria on the tablet supplied:
   a. HAMD-17
   b. HAM-A
   c. Allow subject to complete SHAPS; reviewed by site personnel qualified to oversee completeness
   d. [redacted]
   e. C-SSRS 'Baseline' version with lookback to past year and past 3 months
   f. CGI-S
   g. [redacted]
6. Medical/psychiatric history review
7. Complete the SCID-5-CT (including history of substance or alcohol abuse or dependence)

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8. Prior/concomitant therapies review and reporting
9. Previous and current MDD psychiatric therapy/pharmacologic/nonpharmacologic history
10. Physical examination
11. Vital signs (respiratory rate, BP, and pulse [supine and standing]; temperature)
12. COWS
13. 12-lead ECG
14. Blood and urine collection for central laboratory analysis (including urine drug screen for all subjects and serum pregnancy test for women of child-bearing potential, and blood for thyroid function tests, hepatitis screening, and hepatic monitoring)

The screening visit may be completed over 2 days for scheduling convenience 7 to 28 days prior to Visit 2 (Baseline). The 28-day screening period may be extended to 35 days (after consultation with and approval by the medical monitor), if necessary, for reasons including, but not limited to, discontinuation of a subject's current inefficacious medications or to obtain an ophthalmologic examination. A retest will be allowed for occasional cannabis users (ie, 1 to 2 times/month) only.

The investigator will review the results of the central laboratory tests for inclusion/exclusion criteria and alert the Sponsor-designated medical monitor to confirm eligibility. The investigator will be notified of the medical monitor's decision of the subject's eligibility prior to Visit 2 (Baseline). No subject is to be randomized without prior approval by the CAT team and medical monitor.

After eligibility is confirmed, and before randomization (Visit 2), the following assessments must be performed:

1. Standard ophthalmologic examination
2. Corneal specular microscopy

7.2 Visit 2: Baseline (Day 1)

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

1. Breathalyzer test for blood alcohol level
2. Subject and investigator will complete the following study eligibility criteria on the tablet supplied:
   a. HAMD-17
   b. HAM-A
   c. Allow subject to complete SHAPS; reviewed by site personnel qualified to oversee completeness
   d. [redacted]

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e. C-SSRS 'Since Last Visit' version

f. [redacted]

g. Allow subject to complete HADS; reviewed by site personnel qualified to oversee completeness

h. Allow subject to complete SDS; reviewed by site personnel qualified to oversee completeness

i. CGI-I and CGI-S

j. [redacted]

3. Concomitant therapies review and reporting
4. Physical examination if indicated
5. Weight
6. Vital signs (respiratory rate, BP, and pulse [supine and standing]; temperature)
7. COWS
8. 12-lead ECG
9. Urine drug screen for all subjects and urine pregnancy test for women of child-bearing potential

The investigator will confirm the subject's eligibility prior to completing the remaining randomization visit procedures. After confirmation, it is recommended that the remaining procedures be completed in the following order:

1. Complete randomization via IWRS
2. Collect blood and urine samples for central laboratory analysis, [redacted]
3. Dispense allocated study drug, and instruct subject on the importance of compliance and returning the dispensed bottle with any unused study drug at each visit. Instruct all subjects to take 1 capsule of their assigned study drug daily in the morning and preferably with food; administer the first dose of study drug, which should be taken under the supervision of clinical study personnel. It is recommended that subjects remain in the clinic for 1 hour after taking study drug.
4. AE reporting

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

Prior to Visit 3 (Week 2) (between Days 7 and 14 [+2 days]), a standard ophthalmologic examination should be performed (refer to the Schedule of Procedures and Assessments, footnote "j" and Section 6.3.6).

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7.3 Visit 3: Week 2 (Day 15)

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

1. Breathalyzer test for blood alcohol level
2. Subject and investigator will conduct/administer the following assessments on the tablet supplied:
   a. Allow subject to complete HADS; reviewed by site personnel qualified to oversee completeness
   b. C-SSRS 'Since Last Visit' version
   c. CGI-I
3. Concomitant therapies review and reporting
4. AE reporting
5. Physical examination if indicated
6. Vital signs (respiratory rate, BP, and pulse [supine and standing]; temperature)
7. COWS
8. The subject-returned investigational study drug will be accounted for and subject will be reinstructed if found to be out of compliance
9. Complete IWRS
10. Blood and urine collection for central laboratory analysis (including urine drug screen for all subjects and urine pregnancy test for women of child-bearing potential). Note: blood and urine may be collected earlier in the visit.
11. Dispense allocated study drug and instruct subject on the importance of compliance and returning the dispensed bottle with any unused study drug at each visit.
12. Instruct all subjects to take 1 capsule of their assigned study drug daily in the morning and preferably with food.

The investigator will review the results of the central laboratory tests for safety in a timely manner prior to the next visit.

7.4 Visit 4: Week 4 (Day 29)

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

1. Breathalyzer test for blood alcohol level
2. [redacted]
3. Administer 1 capsule from the bottle assigned to the subject.

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4. Subject and investigator will conduct/administer the following assessments on the tablet supplied:
   a. HAMD-17
   b. HAM-A
   c. Allow subject to complete SHAPS; reviewed by site personnel qualified to oversee completeness
   d. [redacted]
   e. Allow subject to complete HADS; reviewed by site personnel qualified to oversee completeness
   f. Allow subject to complete SDS; reviewed by site personnel qualified to oversee completeness
   g. C-SSRS 'Since Last Visit' version
   h. CGI-I
5. Concomitant therapies review and reporting
6. AE reporting
7. Physical examination, if indicated
8. Weight
9. Vital signs (respiratory rate, BP, and pulse [supine and standing]; temperature)
10. COWS
11. 12-lead ECG
12. The subject-returned investigational study drug will be accounted for and subject will be reinstructed if found to be out of compliance.
13. Complete IWRS
14. Blood and urine collection for central laboratory analysis (including urine drug screen for all subjects and urine pregnancy test for women of child-bearing potential)
15. Dispense allocated study drug and instruct subject on the importance of compliance and returning the dispensed bottle(s) with any unused study drug at each visit.
16. Remind all subjects to take 1 capsule of their assigned study drug daily in the morning and preferably with food starting the day after their clinic visit and continuing until their next clinic visit
17. [redacted]

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

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7.5 Visit 5: Week 6 (Day 43)

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

1. Breathalyzer test for blood alcohol level
2. Subject and investigator will conduct/administer the following assessments on the tablet supplied:
   a. Allow subject to complete HADS; reviewed by site personnel qualified to oversee completeness
   b. Allow subject to complete SDS; reviewed by site personnel qualified to oversee completeness
   c. C-SSRS 'Since Last Visit' version
   d. CGI-I
3. Concomitant therapies review and reporting
4. AE reporting
5. Physical examination if indicated
6. Vital signs (respiratory rate, BP, and pulse [supine and standing]; temperature)
7. COWS
8. The subject-returned investigational study drug will be accounted for and subject will be reinstructed if found to be out of compliance
9. Complete IWRS
10. Blood and urine collection for central laboratory analysis (including urine drug screen for all subjects and urine pregnancy test for women of child-bearing potential)
11. Dispense allocated study drug and instruct subject on the importance of compliance and returning the dispensed bottle with any unused study drug at each visit.
12. Remind all subjects to take 1 capsule of their assigned study drug daily in the morning and preferably with food

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

7.6 Visit 6: Week 8 (Day 57)/End of Treatment

Special effort will be made by the site to encourage subjects who discontinue from the study early to complete all Visit 6 (Week 8) safety assessments (within 2 days of their last dose of study drug), the Safety Follow-up TC, and ophthalmologic examinations (Section 7.8); efficacy assessments are to be performed only if the subject has remained on study drug. If the subject does not attend the discontinuation visit, the C-SSRS should be completed if the site has become aware of a potential suicide-related thought or behavior by other communications.

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Additional ophthalmologic examinations (standard ophthalmologic exams and/or corneal specular microscopies) may be conducted to monitor subject safety.

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

1. Breathalyzer test for blood alcohol level
2. Subject and investigator will conduct/administer the following assessments on the tablet supplied:
   a. HAMD-17
   b. HAM-A
   c. Allow subject to complete SHAPS; reviewed by site personnel qualified to oversee completeness
   d. [redacted]
   e. [redacted]
   f. Allow subject to complete HADS; reviewed by site personnel qualified to oversee completeness
   g. Allow subject to complete SDS; reviewed by site personnel qualified to oversee completeness
   h. C-SSRS 'Since Last Visit' version
   i. CGI-I and CGI-S
   j. [redacted]
   k. [redacted]
3. Concomitant therapies review and reporting
4. AE reporting
5. Physical examination if indicated
6. Weight
7. Vital signs (respiratory rate, BP, and pulse [supine and standing]; temperature)
8. COWS
9. 12-lead ECG
10. Standard ophthalmologic examination (± 2 days) (refer to Section 6.3.6)
11. The subject-returned investigational study drug will be accounted for.
12. Complete IWRS

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13. Blood and urine collection for central laboratory analysis (including urine drug screen for all subjects and urine pregnancy test for women of child-bearing potential)
14. [redacted]

The investigator will review the results of the central laboratory tests for safety in a timely manner.

7.7 Safety Follow-up

7.7.1 Telephone Call (Week 10)

Subjects will receive a TC from the study site approximately 2 weeks after the last dose of study drug to inquire about AEs and concomitant medication usage, and will administer the C-SSRS.

7.7.2 Ocular Examination (Week 12)

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

Additional ophthalmologic examinations (standard ophthalmologic exams and/or corneal specular microscopies) may be conducted to monitor subject safety.

7.8 Early Termination

Subjects who discontinue early from the study should be encouraged to complete the End of Treatment (Visit 6) safety assessments within 2 days after their last dose of study drug; efficacy assessments are to be performed only if the subject has remained on study drug. If the subject does return for an End of Treatment visit, the C-SSRS ‘Since Last Visit’ version should be completed if the site has become aware of a potential suicide-related thought or behavior by other communications (see Section 7.6).

Subjects who discontinue early from the study should plan to complete the Week 12 corneal specular microscopy (see Section 7.7.2).

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8 SAFETY MONITORING AND REPORTING

Investigators are responsible for the detection and documentation of events that meet the definition of an AE, an SAE, suspected adverse reaction, serious suspected adverse reaction, unanticipated problems, or AESIs, as provided in this protocol.

Investigators must review the BTRX-335140 IB to be aware of the safety-related events, which may be anticipated with its use. Investigators will also be versed in the latest standard of care guidelines.

8.1 Adverse Events

8.1.1 Definition of an Adverse Event

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

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

Adverse events beginning with the initial dose of study drug or during the subsequent duration of their enrollment in the study will be considered TEAEs. Treatment-emergent AEs will be recorded on the AE eCRF to include the event, date of onset, whether the AE is associated with an episode of self-harm, severity, frequency, seriousness, date of resolution, action taken with respect to the AE (eg, discontinue study drug, begin concomitant medication, begin nonpharmacological treatment, etc.), outcome, and relationship to the study drug. All TEAEs related to study drug will be followed to a satisfactory resolution or until the event becomes stable or can be explained by another known cause (ie, concurrent condition or medication).

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

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

8.1.2 Definition of a Serious Adverse Event

Serious AEs will be recorded on the AE eCRF from the time of informed consent. An SAE is any AE that results in 1 of the following outcomes, regardless of the investigator's opinion of causation:

- Death
- Initial or prolonged inpatient hospitalization

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

- A life-threatening experience (that is, immediate risk of dying)
- Persistent or significant disability/incapacity
- Congenital anomaly/birth defect
- Other medically important serious event as determined by the investigator (for example, an AE that jeopardizes the subject and may require medical or surgical intervention to prevent 1 of the outcomes listed in this definition)

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

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

8.1.3 Definition of Suspected Adverse Reaction

A suspected adverse reaction is defined as any AE for which there is a reasonable possibility that the AE was caused by the study drug.

8.1.4 Definition of Serious Suspected Adverse Reaction

A serious suspected adverse reaction is any suspected adverse reaction that is determined to be serious, based on the definition of an SAE in Section 8.1.2.

8.2 Classification of Adverse Events

8.2.1 Severity of Adverse Events

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

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- **Mild:** Usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.
- **Moderate:** Usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the subject.
- **Severe:** Interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.

8.2.2 Relationship to Study Drug

The investigator will assess the relationship (ie, causality) of each AE to study drug based on his/her clinical judgment. The investigator's assessment of an AE's relationship to study drug is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All AEs must have their relationship to study agent assessed. The Sponsor's assessment of relationship may differ from the investigator's assessment.

Relationship to study drug will be assessed according to the following guidelines:

- **Possibly related:** The AE is known to occur with the study drug, there is a reasonable possibility that the study drug caused the AE, or there is a temporal relationship between the study drug and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study drug and the AE.
- **Unlikely related**: There is not a reasonable possibility that the administration of the study drug caused the event, there is no temporal relationship between the study drug and event onset, or an alternate etiology has been established.

For initially reporting SAEs, even in situations in which minimal information is available, it is important that the investigator make an assessment of causality for every event. The causality assessment is 1 of the criteria used when determining regulatory reporting requirements. The investigator may change his or her opinion of causality in light of follow-up information and amend the SAE information accordingly in the eCRF or the SAE reporting form, as applicable.

8.3 Time Period and Frequency for Event Assessment and Follow-up

All AEs from the time of informed consent through the last follow-up visit will be recorded in each enrolled subject's eCRF (see Section 6.3.1). For subjects who prematurely discontinue from the study, AEs will continue to be recorded through the last follow-up visit. For subjects who are found to be ineligible for the study during the Screening period and are not enrolled (ie, Screening failures), AEs that are SAEs will be captured. Adverse events beginning with the initial dose of study drug or during the subsequent duration of their enrollment in the study will be considered TEAEs. Treatment-emergent AEs will be recorded on the AE eCRF to include the event, date of onset, whether the AE is associated with an episode of self-harm, severity, frequency, seriousness, date of resolution, action taken with respect to the AE (eg, discontinue study drug, begin concomitant medication, begin

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nonpharmacological treatment, etc.), outcome, and relationship to the study drug. All TEAEs related to study drug will be followed to a satisfactory resolution or until the event becomes stable or can be explained by another known cause (ie, concurrent condition or medication).

- All SAEs that occur after the informed consent is signed will be captured. Serious AEs reported after a subject has taken the last dose of study drug will be collected in the pharmacovigilance system for 30 days after the last dose of study drug. Thereafter, only SAEs that the investigator feels were related to the study drug or a protocol procedure must be reported.

8.4 Adverse Events of Special Interest

Adverse events of special interest (serious or nonserious) are TEAEs of particular safety importance and are required to be reported to the sponsor immediately (and no more than 24 hours after learning of the event, see Section 8.5 for reporting requirements).

Adverse events of special interest for this study are based on nonclinical findings of skin-related and ocular phototoxicity (see Section 1.1.3). Reported TEAEs that are classified in the system organ classes (SOCs) listed below will be considered AESIs:

- Skin and subcutaneous tissue disorders
- Eye disorders

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

Investigators will monitor for dermatologic or ocular events and for any change in visual acuity.

8.5 Reporting Procedures

8.5.1 Reporting Serious Adverse Events and Adverse Events of Special Interest

All SAEs and AESIs must be reported on a study-specific SAE/AESI form in addition to the AE eCRF by electronic data capture (EDC). **Study site personnel must alert the Sponsor-designated medical monitor of the SAE/AESI and must submit the completed study-specific SAE/AESI form to [redacted] within 24 hours of the site's awareness of the SAE/AESI**.

The investigator must also enter the SAE/AESI information into the eCRF as soon as possible thereafter.

In the initial email, the investigator must provide to the Sponsor the SAE/AESI form, completed to the greatest extent possible and the following pages from the eCRF:

- AE record

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- Medical history
- Prior and concomitant medications

Also, the following documents are to be forwarded: any laboratory results, diagnostic test results, or medical reports relevant to the SAE/AESI.

The investigator and supporting personnel responsible for subject care should discuss with the Sponsor-designated medical monitor or designee any need for supplemental investigations of SAEs/AESIs. The results of these additional assessments conducted must be reported to the Sponsor-designated medical monitor.

In the event of a medical emergency in which knowledge of the subject's treatment assignment may influence their clinical care the investigator has the option to unblind the subjects' treatment assignment via the IWRS system. The investigator should make every effort to contact the medical monitor prior to unblinding unless this would adversely delay appropriate medical care. The medical monitor will not be unblinded, they will only provide assistance to the investigator. The reasons for unblinding must be noted in the source documents. The investigator must not disclose the subject's treatment assignment to anyone who does not need the information due to their direct involvement in the subject's clinical care. Disposition of subjects who are unblinded due to a medical emergency will be determined following discussion with the Sponsor.

In the event of death, every effort should be made to obtain a death certificate and if possible, an autopsy report. If the cause of death is unknown, death will be recorded as the event.

The investigator is responsible for safety reporting in compliance with their IRB.

8.5.2 Regulatory Reporting Requirements

The investigator must promptly report all SAEs to the Sponsor in accordance with the procedures detailed in Section 8.5.1. The Sponsor has a legal responsibility to notify, as appropriate, both the local regulatory authority and other regulatory agencies about the safety of a product under clinical investigation. Prompt notification of SAEs by the investigator to the appropriate project contact for SAE receipt is essential so that serious suspected adverse reactions that are either unexpected or observed with increasing occurrence be reported and legal obligations and ethical responsibilities regarding the safety of other subjects are met.

Investigator letters are prepared according to Sponsor policy and are forwarded to the investigators as necessary. An investigator letter is prepared for a serious unexpected suspected adverse reaction (SUSAR) that is attributable to study drug. The purpose of the investigator letter is to fulfill specific regulatory and cGCP requirements regarding the product under investigation.

The investigator, or responsible person according to local requirements, must comply with requirements related to the reporting of SAEs to the IRB.

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The Sponsor is responsible for informing IRB, investigators, and regulatory authorities of any finding that could adversely affect the safety of subjects or affect the conduct of the study. Events will be reported to regulatory authorities in accordance with expedited reporting requirements.

8.5.3 Overdose

Overdose is defined as an accidental or intentional administration of more than the intended dose of study drug. 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 IB.

8.5.4 Pregnancy Reporting

If a female subject or the female partner of a treated male subject becomes pregnant, the subject must notify the investigator within 24 hours of learning of the pregnancy. The investigator must make every effort to ensure that the subject or the pregnant female is aware of the need to notify her healthcare provider regarding her male partner's participation in this clinical study and his potential exposure to BTRX-335140.

The study site must complete a pregnancy form and send to the Sponsor or designee within 24 hours of learning of the pregnancy. The study site will make every effort to follow the pregnancy until outcome is known.

8.6 Safety Oversight

The Sponsor-designated medical monitor and the study team will monitor blinded safety data throughout the course of the study as detailed in the medical monitoring plan. This will include review of AEs, SAEs, AESIs, ECG findings, clinical laboratory results, vital signs, ophthalmologic findings, 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.

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9 STATISTICAL CONSIDERATIONS

9.1 General Considerations

Full details on the statistical methodology to be used will be included in a statistical analysis plan (SAP) to be developed prior to study unblinding.

For the purpose of all safety analyses where applicable, baseline is defined as the last measurement prior to the start of study drug administration. For efficacy analyses, baseline is defined as the arithmetic mean of pretreatment measurements ie, Screening and Baseline, prior to the first administration of study drug, or as the last measurement prior to the first administration of study drug if only single value is available.

Continuous endpoints will be summarized with n, mean, standard deviation (SD), median, minimum and maximum. In addition, change from baseline and percent change from baseline values will be calculated at each time point and summarized descriptively. For categorical endpoints, descriptive summaries will include counts and percentages.

No adjustments will be made for multiple comparisons.

All statistical comparisons will be 2-sided with alpha of 0.05.

SAS version 9.4 or higher will be used to analyze data.

9.2 Analysis Populations

The safety analysis will be conducted on the Safety Population. Efficacy analyses will be conducted on the Efficacy population and the per-protocol (PP) population. The Efficacy population will be used for the primary efficacy analysis. [redacted] Subjects will be analyzed according to randomized treatment assignment for efficacy assessments and according to treatment received for safety assessments.

9.2.1 Safety Population

The Safety population is defined as all subjects who received study drug and will be used to summarize safety data.

9.2.2 Efficacy Population

The Efficacy population includes all randomized subjects who received at least 1 dose of study drug, have a baseline HAMD-17 assessment value, and have at least 1 post-baseline assessment. This population will be used to summarize the primary and other efficacy data.

9.2.3 Per-protocol Population

The PP population includes subjects in the efficacy population who did not experience any major protocol deviations that impact primary efficacy endpoint analysis.

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9.2.4 [redacted]

[redacted]

9.3 Statistical Analysis Methods

9.3.1 Disposition

Frequency counts and percentages of subjects who were screened, randomized, completed the treatment, or discontinued early will be presented by treatment group. Reasons for discontinuation will be summarized.

9.3.2 Demographics and Baseline Characteristics

Demographic and baseline characteristics including age, sex, BMI, race, ethnicity, height, weight, CGI-S, baseline HAMD-17, duration of current depressive episode, antidepressant treatment during the current episode, and other medical history, physical examinations, and prior and concomitant medications will be summarized using descriptive statistics. Prior and concomitant medications will be summarized by World Health Organization Drug Classification (WHODrug) for Drug Statistics Methodology.

9.3.3 Efficacy Analyses

9.3.3.1 Primary Efficacy Analysis

The primary efficacy analysis is a comparison of mean reduction in HAMD-17 scores between BTRX-335140 and placebo at Week 8. The analysis will be conducted using a Mixed-Models Repeated Measures (MMRM) with change from baseline in HAMD-17 score as the dependent variable and will include treatment group, Week, and Week by treatment group interaction as factors, and baseline HAMD-17 score as covariate. Variance estimation will be based on an unstructured covariance matrix. The model-based Week 8 least square (LS) means, standard errors, 95% confidence intervals (CIs), and *p*-values will be reported.

9.3.3.2 Secondary Efficacy Analyses

Similar to the method described above, a MMRM model will be calculated for the change from baseline to each timepoint assessed for comparisons between BTRX-335140 and placebo for the measures listed below. The model-based time points' LS means, standard errors, 95% CIs, and *p*-values will be reported. Alternatively, ANCOVA may be used, as appropriate. The following measures will be analyzed:

- CGI-I
- CGI-S
- SHAPS
- HADS subscales: HADS-A and HADS-D

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- HAM-A total score
- SDS

For CGI-I, an additional analysis comparing the percentage of subjects with responses at Week 4 and Week 8 of 'much improved' or 'very much improved' between BTRX-335140 and placebo will be performed using generalized estimating equations (GEE). The model will include treatment, baseline CGI-S score, week, and week by treatment group interaction. An exchangeable working correlation structure will be assumed.

The change from baseline to Week 8 in CGI-S will be estimated using an ANCOVA model, with main effects for treatment group and a covariate adjustment for baseline score.

The response rates (the percentage of subjects with ≥ 50% decrease in HAMD-17 from baseline to Week 4 and Week 8) will be compared between BTRX-335140 and placebo-treated groups using Cochran-Mantel-Haenszel test. Additional categories may be explored. [redacted].

9.3.4 Safety Analysis

Safety data, including AEs, safety laboratory results, physical examination results, , vital signs, suicidality, COWS scores, and ECG will be summarized by treatment group (placebo and BTRX-335140) and/or listed.

9.3.4.1 Adverse Events

Adverse events occurring after the first dose of study drug will be considered to be TEAEs. The incidence of TEAEs will be summarized by seriousness, severity, and causality to study drug. Treatment-emergent AEs leading to discontinuation will also be summarized.

Adverse events with onset before first study dose and after last study dose will be listed.

9.3.4.2 Adverse Events of Special Interest

Adverse events of special interest for this study are based on nonclinical findings of skin-related and ocular phototoxicity (see Section 1.1.3 and Section 8.4). AESIs will be summarized by SOC/preferred term.

9.3.4.3 Vital Signs

Changes from baseline will be summarized for temperature, pulse (supine and standing), respiratory rate (supine and standing), and BP (supine and standing) by treatment and visit.

9.3.4.4 Physical Examinations

Clinically significant physical examination abnormalities will be listed.

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9.3.4.5 Clinical Laboratory Tests

Changes from baseline will be summarized for each laboratory test by study treatment and visit.

9.3.4.6 Electrocardiography

Electrocardiographic parameters will be listed. Clinically significant abnormalities on ECGs will be listed by subject and visit.

9.3.4.7 Ophthalmology

Ophthalmologic endpoints will be summarized. Clinically significant abnormalities on ophthalmologic exam will be listed by subject and visit.

9.3.4.8 Columbia Suicide Severity Rating Scale

Suicidality will be listed for all subjects.

9.3.4.9 Clinical Opiate Withdrawal Scale

COWS scale items and total scores, along with the numbers of subjects with a score of ≥5, will be summarized at each visit by treatment group.

9.3.4.10 Treatment Compliance with Study Drug

A subject is defined to be compliant if he/she has taken ≥80% and <120% of the number of doses expected to be taken during the study overall. Noncompliant subjects will be listed by treatment group.

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9.3.6 Subgroup Analyses

A subgroup analysis based on gender may be performed. Subgroup analyses based on other characteristics will be considered and included in the SAP.

Efficacy endpoint analyses, which exclude the subjects (n=24) enrolled at the time of the study enrollment shutdown due to COVID-19, will be performed (Section 9.5). Details of these analyses will be included in the SAP.

The impact of the efficacy endpoints ascertainment (in-person vs telephone) will be explored in additional analyses, if a large imbalance in ascertainment method between treated and placebo subjects is present at study visits. Details of these analyses will be included in the SAP.

9.4 Interim Analysis

No interim analyses are planned for this study.

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10 QUALITY ASSURANCE AND QUALITY CONTROL

Quality assurance and quality control systems will be implemented and maintained with Standard Operating Procedures (SOPs) by the Sponsor and/or its designee(s), as appropriate, to ensure that the clinical study is conducted and the data are generated, documented (recorded), and reported in compliance with the protocol, International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH)-GCP E6(R2) guidelines, and applicable regulatory requirements. The accuracy, completeness, and reliability of the study data presented to the Sponsor, however, are the responsibility of the investigator. The investigator or designee must record all required data using the prespecified data collection method defined by the Sponsor or its designee.

The study will be monitored regularly by the Sponsor (Section 8.6) and may be audited or inspected by the Sponsor (or designee), IRB, and/or regulatory authorities at any time during the study or after study completion. In the event of an audit, the investigator agrees to allow the Sponsor, representatives of the Sponsor, the competent authority, or other regulatory agencies direct access to all study records. The investigator will immediately notify the Sponsor of all audits or inspections scheduled by any regulatory authority and promptly forward copies of any audit or inspection reports received to the Sponsor.

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11 REGULATORY AND ETHICAL CONSIDERATIONS

11.1 Regulatory Considerations

This study will be conducted in accordance with 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, the ICH GCP Guideline [E6], and other 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 CRO.

11.2 Ethical Conduct of the Study

This study will comply with the requirements that are enunciated in the European Clinical Trial Directive 2001/20/EC and in the US Code of Federal Regulations (CFR).

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 cGCP.

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.

11.3 Institutional Review Board Approval

This study will be conducted in full compliance with the IRB regulations in 21 CFR 56. Before enrollment of subjects into the study, the protocol, informed consent documents and any subject related advertising will be reviewed and approved by the appropriate IRB and regulatory authority. Amendments to the protocol will be submitted to the same IRB and regulatory authority review requirements as the original protocol. The investigator will promptly notify the IRB and Sponsor of any SAEs or of any other information that might affect the safe use of the investigational product during the study. Institutional Review Board approvals positive opinions and regulatory authorities' approvals must be sent to the Sponsor, or its designee, before initiation of the study or before an amendment is instituted. All correspondence with the IRB and the regulatory authority must be retained in the study regulatory files.

11.4 Informed Consent Process

Written informed consent from each subject must be obtained before any study-specific Screening or Baseline period evaluations are performed. If any new information becomes

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available that might affect patients' willingness to participate in the study, or if any amendments to the protocol require changes to the informed consent/assent form, the Sponsor will provide Investigators with a revised informed consent/assent form. One copy of the signed informed consent documents will be given to the subject; the investigator will retain the original copies of these documents.

The informed consent document, as prepared by the Sponsor or designee, must be reviewed and approved by the IRB before initiation of the study. The informed consent document must contain the basic required elements of consent and additional elements, as applicable, as specified in the 21 CFR 50.25.

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

The ICF will be used to explain the potential risks and benefits of study participation to the subject in simple terms before the subject is entered into the study, and to document that the subject 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 subject. 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 study drug.

11.5 Confidentiality

All information regarding the nature of the proposed investigation that is provided to the investigator by the Sponsor, the Sponsor's designee, or the study monitor, with the exception of information that is required by law or regulations to be disclosed to the IRB/IEC, the subject's parent(s) or legal guardian(s) or the appropriate regulatory authority, must be kept in confidence by the investigator in accordance with current Health Insurance Portability and Accountability Act (HIPAA) standards and/or European standards.

The anonymity of participating subjects will be maintained to the extent required by applicable laws and in accordance with current HIPAA standards. Subjects may be referenced by their initials and an assigned subject identification number on the CRFs and other data collected by the Sponsor. The investigator must maintain all documents related to the study that identify the subject (eg, the signed informed consent document) in strict confidence, except to the extent necessary to allow auditing by the appropriate regulatory authorities, the IRB/IEC, the study monitor, or the Sponsor or its representatives.

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12 STUDY ADMINISTRATION

12.1 Clinical Monitoring

Electronic CRFs will be completed for all study subjects enrolled in the study. At scheduled monitoring visits, eCRFs will be verified against source documentation. In the event that on-site scheduled monitoring cannot be conducted, the monitoring of data in the eCRF will be detailed in the clinical monitoring plan. Upon completion of this review, finalized eCRFs will be submitted to the Sponsor's designated CRO. Any subsequent changes to the eCRFs will be performed in accordance with the CRO's SOPs for editing and clarifying eCRFs.

Data entry will be performed through username and password-protected access to a secure database. All data will be entered using electronic eCRFs. Internally developed programs for plausibility, consistency, and out-of-range data fields will supplement the review of the data. A 100% manual review of AEs, drug accountability and termination summary data, will be performed by Data Management personnel. The Medical Dictionary for Regulatory Activities (MedDRA) coding thesaurus will be used to classify AEs and medical history, and the WHODrug classification will be used to code medications.

After all subjects complete the study and data discrepancies are resolved, and prior to study unblinding, protocol deviations during both enrollment and study execution will be reviewed. Significant protocol deviations and procedural discrepancies will be discussed and major deviations will be identified and reported.

12.2 Source Documents and Record Retention

Essential study documents are among the critical documents required before study enrollment is to occur. Essential documents, as well as supplemental information such as the IB, Pharmacy Manual, CRF Completion Guidelines, final protocol, as specified in the Clinical Operations Manual and/or Regulatory Binder, must be kept on-site in a designated study site file.

The study site files will also contain, including but not limited to, subject accountability records, drug accountability (receipt/dispensing) records, Sponsor/investigator correspondence, IRB/IEC correspondence, deviations, biological sample records, and SAE and IND safety reports/Safety Alert Letters/SUSARs.

12.3 Management of Protocol Amendments and Deviations

All processes and procedures defined in this protocol must be adhered to. Emergency departures from the protocol that eliminate an apparent immediate hazard to a particular subject and that are deemed by the investigator as crucial for the safety and wellbeing of that subject may be instituted for that subject only and documented as deviations. The investigator will contact the medical monitor as soon as possible regarding such a deviation. These departures do not require preapproval by the IRB/IEC; however, the IRB and medical monitor must be notified in writing as soon as possible in accordance with the IRB policies after the departure has been made.

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Study records must be retained for at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. However, these documents should be retained for a longer period if required by applicable regulatory requirements or if agreed to in the Clinical Trial Agreement. It is the responsibility of the Sponsor to inform the site as to when these documents no longer need to be retained.

12.3.1 Protocol Modifications

The protocol cannot be modified except in a formal protocol amendment by the Sponsor.

12.3.2 Protocol Violations and Deviations

Protocol deviations are a change, divergence, or departure from the study design or procedures defined in this protocol. An Important Protocol Deviation is a deviation that may significantly impact the completeness, accuracy, and/or reliability of the study data or that may significantly affect a subject's rights, safety, or well-being. The investigator will notify the IRB of any protocol deviations as required by IRB guidelines and site requirements. Protocol deviations will be documented at the site and in the Sponsor files. In the event of an Important Protocol Deviation, the site will notify the Sponsor or designee. The Sponsor is responsible for notifying the regulatory authorities of any protocol deviations, as required.

12.4 Financial Disclosure

Investigators are required to inform the Sponsor of all disclosable financial interests or arrangements (including those of their spouse and dependent children), prior to study initiation at the site, at study completion, and 1 year after study completion in accordance with 21 CFR Part 54. In addition, the investigator or sub-investigators must promptly notify the Sponsor if there are any reportable changes that occur during the above-described period.

Disclosable financial interests or arrangements, or the absence thereof will be recorded on the Financial Disclosure for Clinical Investigators Form.

Any investigator(s) added as investigational staff to the Food and Drug Administration (FDA) 1572 form must complete the Financial Disclosure for Clinical Investigators Form at the start of his/her participation in the study. The Financial Disclosure for Clinical Investigators Form for any investigator(s) leaving the study prior to completion will also be obtained.

12.5 Stopping Criteria: Suspension or Termination of Study or Investigational Site

The Sponsor may suspend or terminate the study, or part of the study, at any time for any reason. If the study is suspended or terminated, the Sponsor will ensure that applicable sites, regulatory agencies, and IRBs/ECs are notified as appropriate.

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If the Sponsor, investigator, or officials from regulatory agencies discover conditions arising during the study that indicate that the study should be halted or that a study site should be closed, this action may be taken after appropriate consultation between the Sponsor and investigator(s).

12.6 Publication and Information Disclosure Policy

The information that is developed during the conduct of this clinical study is considered to be strictly confidential. This information may be disclosed only as deemed necessary by BlackThorn. At the conclusion of this clinical study, a clinical study report will be prepared. In addition, a manuscript may be prepared for publication in a reputable scientific journal under the direction of the Sponsor. BlackThorn will publish and communicate the clinical study results, irrespective of positive or negative findings. Data generated for this study will be exclusively owned by BlackThorn, as detailed in the Clinical Trial Agreement. The study will be registered on ClinicalTrials.gov. After completion of the study, results will be disseminated through ClinicalTrials.gov.

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APPENDIX 1: GUIDANCE FOR VIRTUAL VISITS (VIA TELEPHONE)

The guidance below is intended for subjects who have been successfully enrolled and randomized into the study and who elect to conduct a virtual visit to reduce subject burden or who are unable to attend in-person clinic visits due to COVID-19 restrictions.

Every reasonable effort should be made to ensure that the subjects continue on study per protocol; however, in the event that a subject cannot come to the clinic after the baseline visit due to self-quarantine, local restrictions, illness, or other reasons as described in the protocol, the site personnel may conduct a virtual (by telephone contact) visit. Clinical laboratory tests are to be performed at a minimum every 4 weeks. Up to 2 nonsequential virtual visits between Visits 3 and 5 may be allowed. If the subject is unable to return for Visit 6, the subject should return to the site, at their earliest convenience, for an End of Treatment visit (see Sections 4.4 and 7.8).

Conducting Study Procedures for the Virtual Visit

The following procedures are to be completed via the telephone (see Table A1-1):

- The following clinician-completed assessments can be performed via the telephone: HAM-A, HAMD-17, CGI-S, CGI-I, and C-SSRS. Raters will need to document, in the tablet, if data were collected via phone (vs in person).
- The following subject-rated scales will be conducted over the phone: [redacted], HADS, SDS, [redacted], and SHAPS. Subjects must have the PDF copy of the SDS with them during the assessment. Following the subject's self-assessment on the SDS, the rater should review the Work/School and Days Lost items to confirm appropriateness of the selected ratings by evaluating the impact of MDD symptoms across those sectors. Site personnel will enter the information directly into the tablet on behalf of the subject and will indicate, in the tablet, that this information was captured over the phone.
- The following will also be discussed with the subject: AEs and concomitant medications, and alcohol and drug use; details will be captured in the source data. As the COWS cannot be fully administered by phone (as some items require direct observations), in capturing AEs site staff should be alert to symptoms that require further enquiry to determine if there is any possibility of opioid withdrawal.
- Study drug compliance will be discussed with the subject; however, pill count will be completed at the time of the next visit when the subject returns to the clinic.

The above assessments are required to be done as listed. [redacted]

When subjects cannot come into the clinic and a virtual visit is performed, all other assessments including laboratory testing, vital signs measurements, and ECG will be noted as unable to be completed.

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Table A1-1: Procedures for Virtual Visit

Period	Virtual Visit	
Visit	3 – 5	Guidance
Procedure and Assessments		
Vital signs	NA	
Physical examination	NA	
Body weight	NA	
12-Lead ECG	NA	
Standard ophthalmologic examination	NA	
Corneal specular microscopy	NA	
Concomitant medication/therapy	X	Review concomitant medications and therapy; document in source
Clinical laboratory tests (hematology, chemistry, urinalysis)	NA	Every effort should be made to have the subject return to the site to obtain clinical laboratory tests at Visit 3
Serum/urine pregnancy test	NA	
Urine drug screen and breathalyzer alcohol screen	NA	Review recreational drug use and alcohol consumption document; document in source
[redacted]	[redacted]	[redacted]
CGI-S	X	Rater to assess over the telephone
CGI-I	X	Rater to assess over the telephone
C-SSRS	X	Rater to assess over the telephone
HAMD-17	X	Rater to assess over the telephone
HAM-A	X	Rater to assess over the telephone
[redacted]	[redacted]	[redacted]
SDS	X	Ensure the subject has access to a PDF copy of the scale. Rater to enter subject scores. Following this assessment, the rater should review the Work/School and Days Lost items to confirm appropriateness of the selected ratings by evaluating the impact of MDD symptoms across those sectors.
HADS	X	Rater to enter subject scores
SHAPS	X	Rater to enter subject scores
[redacted]	[redacted]	
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	
COWS	NA	
Adverse events	X	Investigator to assess; in capturing AEs site staff should be alert to symptoms that require further enquiry to determine if there is any possibility of opioid withdrawal.
Complete IWRS	X	To be done after the subject has confirmed inability to attend the visit in person, then the study drug will be sent to the subject's home
Dispense study drug	X	Dispense as per site specific policy
Study drug compliance	X	Discuss compliance with subject; pill count to be performed at time of the return

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AE = adverse event; CGI-I = Clinical Global Impression of Improvement; CGI-S = Clinical Global Impression of Severity; COWS = Clinical Opiate Withdrawal Scale; C-SSRS = Columbia Suicide Severity Rating Scale; ECG = electrocardiogram; HADS = Hospital Anxiety and Depression Scale; HAM-A = Hamilton Anxiety Rating Scale; HAMD-17 = Hamilton Rating Scale for Depression - 17-Item Version; IWRS = Interactive Web-response System; MDD = major depressive disorder; NA = not applicable; PDF = portable document format; [redacted]; SDS = Sheehan Disability Scale; SHAPS = Snaith-Hamilton Pleasure Scale; [redacted].

Dispensing Study Drug

Subjects may choose to pick up the study drug from the site if that option is available at the site. If subjects are not able to come to the site, the IWRS will be completed in a timely manner to ensure that the subject gets the next bottle of study drug, which will be couriered to the subject's home prior to the current supply running out. Delivery of the study drug will be tracked by the courier and documentation will be provided to the site. The subject will retain their current bottle of medication and any unused supplies, and the prior and new bottle will be returned at their next clinic visit. The courier documentation will be maintained in the pharmacy file to track the distribution of the product, and the subject will be instructed to confirm the receipt of the study drug. This will be documented in the source documents for this subject.

If a subject is required, or is predicted to need, to miss more than 1 sequential clinic visit, they will be instructed to discontinue study drug and asked to return to the clinic for safety evaluation when appropriate and safe to do so.

End of Study Visit

At the early termination visit (see Section 7.8), End of Treatment assessments (see Section 7.6) will be conducted at the clinic. The reason for termination will be documented in EDC and, if appropriate, note that termination was due to the COVID-19 situation (see Section 4.4).

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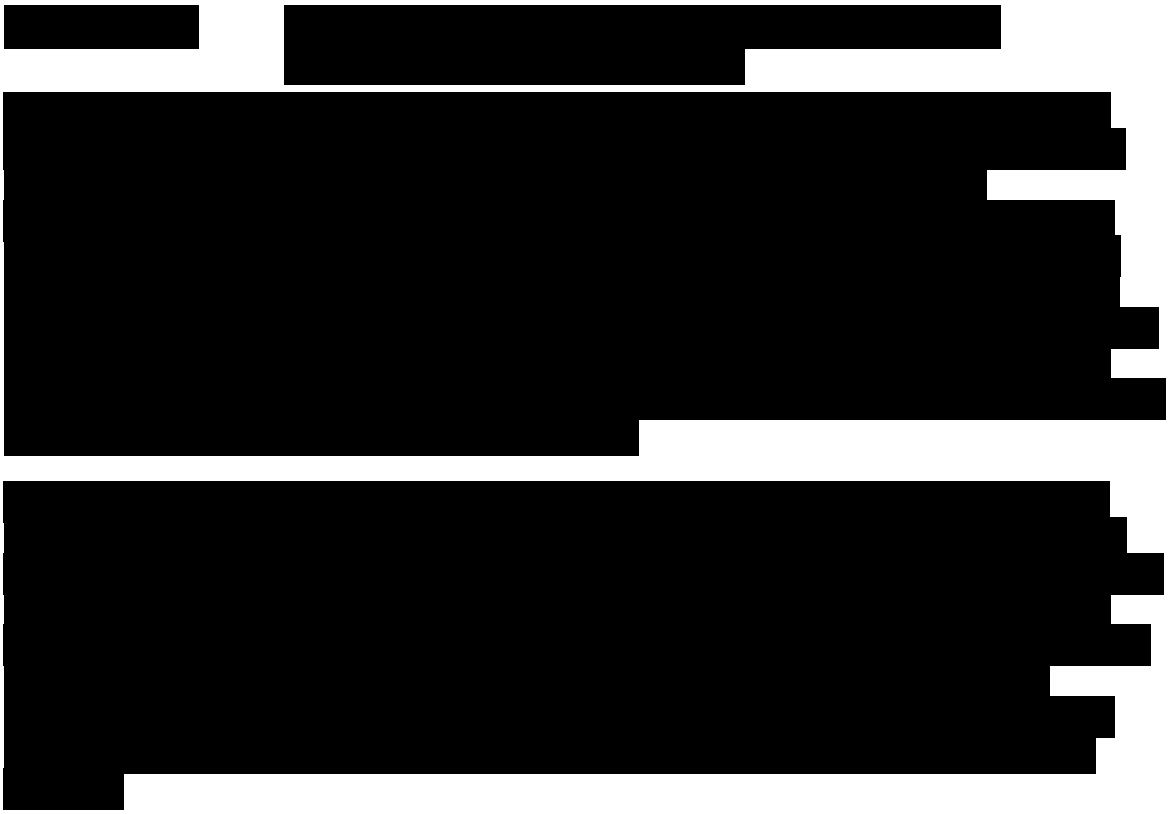

The listed [redacted] below are not exhaustive. See also: http://www.fda.gov/drugs/developmentapprovalprocess/developmentresources/druginteractionslabeling/ucm093664.htm.

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Table A2-1: [redacted]

[redacted]