CLINICAL STUDY PROTOCOL

Protocol Title:	A Biomarker-Guided, Randomized, Double-Blind, Placebo-Controlled Efficacy and Safety Study of Liafensine in Patients with Treatment-Resistant Depression
Protocol Number:	DB104-01
Protocol Name:	ENLIGHTEN
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Study Sponsor:	Denovo Biopharma LLC 10240 Science Center Drive, Suite 120 San Diego, CA 92121 USA
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STATEMENT OF CONFIDENTIALITY

SPONSOR APPROVAL

Sponsor's Approval

The protocol has been approved by Denovo Biopharma LLC.

Responsible Medical Officer: Larry Alphs

DocuSigned by:
Larry Alphs
Signer Name: Larry Alphs
Signing Reason: I approve this document
Signing Time: 02-Sep-2022 | 11:19:16 AM PDT
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02-Sep-2022

Company/Sponsor Signatory
Larry Alphs
Sr VP, CNS Clinical Development
Denovo Biopharma LLC

Date

PROTOCOL SYNOPSIS

Name of Sponsor/Company:

Denovo Biopharma LLC
10240 Science Center Drive, Suite 120
San Diego, CA 92121
USA

Name of Investigational Product: Liafensine **(**DB104; previously BMS-820836)

Name of Active Ingredient: Liafensine

Protocol Number: DB104-01

Title of Study: A Biomarker-Guided, Randomized, Double-Blind, Placebo-Controlled Efficacy and Safety Study of Liafensine in Patients with Treatment-Resistant Depression

Study Center(s): Multiple

Phase of Development: 2b

Rationale:

Major depressive disorder (MDD) is a common psychiatric disorder, with a lifetime prevalence rate of approximately 13-17% in the United States (Hasin 2005, Kessler 2005). Although options for pharmacologic treatment have expanded significantly in the past 25 years, between one-third and two-thirds of patients will not respond to the first antidepressant prescribed, and up to 33% will not respond to multiple interventions (American Psychiatric Association 2013, Berlim 2008, Cain 2007). Novel antidepressants that produce a clinically significant improvement in patients with MDD who have failed to respond to more than one treatment of adequate dose and duration, without producing significant side effects, would represent an important advance in the treatment of treatment-resistant depression (TRD).

For most of the last 30 years, the biogenic amine hypothesis of depression has been driving much of the research into the etiology and treatment of this debilitating psychiatric disorder. Most conventional antidepressants are antagonists at serotonin (5-HT) and/or norepinephrine reuptake sites, and their efficacy correlates reasonably well with their affinity for these receptors. At present, the most commonly used treatments for MDD are the selective serotonin reuptake inhibitors (SSRIs), such as escitalopram, or the serotonin-norepinephrine reuptake inhibitors (SNRIs), such as duloxetine. Recently, a compound with a novel mechanism of action, esketamine (SPRAVATO®), was approved in the United States (US) for the treatment of TRD in conjunction with an oral antidepressant (SPRAVATO 2019). While the precise mechanism of action of esketamine is unknown, the compound is an N-methyl-D-aspartate (NMDA) receptor antagonist. Esketamine use is associated with significant adverse effects including dizziness, nausea, sedation, and psychological effects, including dissociative/perceptual changes. Because of the risks of dissociation with esketamine, the US Food and Drug Administration (FDA) has established a Risk Evaluation and Mitigation Strategy (REMS) requiring that patients be monitored by a healthcare professional for at least 2 hours at each treatment session, concluding with an assessment establishing that the patient is clinically stable and may safely leave the healthcare setting.

This adverse event profile, together with esketamine's REMS requirements, limits its clinical utility, and supports the continued development of novel compounds for the treatment of TRD.

Liafensine (also known as DB104 and previously BMS-820836) is 6-[(4*S*)-1,2,3,4-tetrahydro-2-methyl-4-(2-naphthalenyl)-7-isoquinolinyl]-3-pyridazinamine and it is a potent and selective inhibitor of the reuptake of the 3 monoamines: 5-HT, norepinephrine, and dopamine. Liafensine was developed to incrementally advance antidepressant treatment by synergizing these individual monoaminergic approaches to treating depression.

Liafensine has been tested in 11 Phase 1 studies, and 3 Phase 2b studies (including one rollover study). In the Phase 1 studies, single doses of up to 5 mg, and multiple doses of up to 4 mg liafensine were found to be generally safe and well tolerated. The pharmacokinetic (PK) profile of liafensine was compatible with once-daily (QD) dosing, supporting the potential for liafensine to have low potential for discontinuation symptoms. In the Phase 2b studies, patients who did not experience adequate response to either duloxetine (Study CN162006), or duloxetine or escitalopram (Study CN162007), were randomized to receive either liafensine or duloxetine (CN162006), or duloxetine or escitalopram (CN162007), for 6 weeks. In Study CN162006, a flexible dose of liafensine was used (0.5-2 mg QD). In Study CN162007, several fixed dosage levels of liafensine were used (0.25, 0.5, 1, and 2 mg QD). In these Phase 2b studies, liafensine was found to be generally safe and well tolerated. In Study CN162006, the most common adverse events (AEs) reported by at least 5% of patients treated with liafensine were headache (7.0%), nausea (7.0%), constipation (5.3%), and anxiety (5.3%). In Study CN162007, the most common AEs reported by at least 5% of patients treated with any dose of liafensine were headache (14.3%), nausea (12.0%), nasopharyngitis (8.8%), upper respiratory tract infection (8.0%), dry mouth (8.0%), constipation (7.7%), dizziness (7.0%), diarrhea (5.5%), fatigue (5.9%), and urinary tract infection (5.5%).

In the Phase 2b studies conducted by Bristol-Myers Squibb (BMS), there were no significant differences noted between the treatment groups in the decrease (improvement) of the mean Montgomery-Åsberg Depression Rating Scale (MADRS) total score. In an effort to identify a potential pharmacogenomic biomarker of response to liafensine, Denovo Biopharma LLC (Denovo) has conducted a genome-wide scan with human whole genome single nucleotide polymorphism (SNP) arrays using germline deoxyribonucleic acid (DNA) extracted from blood samples of patients in BMS Studies CN162006 and CN162007. From this genome-wide screening, SNP rs12217173 was identified as strongly associated with treatment response to liafensine ($p = 6.6 \times 10^{-8}$). Patients with a GG genotype at rs12217173 showed significantly better response to liafensine compared to patients with an AA or AG genotype, or patients in the control arm (duloxetine or escitalopram). These findings were replicated using a separate validation sample set. Liafensine treatment effects in patients with the GG genotype were comparable for the 0.5 to 2 mg QD flexible doses in the CN162006 study and for the 1 mg QD and 2 mg QD doses in the CN162007 study. These combined data showed that patients with the GG genotype had a 4.7-point difference in MADRS total score decrease from baseline in the liafensine arm vs the control arm ($p = 0.025$), while patients with the AA or AG genotype at rs12217173 did not show an improved treatment effect in the liafensine arm vs the control arm (MADRS 0.3-point difference, $p = 0.74$). Moreover, patients carrying different genotypes at rs12217173 did not exhibit different treatment responses to duloxetine or escitalopram. Thus, SNP rs12217173 appears to be a pharmacogenomic biomarker specific for liafensine; Denovo has termed rs12217173 as "DGM4" (Denovo Genomic Marker 4) and the GG genotype of DGM4 is defined as DGM4 positive, whereas the AA or AG genotype of DGM4 is defined as DGM4 negative.

Objectives:

Primary Efficacy Objective

- To demonstrate that liafensine is superior to placebo in DGM4-positive patients with TRD as assessed by the change in MADRS total score from baseline to Day 42 of double-blind treatment

Key Secondary Efficacy Objective

- To evaluate the change from baseline to Day 42 in DGM4-positive patients with TRD treated with liafensine vs placebo on the Clinical Global Impressions Scale-Severity (CGI-S)

Safety Objectives

- To compare the safety and tolerability of liafensine vs placebo in all randomized patients with TRD who received at least one dose of study drug during double-blind treatment
- To evaluate safety for liafensine 1 mg QD and 2 mg QD vs placebo in both DGM4-positive and DGM4-negative patients

Other Secondary Efficacy Objectives

- To evaluate the Clinical Global Impressions Scale-Improvement (CGI-I) at Day 42 in DGM4-positive patients with TRD treated with liafensine vs placebo
- To evaluate the change from baseline to Day 42 in DGM4-positive patients with TRD treated with liafensine vs placebo on the patient-reported functionality with Sheehan Disability Scale (SDS)

Exploratory Objectives

- To compare the proportion of DGM4-positive patients with TRD who respond to liafensine after 6 weeks of treatment vs placebo (response is defined as ≥ 50% improvement from baseline in MADRS total score at Day 42)
- To compare the proportion of DGM4-positive patients with TRD who are in remission after 6 weeks of treatment with liafensine vs placebo (remission is defined as MADRS total score ≤ 10 at Day 42)
- To evaluate the change from baseline to Day 42 in DGM4-positive patients with TRD treated with liafensine vs placebo on MADRS anhedonia subscale and individual items
- To compare the efficacy of liafensine vs placebo in DGM4-negative patients with TRD, as assessed by change in MADRS total score from baseline to Day 42
- To evaluate efficacy for liafensine 1 mg QD and 2 mg QD vs placebo in both DGM4-positive and DGM4-negative patients
- To evaluate the PK of liafensine as part of the population PK analyses

Endpoints:

Primary Endpoint

- Change in MADRS total score from baseline to Day 42, in DGM4-positive patients

Key Secondary Endpoint

- Change in CGI-S from baseline to Day 42, in DGM4-positive patients

Safety Endpoints

- Adverse events as characterized by type, frequency, severity, timing, seriousness, and relationship to study therapy
- Laboratory abnormalities as characterized by type, frequency, severity, and timing
- Vital signs including supine (after at least 5 min rest) and standing (after standing 1 min and 3 min), blood pressure and heart rate, weight, electrocardiogram (ECG), clinical laboratory evaluation, Discontinuation-Emergent Signs and Symptoms scale (DESS) scale, and Columbia Suicide Severity Rating Scale (C-SSRS) data

Other Secondary Endpoints

- CGI-I at Day 42, in DGM4-positive patients
- Change in SDS from baseline to Day 42, in DGM4-positive patients

Exploratory Endpoints

- Treatment response (defined as ≥ 50% improvement from baseline in MADRS total score at Day 42) in DGM4-positive patients
- Remission (defined as MADRS total score ≤ 10 at Day 42) in DGM4-positive patients
- Change in MADRS anhedonia subscale and individual items from baseline to Day 42, in DGM4-positive patients
- Change in MADRS total score from baseline to Day 42, in DGM4-negative patients
- Change in MADRS total score from baseline to Day 42 in 1 mg QD group and 2 mg QD group, in both DGM4-positive and DGM4-negative patients

Study Design:

This study will be conducted as a multicenter, randomized, double-blind, placebo-controlled Phase 2b trial to assess the efficacy, safety, tolerability, and PK of liafensine in DGM4-stratified TRD patients. The study includes an up to 21-day screening period.

Approximately 180 patients with TRD who meet all eligibility criteria will be randomized in a 1:1:1 ratio to receive either liafensine 1 mg QD, liafensine 2 mg QD, or placebo QD for 6 weeks. Approximately 150 randomized patients carrying genotype GG at DGM4 (DGM4-positive) and approximately 30 randomized patients carrying genotype AA or AG at DGM4 (DGM4-negative) will be enrolled in this study. DGM4 status (positive vs negative) of randomized patients will be blinded to the sites, the patients, and sponsor. An Interactive Response Technology (IRT) system will be used for blinding DGM4 status, and to achieve the planned randomization ratio of DGM4-positive:DGM4-negative patients by randomly excluding most screened DGM4-negative patients (~80%). Randomization will be stratified by DGM4 status (positive vs negative) and region (North America vs Other). Patients who complete the study or discontinue after randomization will have two post-study follow-up visits (14 days after the last administered dose of study drug, and 28 days after the last administered dose of study drug); the 2nd follow-up visit will be conducted via phone call. Every effort will be made to ensure that the protocol-required tests and procedures are completed.

During the study, safety will be assessed on the basis of spontaneously reported AEs; the DESS; physical, neurological, and psychiatric examinations (including the C-SSRS); vital signs including supine and standing blood pressure and heart rate; weight; 12-lead ECGs; clinical laboratory test results; serum and urine pregnancy tests (females of childbearing potential only); and concomitant medication assessments. In addition to blood samples collected for PK, additional blood samples may be collected for determination of plasma concentrations of liafensine and its primary metabolite in association with the evaluation of safety signals.

The overall study design is shown in Figure 1. The schedule of study assessments is presented in Table 1 with the clinical laboratory tests shown in Table 3.

To ensure the safety of patients enrolled in this study, an Independent Data Monitoring Committee (IDMC) will monitor data on an ongoing basis. The IDMC will comprise individuals external to the sponsor and will consist of at least one medical expert in the relevant therapeutic area, at least one hepatologist, and at least one statistician.

Methodology:

This is a randomized, double-blind, placebo-controlled Phase 2b trial to assess the efficacy, safety, tolerability, and PK of liafensine. The primary endpoint is change in MADRS total score from baseline (Visit 3, Day -1) to Visit 7 (Day 42) in DGM4-positive patients. The primary analysis will be conducted to assess the superiority of liafensine treatment (combined liafensine 1 mg QD and 2 mg QD doses) vs placebo in randomized patients who are DGM4-positive.

Sample Size Justification:

Eligible patients will be randomized 1:1:1 to receive liafensine 1 mg QD, liafensine 2 mg QD, or placebo QD. The primary analysis is to assess treatment difference in MADRS total score change from baseline (Visit 3, Day -1) to Visit 7 (Day 42), between the combined liafensine 1 mg QD and 2 mg QD doses vs placebo, in DGM4-positive patients. A sample size of 47 randomized DGM4-positive patients per arm is sufficient to detect a 4.5-unit difference between the combined liafensine 1 mg QD and 2 mg QD treatment groups and the placebo treatment group in the change in MADRS total score with 80% power at the 2-sided 0.05 alpha level, assuming a standard deviation of 9 units.

To compensate for potential loss of power due to randomized patients who do not take at least one dose of study drug or who do not have a post-randomization efficacy evaluation (~5%), a total of approximately 150 DGM4-positive patients (50 DGM4-positive patients per arm) will be randomized.

To examine trend in the total patient population and in the subgroup of DGM4-negative patients in order to evaluate DGM4 as a potential biomarker for predicting response to liafensine, approximately 20% additional DGM4-negative patients (10 per arm, total approximately 30) will also be randomized. The sample size of 30 randomized patients in the DGM4-negative group is not estimated based on formal statistics in terms of power and alpha level.

Sample size re-estimation may be conducted if the percentage of missing data at Day 42 visit is higher than expected and/or if the observed variance is higher than the one assumed in the sample size estimation.

Number of Patients (planned):

~180

Diagnosis and Criteria for Inclusion:

Subject Inclusion Criteria:

Patients must meet all of the following inclusion criteria to be eligible for enrollment into the study:

1. Provide signed informed consent which includes pharmacogenomic (PGx) testing.
2. Have a diagnosis of MDD without psychotic features, according to the *Diagnostic and Statistical Manual of Mental Disorders,* 5th Edition (DSM-5) criteria, based on clinical assessment and confirmed by the Mini International Neuropsychiatric Interview (MINI).
3. TRD documented in the Massachusetts General Hospital (MGH) Antidepressant Treatment Response Questionnaire (ATRQ) (5-year version). That is:
   a. clinically meaningful inadequate response (estimated < 50% improvement per investigator/patient consensus and documented by the investigator) with:
      1) at least 2 treatment courses with antidepressant regimens;
      2) used at least 2 different pharmacologic treatment classes (eg, SSRI, SNRI, TCA, MAOI, atypical); or if only one class treatment is used, at least one combination treatment course;
      3) the doses have been given at accepted therapeutic doses for an adequate duration (at least 6 weeks), per product label;
      4) One of these inadequate response must occur within the current episode.

   Note: non-pharmacological treatment (eg, cognitive behavioral therapy, electroconvulsive therapy, repetitive transcranial magnetic stimulation, vagus nerve stimulation, acupuncture) are not counted as treatment regimen.
4. To be eligible, patients must have DGM4 genotype results obtained from the designated Clinical Laboratory Improvement Amendments (CLIA) lab, and all eligible DGM4-positive

patients and about 20% DGM4-negative patients will be randomly included by an IRT system in order to achieve the appropriate randomization ratio of DGM4-positive vs negative patients.

5. Pregnancy conception limitations
   - Female patients must be postmenopausal or surgically sterile or, if of childbearing potential and the partner is not vasectomized (6 months minimum), must agree to use a medically acceptable form of contraception from the time of signing the informed consent form (ICF) through at least 60 days following the last administration of study drug. If only the barrier method is used, a double barrier must be employed. Postmenopausal women must have had ≥ 24 months of spontaneous amenorrhea. Surgically sterile women are defined as those who have had a hysterectomy, bilateral ovariectomy, or bilateral tubal ligation. All women of childbearing potential must have a negative pregnancy test result before administration of study drug.
   - Male patients must be biologically incapable of having children (eg, vasectomized) or must agree to use the above forms of birth control for themselves and their partner from the time of signing the ICF through at least 120 days following the last administration of study drug.
6. Be fluent in the local language.
7. Male or female aged 18 to 70, inclusive, at time of Visit 1.
8. Have a HAMD-17 total score ≥ 21 at screening and baseline.
9. Be willing to discontinue the use of antidepressant drugs (including over-the-counter medications to treat depression [eg, St John's Wort]) at least 5 half-lives (or at least 1 week for herbal or other over-the-counter medications for depression) prior to baseline (Day -1). For fluoxetine, a washout period of at least 3 weeks for ≤ 20 mg/day and at least 4 weeks for > 20 mg/day is required.

Subject Exclusion Criteria:

Patients with any of the following characteristics/conditions will be excluded from the study:

1. Prior participation in a study with liafensine.
2. Used any investigational drug product, device, or biologic within 3 months or 5 half-lives (whichever is longer) prior to baseline (Day -1).
3. A positive pregnancy test result or currently breastfeeding.
4. Clinically significant and/or unstable illness (including chronic, persistent, or acute infection), medical/surgical procedure, or trauma within 30 days prior to screening or between screening and baseline (Day -1) as determined by the investigator.
5. A history or presence of a clinically significant and/or unstable hepatic, renal, gastrointestinal, cardiovascular, endocrine, respiratory, immunologic, hematologic, dermatologic, or neurologic abnormality, or any other condition, that in the investigator's opinion, represents potential risk to the patient's safety and ability to fully participate in the study, or affects the absorption, distribution, metabolism, or excretion of liafensine.
6. Presence of autoimmune hepatitis, primary sclerosing cholangitis, untreated hepatitis C, active hepatitis B, or any other uncontrolled or unstable liver disease according to local guidance.
7. Uncontrolled human immunodeficiency virus (HIV) infection according to local guidance.
8. Uncontrolled abnormal thyroid function according to local guidance.

9. One or more clinical laboratory evaluations are outside the reference range, at screening, that are in the investigator's opinion, of potential risk to the patient's safety.
10. Has at the Screening Visit:
    - Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) levels > 1.5x the upper limit of normal (ULN) at screening.
    - Total bilirubin (TBL) > 2 mg/dL (34.2 μmol/L) at screening, unless there is an explained indirect hyperbilirubinemia, eg, Gilbert's syndrome.
    - Alkaline phosphatase (ALP) > 1.5x the ULN at screening.

    Note: Laboratory tests can be repeated to see if values return to normal range, but any such laboratory abnormality must be resolved by the Baseline Visit (Day -1).
11. Clinically significant vital sign abnormality at screening. This includes, but is not limited to, the following, in the supine (after at least 5 min rest) and standing (after 1 min and 3 min standing): systolic blood pressure ≥ 140 mmHg; diastolic blood pressure ≥ 90 mmHg; or heart rate < 50 or > 90 beats per minute. If the initial blood pressure is ≥ 140/90 mmHg, the lowest value from up to 3 additional attempts, which also must not be ≥ 140/90 mmHg, should be used. Patients with symptomatic orthostatic hypotension, at the discretion of investigator, will be excluded.
12. Corrected QT interval measurement according to the Fridericia rule (QT_cF) > 450 msec for men and > 470 msec for women during controlled rest at screening, or history of long-QT syndrome.
13. ECGs containing any of the following readings:
    - Left bundle branch block
    - Right bundle branch block with QRS duration > 140 ms
    - Intraventricular conduction defect with QRS duration > 140 ms
    - Long QT syndrome
14. History of seizure, other than childhood febrile seizures.
15. History of clinically significant head trauma, including closed head injury with loss of consciousness, that is, in the opinion of the investigator, likely to affect central nervous system function.
16. History of clinically significant symptomatic orthostatic hypotension (ie, postural syncope).
17. History of narrow angle glaucoma.
18. History of cancer within 2 years prior to screening or between screening and baseline (Day -1), except for non-metastatic basal and/or squamous cell carcinoma of the skin.
19. Use of prescription or nonprescription medications for attention-deficit hyperactivity disorder (ADHD), narcolepsy, or cognitive enhancement (eg, methylphenidate, atomoxetine, modafinil, ginkgo biloba, and huperzine A) within 30 days prior to screening or between screening and baseline (Day -1).

20. Regular consumption of (eg, more days than not) excessive quantities of xanthine-containing beverages (eg, more than five cups of coffee or the equivalent per day) within 30 days prior to screening or between screening and baseline (Day -1).
21. Urine drug screen (UDS) positive for a drug of abuse, with the exception of cannabis in countries where it is legally available (see Table 3 for list of drugs of abuse). Where legal, prior use of cannabis is permitted provided the patient agrees to abstain from smoking or ingesting cannabis or cannabis products during the study.
22. Use of potent inducers of CYP3A4 (eg, rifampin, rifabutin, phenytoin, carbamazepine, or phenobarbital) within 2 weeks prior to baseline (Day-1).
23. Current diagnosis or history of a psychotic disorder, MDD with psychotic features, manic or hypomanic episode of bipolar or related disorders.
24. Current diagnosis of anxiety disorder (if primary), post-traumatic stress disorder, obsessive-compulsive disorder (if primary), intellectual disability (DSM-5 diagnostic code 319), borderline personality disorder, antisocial personality disorder, histrionic personality disorder, or narcissistic personality disorder according to the DSM-5 criteria, or any other psychiatric or neurologic disorder or symptom due to a general medical condition, that, in the judgement of the investigator, could pose undue risk to the patient or compromise the study.
25. Hospitalized or discharged from psychiatric ward within 8 weeks prior to the screening visit and planned hospitalization for any condition(s) during the study.
26. Moderate or severe alcohol use disorder or other substance use disorder (except nicotine or caffeine), within 6 months prior to screening, according to the DSM-5 criteria.
27. Significant risk of suicide determined by:
    a. Acute suicidality as evidenced by answering “yes” to Question 5 (“In the Past Year”) on the C-SSRS, indicating active suicidal ideation with specific plan and intent for suicide, at screening, or baseline (Day -1); or
    b. History of suicidal behavior as indicated by a “yes” response on the Suicidal Behavior section of the C-SSRS (“In the past year”) or
    c. A score ≥ 5 on Item 10 (suicidal thoughts) of the MADRS at screening or baseline (Day -1); or
    d. Has attempted suicide within 6 months prior to the initial screening visit.
28. Previous allogenic bone marrow transplant.
29. Received non-leukocyte-depleted whole blood transfusion within 4 months prior to PGx testing at Screening.
30. Currently employed by the sponsor or by a clinical trial site participating in this study, or a first-degree relative of an employee of the sponsor or of an employee at a participating clinical trial site.

Concomitant Treatment and Study Restrictions:

Prohibited concomitant treatments and study restrictions during the study from baseline to 14 days after the last dose of study drug (the first half of the follow-up) include:

1. Any investigational drug product, device, or biologic.
2. Any prescription or nonprescription medication for ADHD, narcolepsy, or cognitive enhancement (eg, methylphenidate, atomoxetine, modafinil, ginkgo biloba, and huperzine A).
3. Any psychopharmacologic drug (including antidepressants and over-the-counter medications to treat depression (eg, herbal medicines like St John's Wort, or other local herbal medicines).
4. Potent inducers of CYP3A4 (eg, rifampin, rifabutin, phenytoin, carbamazepine, or phenobarbital).

Permitted concomitant treatments, provided they meet the specific requirements detailed below, during the study and during the indicated periods include:

1. Up to 3 g/day paracetamol/acetaminophen.
2. Non-psychopharmacologic drugs provided the dose and condition being treated have been relatively stable for at least 2 months prior to screening and between screening and baseline (Day -1) and are not expected to change meaningfully during the study.
3. Soporific drugs may be used provided the dose and condition being treated have been relatively stable for at least 2 months prior to screening and between screening and baseline (Day -1) and are not expected to change meaningfully during the study.
4. Psychotherapy (including cognitive behavioral therapy) is permitted provided psychotherapy was initiated at least 2 months prior to screening and is expected to remain relatively stable during the study.
5. Anxiolytic drugs which do not affect 5-HT, norepinephrine, and dopamine activity may be used provided the dose and condition being treated have been relatively stable for at least 2 months prior to screening and between screening and baseline (Day -1) and are not expected to change meaningfully during the study.

Note 1: if a pro re nata (PRN) use has been established prior to the study, and not expected to change during the trial, this is considered stable treatment.

Note 2: Medication considered necessary for the patient's safety and well-being may be given at the discretion of the investigator and recorded in the appropriate sections of the electronic Case Report Form (eCRF). If a psychopharmacologic medication that is otherwise prohibited is administered the study measurements such as MADRS and CGI should be collected prior to dosing when possible. If any medication that is otherwise prohibited is administered to a patient, the investigator should inform the medical monitor prior to administration of medication when possible.

Other restrictions include:

1. Patients will be required to maintain their normal intake of xanthine-containing foods or beverages, and, if permitted, vitamin and herbal supplements, during the study, including the screening and follow-up periods.
2. Patients should not provide blood or plasma donations between screening and baseline (Day -1), during the study and for 6 weeks after the last dose of study drug.

Investigational Product, Dosage, and Mode of Administration:

Investigational product (study drug) will be supplied as tablets containing 1 mg liafensine or matching placebo. Study drug will be taken orally, once daily in the morning (AM). Patients will be instructed to take the study drug whole, and not to chew, divide, or crush the tablets.

Randomization Phase:

Liafensine 1 mg QD

- Day 1 through Day 42: one 1 mg liafensine tablet and one matching placebo tablet QD

Liafensine 2 mg QD

- Day 1 through Day 7: one 1 mg liafensine tablet and one matching placebo tablet QD
- Day 8 through Day 42: two 1 mg liafensine tablets QD

Placebo QD

- Day 1 through Day 42: two placebo tablets QD

Further details regarding the packaging, storage, handling, and administration of study drug will be provided in the study Pharmacy Manual.

Duration of Study Participation:

The duration of participation for individual patients will be up to 91 days or longer, including an up to 21-day screening period (screening period may exceed 21 days if a prolonged washout of prior drug is required), a 6-week double-blind treatment period, and 2 post-study follow-up visits (at 14 days and 28 days after the last dose of study drug).

Reference Therapy, Dosage and Mode of Administration:

None.

Criteria for Evaluation:

Efficacy:

The primary efficacy outcome measure is the change in MADRS total score from baseline (Visit 3, Day -1) to Visit 7 (Day 42) in DGM4-positive patients.

Other assessments include CGI-S, CGI-I and SDS. See Table 1 for the overall schedule of efficacy assessments. Note that CGIs should be completed only after the Patient Rated Depression and Functioning Checklist (PRDF Checklist) has been completed as instructed.

Pharmacokinetics:

Blood samples will be collected for determination of concentrations of liafensine and its primary metabolite on Day 28 (Visit 6) and on Day 42 (Visit 7) prior to dosing. The time of prior dose and time of blood collection will be recorded.

If the patient discontinues before Day 42 (Visit 7), a blood sample will be collected for PK on the last day the patient takes a full dose of study drug or as soon as possible thereafter, but within 3 days after the last dose in any case. When possible, and if medically appropriate, blood samples should be collected for PK from patients who experience a serious adverse event (SAE) or overdose.

Pharmacogenomics:

PGx testing is required to determine eligibility for the study. PGx analysis may determine the association between genetic variations and the patient's response to treatment with liafensine.

Safety:

Safety will be assessed based on spontaneously reported AEs; the DESS scale; physical, neurological, and psychiatric examinations (including C-SSRS); vital signs including orthostatic changes in blood pressure and heart rate; weight; 12-lead ECGs; clinical laboratory test results; serum and urine pregnancy tests (females of childbearing potential only); and concomitant medication assessments. Safety assessments in addition to those specified may be performed to ensure patient safety if judged appropriate by the investigator.

See Table 1 for the overall schedule of safety assessments.

Psychiatric assessments will be performed by a psychiatrist or trained and certified clinical staff member. Neurologic assessments will be performed by an experienced clinician.

Patients fulfilling Hy's law, defined as [ALT or AST ≥ 3 × ULN] and [TBL ≥ 2 × ULN], in the absence of significant increase in ALP and in the absence of an alternative diagnosis that explains the increase in total bilirubin, will be discontinued, with medical follow-up as appropriate.

An IDMC will be established to monitor data on an ongoing basis to ensure the continuing safety of patients enrolled in this study. The IDMC will comprise individuals external to the sponsor and will consist of at least one medical expert in the relevant therapeutic area, at least one hepatologist, and at least one statistician. The IDMC's responsibilities, authorities, and procedures, with details of decision-making guidance and dissemination of the results, will be documented in the IDMC charter.

Statistical Methods:

Efficacy Analyses:

Efficacy analyses will be performed on the Full Analysis Set (FAS), which includes all randomized patients who took at least one dose of study drug and had a post-randomization efficacy evaluation. The FAS population will be analyzed by treatment randomized regardless of the actual treatment received.

The primary and secondary efficacy results of the study will be based on analysis in FAS patients with DGM4-positive biomarker.

The primary efficacy endpoint is the change in MADRS total score from baseline (Visit 3, Day -1) to Day 42 (Visit 7), in DGM4-positive patients. The primary efficacy analysis will be performed at 2-sided significance level of 0.05 using a mixed model for repeated measures (MMRM) that includes the fixed effects of treatment, visit (categorical covariate), treatment-by-visit interaction, baseline MADRS total score (continuous covariate), and region. An unstructured variance-covariance matrix will be used. The least-squares (LS) mean change in MADRS total score at Day 42 from baseline for the combined liafensine treatment arms (1 mg QD and 2 mg QD) and placebo arm will be estimated from the MMRM model. Prior work suggests that both doses of liafensine may be efficacious, therefore, the treatment effect for the combined liafensine treatment arms vs placebo will be estimated using the LS means' difference at Day 42 from the MMRM model. The comparison of the change in MADRS total score from baseline to Day 42 between each of the 2 liafensine treatment arms and placebo will be carried out using the same approach.

The key secondary efficacy endpoint will be the change from baseline in CGI-S at Day 42 in DGM4-positive patients. CGI-S will be analyzed using the same MMRM model as for the primary efficacy endpoint.

Other secondary efficacy endpoints include CGI-I and SDS at Day 42 as continuous endpoints. These endpoints will be analyzed using the same MMRM model as for the primary efficacy endpoint.

The exploratory efficacy results of the study will also be based on analysis in FAS patients.

Response rate, remission rate, and corresponding 95% confidence interval (CI) for each treatment

group will be derived by using Clopper-Pearson method. The comparison of response rates and remission rates between treatment groups at Day 42 will be carried out using the Cochran–Mantel–Haenszel (CMH) test.

Subscales, individual items, and domains of endpoints will also be analyzed in an exploratory manner, using the same MMRM model as for the primary efficacy endpoint.

The change in MADRS total score from baseline in DGM4-negative patients will be analyzed using the same approach as described for the primary endpoint analysis in the DGM4-positive patients. The LS mean of the change in MADRS total score for each treatment group and the treatment difference in DGM4-negative patients will be estimated. The treatment effects between the DGM4-positive and negative patients will be compared primarily in descriptive fashion to examine the trend, and may be evaluated by including the interaction of DGM4 status and treatment group in the mixed model.

Safety Analyses:

Safety analyses will be conducted in the safety population, which includes all randomized patients who have received at least one dose of study drug. The safety population will be analyzed by the actual treatment received regardless of treatment randomized.

The incidence and severity of treatment-emergent adverse events (TEAEs) will be summarized by treatment. Vital signs including orthostatic changes in blood pressure and heart rate, weight, ECG, clinical laboratory evaluation, DESS scale, and C-SSRS data will be summarized with descriptive statistics, by visit and by treatment.

Safety and tolerability data will be summarized descriptively, including tables, listings, and graphs, as appropriate. Unless otherwise stated, descriptive summary statistics for continuous variables will include number (N), mean (ie, arithmetic mean), standard deviation (SD), minimum, median, and maximum. Descriptive summary statistics for categorical data will include frequency and proportion.

All data will be presented by treatment regimen.

Individual data for vital signs including supine and standing blood pressure and heart rate, weight, clinical laboratory measures, and ECG assessments will be listed and summarized for any potentially clinically important (PCI) values according to predetermined PCI criteria.

There will be no imputation (substitution) for missing data unless explicitly specified otherwise in this protocol or in the statistical analysis plan.

Pharmacokinetics:

PK data collected in this study will be included in a population PK analysis of plasma concentrations of liafensine and its primary metabolite. It will be performed using a nonlinear mixed-effects modeling approach. The analysis will evaluate the effects of age, sex, ethnicity, and other covariates on the PK profile of liafensine. The results of this analysis with be reported separately.

Statistical Analysis Plan:

Additional details of the analyses will be provided in the statistical analysis plan which will be finalized prior to unblinding.

Ethical Considerations:

This study will be conducted in accordance with applicable country-specific laws and regulations, including, but not limited to, International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) guidelines and the ethical principles that have their origins in the Declaration of Helsinki. The institutional review board (IRB)/independent ethics committee (IEC) must review and approve the protocol and ICF before any patients are enrolled. Before any procedures specified in the protocol are performed, the patient must sign and date the IRB/IEC-approved ICFs.

Table 1: Schedule of Assessments

Study Procedures [a]	Screening 1	Screening 2	Baseline	Double-Blind Treatment					Follow-up[b]	
	Visit 1	Visit 2 [c]	Visit 3	(No visit)	Visit 4	Visit 5	Visit 6	Visit 7 EOT	14 days post-last dose	28 days post-last dose
	Prior to Day -21	Day -21 to Day -2 [d]	Day -1[e]	Day 1[e]	Day 7	Day 14	Day 28	Day 42 [f]		
Visit window (day)			+ 3		± 3	± 3	± 3	± 3	± 3	± 3
Brief informed consent	X[g]									
Full informed consent[g]		X[g]								
Preliminary Inclusion/exclusion	X[h]									
Inclusion/exclusion		X	X							
Medical/surgical history	X									
Prior medications		X								
Demographics	X									
Psychiatric evaluation via MINI (selected or full modules)		X								
ATRQ (5 Year)		X								
Physical examination		X						X		
Neurological examination		X						X		
Blood sampling for PGx[i]	X					X				
Randomization			X							
Study drug dispensing			X		X	X	X			
Study treatment				X----------				----------X		
MADRS[rater 1]		X	X		X	X	X	X		
HAMD-17[rater 1]		X	X							
SDS			X					X		
PRPS			X							
PRDF Checklist[j]		X	X		X	X	X	X		
CGI-S[rater 2]		X	X		X	X	X	X		
CGI-I[rater 2]					X	X	X	X		
C-SSRS		X[k]	X[l]		X[l]	X[l]	X[l]	X[l]	X[l]	X[l]
DESS[m]									X	

Study Procedures [a]	Screening 1	Screening 2	Baseline	Double-Blind Treatment					Follow-up [b]	
	Visit 1	Visit 2 [c]	Visit 3	(No visit)	Visit 4	Visit 5	Visit 6	Visit 7 EOT	14 days post-last dose	28 days post-last dose
	Prior to Day -21	Day -21 to Day -2 [d]	Day -1[e]	Day 1[e]	Day 7	Day 14	Day 28	Day 42 [f]		
Visit window (day)			+ 3		± 3	± 3	± 3	± 3	± 3	± 3
Vital signs[n]		X	X		X	X	X	X	X	
Body weight		X						X		
Height		X								
12-lead ECG[o]		X	X					X		
Clinical laboratory tests[p]		X	X[q]			X	X	X	X	
Urinalysis (on-site/central labs in the event of macroscopic findings)		X	X[q]					X		
Urine drug screen (on-site)		X	X[q]					X		
Pregnancy test[r]	X		X					X		
Blood sampling for PK[s]							X	X		
Concomitant medications			X----------							----------X
Collection of AEs	X----------									----------X

Abbreviations: AE = adverse event; ATRQ = Antidepressant Treatment Response Questionnaire; BP = blood pressure; CGI-I = Clinical Global Impressions–Improvement Scale; CGI-S = Clinical Global Impressions–Severity Scale; C-SSRS = Columbia–Suicide Severity Rating Scale; DESS = Discontinuation-Emergent Signs and Symptoms scale; ECG = electrocardiogram; EOT = End-of-Treatment; FT_3 = free triiodothyronine; FT_4 = free thyroxine; HAMD-17 = Hamilton Depression Rating Scale - 17 Item; HIV = human immunodeficiency virus; MADRS = Montgomery-Åsberg Depression Rating Scale; MINI = Mini International Neuropsychiatric Interview; PGx = pharmacogenomics; PK = pharmacokinetics; PRDF Checklist = Patient Rated Depression and Functioning Checklist; SAE = serious adverse event; PRPS = Placebo Response Propensity Scale; SDS = Sheehan Disability Scale; TSH = thyroid-stimulating hormone.

[a] Every effort should be made for visits to occur on the designated study days. A ± 3-day visit window is permitted at all visits except for Screening and Baseline (Day -1). The overall treatment period in the protocol should be maintained (ie, visits should be scheduled based on the baseline visit rather than the previous visit). Study procedures designated for a specific visit should be performed on 1 day whenever this is possible and in the best interest of the patient. If this is not possible and/or not in the best interest of the patient, study procedures may be performed across more than 1 day but within the visit window.

[b] Randomized patients who complete the study or discontinue early will have two follow-up visits (14 and 28 days after the last dose of study drug), the 2nd follow-up visit will be conducted via phone call.

[c] Visit 2 activities are performed only after the confirmation of initial eligibility including PGx. Patients who screen fail due to PGx status are not eligible for re-screening.

	Prior to Day -21	Day -21 to Day -2 [d]	Day -1[e]	Day 1[e]	Day 7	Day 14	Day 28	Day 42 [f]		

[d] Screening period may exceed 20 days if a prolonged washout is required.

[e] Baseline evaluations will be performed on Day -1. Patients will be instructed to take the first dose of double-blind study drug the day after baseline evaluations (ie, on Day 1), and will be instructed to take double-blind study drug for the remainder of the 6-week treatment period.
[f] For patients who discontinue early from the study, safety and efficacy determinations designated for Day 42 will be performed on the last day the patient takes a full dose of study drug or as soon as possible thereafter, but within 3 days of their last dose in any event.
[g] At Visit 1, two brief ICFs will be obtained for the purpose of genotyping and biobanking, separately. A full ICF must be obtained at Visit 2.
[h] Refer to Appendix 2 Preliminary Diagnosis Checklist
[i] A separate blood sample (6 mL) will be collected for PGx (eg, determination of DGM4 status at Screening and for future research at Visit 5).
[j] The PRDF Checklist must be completed by the patient and reviewed by the rater 2 prior to CGI-S assessment. The baseline checklist entry must be compared by rater 2 against post-baseline entry to refresh the knowledge of patient's baseline condition prior to CGI-I assessment.
[k] C-SSRS baseline/screening version
[l] C-SSRS since-last-visit version
[m] For randomized patients who complete the study or discontinue early, the DESS scale will be completed daily by the patient for 14 days post-last dose.
[n] Vital signs should be taken prior to blood draws when applicable and include supine (after at least 5 min rest) and standing (after 1 min and 3 min standing), BP and heart rate, respiration rate, and temperature. Vital signs can be repeated for up to 3 occurrences after Visit 2 to see if values return to normal range, but any abnormality must be resolved by the Baseline Visit.
[o] ECG recordings will be completed prior to blood draws when applicable and will be performed in triplicate (at 1-minute intervals between each of the three recordings) for baseline. Single ECG will be performed for rest of the timepoints.
[p] Hematology, blood chemistry, and urinalysis will be performed at all visits with a laboratory determination. Serologic tests for HIV, hepatitis B core antibody, hepatitis B surface antigen, and hepatitis C virus antibody, and a thyroid panel (TSH, FT3, and FT4) will be completed only at Screening. Laboratory tests can be repeated for up to 3 occurrences after Visit 2 to see if values return to normal range, but any abnormality must be resolved by the Baseline Visit.
[q] To be conducted on Day -1 only if (1) the screening period exceeds 21 days; (2) a longer than 21-day prior medication washout is required, eg, fluoxetine > 20 mg/day; (3) per investigator's judgement. For women of pre-menopause, an inquiry of menses status should be made in the presence of macroscopic blood detection before submitting the sample to the central labs for microscopic examination.
[r] Serum pregnancy testing will be performed in females of childbearing potential at Screening 1 and Day 42 (Visit 7). Urine pregnancy testing will be performed at Baseline (Day -1, dip stick).
[s] Blood samples for PK will be collected on Day 28 (Visit 6) and on Day 42 (Visit 7) prior to dose administration, or at the time of early withdrawal, if within 3 days of last dose. Time of last dose and time of blood collection will be recorded. When possible, and if medically appropriate, blood samples should be collected for PK from patients who experience an SAE or overdose.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

The following abbreviations and specialist terms are used in this study protocol.

Abbreviation or Specialist Term	Explanation
β-hCG	β-human chorionic gonadotropin
5-HT	serotonin
ADHD	attention-deficit/hyperactivity disorder
AE	adverse event
AESI	adverse event of special interest
ALP	alkaline phosphatase
ALT (SGPT)	alanine aminotransferase (serum glutamic-pyruvic transaminase)
aPTT	activated prothrombin time
AST (SGOT)	aspartate aminotransferase (serum glutamic-oxaloacetic transaminase)
ATRQ	Antidepressant Treatment Response Questionnaire
AUC	area under the curve
BMS	Bristol-Myers Squibb
BP	blood pressure
BUN	blood urea nitrogen
CGI-I, -S	Clinical Global Impressions Scale-Improvement, -Severity
CI	confidence interval
CK	creatine kinase
CLIA	Clinical Laboratory Improvement Amendments
C_{max}	maximum (peak) plasma drug concentration
CMH	Cochran-Mantel-Haenszel
CNS	central nervous system
CPK	serum creatine phosphokinase
CRO	contract research organization
CV	curriculum vitae
C-SSRS	Columbia-Suicide Severity Rating Scale
DESS	Discontinuation-Emergent Signs and Symptoms (scale)
DGM4	Denovo Genomic Marker 4, a genetic biomarker for DB104 (liafensine)
DILI	drug-induced liver injury
DNA	deoxyribonucleic acid
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, 5th Edition
ECG	electrocardiogram
eCRF	electronic Case Report Form
EDC	electronic data capture (system)

Abbreviation or Specialist Term	Explanation
EOT	End-of-Treatment
FAS	Full Analysis Set
FDA	(United States) Food and Drug Administration
FT_3	free triiodothyronine
FT_4	free thyroxine
GCP	Good Clinical Practice
HAMD-17	Hamilton Depression Rating Scale - 17 Item
HBsAg	hepatitis B surface antigen
HCV	hepatitis C virus
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
IB	Investigator's Brochure
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICF	informed consent form
IDMC	Independent Data Monitoring Committee
IEC	Independent Ethics Committee
IgM	immunoglobulin M (antibodies)
IMP	investigational medicinal product
IRB	Institutional Review Board
IRT	Interactive Response Technology
IVD	in vitro diagnostic
LS	least-squares
MAD	multiple ascending dose
MADRS	Montgomery-Åsberg Depression Rating Scale
MAOI	monoamine oxidase inhibitor
MCH(C)	mean corpuscular hemoglobin (concentration)
MCV	mean corpuscular volume
MDD	major depressive disorder
MDMA	3,4-methylenedioxy-N-methylamphetamine ("Ecstasy")
(MGH) ATRQ	(Massachusetts General Hospital) Antidepressant Treatment Response Questionnaire
MINI	Mini International Neuropsychiatric Interview
NMDA	N-methyl-D-aspartate
PCI	potentially clinically important
PGx	pharmacogenomics

Abbreviation or Specialist Term	Explanation
PI	principal investigator The investigator who leads the study conduct at an individual study center. Every study center has a principal investigator.
PK	pharmacokinetic
PRDF	Patient Rated Depression and Functioning (Checklist)
PRPS	Placebo Response Propensity Scale
SAD	single ascending dose
SAP	statistical analysis plan
SDS	Sheehan Disability Scale
SNP	single nucleotide polymorphism
QD	once daily
QT_cF	corrected QT interval measurement according to the Fridericia rule
RBC	red blood cell (count)
REMS	Risk Evaluation and Mitigation Strategy
SAE	serious adverse event
SNP	single nucleotide polymorphism
SNRI	serotonin norepinephrine reuptake inhibitor
SOA	schedule of assessments
SSRI	selective serotonin reuptake inhibitor
SUSAR	suspected unexpected serious adverse reactions
TBL	total bilirubin
TCA	tricyclic antidepressants
TEAE	treatment-emergent adverse event
TRD	treatment-resistant depression
TSH	thyroid-stimulating hormone
UDS	urine drug screen
ULN	upper limit of normal
US	United States
WBC	white blood cell (count)

1. INTRODUCTION

1.1. Background

Major depressive disorder (MDD) is a common psychiatric disorder, with a lifetime prevalence rate of approximately 13-17% in the United States (Hasin 2005, Kessler 2005). Although options for pharmacologic treatment have expanded significantly in the past 25 years, between one-third and two-thirds of patients will not respond to the first antidepressant prescribed, and up to 33% will not respond to multiple interventions (American Psychiatric Association 2013, Berlim 2008, Cain 2007). Novel antidepressants that produce a clinically significant improvement in patients with MDD who have failed to respond to more than one treatment of adequate dose and duration, without producing significant side effects, would represent an important advance in the treatment of treatment-resistant depression (TRD).

For most of the last 30 years, the biogenic amine hypothesis of depression has been driving much of the research into the etiology and treatment of this debilitating psychiatric disorder. Most conventional antidepressants are antagonists at serotonin (5-HT) and/or norepinephrine reuptake sites, and their efficacy correlates reasonably well with their affinity for these receptors. At present, the most commonly used treatments for MDD are the selective serotonin reuptake inhibitors (SSRIs), such as escitalopram, or the serotonin-norepinephrine reuptake inhibitors (SNRIs), such as duloxetine.

Despite a plethora of pharmacologic and non-pharmacologic treatments, there is significant unmet need for better treatments for persons with depression. Many patients remain inadequately responsive to treatment. The STAR*D study found that only 37% of depressed patients responded to a first step in treatment with decreasing likelihood of response in later steps (31%, 14%, and 13%) (Rush 2006). As many as 30% of patients remain treatment resistant after 4 different treatment steps. Nor was there any rational progression for selecting an initial treatment.

Recently, a compound with a novel mechanism of action, esketamine (SPRAVATO®), was approved in the United States (US) for the treatment of TRD in conjunction with an oral antidepressant (SPRAVATO 2019). While the precise mechanism of action of esketamine is unknown, the compound is an N-methyl-D-aspartate (NMDA) receptor antagonist. Esketamine is administered intranasally through a restricted distribution system, under a Risk Evaluation and Mitigation Strategy (REMS), and its use is associated with significant adverse effects including dizziness, nausea, sedation, and psychological effects. The most common psychological effects of esketamine are dissociative/perceptual changes (including distortion of time and space, and illusions), derealization, and depersonalization. Patients may describe these symptoms as feeling disconnected from themselves, their thoughts and feelings, space, and time. Because of the risks of dissociation with esketamine, the US Food and Drug Administration (FDA) has established a REMS requiring that patients be monitored by a healthcare professional for at least 2 hours at each treatment session, concluding with an assessment establishing that the patient is clinically stable and may safely leave the healthcare setting. The persistent high numbers of treatment-resistant patients, the adverse event profile and difficulty accessing both existing and newer treatments, and the need to find better ways of driving treatment selection to treatment-responsive patients support the biomarker-driven development of liafensine for TRD.

1.2. Liafensine

Liafensine (also known as DB104 and BMS-820836[1]) is 6-[(4*S*)-1,2,3,4-tetrahydro-2-methyl-4-(2-naphthalenyl)-7-isoquinolinyl]-3-pyridazinamine and it is a potent and selective inhibitor of the reuptake of the 3 monoamines: 5-HT, norepinephrine, and dopamine. Liafensine was developed to incrementally advance antidepressant treatment by synergizing these individual monoaminergic approaches to treating depression.

1.2.1. Study Rationale

Liafensine has been tested in 11 Phase 1 studies, and 3 Phase 2b studies (including one rollover study). In the Phase 1 studies, single doses of up to 5 mg, and multiple doses of up to 4 mg liafensine were found to be generally safe and well tolerated. The pharmacokinetic (PK) profile of liafensine was compatible with once-daily (QD) dosing, supporting the potential for liafensine to have low potential for discontinuation symptoms. In the Phase 2b studies, patients who did not experience adequate response to either duloxetine (Study CN162006), or duloxetine or escitalopram (Study CN162007), were randomized to receive either liafensine or duloxetine (CN162006), or duloxetine or escitalopram (CN162007), for 6 weeks. In Study CN162006, a flexible dose of liafensine was used (0.5-2 mg QD). In Study CN162007, several fixed dosage levels of liafensine were used (0.25, 0.5, 1, and 2 mg QD). In these Phase 2b studies, liafensine was found to be generally safe and well tolerated. In Study CN162006, the most common adverse events (AEs) reported by at least 5% of patients treated with liafensine were headache (7.0%), nausea (7.0%), constipation (5.3%), and anxiety (5.3%). In Study CN162007, the most common AEs reported by at least 5% of patients treated with any dose of liafensine were headache (14.3%), nausea (12.0%), nasopharyngitis (8.8%), upper respiratory tract infection (8.0%), dry mouth (8.0%), constipation (7.7%), dizziness (7.0%), diarrhea (5.5%), fatigue (5.9%), and urinary tract infection (5.5%).

In the Phase 2b studies conducted by Bristol-Myers Squibb (BMS), there were no significant differences noted between the treatment groups in the decrease (improvement) of the mean Montgomery-Åsberg Depression Rating Scale (MADRS) total score. In an effort to identify a potential pharmacogenomic biomarker of response to liafensine, Denovo Biopharma LLC (Denovo) has conducted a genome-wide scan with human whole genome single nucleotide polymorphism (SNP) arrays using germline deoxyribonucleic acid (DNA) extracted from blood samples of patients in BMS Studies CN162006 and CN162007. From this genome-wide screening, SNP rs12217173 was identified as strongly associated with treatment response to liafensine ($p = 6.6 \times 10^{-8}$). Patients with a GG genotype at rs12217173 showed significantly better response to liafensine compared to patients with an AA or AG genotype, or patients in the control arm (duloxetine or escitalopram). These findings were replicated using a separate validation sample set. Liafensine treatment effects in patients with the GG genotype were comparable for the 0.5 to 2 mg QD flexible doses in the CN162006 study and for the 1 mg QD and 2 mg QD doses in the CN162007 study. These combined data showed that patients with the GG genotype had a 4.7-point difference in MADRS total score decrease from baseline in the liafensine arm vs the control arm ($p = 0.025$), while patients with the AA or AG genotype at rs12217173 did not show an improved treatment effect in the liafensine arm vs the control arm

[1] BMS-820836-03 was selected for the dosage form development and is also referred to as BMS-820836.

(MADRS 0.3-point difference, p = 0.74). Moreover, patients carrying different genotypes at rs12217173 did not exhibit different treatment responses to duloxetine or escitalopram. Thus, SNP rs12217173 appears to be a pharmacogenomic biomarker specific for liafensine; Denovo has termed rs12217173 as "DGM4" (Denovo Genomic Marker 4) and the GG genotype of DGM4 is defined as DGM4 positive, whereas the AA or AG genotype of DGM4 is defined as DGM4 negative.

DGM4 is located at the *ANK3* gene locus, and the *ANK3* gene has been found to be associated with depression and other central nervous system (CNS) disorders, including bipolar disorder (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014, Craddock 2013, Mühleisen 2014, Rangaraju 2016). Additionally, Zhu et al. found that in a genetic mouse model with conditional disruption of forebrain Ank3 pyramidal neurons (Ank3 cKO), repeated social defeat stress resulted in repeated episodes of dramatic behavioral changes ranging from hyperactivity to "depression-like" behavior (Zhu 2017).

Taken together, these data strongly support the potential utility and further study of liafensine in DGM4-positive patients with TRD.

1.2.2. Summary of Relevant Prior Animal Studies

Background information in addition to that presented below is available in the Investigator's Brochure.

1.2.2.1. Absorption, Metabolism, and Drug Interactions

Based on in vitro studies, the metabolism of liafensine is judged to be primarily mediated by CYP3A4 with additional contributions from CYP1A1/2, CYP2D6, CYP2C9, and CYP2C19.

In vitro, liafensine exhibited some inhibition of CYPs 2C9, 2C19, and 2D6, with IC_{50} estimates of > 1 μM. Liafensine was a time-dependent inhibitor of CYP3A4 and does not induce relevant human CYPs. Liafensine was a weak inhibitor of P-gp with an IC_{50} of ~20 μM (~7.3 μg/mL). Clinically relevant concentrations of liafensine are in the low ng/mL range, suggesting a low potential for drug interactions involving P-gp inhibition, CYP induction, or CYP inhibition at the doses examined. Similarly, low plasma concentrations of its active metabolite, 821007, in vivo suggest low potential for drug interactions at clinically relevant concentrations.

Because liafensine is a substrate for CYP3A4, exposure to liafensine may be altered by co-administered drugs that are inhibitors or inducers of this enzyme.

1.2.2.2. Preclinical Toxicity

Liafensine has a moderate potential for acute toxicity. Single oral doses up to 20 mg/kg were tolerated in the rat, and a dose of 0.6 mg/kg was tolerated in monkeys, with clinical findings consistent with targeted inhibition of monoamine reuptake including stereotypic behaviors and elevations in body temperature; increased activity, abnormal respiration in rats; and decreased feeding behavior and evidence of skeletal muscle injury in monkeys.

After repeated dosing, liafensine was clinically tolerated up to 3 mg/kg/day in rats and 1 mg/kg/day in monkeys for up to 3 months. The primary treatment-related findings were pharmacologically-mediated effects including decreases in food consumption and body weight,

increased activity, and stereotypic behaviors at all doses. Target organs were limited to skeletal muscle in rats and monkeys after 2 weeks of dosing and the liver in rats after chronic dosing.

Liafensine-related skeletal muscle injury and repair, which may have been a consequence of pharmacologically mediated increases in activity and/or direct adrenergic stimulation, were observed only at higher doses in rats (up to 6 mg/kg/day) and monkeys (up to 5 mg/kg/day) after dosing (area under the curve [AUC] multiples at ≥ 12-fold in the rat and 3.8-fold in the monkey). The generally minimal severity and limited distribution of these drug-related skeletal muscle changes likely accounted for the absence of correlative increases in serum muscle-related enzymes (AST, CK). There was no skeletal muscle injury noted in rats or monkeys after 3 months of dosing at systemic exposure multiples up to 61- and 4-fold, respectively. The lack of liafensine-related effects on the skeletal muscle after 3 months suggests accommodation with repeat dosing. Routine evaluation of serum AST and CK activities are anticipated to be adequate for monitoring for the development of skeletal muscle changes in humans in the clinical studies.

There was liafensine-related hepatotoxicity in rats after 3 and 6 months of dosing, which included the dose-related presence of multinucleate hepatocytes, Kupffer cell hypertrophy and pigment accumulation, and increases (1.2 to 4-fold) in liver transaminases. A lack of progression of the hepatotoxicity in rats was noted, comparing incidence and severity from subchronic to chronic studies. Moreover, there was no evidence of hepatic inflammation or fibrosis associated with chronic treatment in rats. Evidence of partial or complete recovery of most liafensine-related findings including all target organ effects were noted in rats. Test article-related hepatic changes were not associated with increased hepatocellular proliferation or ultrastructural changes indicative of abnormal cell division.

There were no liafensine-related effects on the liver in monkeys after subchronic (3 months) or chronic (9 months) of dosing, where systemic exposures overlapped with those that produced liver changes in rats. Moreover, there were no liafensine-related effects on the liver in mice treated for up to 3 months. Collectively, the absence of drug-related hepatic changes at overlapping or higher systemic exposures in monkeys and mice treated subchronically/ chronically with liafensine suggests the treatment-related hepatotoxicity is rat specific.

1.2.3. Pharmacokinetics in Humans

The absorption of liafensine from either oral solution or tablet formulation was relatively slow. From the oral solution, the median t_{max} was approximately 5.0–7.2 hours postdose after single doses (0.025 to 5 mg), and 4–5 hours after once-daily doses for 14 days (0.1 to 4 mg). In the relative bioavailability study (CN162004), the median t_{max} was 7 hours from both the solution and tablet. Additionally, the PK of liafensine from the tablet formulation given with a high-fat meal was comparable to that under fasting conditions. The adjusted geometric means ratio of the C_{max} and $AUC_{(0\text{-}72h)}$ from the tablet with the high-fat meal vs tablet under fasting conditions was 1.021 (90% CI: 0.955, 1.092) and 1.018 (90% CI: 0.966, 1.071), respectively. Therefore, liafensine may be administered without regard to food.

Steady state of liafensine was achieved after approximately 10 days of QD dosing (0.1 to 2 mg QD), and by Day 12 after 3 mg titration. The accumulation of liafensine AUC_t after 14 days of once daily dosing ranged from 2.67 to 4.59.

Liafensine exhibited low to moderate variability in plasma exposures. The variability (% coefficient of variation [CV]) of C_{max} and AUC ranged from 14% to 29% and 12% to 44%,

respectively, in the single ascending dose (SAD) study, and 6% to 40% and 8% to 40%, respectively, in the multiple ascending dose (MAD) studies.

Based on in vitro studies, liafensine is primarily metabolized by multiple CYP enzymes, including CYP3A4 as a predominant enzyme, with additional contributions from CYP1A1/2, CYP2C9/19, and CYP2D6. The elimination of liafensine was relatively slow. The mean $t_{½}$ ranged from 34–57 hours after single doses, and 44–74 hours after repeat daily doses for 14 days. Following a single oral dose administration of [^{14}C]liafensine in adult male healthy volunteers, 63% and 78% of the dose was recovered by Day 22 and Day 64, respectively. When extrapolated to infinity, approximately 83% of the dose would have been recovered. The radioactive dose was mainly excreted in the feces (62%) compared to 21% in the urine.

The C_{max} and AUC-time curve extrapolated to infinity (AUC_{∞}) of liafensine increased slightly greater than in proportion to dose after single doses ranging from 0.025 to 5 mg. After once-daily doses (0.1 to 2 mg QD), the C_{max} and AUC_t of liafensine increased slightly less than proportional to dose on Day 14. There has been no evidence of time-dependent PK after repeat dosing of liafensine due to either autoinhibition or autoinduction. The observed degree of accumulation in liafensine is consistent with the elimination $t_{½}$ of liafensine.

There were no apparent sex differences in the PK of liafensine and its active metabolite 821007 in the completed Phase 1 studies. In the MAD study (CN162002), although the geometric means of liafensine C_{max} and AUC_t in the female subjects appeared to be lower than those in the male subjects in the 1 mg dose groups, there were marked overlaps in these PK parameters between the female and male subjects.

The PK of liafensine in healthy elderly subjects was evaluated in a ketoconazole DDI study (CN162015). The elderly population in the study included 12 healthy male (N = 8) and female (N = 4) subjects 65 to 85 years old, inclusive, and received a single 1 mg oral dose of liafensine administered alone. Dose-normalized analysis showed that liafensine C_{max} was 38% lower and AUC_{∞} was 19% higher in the elderly subjects than the young subjects. $t_{½}$ was longer in the elderly compared to young subjects (84 hours vs 49 hours). No meaningful difference was observed in the metabolite-to-parent C_{max} ratio between the two populations. 821007 $t_{½}$ could not be characterized in the elderly due to insufficient sampling in the terminal phase of the profile. The C_{max} and AUC_{∞} values of liafensine were comparable in the elderly male and female subjects.

Co-administration of liafensine with the strong CYP3A4 inhibitor ketoconazole (CN162015) increased C_{max} and AUC_{∞} of liafensine by 15% and 32%, respectively, compared to administration of liafensine alone. Coadministration with ketoconazole also increased C_{max} and AUC_{∞} of 821007, the active metabolite of liafensine, by 16% and 25%, respectively, compared to administration of liafensine alone. Because of the observed small increases in liafensine exposures when co-administered with the potent CYP3A inhibitor ketoconazole, CYP3A4 inhibitors will not be excluded in this study.

Co-administration of liafensine with rifampin (Study CN162017) decreased C_{max} and AUC_{∞} of liafensine by 34% and, 66%, respectively, compared to administration of liafensine alone. Therefore, in this study, liafensine should not be taken with strong inducers of CYP3A4 and those may lower liafensine exposure and reduce its effectiveness.

1.2.4. Clinical Studies of Liafensine

The Investigator's Brochure (IB) lists 14 completed studies in which liafensine was administered to 470 subjects in 11 Phase 1 studies and 1,264 subjects in 3 Phase 2b studies including a rollover study. These studies are summarized in the Investigator's Brochure.

1.2.5. Potential Risks and Benefits

Please see the Investigator's Brochure for additional discussion and information for the following section.

A number of safety monitoring practices are being employed by this protocol (including, but not limited to, physical examinations, vital signs including orthostatic changes in blood pressure and heart rate monitoring at specified intervals, triplicate 12-lead ECGs, clinical laboratory evaluations, and AE collection) in order to ensure the patients' safety.

There is an anticipated health benefit for study patients from receipt of study drug. The previous Phase 2b studies conducted by Bristol-Myers Squibb (BMS) did not demonstrate clinically meaningful and statistically significant improvement in depression symptoms in general TRD patient population. However, the pharmacogenomic analysis conducted by Denovo indicates a strong correlation between DGM4 status and symptom improvement in liafensine-treated patients, suggesting that liafensine may provide significant treatment benefit in DGM4-positive TRD patients.

2. OBJECTIVES AND ENDPOINTS

2.1. Objectives

2.1.1. Primary Efficacy Objective

- To demonstrate that liafensine is superior to placebo in DGM4-positive patients with TRD as assessed by the change in MADRS total score from baseline to Day 42 of double-blind treatment

2.1.2. Key Secondary Efficacy Objective

- To evaluate the change from baseline to Day 42 in DGM4-positive patients with TRD treated with liafensine vs placebo on the Clinical Global Impressions Scale-Severity (CGI-S)

2.1.3. Safety Objectives

- To compare the safety and tolerability of liafensine vs placebo in all randomized patients with TRD who received at least one dose of study drug during double-blind treatment
- To evaluate safety for liafensine 1 mg QD and 2 mg QD vs placebo in both DGM4-positive and DGM4-negative patients

2.1.4. Other Secondary Efficacy Objectives

- To evaluate Clinical Global Impressions Scale-Improvement (CGI-I) at Day 42 in DGM4-positive patients with TRD treated with liafensine vs placebo
- To evaluate the change from baseline to Day 42 in DGM4-positive patients with TRD treated with liafensine vs placebo on the patient-reported functionality with Sheehan Disability Scale (SDS)

2.1.5. Exploratory Objectives

- To compare the proportion of DGM4-positive patients with TRD who respond to liafensine after 6 weeks of treatment vs placebo (response is defined as $\geq$ 50% improvement from baseline in MADRS total score at Day 42)
- To compare the proportion of DGM4-positive patients with TRD who are in remission after 6 weeks of treatment with liafensine vs placebo (remission is defined as MADRS total score $\leq$ 10 at Day 42)
- To evaluate the change from baseline to Day 42 in DGM4-positive patients with TRD treated with liafensine vs placebo on MADRS anhedonia subscale and individual items
- To compare the efficacy of liafensine vs placebo in DGM4-negative patients with TRD, as assessed by change in MADRS total score from baseline to Day 42

- To evaluate efficacy for liafensine 1 mg QD and 2 mg QD vs placebo in both DGM4-positive and DGM4-negative patients
- To evaluate the PK of liafensine as part of the population PK analyses

2.2. Endpoints

2.2.1. Primary Endpoint

- Change in MADRS total score from baseline to Day 42, in DGM4-positive patients

2.2.2. Secondary Endpoint

- Change in CGI-S from baseline to Day 42, in DGM4-positive patients

2.2.3. Safety Endpoints

- Adverse events as characterized by type, frequency, severity, timing, seriousness, and relationship to study therapy
- Laboratory abnormalities as characterized by type, frequency, severity, and timing
- Vital signs including supine (after at least 5 min rest) and standing (after standing 1 min and 3 min), blood pressure and heart rate, weight, electrocardiogram (ECG), clinical laboratory evaluation, Discontinuation-Emergent Signs and Symptoms scale (DESS) scale, and Columbia Suicide Severity Rating Scale (C-SSRS) data

2.2.4. Other Secondary Endpoints

- CGI-I at Day 42, in DGM4-positive patients
- Change in SDS from baseline to Day 42, in DGM4-positive patients

2.2.5. Exploratory Endpoints

- Treatment response (defined as ≥ 50% improvement from baseline in MADRS total score at Day 42) in DGM4-positive patients
- Remission (defined as MADRS total score ≤ 10 at Day 42) in DGM4-positive patients
- Change in MADRS anhedonia subscale and individual items from baseline to Day 42, in DGM4-positive patients
- Change in MADRS total score from baseline to Day 42, in DGM4-negative patients
- Change in MADRS total score from baseline to Day 42 in 1 mg QD group and 2 mg QD group, in both DGM4-positive and DGM4-negative patients

3. INVESTIGATIONAL PLAN

3.1. Overall Study Design

This study will be conducted as a multicenter, randomized, double-blind, placebo-controlled Phase 2b trial to assess the efficacy, safety, tolerability, and PK of liafensine. The study includes an up to 21-day screening period (the screening period may exceed 21 days if a prolonged washout is required).

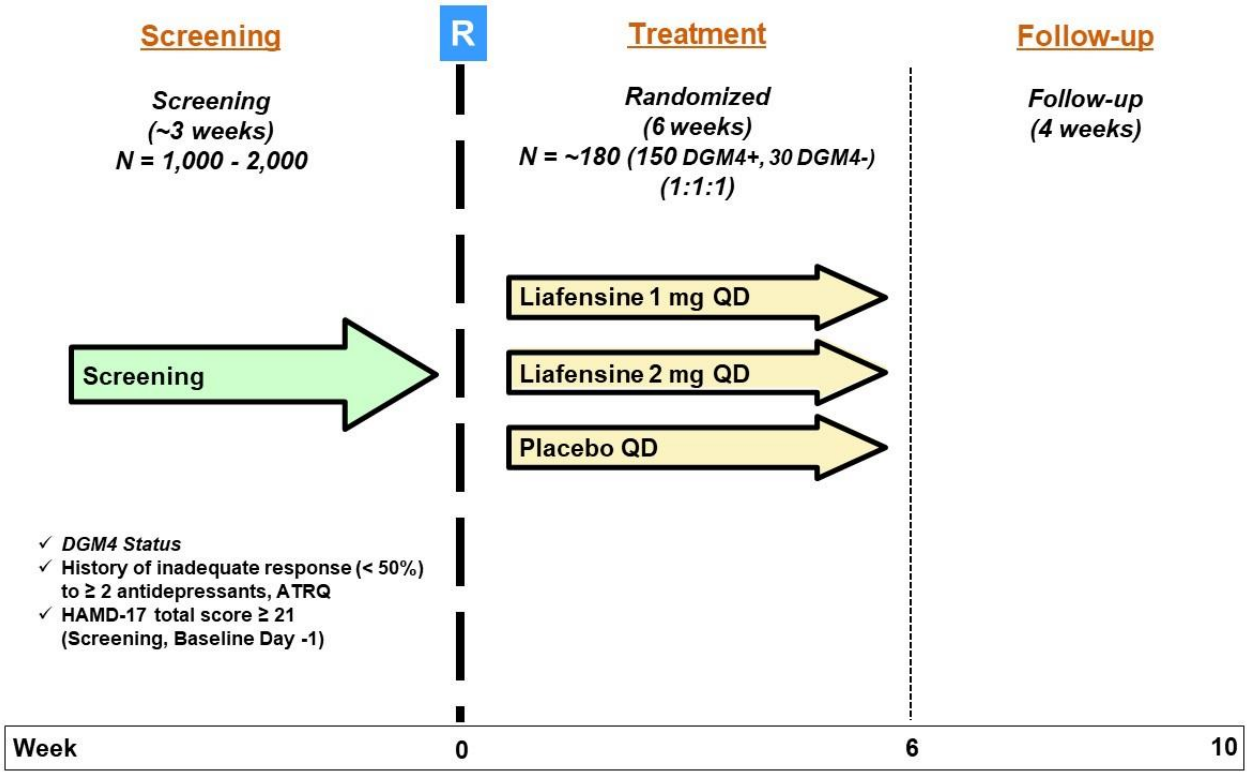
Approximately 180 patients with TRD who meet all eligibility criteria will be randomized in a 1:1:1 ratio to receive either liafensine 1 mg QD, liafensine 2 mg QD, or placebo QD for 6 weeks. Approximately 150 randomized patients will be genotype GG for DGM4 (DGM4-positive) and approximately 30 randomized patients will be genotype AA or AG for DGM4 (DGM4-negative). DGM4 status (positive vs negative) of randomized patients will be blinded to the sites, patients, and sponsor. An Interactive Response Technology (IRT) system will be used for blinding to DGM4 status, and to achieve the correct randomization ratio of DGM4-positive:DGM4-negative patients by randomly excluding most screened DGM4-negative patients (~80%). Randomization will be stratified by DGM4 status (positive vs negative) and region (North America vs Other). Patients who complete the study or discontinue after randomization will have 2 post-study follow-up visits (14 days after the last administered dose of study drug, and 28 days after the last administered dose of study drug); the 2nd follow-up visit will be conducted via phone call.

During the study, safety will be assessed on the basis of spontaneously reported AEs; the Discontinuation-Emergent Signs and Symptoms scale (DESS); physical, neurological, and psychiatric examinations (including the Columbia-Suicide Severity Rating Scale [C-SSRS]); vital signs including orthostatic changes in blood pressure and heart rate; weight; 12-lead electrocardiograms (ECGs); clinical laboratory test results; serum and urine pregnancy tests (females of childbearing potential only); and concomitant medication assessments. In addition to blood samples collected for PK, additional blood samples may be collected for determination of plasma concentrations of liafensine and its primary metabolite in association with the evaluation of safety signals.

The overall study design is shown in Figure 1. The schedule of study assessments is presented in Table 1 with the clinical laboratory tests shown in Table 3.

To ensure the safety of patients enrolled in this study, an Independent Data Monitoring Committee (IDMC) will monitor data on an ongoing basis (Section 8.3.8). The IDMC will comprise individuals external to the sponsor and will consist of at least one medical expert in the relevant therapeutic area, at least one hepatologist, and at least one statistician.

Figure 1: Overall Study Design



Abbreviations: ATRQ = Antidepressant Treatment Response Questionnaire; DGM4 = Denovo Genomic Marker 4; HAMD-17 = Hamilton Depression Rating Scale - 17 Item; QD = once daily; R = randomization.

3.2. Methodology

This is a randomized, double-blind, placebo-controlled Phase 2b trial to assess the efficacy, safety, tolerability, and PK of liafensine. The primary endpoint is change in MADRS total score from baseline (Visit 3, Day -1) to Visit 7 (Day 42) in DGM4-positive patients. The primary analysis will be conducted to assess the superiority of liafensine treatment (combined liafensine 1 mg QD and 2 mg QD doses) vs placebo in randomized patients who are DGM4-positive.

3.3. Study Duration and Follow-up

The duration of participation for individual patients will be up to 91 days or longer, including an up to 21-day screening period (screening period may exceed 21 days if a prolonged washout is required), a 6-week double-blind treatment period, and 2 post-study follow-up visits (at 14 days and 28 days after the last dose of study drug); the 2nd follow-up visit will be conducted via phone call (Figure 1).

3.4. Sample Size Justification

Eligible patients will be randomized 1:1:1 to receive liafensine 1 mg QD, liafensine 2 mg QD, or placebo QD. The primary analysis is to assess treatment difference in MADRS total score change from baseline (Visit 3, Day -1) to Visit 7 (Day 42), between the combined liafensine 1 mg QD and 2 mg QD doses vs placebo, in DGM4-positive patients. A sample size of 47 randomized DGM4-positive patients per arm is sufficient to detect a 4.5-unit difference between the combined liafensine 1 mg QD and 2 mg QD treatment groups and the placebo treatment group in the change in MADRS total score with 80% power at the 2-sided 0.05 alpha level, assuming a standard deviation of 9 units.

To compensate for potential loss of power due to randomized patients who do not take at least one dose of study drug or who do not have a post-randomization efficacy evaluation (~5%), a total of approximately 150 DGM4-positive patients (50 DGM4-positive patients per arm) will be randomized.

To examine trend in the total patient population and in the subgroup of DGM4-negative patients in order to evaluate DGM4 as a potential biomarker for predicting response to liafensine, approximately 20% additional DGM4-negative patients (10 per arm, total approximately 30) will also be randomized. The sample size of 30 randomized patients in the DGM4-negative group is not estimated based on formal statistics in terms of power and alpha level.

Sample size re-estimation may be conducted if the percentage of missing data at Day 42 visit is higher than expected and/or if the observed variance is higher than the one assumed in the sample size estimation.

3.5. Number of Subjects

Approximately 180 patients will be enrolled in the study.

3.6. Treatment Assignment

Patients who meet all of the inclusion criteria and none of the exclusion criteria will be randomized through an IRT system in a 1:1:1 ratio to receive either liafensine 1 mg QD, liafensine 2 mg QD, or placebo QD for 6 weeks.

3.7. Criteria for Study Termination

The sponsor may decide to terminate the study at any time. Furthermore, the sponsor will consider recommendations provided by an external IDMC with regards to study continuation, modification, and/or termination (Section 7.6).

4. SELECTION AND WITHDRAWAL OF PATIENTS

This study can fulfill its objectives only if appropriate patients are enrolled. The following eligibility criteria are designed to select patients for whom participation in the study is considered appropriate. All relevant medical and nonmedical conditions should be taken into consideration when deciding whether a patient is suitable for this protocol.

Patient eligibility should be reviewed and documented by an appropriate member of the investigator's study team before patients are included in the study.

4.1. Subject Inclusion Criteria

Patients must meet all the following inclusion criteria to be eligible for enrollment into the study:

1. Provide signed informed consent which includes pharmacogenomic (PGx) testing.
2. Have a diagnosis of MDD without psychotic features, according to the *Diagnostic and Statistical Manual of Mental Disorders,* 5th Edition (DSM-5) criteria, based on clinical assessment and confirmed by the Mini International Neuropsychiatric Interview (MINI).
3. TRD documented in the Massachusetts General Hospital (MGH) Antidepressant Treatment Response Questionnaire (ATRQ) (5-year version). That is:
   a. clinically meaningful inadequate response (estimated < 50% improvement per investigator/patient consensus and documented by the investigator) with:
      1) at least 2 treatment courses with antidepressant regimens;
      2) used at least 2 different pharmacologic treatment classes (eg, SSRI, SNRI, TCA, MAOI, atypical); or if only one class treatment is used, at least one combination treatment course;
      3) the doses have been given at accepted therapeutic doses for an adequate duration (at least 6 weeks), per product label;
      4) One of these inadequate response must occur within the current episode.

   Note: non-pharmacological treatment (eg, cognitive behavioral therapy, electroconvulsive therapy, repetitive transcranial magnetic stimulation, vagus nerve stimulation, acupuncture) are not counted as treatment regimen.
4. To be eligible, patients must have DGM4 genotype results obtained from the designated Clinical Laboratory Improvement Amendments (CLIA) lab, and all eligible DGM4-positive patients and about 20% DGM4-negative patients will be randomly included by an IRT system in order to achieve the appropriate randomization ratio of DGM4-positive vs negative patients.
5. Pregnancy conception limitations
   - Female patients must be postmenopausal or surgically sterile or, if of childbearing potential and the partner is not vasectomized (6 months minimum), must agree to use a medically acceptable form of contraception from the time of signing the informed consent form (ICF) through at least 60 days following the last administration of study drug. If only the barrier method is used, a double barrier must be employed. Postmenopausal women must have had ≥ 24 months of spontaneous amenorrhea. Surgically sterile women are defined as those who have had a hysterectomy, bilateral ovariectomy, or bilateral tubal ligation. All women of childbearing potential must have a negative pregnancy test result before administration of study drug.
   - Male patients must be biologically incapable of having children (eg, vasectomized) or must agree to use the above forms of birth control for themselves and their partner from the time of signing the ICF through at least 120 days following the last administration of study drug.

6. Be fluent in the local language.
7. Male or female aged 18 to 70, inclusive, at time of Visit 1.
8. Have a HAMD-17 total score ≥ 21 at screening and baseline.
9. Be willing to discontinue the use of antidepressant drugs (including over-the-counter medications to treat depression [eg, St John's Wort]) at least 5 half-lives (or at least 1 week for herbal or other over-the-counter medications for depression) prior to baseline (Day -1). For fluoxetine, a washout period of at least 3 weeks for ≤ 20 mg/day and at least 4 weeks for > 20 mg/day is required.

4.2. Subject Exclusion Criteria

Patients with any of the following characteristics/conditions will be excluded from study:

1. Prior participation in a study with liafensine.
2. Used any investigational drug product, device, or biologic within 3 months or 5 half-lives (whichever is longer) prior to baseline (Day -1).
3. A positive pregnancy test result or currently breastfeeding.
4. Clinically significant and/or unstable illness (including chronic, persistent, or acute infection), medical/surgical procedure, or trauma within 30 days prior to screening or between screening and baseline (Day -1) as determined by the investigator.
5. A history or presence of a clinically significant and/or unstable hepatic, renal, gastrointestinal, cardiovascular, endocrine, respiratory, immunologic, hematologic, dermatologic, or neurologic abnormality, or any other condition, that in the investigator's opinion, represents potential risk to the patient's safety and ability to fully participate in the study, or affects the absorption, distribution, metabolism, or excretion of liafensine.
6. Presence of autoimmune hepatitis, primary sclerosing cholangitis, untreated hepatitis C, active hepatitis B, or any other uncontrolled or unstable liver disease according to local guidance.
7. Uncontrolled human immunodeficiency virus (HIV) infection according to local guidance.
8. Uncontrolled abnormal thyroid function according to local guidance.
9. One or more clinical laboratory evaluations are outside the reference range, at screening, that are in the investigator's opinion, of potential risk to the patient's safety.
10. Has at the Screening Visit:
    - Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) levels > 1.5x the upper limit of normal (ULN) at screening.
    - Total bilirubin (TBL) > 2 mg/dL (34.2 μmol/L) at screening, unless there is an explained indirect hyperbilirubinemia, eg, Gilbert's syndrome.
    - Alkaline phosphatase (ALP) > 1.5x the ULN at screening.

    Note: Laboratory tests can be repeated to see if values return to normal range, but any such laboratory abnormality must be resolved by the Baseline Visit (Day -1).
11. Clinically significant vital sign abnormality at screening. This includes, but is not limited to, the following, in the supine (after at least 5 min rest) and standing (after 1 min and 3 min standing): systolic blood pressure ≥ 140 mmHg; diastolic blood pressure ≥ 90 mmHg; or heart rate < 50 or

> 90 beats per minute. If the initial blood pressure is ≥ 140/90 mmHg, the lowest value from up to 3 additional attempts, which also must not be ≥ 140/90 mmHg, should be used. Patients with symptomatic orthostatic hypotension, at the discretion of investigator, will be excluded.

12. Corrected QT interval measurement according to the Fridericia rule (QT_cF) > 450 msec for men and > 470 msec for women during controlled rest at screening, or history of long-QT syndrome.
13. ECGs containing any of the following readings:
    - Left bundle branch block
    - Right bundle branch block with QRS duration > 140 ms
    - Intraventricular conduction defect with QRS duration > 140 ms
    - Long QT syndrome
14. History of seizure, other than childhood febrile seizures.
15. History of clinically significant head trauma, including closed head injury with loss of consciousness, that is, in the opinion of the investigator, likely to affect central nervous system function.
16. History of clinically significant symptomatic orthostatic hypotension (ie, postural syncope).
17. History of narrow angle glaucoma.
18. History of cancer within 2 years prior to screening or between screening and baseline (Day -1), except for non-metastatic basal and/or squamous cell carcinoma of the skin.
19. Use of prescription or nonprescription medications for attention-deficit hyperactivity disorder (ADHD), narcolepsy, or cognitive enhancement (eg, methylphenidate, atomoxetine, modafinil, ginkgo biloba, and huperzine A) within 30 days prior to screening or between screening and baseline (Day -1).
20. Regular consumption of (eg, more days than not) excessive quantities of xanthine-containing beverages (eg, more than five cups of coffee or the equivalent per day) within 30 days prior to screening or between screening and baseline (Day -1).
21. Urine drug screen (UDS) positive for a drug of abuse, with the exception of cannabis in countries where it is legally available (see Table 3 for list of drugs of abuse). Where legal, prior use of cannabis is permitted provided the patient agrees to abstain from smoking or ingesting cannabis or cannabis products during the study.
22. Use of potent inducers of CYP3A4 (eg, rifampin, rifabutin, phenytoin, carbamazepine, or phenobarbital) within 2 weeks prior to baseline (Day-1).
23. Current diagnosis or history of a psychotic disorder, MDD with psychotic features, manic or hypomanic episode of bipolar or related disorders.
24. Current diagnosis of anxiety disorder (if primary), post-traumatic stress disorder, obsessive-compulsive disorder (if primary), intellectual disability (DSM-5 diagnostic code 319), borderline personality disorder, antisocial personality disorder, histrionic personality disorder, or narcissistic personality disorder according to the DSM-5 criteria, or any other psychiatric or neurologic disorder or symptom due to a general medical condition, that, in the judgement of the investigator, could pose undue risk to the patient or compromise the study.

25. Hospitalized or discharged from psychiatric ward within 8 weeks prior to the screening visit and planned hospitalization for any condition(s) during the study.
26. Moderate or severe alcohol use disorder or other substance use disorder (except nicotine or caffeine), within 6 months prior to screening, according to the DSM-5 criteria.
27. Significant risk of suicide determined by:
    a. Acute suicidality as evidenced by answering "yes" to Question 5 ("In the Past Year") on the C-SSRS, indicating active suicidal ideation with specific plan and intent for suicide, at screening, or baseline (Day -1); or
    b. History of suicidal behavior as indicated by a "yes" response on the Suicidal Behavior section of the C-SSRS ("In the past year") or
    c. A score ≥ 5 on Item 10 (suicidal thoughts) of the MADRS at screening or baseline (Day -1); or
    d. Has attempted suicide within 6 months prior to the initial screening visit.
28. Previous allogenic bone marrow transplant.
29. Received non-leukocyte-depleted whole blood transfusion within 4 months prior to PGx testing at Screening.
30. Currently employed by the sponsor or by a clinical trial site participating in this study, or a first-degree relative of an employee of the sponsor or of an employee at a participating clinical trial site.

4.3. Contraceptive Guidelines

Female patients must be postmenopausal or surgically sterile or, if of childbearing potential and the partner is not vasectomized (6 months minimum), must agree to use a medically acceptable form of contraception from the time of signing the ICF through at least 60 days following the last administration of study drug. If only the barrier method is used, a double barrier should be employed. Postmenopausal women must have had ≥ 24 months of spontaneous amenorrhea. Surgically sterile women are defined as those who have had a hysterectomy, bilateral ovariectomy, or bilateral tubal ligation. All women of childbearing potential must have a negative pregnancy test result before administration of study drug.

Male patients must be biologically incapable of having children (eg, vasectomized) or must agree to use the above forms of birth control for themselves and their partner from the time of signing the ICF through at least 120 days following the last administration of study drug.

4.4. Patient Withdrawal Criteria

Patients have the right to withdraw from study at any time for any reason.

4.4.1. Withdrawal from Study Treatment or Study Participation

Patients must be discontinued from **study treatment** for any of the following reasons:

- Unacceptable toxicity in the opinion of treating investigator that the risk outweighs potential benefit for the subject. For laboratory abnormality meeting withdrawal criteria, please refer to Section 7.6.1.8.

- Pregnancy

Patients may be discontinued from **study participation** for any of the following reasons:

- Intercurrent illness
- Withdrawal of consent
- Death
- Study terminated by the sponsor, relevant regulatory agencies, or institutional review board (IRB)/independent ethics committee (IEC)

All patients who discontinue from study treatment are encouraged to continue the study participation, and at a minimum undergo the End-of-Treatment (EOT) assessments, unless consent is withdrawn, as indicated in the schedule of events (Table 1). If a patient does not return for a scheduled visit, every effort should be made to contact the patient.

4.4.2. Withdrawal from Follow-up

Patients may withdraw or may be withdrawn from study follow up for any of the following reasons:

- Consent withdrawn/refuses follow up
- Death
- Study terminated by the sponsor
- Lost to Follow-up
- Other, specify

5. TREATMENT OF SUBJECTS

5.1. Description of Study Treatment

Patients will be randomly assigned through an IRT system in a 1:1:1 ratio to receive either liafensine 1 mg QD, liafensine 2 mg QD, or placebo QD for 6 weeks (Table 2).

Randomization Phase:

Liafensine 1 mg QD

- Day 1 through Day 42: one 1 mg liafensine tablet and one matching placebo tablet QD

Liafensine 2 mg QD

- Day 1 through Day 7: one 1 mg liafensine tablet and one matching placebo tablet QD
- Day 8 through Day 42: two 1 mg liafensine tablets QD

Placebo QD

- Day 1 through Day 42: two placebo tablets QD

Table 2: Investigational Product

Product Name:	**Liafensine Tablets 1 mg**	**Placebo for Liafensine Tablets 1 mg**
Dosage Form:	Tablet	Tablet
Unit Dose	1 mg	N/A
Route of Administration	Oral	Oral
Physical Description	White to off-white round film-coated tablet	White to off-white round film-coated tablet
Manufacturer	Patheon Inc. (Toronto, Canada)	Patheon Inc. (Toronto, Canada)

5.2. Administration of Study Treatment

Investigational product (study drug) will be supplied as tablets containing 1 mg liafensine or matching placebo. Study drug will be taken orally, once daily. Patients will be instructed to take the study drug whole, and not to chew, divide, or crush the tablets and take the study drug at the same approximate time each day in the morning.

5.3. Concomitant Medications

Prohibited concomitant treatments and study restrictions during the study from baseline to 14 days after the last dose of study drug (the first half of the follow-up) include:

1. Any investigational drug product, device, or biologic.
2. Any prescription or nonprescription medication for ADHD, narcolepsy, or cognitive enhancement (eg, methylphenidate, atomoxetine, modafinil, ginkgo biloba, and huperzine A).

3. Any psychopharmacologic drug (including antidepressants and over-the-counter medications to treat depression (eg, herbal medicines like St John's Wort, or other local herbal medicines).
4. Potent inducers of CYP3A4 (eg, rifampin, rifabutin, phenytoin, or carbamazepine, phenobarbital).

Permitted concomitant treatments, provided they meet the specific requirements detailed below, during the study and during the indicated periods include:

1. Up to 3 g/day paracetamol/acetaminophen.
2. Non-psychopharmacologic drugs provided the dose and condition being treated have been relatively stable for at least 2 months prior to screening and between screening and baseline (Day -1) and are not expected to change meaningfully during the study.
3. Soporific drugs may be used provided the dose and condition being treated have been relatively stable for at least 2 months prior to screening and between screening and baseline (Day -1) and are not expected to change meaningfully during the study.
4. Psychotherapy (including cognitive behavioral therapy) is permitted provided psychotherapy was initiated at least 2 months prior to screening and is expected to remain relatively stable during the study.
5. Anxiolytic drugs which do not affect 5-HT, norepinephrine, and dopamine activity may be used provided the dose and condition being treated have been relatively stable for at least 2 months prior to screening and between screening and baseline (Day -1) and are not expected to change meaningfully during the study.

Note 1: if a pro re nata (PRN) use has been established prior to the study, and not expected to change during the trial, this is considered stable treatment.

Note 2: Medication considered necessary for the patient's safety and well-being may be given at the discretion of the investigator and recorded in the appropriate sections of the electronic Case Report Form (eCRF). If a psychopharmacologic medication that is otherwise prohibited is administered the study measurements such as MADRS and CGI should be collected prior to dosing when possible. If any medication that is otherwise prohibited is administered to a patient, the investigator should inform the medical monitor prior to administration of medication when possible.

Other restrictions include:

1. Patients will be required to maintain their normal intake of xanthine-containing foods or beverages, and, if permitted, vitamin and herbal supplements, during the study, including the screening and follow-up periods.
2. Patients should not provide blood or plasma donations between screening and baseline (Day -1), during the study and for 6 weeks after the last dose of study drug.

5.4. Treatment Compliance

Compliance with study drug must be stressed and assessed at each visit. Study drug compliance will be assessed by the investigator and/or study personnel at designated visits by direct questioning and recording tablet counts from the previously dispensed bottles (ie, returned tablet counts). In the events of poor compliance, eg, < 80%, investigator and/or study personnel should follow up closely with patients and make every effort to improve patients' treatment compliance.

All study drug dispensed and returned must be recorded in the Drug Accountability Log.

The study site will keep a record of all drugs dispensed to and returned by patients throughout the study. Start and end dates of doses of study drug administered since the last dispensing visit will be recorded in the eCRFs (study drug dosage administration record) at visits specified in the table of assessments (Table 1). Deviations from the prescribed dosage regimen should be recorded in the eCRF.

Each patient should be instructed to return all study drug packaging and unused material to the study site at each visit. Study site personnel will destroy unused medication unless it is the site's policy to return to Denovo or its designee at the end of the study.

5.5. Randomization and Blinding

DGM4 status (positive vs negative) of randomized patients will be blinded to the sites, the patients, and sponsor. An IRT system will be used for blinding to DGM4 status, and to achieve the correct randomization ratio of DGM4 positive:DGM4-negative patients by randomly excluding most screened DGM4-negative patients (~80%).

The study team from the sponsor will also be blinded for DGM4 status of individual patients; however, the sponsor will be informed of the total number of patients enrolled in the DGM4-positive group by an independent unblinding team who supports the IDMC meetings in order to ensure the target enrollment is achieved in this group.

Treatment randomization will be stratified by DGM4 status (positive vs negative) and region (North America vs Other). Treatment assignment is blinded to the study site, patients, and the sponsor.

5.6. Emergency Treatment Unblinding

Unblinding during the study is discouraged and should only happen if knowledge of the patient's study drug would affect subsequent treatment and such knowledge is essential for the clinical management of the patient. In such a case, the investigator should contact the sponsor's medical monitor before breaking the treatment code whenever possible. The treatment assignment shall only be shared, if necessary and feasible, with personnel involved in the clinical management of the patient. Personnel exclusively involved in the clinical trial conduct, eg, study coordinators, study manager, and representatives from the contract research organization (CRO)/sponsor, shall remain blinded. The sponsor will be notified once the treatment code is broken and should remain blinded to the treatment assignment.

6. STUDY DRUG MATERIALS AND MANAGEMENT

6.1. Liafensine/Placebo

Investigational product (study drug) will be supplied by Denovo as tablets containing 1 mg of liafensine or matching placebo. The tablets must not be crushed or broken for administration.

Details of study drug packaging, labeling, and storage conditions will be provided in the study Pharmacy Manual.

The pharmacist or designee will dispense study drug kit(s) containing an adequate number of tablets, including overage, for each dosing cycle. Details of study drug dispensing and compliance will be provided in the Pharmacy Manual.

7. STUDY PROCEDURES AND ASSESSMENTS

7.1. Study Procedures

7.1.1. Informed Consent

Written informed consent must be obtained by signing an IRB or IEC approved ICF. Informed consent may be obtained more than 21 days prior to randomization.

Informed consent for study procedures will be obtained over 2 visits in the following manner:

Visit 1: Two separate brief ICFs will be completed, including

1. A brief ICF to explain sampling for PGx testing for the purpose of genotyping only.
2. A brief ICF to explain future scientific research biobanking; includes the option (checkbox for participation) to participate in the future scientific research program for all subjects.

Visit 2: A full ICF that explains the study procedures from V2 to EOS.

Results from physical exam, laboratory test, or other routine medical care performed prior to the date of informed consent, but within the allowed timeframe for screening procedures, can be used for determining the patient's eligibility if obtained as part of the patient's standard of care. If such results or evaluations are used in the determination of eligibility, this must be clearly documented in the patient's source documents.

All materials used for the baseline assessments and follow-up of patients, or for the investigation of AEs, may be duplicated and made available to the sponsor and/or an authorized independent body for review.

7.1.2. Study Treatment Evaluations and Procedures

7.1.2.1. Screening 1/Screening 2 Procedures (Visit 1 and Visit 2)

Unless otherwise noted below, all screening procedures, aside from DGM4 genotyping (performed prior to Day -21), must be performed within 21 days prior to randomization.

Screening 1 and Screening 2 procedures will be conducted over two visits as detailed in Table 1.

Screening 2 will only be performed after the initial eligibility including PGx (DGM4 status) is confirmed.

Laboratory testing and the assessment of vital signs can be repeated for up to 3 occurrences after Visit 2 to see if values return to normal range, but any abnormality must be resolved by the Baseline Visit.

All procedures as presented in Table 1 must be completed, including:

Visit 1

- Brief informed consents
- Inclusion/exclusion criteria – preliminary determination (not include MINI and ATRQ)
- A complete medical history

- Demographics
- Preliminary diagnosis checklist
- Biomarker: blood draw for DGM4 status
- Pregnancy test
- An assessment of adverse events

Visit 2

- Full informed consent
- Inclusion/exclusion criteria determination (continued)
- Psychiatric evaluations (MINI and ATRQ)
- Blood draw for clinical laboratory evaluations
- Complete physical and neurological examinations
- PRDF Checklist
- CGI-S
- MADRS
- HAMD-17
- C-SSRS, baseline/screening version
- Vital signs including supine (after at least 5 min rest) and standing (after 1 min and 3 min standing), blood pressure and heart rate
- Body weight and height
- Single 12-lead ECG will be performed
- Urinalysis (on-site unless macro-findings are observed and central lab assessment is required)
- Urine drug screen (on-site)
- Prior medications
- Assessment of adverse events

Note: Rater 1 is to assess HAMD-17 and MADRS; rater 2 is to assess CGI-S after reviewing PRDF Checklist; the rest of scales maybe collected by either rater.

7.1.2.2. Evaluations on Study From Baseline to End-of-Treatment

Please see Table 1 for all study procedures and timing.

- Continuation of inclusion/exclusion criteria determination
- Complete physical and neurological examinations
- Patient questionnaires and scales including PRPS, MADRS, HAMD-17, PRDF Checklist, CGI-S, CGI-I, SDS and C-SSRS (since-last-visit version)
- Vital signs including supine (after at least 5 min) and standing (after 1 min and 3 min standing), blood pressure and heart rate

- ECGs will be performed. 12-lead ECG recordings will be performed in triplicate (at 1-minute intervals between each of three recordings) at baseline. Single ECG will be performed at other visits.
- Clinical laboratory tests will be performed
- Urine drug screen
- Urine pregnancy test at baseline
- Blood samples for PK per patient will be collected. Time of last dose and time of blood collection will be recorded. When possible, and if medically appropriate, blood samples should be collected for PK from patients who experience an SAE or overdose.
- Concomitant medications
- Assessment of adverse events

Note: Rater 1 is to assess HAMD-17 and MADRS; rater 2 is to assess CGI-S after reviewing PRDF Checklist, and CGI-I after reviewing current and baseline PRDF Checklist and other baseline symptoms; the rest of scales maybe collected by either rater.

7.1.3. End-of-Treatment and Follow-up Evaluations and Procedures

Please see Table 1 for Day 42 EOT and Follow-up procedures and timing.

- Physical and neurological examinations
- Patient questionnaires and scales including MADRS, PRDF Checklist, CGI-S, CGI-I , SDS, C-SSRS (since-last-visit version), and DESS.
- Vital signs including supine (after at least 5 min rest) and standing (after 1 min and 3 min standing), blood pressure, heart rate, and body weight.
- ECGs will be performed on Day 42 (Visit 7) or at the time of early withdrawal, if within 3 days of last dose. Single 12-lead ECG will be performed.
- Clinical laboratory tests (including hematology, blood chemistry)
- Urinalysis (on-site unless macro-findings are observed and central lab assessment is required)
- Urine drug screen
- Serum pregnancy test
- Blood samples for PK per patient will be collected on Day 42 (Visit 7) or at the time of early withdrawal, if within 3 days of last dose. Time of last dose and time of blood collection will be recorded. When possible, and if medically appropriate, blood samples should be collected for PK from patients who experience an SAE or overdose.
- Assessment of adverse events
- Concomitant medications

Note: Rater 1 is to assess HAMD-17 and MADRS; rater 2 is to assess CGI-S after reviewing PRDF Checklist, and CGI-I after reviewing current and baseline PRDF Checklist and other baseline symptoms; the rest of scales maybe collected by either rater.

7.2. Assessment of Efficacy

The primary efficacy outcome measure is the change in MADRS total score from baseline (Visit 3, Day -1) to Visit 7 (Day 42) in DGM4-positive patients. Other efficacy assessments include CGI-S, CGI-I, and SDS.

The overall schedule of efficacy assessments is provided in Table 1. Note that CGIs should be completed only after the Patient Rated Depression and Functioning Checklist (PRDF Checklist) has been completed as instructed.

Montgomery-Åsberg Depression Rating Scale (MADRS)

The Montgomery-Åsberg Depression Rating Scale (MADRS) is a 10-item clinician-administered scale, designed to be particularly sensitive to antidepressant treatment effects in patients with major depression (Montgomery 1979). Each MADRS item is rated on a 0 to 6 scale. The MADRS total score is calculated as the sum of the 10 individual item scores; the total score can range from 0 to 60. Higher MADRS scores indicate higher levels of depressive symptoms.

A structured interview guide for Montgomery-Asberg (SIGMA) will be provided.

Clinical Global Impressions Scale-Severity (CGI-S)

The CGI-Severity (CGI-S) asks the clinician one question: “Considering your total clinical experience with this particular population, how mentally ill is the patient at this time?” which is rated on the following seven-point scale: 1 = normal, not at all ill; 2 = borderline mentally ill; 3 = mildly ill; 4 = moderately ill; 5 = markedly ill; 6 = severely ill; 7 = among the most extremely ill patients (Guy 1976).

This rating is based upon observed and reported symptoms, behavior, and function in the past seven days. Clearly, symptoms and behavior can fluctuate over a week; the score should reflect the average severity level across the seven days.

Clinical Global Impressions Scale-Improvement (CGI-I)

The CGI-Improvement (CGI-I) is similarly simple in its format (Guy 1976). Each time the patient is seen after medication has been initiated, the clinician compares the patient's overall clinical condition to the one-week period just prior to the initiation of medication use (the so-called baseline visit). The CGI-S score obtained at the baseline (initiation) visit serves as a good basis for making this assessment. Again, only the following one query is rated on a seven-point scale: “Compared to the patient's condition at admission to the project (prior to medication initiation), this patient's condition is: 1 = very much improved since the initiation of treatment; 2 = much improved; 3 = minimally improved; 4 = no change from baseline (the initiation of treatment); 5 = minimally worse; 6 = much worse; 7 = very much worse since the initiation of treatment.”

The CGI-I score generally tracks with the CGI-S such that improvement in one follows the other. Anchors for scoring, however, are quite different, and the CGI-I is based upon changes from the initiation of treatment in contrast to changes from the preceding week of treatment. Consequently, the two CGI scores can occasionally be dissociated such that a clinician may notice changes in the CGI-I relative to baseline despite no recent changes in the overall CGI severity score or vice versa.

Patient Rated Depression and Functioning Checklist (PRDF Checklist)

PRDF Checklist is used to assess overall depression symptoms and the psychosocial impact over the last week. It will be completed by patients and reviewed with the clinician before finalizing. The post-baseline entry must be compared to baseline checklist entry to refresh the knowledge of patient's baseline condition. The checklist must be administered prior to the administration of CGIs.

Sheehan Disability Scale (SDS)

The SDS will be used to assess functional impairment in three major life domains: work, social life/leisure activities, and family life/home responsibilities (Sheehan 2008). It is a five-item scale that will be completed by the participant. Three items use a Likert scale with a 10-point range (0 / Moderately to 10 / Extremely) and two items are open-ended (eg, "On how many days in the last week did you…?"). The scale is expected to take about 5 minutes to complete.

7.3. Other Assessments

Hamilton Depression Rating Scale – 17 Item (HAMD-17)

The Hamilton Rating Scale for Depression – 17 Item (HAMD-17) is used to quantify the severity of symptoms of depression and is one of the most widely used and accepted instruments for assessing depression (Hamilton 1960). The standard version of the HAMD-17 is designed to be administered by a trained clinician, and it contains 17 items rated on either a 3- or 5-point scale, with the sum of all items making up the total score. The HAMD-17 may be a useful scale for cognitively impaired patients who have difficulty with self-report instruments.

The HAMD-17 may be used to monitor the patient's progress during treatment, after the diagnosis of major depression has been established. This clinician-administered scale exists in several versions, ranging from 6 to 31 items; answers by patients are scored from 0 to 2 or 0 to 4 and tallied to obtain an overall score. A decrease of 50% or more in the HAMD-17 score is often considered to indicate a positive treatment response, whereas a score of 7 or less is considered equivalent to a remission.

In the current study, the HAMD-17 is utilized for eligibility only at screening and baseline visit.

Massachusetts General Hospital (MGH) Antidepressant Treatment Response Questionnaire (ATRQ) (5-year version)

Massachusetts General Hospital (MGH) Antidepressant Treatment Response Questionnaire (ATRQ) (5-year version) is a self-rated scale used to determine treatment resistance in major depressive disorder (MDD) (Chandler 2010). The MGH ATRQ defines a treatment period for an adequate duration of treatment and provides specific operational criteria for adequate dosage for each of the most commonly used antidepressants.

Placebo Response Propensity Scale (PRPS)

The Placebo Response Propensity Scale (PRPS) was developed to assess the conceptual constructs which may influence placebo response in a clinical trial.

7.4. Pharmacokinetics

Blood samples will be collected for determination of concentrations of liafensine and its primary metabolite on Day 28 (Visit 6) and on Day 42 (Visit 7) prior to dose administration. The time of last dose and time of blood collection will be recorded.

If the patient discontinues before Day 42 (Visit 7), a blood sample will be collected for PK on the last day the patient takes a full dose of study drug or as soon as possible thereafter, but within three days after the last dose in any case. When possible, and if medically appropriate, blood samples should be collected for PK from patients who experience a serious adverse event (SAE) or overdose.

7.5. Pharmacogenomics

Blood will be collected for DGM4 assessment by central laboratory. Any remaining samples not used for DGM4 assessment will be stored for potential future biomarker/pharmacogenomic research as described below.

The DGM4 biomarker status for each patient will be blinded to the sponsor, investigative site, and patient. Blood will also be collected to be stored at a contracted laboratory for future biomarker/pharmacogenomic research.

The biomarker/pharmacogenomic research includes studying genetic variations such as polymorphisms on serotonin, dopamine, or norepinephrine transporters, as well as CYP450 genes), which may help to understand the varied responses due to liafensine among different patients. The blood samples can also be used for developing DGM4 in vitro diagnostic (IVD) assay which will be used as a companion diagnostic in the future.

To protect patients' confidentiality, the stored biospecimens and data generated from them will be coded with the patients' study number. Biospecimens will be stored indefinitely unless a time limit is required by local regulations or ethical requirements. Patients can withdraw their consent for the use of their biospecimens at any time by making a request to the investigator, in which case any remaining biospecimen will be destroyed; data already generated from the biospecimens will continue to be stored to protect the integrity of existing analyses. Results generated from the biospecimen will not be provided to the patients (unless required by local regulations), and their family members, the investigators, or other physicians. Patients will not be compensated if the use of the biospecimens results in commercially viable products.

Instructions for sample collection, processing, storage, and shipment will be provided in a separate document.

7.6. Assessment of Safety

Safety will be assessed on the basis of spontaneously reported AEs; the DESS scale; physical, neurological, and psychiatric examinations (including C-SSRS); vital signs including orthostatic changes in blood pressure and heart rate; weight; 12-lead ECGs; clinical laboratory test results; serum and urine pregnancy tests (females of childbearing potential only); and concomitant medication assessments. Safety assessments in addition to those specified may be performed to ensure patient safety if judged appropriate by the investigator.

See Table 1 for the overall schedule of safety assessments.

Psychiatric assessments will be performed by a psychiatrist or trained and certified clinical staff member, and neurologic assessments will be performed by an experienced clinician.

Patients fulfilling Hy's law, defined as [ALT or AST ≥ 3 × ULN] and [TBL ≥ 2 × ULN], in the absence of significant increase in ALP and in the absence of an alternative diagnosis that explains the increase in total bilirubin, will be discontinued, with medical follow-up as appropriate.

An IDMC will be established to monitor data on an ongoing basis to ensure the continuing safety of patients enrolled in this study (Section 8.3.8). The IDMC will comprise of individuals external to the sponsor and will consist of at least one medical expert in the relevant therapeutic area, at least one hepatologist, and at least one statistician. The IDMC's responsibilities, authorities, and procedures, with details of decision-making guidance and dissemination of the results, will be documented in the IDMC charter.

7.6.1. Safety Parameters

Every effort should be made to ensure that the protocol-required tests and procedures are completed. Safety assessments will include collection of AEs, vital signs including supine and standing blood pressure and heart rate, physical examinations, neurological examinations, electrocardiograms, laboratory tests, and concomitant medications.

7.6.1.1. Adverse Events

Assessment of AEs will include the type, incidence, severity, timing, seriousness, and relatedness.

Adverse events reporting requirements are provided in Section 7.6.2.

7.6.1.2. Medical History and Demographics

Demographic and baseline variables include the following: age, sex, ethnicity, and race. Other baseline characteristics include the following: relevant medical history and neuro-oncology history.

7.6.1.3. Vital Signs

Vital signs will include measurement of temperature, supine (after at least 5 min rest) and standing (after1 min and 3 min standing), blood pressure (BP) and heart rate.

7.6.1.4. Physical Examination and Neurological Examination

The physical and neurological assessments will be performed by an experienced clinician.

The physical examination includes height and weight, and also examination of the heart, lungs, and abdomen at a minimum.

The neurological examination includes evaluation of cranial nerves, muscle strength, tone and bulk, reflexes, coordination, sensory function and gait.

7.6.1.5. Psychiatric Assessments

Psychiatric assessments will be performed by a psychiatrist or appropriately qualified clinical staff member.

Columbia-Suicide Severity Rating Scale (C-SSRS)

The Columbia-Suicide Severity Rating Scale (C-SSRS) was designed to quantify the severity of suicidal ideation and behavior (Posner 2011). C-SSRS is a measure used to identify and assess individuals at risk for suicide. Questions are phrased for use in an interview format, but can be completed as a self-report measure if necessary. C-SSRS measures four constructs: the severity of ideation, the intensity of ideation, behavior, and lethality. It includes "stem questions," which if endorsed, prompt additional follow-up questions to obtain more information. There are four versions of the scale available, including: 1) Baseline/screening version, which allows practitioners to gather a lifetime and recent 12 months history of suicidal ideation and/or behavior; 2) Since Last Visit version for assessment of suicidal thoughts and behaviors since C-SSRS was last administered; 3) Screen version, a shortened version of the full form (3-6 questions) most commonly used in clinical triage settings; 4) Risk Assessment Page, which provides a checklist of protective and risk factors of suicidality.

Discontinuation-Emergent Signs and Symptoms scale (DESS)

The DESS scale will be used to monitor patients for discontinuation symptoms (Rosenbaum 1998). It includes 43 signs or symptoms for which the patient indicates whether there has been any change since their last visit – indicating new symptom, old symptom but worse, old symptom but improved, old symptom but unchanged, symptom not present. The DESS total score is calculated as the count of items with new symptoms or old symptoms but worse. The total score can range from 0 to 43.

Mini International Neuropsychiatric Interview (MINI)

The Mini International Neuropsychiatric Interview (MINI) is a structured diagnostic interview designed to meet the need for an accurate psychiatric interview in multicenter trials, with a short administration time (~26 minutes) (Sheehan 1998). The MINI was developed to explore 17 disorders according to Diagnostic and Statistical Manual diagnostic criteria, focusing on the existence of current disorders. For each disorder, one or two screening questions rule out the diagnosis when answered negatively. Probes for severity, disability or medically explained symptoms are not explored symptom-by-symptom.

7.6.1.6. Electrocardiogram

Electrocardiogram (ECG): 12 lead (with a 10 second rhythm strip) tracing will be used for all ECGs. It is preferable that the machine used has a capacity to calculate the standard intervals automatically. 12-lead ECG recordings will be performed in triplicate (at approximately 1-minute intervals between each of the three recordings) at baseline, and single ECG for rest of visits.

QT corrected by Fridericia's formula is recommended: $QTcF = QT/RR^{0.33}$.

7.6.1.7. Laboratory Assessments

Clinical laboratory assessment will be performed at the time points indicated in Table 1. Table 3 provides the minimum required laboratory parameters for this study.

Table 3: Laboratory Tests

Blood chemistry
- Alanine aminotransferase (ALT/SGPT)
- Alkaline phosphatase (ALP)
- Aspartate aminotransferase (AST/SGOT)
- Bilirubin (direct and total)
- Blood urea nitrogen (BUN)
- Calcium
- Chloride
- Cholesterol (total)
- Creatine kinase (CK)
- Creatinine
- Glucose (serum)
- Phosphorus
- Potassium
- Protein (albumin and total)
- Sodium

Thyroid panel (Screening only)
- Thyroid-stimulating hormone (TSH)
- Free triiodothyronine (FT_3)
- Free thyroxine (FT_4)

Urinalysis (routine)
- On-site macroscopic examination Central lab microscopic examination (in the event of protein, blood, nitrite and/or leukocyte esterase noted in macroscopic)
- Bilirubin
- Blood (occult and cells)
- Ketones
- Leukocytes
- Nitrite
- pH
- Protein
- Specific gravity
- Urobilinogen

Hematology
- Hematocrit
- Hemoglobin
- MCH concentration (MCHC)
- Mean corpuscular hemoglobin (MCH)
- Mean corpuscular volume (MCV)
- Platelet count
- Red blood cell (RBC) count
- White blood cell (WBC) differential
  - Neutrophil count (absolute and %)
  - Lymphocyte count (absolute and %)
  - Monocyte count (absolute and %)
  - Eosinophil count (absolute and %)
- Basophil count (absolute and %)
- WBC count

Pregnancy tests (females of childbearing potential only)
- Serum β-hCG test
- Urine β-hCG test

Virology/Serology (Screening only)
- Hepatitis C virus (HCV)
- Hepatitis C virus antibody
- Hepatitis B surface antigen (HBsAg)
- Hepatitis B core antibody (HBcAb)
- HIV antibodies

Urine drug screen
- Amphetamines
- Barbiturates
- Cocaine
- MDMA (“Ecstasy”)
- Methadone
- Methamphetamine
- Opiates
- Oxycodone
- Phencyclidine (“PCP”)
- Tetrahydrocannabinol

Abbreviations: b-hCG = b-human chorionic gonadotropin; HIV = human immunodeficiency virus; MDMA = 3,4-methylenedioxy-N-methylamphetamine; PCP = 1-(1-phenylcyclohexyl) piperidine; SGOT = serum glutamic-oxaloacetic transaminase; SGPT = serum glutamic-pyruvic transaminase.

7.6.1.8. Laboratory Abnormalities

An increase of serum aminotransferases of > 3 x ULN must be followed by repeat testing of all four of the AT serum measures (ALT, AST, ALP, and TBL) within 48 to 72 hours to confirm the abnormalities and to determine if they are increasing or decreasing. In the urgent event that a local laboratory must be used, the test results must include the normal range and units. These results must be collected on a CRF and entered in the electronic data capture (EDC) system.

For patients with ALT or AST > 8 × ULN, ALT or AST > 5 × ULN for more than 2 weeks, ALT or AST > 3 × ULN and TBL > 2 × ULN, or ALT or AST > 3 × ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (> 5%), treatment must be discontinued and the patient should be monitored, at a minimum, weekly until liver chemistry results (ALT, AST, ALP, and bilirubin) resolve, stabilize, or return to baseline values.

In all the above situations involving elevations in liver enzymes, every attempt should be made to obtain the following:

- Viral hepatitis serology including:
  - Hepatitis A immunoglobulin M (IgM) antibody
  - Hepatitis B surface antigen and hepatitis B core antibody (IgM)
  - Hepatitis C RNA
- Blood sample for plasma PK analysis. The date and time of the PK blood sample collection and dates and times of the 2 previous doses of study medication must be recorded.
- Serum creatine phosphokinase (CPK)
- Fractionate bilirubin, if total bilirubin is ≥ 1.5 × ULN
- Record of the appearance or worsening of clinical symptoms of hepatitis, including fatigue, decreased appetite, nausea, vomiting, abdominal pain, jaundice, fever, or rash on the AE report form.
- Record of the use of concomitant medications, acetaminophen, herbal remedies, other over-the-counter medications, putative hepatotoxins, or alcohol on the concomitant medication form.
- If these are identified, consultation with a gastrointestinal or hepatology specialist should be considered.

The following are required for patients with ALT or AST ≥ 3 × ULN and total bilirubin ≥ 1.5 × ULN but are optional for other liver chemistry results:

- Anti-nuclear antibody, anti-smooth muscle antibody, and Type I anti-liver kidney microsomal antibodies
- Liver imaging (ultrasound, magnetic resonance imaging, or computed tomography scan) to evaluate liver disease

Hy's Law

Hepatic function abnormality defined by an increase in AST and/or ALT to ≥ 3 × ULN concurrent with an increase in TBL to ≥ 2 × ULN but without increase in alkaline phosphatase (ie, ALP < 2 × ULN) meets the criteria for Hy's Law and raises the concern for drug-induced liver injury (DILI) when no other cause of the abnormal laboratory results is identified. Follow-up investigations and inquiries will be initiated promptly by the investigational site to determine whether the findings are reproducible and/or whether there is objective evidence that clearly supports causation by a disease (eg, cholelithiasis and bile duct obstruction with distended gallbladder) or an agent other than the investigational product.

Cases meeting Hy's Law are considered an AESI and must be reported as SAEs. Study drug should be permanently discontinued for a Hy's Law case.

7.6.1.9. Pregnancy Screen

Serum pregnancy tests are required for female patients of childbearing potential. Serum pregnancy testing will occur at the timepoints indicated in Table 1.

If the patient is found to be pregnant, the patient will be withdrawn from study treatment immediately and the sponsor or sponsor representative notified within 24 hours of awareness. If the patient's partner is found to be pregnant the same procedure of reporting and follow up should be followed but the patient can continue study treatment.

7.6.2. Adverse and Serious Adverse Events

7.6.2.1. Definition of an Adverse Event

An adverse event (AE) is any untoward medical occurrence associated with the use of a drug in patients, whether or not considered drug related. It can be any unfavorable and unintended sign (including laboratory abnormalities), symptom, or disease temporally associated with the use of a drug, regardless of causal relationship to the drug.

Examples of AEs include the following:

- Significant or unexpected worsening or exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment or a concomitant medication.
- Any abnormal laboratory test results if:
  - Accompanied by clinical symptoms
  - Leads to a discontinuation of study treatment or change in study dosing
  - Requires significant additional concomitant medication or other therapy
  - Requires additional diagnostic testing (does not include repeat testing of an abnormal test) or medical intervention (does not include replacing of electrolytes)

Anticipated day-to-day fluctuations of pre-existing diseases(s) or conditions(s) present or detected at the start of the study that do not worsen are not considered AEs.

7.6.2.2. Serious Adverse Event

A serious adverse event (SAE) is an AE that meets at least one of the following criteria:

- Results in death
- Is life-threatening (AE that places the patient at immediate risk of death from the reaction as it occurred; it does not include a reaction that, had it occurred in a more severe form, might have caused death)
- Requires inpatient hospitalization or prolongation of existing hospitalization (see exceptions below)
- Results in persistent or significant disability/incapacity (substantial disruption of the ability to conduct normal life functions)
- Results in congenital anomaly/birth defect
- Other important medical events that may not result in death, be life-threatening or require hospitalization may be considered SAEs when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias, or convulsions that do not result in patient hospitalization, or the development of drug dependency or drug abuse

Hospitalization

Hospitalization is defined as any initial admission (even less than 24 hours) in a hospital or equivalent healthcare facility or any prolongation of an existing admission. An emergency room visit does not necessarily constitute a hospitalization; however, the event leading to the emergency room visit should be assessed for medical importance.

Hospitalization or prolongation of hospitalization due to the following reasons or conditions should not be reported as an SAE. Examples include but not limited to:

- Admission for treatment of a pre-existing condition not associated with the development of a new AE or with a worsening of the pre-existing condition
- Social, administrative for insurance reimbursement, or convenience admission
- Hospitalization for elective procedures for the treatment of pre-existing conditions (eg, hip replacement for pre-existing osteoarthritis)

Admission to rehabilitation facilities, respite care, hospice facilities, nursing homes, skilled nursing facilities, and same-day surgeries are not considered hospitalization.

7.6.2.3. Adverse Events of Special Interest

Potential liver injury, ie, suspected and/or confirmed Hy's Law, DILI cases will be assessed as adverse events of special interest (AESI) and must be reported as SAE. Information of abnormal laboratory test results including the course and evolvement, clinical signs and symptoms, detailed medical history and concurrent medication, history and current lifestyle including alcoholic assumption, history of exposure to environmental chemical agents will be used to assess the case(s).

7.6.2.4. Relationship to Study Treatment

Liafensine is an investigational medicinal product (IMP). The causality relationship to liafensine for all AEs (serious and non-serious) will be assessed by the investigator. The causal relationship of an AE to the study treatment will be assessed as either:

Related

There is as at least a reasonable possibility that the drug caused the AE (ie, there is evidence or arguments to suggest a causal relationship between the drug and AE)

Unrelated

- Does not follow a reasonable temporal sequence from administration of drug
- Does not appear to worsen when the drug is re-administered
- There is not a reasonable possibility that the drug caused the AE
- An alternate etiology has been established, ie, the AE is more likely explained by another cause (eg, other study treatments or procedures, underlying medical conditions, concomitant medications, etc) than the study treatment.

7.6.2.5. Recording Adverse Events

Adverse events should only be recorded by an investigator or by a healthcare provider qualified by training and experience. Patients should be asked in an open-ended manner about the occurrence of AEs. All AEs are recorded in the electronic CRF (eCRF).

It is generally not necessary to record both a diagnosis and its associated symptoms and laboratory abnormalities. For example, if "acute renal failure" is recorded as an AE, "creatinine 5 mg/dL" need not be recorded.

If an AE necessitates a procedure, the description of the event (eg, appendicitis) rather than the procedure (appendectomy) should be listed as the AE.

Any ongoing AEs and SAEs should be followed during study visits until resolution, or until the event is otherwise explained or judged by the investigator as stable or no longer clinically significant (unless the patient is lost to follow-up or withdraws consent).

7.6.2.6. Severity Assessment of Adverse Events

Adverse events will be reported, and severity graded, according to the following guidelines:

Mild	Awareness of signs or symptoms but easily tolerated
Moderate	Discomfort sufficient to cause interference with normal everyday activities
Severe	Incapacitating with inability to work or perform normal activities

In the event of the change in severity, the existing AE should be updated accordingly.

7.6.2.7. Reporting Adverse Events

All AEs regardless of causality will be recorded from the time a patient signs an ICF and through Follow-up period.

All SAEs regardless of causality will be recorded from the time a patient signs an ICF until 30 days after the last administration of study treatment. If SAEs are determined to be related to the study treatment by the investigators, these SAEs need to be reported beyond 30 days.

7.6.2.8. Serious Adverse Event Reporting Requirements

If an SAE occurs, the sponsor or designee is to be notified within 24 hours of investigator's awareness of the event. The time frame also applies to follow-up information on a previously reported SAE and pregnancies. This can be done by completing SAE entry through eCRF in the EDC. In the event of EDC outage, the investigator must complete the paper SAE reporting form and email or fax it to the sponsor or designee.

Investigators may be requested to obtain additional information on an SAE to allow for a complete assessment of the event.

Adverse event (serious and non-serious) reporting to regulatory authorities, including Suspected Unexpected Serious Adverse Reactions (SUSARs), will be carried out in accordance with applicable local regulations by the investigator, sponsor or designee.

All SAEs should be followed to resolution or stabilization.

7.6.3. Pregnancy Reporting

Pregnancy is not considered an adverse event. However, any patient who becomes pregnant during the study must be withdrawn from the study immediately. The investigator must report all pregnancies in the patient or partner of the patient within 24 hours of the investigator becoming aware of the event.

Pregnancy and maternal information will be collected until completion or termination of pregnancy including outcome of pregnancy and details of birth (if applicable).

If the outcome of the pregnancy meets the criteria for an SAE (eg. ectopic pregnancy, spontaneous abortion, intrauterine fetal demise, neonatal death, or congenital anomaly/birth defect) the investigator should follow the procedures for reporting SAEs.

8. STATISTICS

Additional details of the analyses will be provided in the statistical analysis plan (SAP) which will be finalized prior to breaking the blind.

8.1. Study Design

This study will be conducted as a multicenter, a randomized, double-blind, placebo-controlled Phase 2b trial to assess the efficacy, safety, tolerability, and PK of liafensine. The primary endpoint is change in MADRS total score from baseline (Visit 3, Day -1) to Visit 7 (Day 42) in DGM4-positive patients. The primary analysis will be conducted to assess liafensine treatment effect for combined liafensine 1 mg QD and 2 mg QD doses vs placebo in randomized patients who are DGM4-positive.

8.2. Determination of Sample Size and Statistical Rationale

Eligible patients will be randomized 1:1:1 to receive liafensine 1 mg QD, liafensine 2 mg QD, or placebo QD. The primary analysis is to assess treatment difference in MADRS total score change from baseline (Visit 3, Day -1) to Visit 7 (Day 42) between the combined liafensine 1 mg QD and 2 mg QD doses vs placebo, in DGM4-positive patients. A sample size of forty-seven (47) randomized DGM4-positive patients per arm is sufficient to detect a 4.5-unit difference between the combined liafensine 1 mg QD and 2 mg QD treatment groups and the placebo treatment group in the change in MADRS total score from baseline to Visit 7 with 80% power at the 2-sided 0.05 alpha level, assuming a standard deviation of 9 units.

To compensate for potential loss of power due to randomized patients who do not take at least one dose of study drug or who do not have a post randomization efficacy evaluation (~5%), a total of approximately 150 DGM4-positive patients (50 DGM4-positive patients per arm) will be randomized.

To examine trend in the total patient population and in the subgroup of DGM4-negative patients in order to evaluate DGM4 as a potential biomarker for predicting response to liafensine, approximately 20% additional DGM4-negative patients (10 per arm, total 30) will also be randomized. The sample size of 30 randomized patients in the DGM4 negative group is not estimated based on formal statistics in terms of power and alpha level.

The sample size may be re-estimated/re-adjusted if more than expected missing data are observed at Day 42.

8.3. Statistical Methods

8.3.1. Analysis Population

The Full Analysis Set (FAS) includes all randomized patients who took at least one dose of study drug and had a post-randomization efficacy evaluation. The FAS population will be analyzed by treatment randomized regardless of the actual treatment received. The primary and secondary efficacy results of the study will be based on analysis in FAS patients with positive DGM4 biomarker. Safety Population includes all patients who took at least one dose of study drug. The Safety population will be analyzed by the actual treatment received regardless of treatment randomized.

8.3.2. Primary Estimand

The primary estimand, the main clinical quantity of interest to be estimated in the study, is defined by the following three components:

- Population: patients with treatment-resistant depression with the DGM4-positive biomarker
- Endpoint: change in MADRS total score from baseline to Day 42
- Measure of Intervention: the effect of the initially randomized treatment that would have been observed had all patients remained on their treatment throughout the double-blind phase

The primary analysis will be based on the full analysis set, as described above, and the MADRS total scores collected during the double-blind treatment period.

8.3.3. Efficacy Analyses

The primary efficacy endpoint is the change in MADRS total score from baseline (Visit 3, Day -1) to Day 42 (Visit 7). The primary efficacy analysis will be performed in DGM4-positive patients at 2-sided significance level of 0.05 using a mixed model for repeated measures (MMRM) that includes the fixed effects of treatment, visit (categorical covariate), treatment-by-visit interaction, baseline MADRS total score (continuous covariate), and region. An unstructured variance-covariance matrix will be used. The least-squares (LS) mean change in MADRS total score at Day 42 from baseline for the combined liafensine treatment arms (1 mg QD and 2 mg QD) and placebo arm will be estimated from the MMRM model. The treatment effect for the combined liafensine treatment arms vs placebo will be estimated using the LS mean difference at Day 42 from the MMRM model. The comparison of the change in MADRS total score from baseline to Day 42 between each of the 2 liafensine treatment arms and placebo will be carried out using the same approach. The key secondary efficacy endpoint for this study is the change in CGI-S from baseline (Visit 3, Day -1) to Day 42 (Visit 7), in DGM4-positive patients.

Other secondary and exploratory efficacy endpoints include the CGI-I, SDS, response (defined as $\geq$ 50% improvement from baseline in MADRS total score at Day 42), and remission (defined as MADRS total score $\leq$ 10 at Day 42), in DGM4-positive patients. CGI-S, CGI-I, and SDS will be analyzed using the same MMRM model as for the primary efficacy endpoint. Response rate, remission rate, and corresponding 95% confidence interval (CI) for each treatment group will be derived by using Clopper-Pearson method. The comparison of response rate and remission rate between treatment groups will be carried out using the Cochran–Mantel–Haenszel (CMH) test.

Subscales, individual items, and domains of endpoints will also be analyzed in an exploratory manner, using the same MMRM model as for the primary efficacy endpoint.

The change in MADRS total score from baseline in DGM4-negative patients will be analyzed using the same approach as described for the primary endpoint analysis in the DGM4-positive patients. The LS mean of the change in MADRS total score for each treatment group and the treatment difference in DGM4-negative patients will be estimated. The treatment effects between the DGM4positive and negative patients will be compared primarily in descriptive fashion to examine the trend, and may be evaluated by including the interaction of DGM4 status and treatment group in the mixed model.

8.3.3.1. Type I Error Control for Multiple Comparisons

The family-wise type I error rate will be controlled at a fixed 1-sided alpha level of 0.025 using a closed testing procedure for multiple dose comparisons and multiple endpoint comparisons in DGM4-positive patients (Marcus 1976). In the closed testing procedure, the average effect of 1 mg and 2 mg dose groups will be first compared with the placebo group on change in MADRS total score from baseline to Day 42 in DGM4-positive subjects. If the one-sided p-value was ≤ 0.025, then the one-sided p-values are calculated for the comparisons of 1 mg dose vs placebo and 2 mg dose vs placebo, respectively. If both individual p-values are ≤ 0.025, a significant treatment effect is declared for both dose groups. If only one p-value is ≤ 0.025, a significant treatment effect is declared for the corresponding dose group. All other efficacy endpoints will not be controlled for multiplicity with nominal p-values provided for the exploratory purpose only. Additional details will be specified in the SAP.

8.3.4. Safety Analyses

The safety analyses will be conducted in the safety population, which includes all randomized patients who have received at least one dose of study drug. The safety population will be analyzed by the actual treatment received regardless of treatment randomized.

Adverse events will be coded using the current version of the Medical Dictionary for Regulatory Activities (MedDRA) classification system. Listings will include the verbatim term, preferred term, and system organ class. The incidence and severity of treatment-emergent adverse events (TEAEs) will be summarized by treatment. Vital signs including supine (after at least 5 min rest) and standing (after 1 min and 3 min standing), blood pressure and heart rate and heart rate, weight, ECG, clinical laboratory evaluation, DESS scale, and C-SSRS data will be summarized with descriptive statistics, by visit and by treatment.

Safety and tolerability data will be summarized descriptively, including tables, listings, and graphs, as appropriate. Unless otherwise stated, descriptive summary statistics for continuous variables will include number (N), mean (ie, arithmetic mean), standard deviation (SD), minimum, median, and maximum. Descriptive summary statistics for categorical data will include frequency and proportion.

All data will be presented by treatment regimen.

Individual data for vital signs including supine (after at least 5 min rest) and standing (after 1 min and 3 min standing), blood pressure and heart rate, weight, clinical laboratory measures, and ECG assessments will be listed and summarized for any potentially clinically important (PCI) values according to predetermined PCI criteria.

There will be no imputation (substitution) for missing data unless explicitly specified otherwise in this protocol or in the statistical analysis plan.

8.3.5. Pharmacokinetics

Plasma concentrations of liafensine and its metabolite will be listed by subject and time of collection. A population PK analysis of plasma concentrations of liafensine and its primary metabolite will be performed using a nonlinear mixed-effects modeling approach. The analysis will evaluate the effects of age, sex, ethnicity, and other covariates on the PK profile of liafensine. This analysis will be reported separately.

8.3.6. Subgroup Analyses

Subgroup analyses will be considered for gender, ethnicity, and region, and may be explored for other factors (such as age and baseline severity in term of MADRS total score). Summary statistics will be provided for efficacy measures by treatment arm for each subgroup.

8.3.7. Statistical Analysis Plan

Additional details of the analyses will be provided in the statistical analysis plan which will be finalized prior to unblinding.

8.3.8. Independent Data Monitoring Committee

An IDMC will be established to monitor data on an ongoing basis to ensure the continuing safety of patients enrolled in this study. The IDMC will comprise individuals external to the sponsor and will consist of at least one medical expert in the relevant therapeutic area, at least one hepatologist, and at least one statistician. The IDMC's responsibilities, authorities, and procedures, with details of decision-making guidance and dissemination of the results, will be documented in the IDMC charter.

9. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

9.1. Study Monitoring

Before an investigational site can enter a patient into the study, a representative of Denovo will visit the investigational study site to:

- Determine the adequacy of the facilities
- Discuss with the investigator(s) and other personnel their responsibilities with regard to protocol adherence, and the responsibilities of Denovo or its representatives. This will be documented in a Clinical Trial/Study Agreement between Denovo and the investigator.

During the study, a monitor from Denovo or representative will have regular contacts with the investigational site, for the following:

- Provide information and support to the investigator(s)
- Confirm that facilities remain acceptable
- Confirm that the investigational team is adhering to the protocol, that data are being accurately recorded in the case report forms, and that investigational product accountability checks are being performed
- Perform source data verification. This includes a comparison of the data in the case report forms with the patient's medical records at the hospital or practice, and other records relevant to the study. This will require direct access to all original records for each patient (eg, clinic charts, electronic medical record, shadow charts).
- Record and report any protocol deviations not previously sent to Denovo.
- Confirm AEs and SAEs have been properly documented on eCRFs and confirm any SAEs have been forwarded to Denovo and those SAEs that met criteria for reporting have been forwarded to the IRB/IEC.

The monitor will be available between visits if the investigator(s) or other staff needs information or advice.

9.2. Audits and Inspections

Authorized representatives of Denovo, a regulatory authority, an IRB, or an IEC may visit the site to perform audits or inspections, including source data verification. The purpose of a Denovo audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, ICH GCP guidelines, and any applicable regulatory requirements. The investigator should contact Denovo immediately if contacted by a regulatory agency about an inspection.

9.3. Institutional Review Board

The principal investigator (PI) must obtain IRB/IEC approval for the investigation. Initial IRB/IEC approval, and all materials approved by the IRB/IEC for this study including the patient consent form and recruitment materials must be maintained by the investigator and made available for inspection.

9.4. Curriculum Vitae and Medical Licenses

The PI is responsible for ensuring that the study is being conducted by qualified personnel. Documentation of these qualifications must be maintained within the Regulatory Binder, and includes the following:

Curriculum Vitae (CV): CVs for the PI and all sub-investigators listed on the FDA Form 1572 must be signed and dated. These CVs must show affiliation with the institution conducting the study and be current within two years of the personnel initiating their participation in the study.

Medical Licenses: Medical licenses (eg, physicians, physician assistants, nurses) listed on the FDA Form 1572 must be kept current, and copies must be maintained in the Regulatory binder during the entire period of the person's participation in the study.

9.5. Financial Disclosure

Documentation of each investigator's proprietary or financial interest in Denovo is required by the US Code of Federal Regulations (21 CFR 54). A financial disclosure form provided by the sponsor must be completed, signed, and dated by the PI and each sub-investigator listed on the FDA Form 1572. This form must be executed prior to the personnel's participation in the study. Each investigator must inform the sponsor of any change in his/her financial interest in the sponsor for up to 1 year after the end of the study.

10. QUALITY CONTROL AND QUALITY ASSURANCE

To ensure compliance with GCP and all applicable regulatory requirements, Denovo or its representative will monitor for compliance closely and may conduct a quality assurance audit.

11. ETHICS

11.1. Local Regulations

The study must fully adhere to the principles outlined in the ICH GCP E6 Guideline (2016) and remain consistent with the most recent version of the Declaration of Helsinki. The investigator will ensure that the conduct of the study complies with the basic principles of GCP as outlined in the current version of 21 CFR, subpart D, Part 312, "Responsibilities of Sponsors and Investigators," Part 50, "Protection of Human Subjects," and Part 56, "Institutional Review Boards."

11.2. Ethics Review

This study is being conducted under a United States (US) Investigational New Drug application and other regulatory applications, as applicable. This protocol (and any modifications) and appropriate consent procedures must be reviewed and approved by an IRB/IEC. This board must operate in accordance with the current federal regulations. The investigator will send a letter or certificate of IRB/IEC approval to the sponsor (or designee) before patient enrollment and whenever subsequent modifications to the protocol are made.

11.3. Ethical Conduct of the Study

This study will be conducted in accordance with applicable country-specific laws and regulations, including, but not limited to, ICH GCP guidelines and the ethical principles that have their origins in the Declaration of Helsinki. The IRB/IEC must review and approve the protocol and ICF before any patients are enrolled. Before any procedures specified in the protocol are performed, the patient must sign and date the IRB/IEC-approved ICF.

Study personnel involved in conducting this trial will be qualified by education, training, and experience to perform their respective task(s). This trial will not use the services of study personnel for whom sanctions have been invoked or there has been scientific misconduct or fraud (eg, loss of medical licensure, debarment, etc.).

11.4. Written Informed Consent

Investigators must ensure that patients, or, in those situations where consent cannot be given by patients, their legally acceptable representatives, are clearly and fully informed about the purpose, potential risks, and other critical issues regarding clinical studies in which they volunteer to participate.

Denovo will provide the investigator with an appropriate (ie, global or local) sample ICF which will include all elements required by ICH GCP and applicable regulatory requirements. The sample ICF will adhere to the ethical principles that have their origin in the Declaration of Helsinki.

Investigators must:

1. Provide a copy of the consent form and written information about the study in the language in which the patient is most proficient prior to clinical study participation. The language must be non-technical and easily understood.
2. Allow time necessary for patient to inquire about the details of the study.
3. Obtain an informed consent signed and personally dated by the patient and by the person who conducted the informed consent discussion, and provide a copy of the signed consent to the patient.
4. Obtain the IRB/IEC's written approval/favorable opinion of the written ICF and any other information to be provided to the patients, prior to the beginning of the study, and after any revisions are completed for new information.
5. Revise the informed consent whenever important new information becomes available that is relevant to the patient's consent. The investigator, or a person designated by the investigator, should fully inform the patient of all pertinent aspects of the study and of any new information relevant to the patient's willingness to continue participation in the study. This communication should be documented.

The consent form must also include a statement that Denovo and regulatory authorities have direct access to patient records.

The rights, safety, and well-being of the study patients are the most important considerations and should prevail over interests of science and society.

11.5. Patient Confidentiality

The confidentiality of records that could identify patients must be protected, respecting the privacy and confidentiality rules applicable to regulatory requirements, the patients' signed ICF and, in the US, the patients' signed HIPAA Authorization.

All clinical information is confidential, but data generated for this study must be available for inspection on request to representatives of the US FDA, other national or local regulatory or health authorities, Denovo representatives, and the associated IRB/IEC.

All records must be kept in a secured area.

11.6. Patient Recruitment

Advertisements approved by IRBs/IECs and investigator databases may be used as recruitment procedures. However, Denovo will have an opportunity to review and approve the content of any study recruitment materials directed to potential study patients before such materials are used.

11.7. Reporting of Safety Issues and Serious Breaches of the Protocol or ICH GCP

Progress reports and notifications of serious adverse drug reactions will be provided to the IRB or IEC according to local regulations and guidelines.

12. DATA HANDLING AND RECORD KEEPING

12.1. Data/Document

The investigator must ensure that the records and documents pertaining to the conduct of the study and the distribution of study treatment are complete, accurate, filed, and retained. Examples of source documents include hospital records, clinic and office charts, laboratory notes, memoranda, patient's diaries or evaluation checklists, dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiche, X-ray film and reports, records kept at the pharmacy and the laboratories.

12.2. Data Management

Data will be collected via eCRF. The term "case report form" includes as applicable paper forms and/or electronic data capture screens or forms for studies that utilize electronic data capture. For randomized patients, all and only data for the procedures and assessments specified in this protocol and required by the case report forms should be submitted on the appropriate eCRF (unless transmitted to the sponsor or a designee electronically, eg, laboratory data). Data from some procedures required by the protocol, such as physical exams or some types of laboratory tests, will be recorded only on the source documents. Additional procedures and assessments may be performed as part of the investigator's institution or medical practice standard of care. Data for unscheduled or additional assessments should remain in the patient's medical record and should not be recorded on eCRFs unless specifically requested.

The eCRF must be completed and signed by the investigator or authorized delegate from the study staff. This also applies to records for those patients who fail to complete the study or are randomized and never treated. If a patient stops dosing or terminates from the study, the dates and reasons must be noted on the eCRF.

All paper forms should be typed or filled out using indelible ink and must be legible. Errors should be crossed out but not obliterated, the correction inserted, and the change initialed and dated by the investigator or his or her authorized delegate. Any discrepancies in the data will be brought to the attention of the clinical team, and investigational site personnel, if necessary. Resolutions to these issues will be reflected in the database. An audit trail within the system will track all changes to the data.

The investigator should ensure the accuracy, completeness, legibility, and timeliness of the data reported to the sponsor in the eCRF and in all required reports.

12.3. Inspection of Records

Denovo will be allowed to conduct site visits to the investigation facilities for the purpose of monitoring any aspect of the study. The investigator should understand that source documents for this study should be made available to appropriately qualified personnel from Denovo Quality Assurance (or designee), or to health authority inspectors after appropriate notification.

The investigator agrees to allow the monitor to inspect the drug storage area, study treatment stocks, drug accountability records, patient charts and study source documents, and other records relative to study conduct. The verification of the eCRF data must be by direct inspection of source documents.

Upon request, the investigator will supply Denovo (or designee) with any required background data from the study documentation or clinic records. This is particularly important when eCRFs (if paper) are illegible or when errors in data transcription are suspected. In case of special problems or governmental queries or requests for audit inspections, it is also necessary to have access to the complete study records, provided that patient confidentiality is protected.

12.4. Retention of Records

The investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should be classified into two separate categories as follows: (1) investigator's study file and (2) patient clinical source documents.

The investigator's study file will contain as applicable the protocol and protocol amendments, eCRFs, query forms, IRB/IEC and governmental approval with correspondence, sample informed consent, drug records, staff curriculum vitae and authorization forms, and other appropriate documents and correspondence.

Patient clinical source documents (usually predefined by the project to record key efficacy and safety parameters independent of the eCRFs) may include, but are not limited to, patient hospital/clinic records, physician's and nurse's notes, appointment book, original laboratory reports, ECG, electroencephalogram, computed tomography/magnetic resonance imaging scan, bone scan, X-ray, pathology and special assessment reports, signed ICFs, patient diaries, consultant letters, and patient screening and enrollment logs.

The investigator must keep these two categories of documents on file for at least the latest of 2 years following the marketing application approval date for the study treatment in the indication being investigated, 2 years after the investigation is completed or discontinued, or for a time consistent with local regulatory requirements. After that period of time, the documents may be destroyed subject to local regulations with prior written permission from the sponsor. If the investigator wants to assign the study records to another party or move them to another location, the sponsor must be notified in advance.

If the investigator cannot guarantee the archiving requirement at the study site for any or all of the documents, special arrangements must be made between the investigator and the sponsor to store these in a sealed container outside of the study site so that they can be returned sealed to the investigator in case of a regulatory audit. When source documents are required for the continued care of the patient, appropriate copies should be made for storing outside of the study site.

13. PUBLICATION POLICY

13.1. Communication of Results by Denovo

All information obtained as a result of this study should be regarded as confidential. Denovo fulfills its commitment to publicly disclose clinical trial results through posting the results of studies to public registries in accordance with applicable local laws/regulations.

13.2. Publications by Investigators

Information regarding use or publication of study related information will be provided to the investigator in the Clinical Trial Agreement.

14. INVESTIGATOR PROTOCOL SIGNATURE PAGE

I understand that all information supplied to me by Denovo Biopharma LLC is confidential.

I understand that this protocol and all amendments must be submitted to the appropriate institutional review board (IRB)/independent ethics committee IEC.

I have read protocol DB104-01 and its appendices and agree to adhere to all requirements in the conduct of the study.

In signing below, I agree:

- To assume responsibility for the proper conduct of the study at my site
- To conduct the study in compliance with this protocol, any future amendments, and with any other study procedures provided by Denovo Biopharma LLC
- That I am aware of, and will comply with Good Clinical Practices (GCP) and all applicable regulatory requirements
- To ensure that all persons assisting me with the study are adequately informed about the study and of their study-related duties as delegated by me
- Not to implement any changes to the protocol without written agreement from Denovo Biopharma LLC and prior review and written approval from the IRB/IEC except where necessary to eliminate an immediate hazard to study patients

I acknowledge that failure to adhere to these stipulations may constitute a breach of governmental regulations, may invalidate the data, and may result in termination of the study at my site.

Principal Investigator's Signature	Date
Principal Investigator's Printed Name	
Institution Name	

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16. APPENDICES

APPENDIX 1. GUIDANCE TO STUDY SITES ON STUDY AND PATIENT MANAGEMENT IN CASE OF A PUBLIC HEALTH EMERGENCY

In the case of a public health emergency, Denovo institutes minimum study requirements, in the event of travel restriction, to safeguard the rights, welfare, and safety of study subjects and investigative site staff. These measures align with the current guidance issued the US FDA in March 2020 and updated 30 August 2021 (FDA 2021) in regard to the impact of COVID-19 on the management of clinical trials.

Also addressed are issues in study conduct that may be impacted by a public health emergency such as patient visits, documentation of deviations to the protocol, and recording of data. Changes that may be made to the protocol and clinical trial conduct as a result of any public health emergency are to be considered temporary and will only remain in effect until a future Administrative Letter to Investigators notifies study sites to resume study conduct in accordance with the full protocol and normal practices.

Where feasible, sites should continue to follow the full schedule of assessments. All assessments missed as a result of any public health emergency, including COVID-19, will be captured in the CRFs.

Adjustments to Visit Windows and Study Assessments

Study assessments associated with ± 3-day visit windows are assigned a ± 5-day window. Visits conducted within the expanded window are not considered protocol deviations. Visits outside of the expanded window may be considered protocol deviations. Data should be recorded on the CRF associated with the nominal visit day.

Study procedures that cannot be conducted due to institutional restrictions will not be reported as protocol deviations if the sponsor is made aware in advance on a case-by-case basis. Such procedures may include PK blood sample collection and ECGs.

Remote Patient Visits

- **If permitted by institutional guidelines,** remote patient visits may be conducted with site staff by telephone or using video technology and/or with a visit by a home healthcare professional. Depending on local IRB/IEC policy, a revised ICF may be needed to allow remote patient visits.
- If new local and/or central laboratories are used during remote visits for safety laboratory assessments (eg, hematology and chemistry), ensure all test results, laboratory certifications, and reference normal laboratory values and units are collected and documented in the site records consistent with institutional guidelines. The laboratory normal ranges should be provided to the sponsor for entry into the clinical database.
- Ensure all details of remote patient visits are documented in patient site records.

Shipment of Clinical Trial Material to Patients

Study drug (capsules or tablets) may be shipped to the homes of study participants being visited by home health professionals under three conditions: 1) such clinical trial material shipment is permitted by local institution policy; 2) shipment is by a secure, trackable method, and documentation of shipment and receipt is retained with the patient or pharmacy records;

and 3) the material is shipped and stored per the requirements in Pharmacy Manual and the conditions stated on the study drug label. Clinical trial material exposed to temperature excursions should be replaced. In the patient's home, clinical trial material must be stored under the conditions stated on the container labels and the Pharmacy Manual. Unused material and empty containers should be returned to the study site to enable the pharmacy to maintain accountability records.

If any public health emergency impacts sites and subjects, these measures should be implemented immediately and remain in place until the impact from the public health emergency subsides.

Missed Study Drug Administration

If study drug administration is missed due to inability to obtain study drug during the public health emergency, this reason should be recorded in the patient site record and reported in the CRF as the reason for missed dose and will be documented as a protocol deviation.

APPENDIX 2. PRELIMINARY DIAGNOSIS CHECKLIST

Guide to Completion

The checklist is to be completed at Visit 1 PRIOR to collecting blood samples for genotyping.

Item	**Outcome**	**Source (Select all that apply)**
Does patient have depression?	□ Yes □ No □ Not sure □ Further information required	□ Written/digital medical records □ Notes from treating physician □ Oral report by patient □ Other, please specify_____
Does patient meet criteria for Treatment Resistant Depression (TRD)* within the past 5 years?	□ Yes □ No □ Not sure □ Further information required	□ Written /digital medical records □ Notes from treating physician □ Oral report by patient □ Pharmacy records are available □ Other, please specify_____
How many treatment course(s) with antidepressants given > 6 weeks has the patient *failed* within the past 5 years?	□ One course □ Two courses □ Three courses □ More than 3 courses	□ Written /digital medical records □ Notes from treating physician □ Oral report by patient □ Pharmacy records are available □ Other, please specify_____
How many *augmentation(s) of antidepressant therapy* which lasted >6 weeks has the patient experienced in past 5 years? Note: augmentation can be considered failed treatment regimen.	□ None □ One augmentation □ Two augmentations □ Three augmentations □ More than 3 augmentations	□ Written /digital medical records □ Notes from treating physician □ Oral report by patient □ Pharmacy records are available □ Other, please specify _____

Item	Outcome	Source (Select all that apply)
Do you consider that this patient meets preliminary diagnosis of TRD and should be assessed with PGx?	□ Yes □ No □ Not sure □ Further information required	
Has the patient taken his/her prescribed antidepressant medication reliably in the past year?	□ Yes □ No □ Not sure	

*TRD is defined as within the past 5 years patient must have had a clinically meaningful inadequate response (estimated < 50% improvement per investigator/patient consensus and documented by the investigator to at least two treatment courses with antidepressant regimens. These must involve at least two different pharmacologic treatment classes and have been given at accepted therapeutic doses for an adequate duration (at least 6 weeks). One of these treatment failures must have occurred within the current episode.

APPENDIX 3. PATIENT RATED DEPRESSION AND FUNCTIONING CHECKLIST

Completed by patient based on patient experience in the past week. To be reviewed with clinician prior to finalization.

Guide to Completion:(Consider the current impact of your symptoms compared to when you were NOT depressed and/or felt okay)

- **No Impact** — Your behavior or functioning related to these symptoms is **not impaired** as compared to when you were not depressed
- **Minimal Impact** — There has been minimal if any impact on your behavior or functioning related to these symptoms as compared to when you were not depressed
- **Mild Impact** — Your behavior or functioning related to these symptoms is **mildly impaired** as compared to when you were not depressed
- **Moderate Impact** — Your behavior or functioning related to these symptoms is **moderately impaired** as compared to when you were not depressed
- **Severe Impact** — Your behavior or functioning related to these symptoms is **markedly impaired** as compared when you were not depressed
- **Very Severe Impact** — Your behavior or functioning related to these symptoms is **very severely impaired** as compared to when you were not depressed

Note: Changes in your behavior or function refer to all aspects of your life. It includes maintaining expectations you or others have for you about your job or primary life role, your social life, and your personal interests/activities.

Symptom	**Questions**	**Frequency of symptom in PAST WEEK**	**Impact on <u>your emotions, activities, and your relationships with others</u> in the PAST WEEK**
Depressed thoughts/sadness	In the past week how often have you had upsetting sad or depressed thoughts? What has been their impact on your emotions, activities and relationships with others?	□ Never □ Seldom □ Occasional □ Often □ Very often □ All the time	□ No impact in past week □ Minimal impact in past week □ Mild impact in past week □ Moderate impact in past week □ Severe impact in past week □ Very severe impact in past week
Crying	In the past week how often have your thoughts, or the actions of others upset you and caused you to cry (outwardly or inwardly). What has been the impact on your life?	□ Never □ Seldom □ Occasional □ Often □ Very often □ All the time	□ No impact in past week □ Minimal impact in past week □ Mild impact in past week □ Moderate impact in past week □ Severe impact in past week □ Very severe impact in past week
Low energy, easily get tired, **fatigue, feeling "slowed down"**	In the past week how often have you found that you **don't have the energy level to complete your daily activ**ities? What has been the impact on your life?	□ Never □ Seldom □ Occasional □ Often □ Very often □ All the time	□ No impact in past week □ Minimal impact in past week □ Mild impact in past week □ Moderate impact in past week □ Severe impact in past week □ Very severe impact in past week

Symptom	**Questions**	**Frequency of symptom in PAST WEEK**	**Impact on <u>your emotions, activities, and your relationships with others</u> in the PAST WEEK**
Impaired communication	In the past week how often have you found that you are **not communicating with others (by phone, internet or in person)?** What has been the impact on your life?	□ Never □ Seldom □ Occasional □ Often □ Very often □ All the time	□ No impact in past week □ Minimal impact in past week □ Mild impact in past week □ Moderate impact in past week □ Severe impact in past week □ Very severe impact in past week
Impaired social interactions with others	In the past week how often have you found that you **don't want to spend time with others** (in person, on the phone or on the internet)? What has been the impact on your life?	□ Never □ Seldom □ Occasional □ Often □ Very often □ All the time	□ No impact in past week □ Minimal impact in past week □ Mild impact in past week □ Moderate impact in past week □ Severe impact in past week □ Very severe impact in past week
Impaired ability to experience a wide range of emotions	In the past week how often have you felt that you were NOT experiencing a full range of positive emotions like **happiness, joy, satisfaction, and pride?** What has been the impact on your life?	□ Never □ Seldom □ Occasional □ Often □ Very often □ All the time	□ No impact in past week □ Minimal impact in past week □ Mild impact in past week □ Moderate impact in past week □ Severe impact in past week □ Very severe impact in past week

Symptom	**Questions**	**Frequency of symptom in PAST WEEK**	**Impact on <u>your emotions, activities, and your relationships with others</u> in the PAST WEEK**
Impairment in your interests or activities	In the past week how often have you found that **your job, hobbies, sports, music, etc. are not interesting or fulfilling**? What has been the impact on your life?	□ Never □ Seldom □ Occasional □ Often □ Very often □ All the time	□ No impact in past week □ Minimal impact in past week □ Mild impact in past week □ Moderate impact in past week □ Severe impact in past week □ Very severe impact in past week
Impaired memory/ concentration/ attention/ability to make decisions	In the past week how often have you found that **your ability to remember or maintain your attention on tasks or your ability to make decisions?** What has been the impact on your life?	□ Never □ Seldom □ Occasional □ Often □ Very often □ All the time	□ No impact in past week □ Minimal impact in past week □ Mild impact in past week □ Moderate impact in past week □ Severe impact in past week □ Very severe impact in past week
Increased irritability/ feelings of anger hostility	In the past week how often have you found that you **get angry or are easily irritated by others?** Think about the number arguments you have had with others. What has been the impact on your life?	□ Never □ Seldom □ Occasional □ Often □ Very often □ All the time	□ No impact in past week □ Minimal impact in past week □ Mild impact in past week □ Moderate impact in past week □ Severe impact in past week □ Very severe impact in past week

Symptom	**Questions**	**Frequency of symptom in PAST WEEK**	**Impact on <u>your emotions, activities, and your relationships with others</u> in the PAST WEEK**
Suicidality/thoughts of death	In the past week how often have you felt **that life was not worth living, that you wished you were dead or, even, that you wanted to take your life**? What has been the impact on your life?	□ Never □ Seldom □ Occasional □ Often □ Very often □ All the time	□ No impact in past week □ Minimal impact in past week □ Mild impact in past week □ Moderate impact in past week □ Severe impact in past week □ Very severe impact in past week
Decreased interest in physical or emotional intimacy with others	In the past week how often have you found that your **interest in being intimate with others has diminished**? What has been the impact on your life?	□ Never □ Seldom □ Occasional □ Often □ Very often □ All the time	□ No impact in past week □ Minimal impact in past week □ Mild impact in past week □ Moderate impact in past week □ Severe impact in past week □ Very severe impact in past week
Decreased sexual desire and/or interest in sexual activities	In the past week how often have you found that your **sexual desire and activities have diminished**? What has been the impact on your life?	□ Never □ Seldom □ Occasional □ Often □ Very often □ All the time	□ No impact in past week □ Minimal impact in past week □ Mild impact in past week □ Moderate impact in past week □ Severe impact in past week □ Very severe impact in past week

Symptom	**Questions**	**Frequency of symptom in PAST WEEK**	**Impact on <u>your emotions, activities, and your relationships with others</u> in the PAST WEEK**
Increased anxiety/ tension	In the past week how often have you found that you are **anxious, tense or that you are worrying about things**? What has been the impact on your life?	□ Never □ Seldom □ Occasional □ Often □ Very often □ All the time	□ No impact in past week □ Minimal impact in past week □ Mild impact in past week □ Moderate impact in past week □ Severe impact in past week □ Very severe impact in past week
Feelings of worthlessness, helplessness, hopelessness, or pessimism	In the past week how often have you **felt pessimistic about your life with feelings of helplessness or worthlessness**? What has been the impact on your life?	□ Never □ Seldom □ Occasional □ Often □ Very often □ All the time	□ No impact in past week □ Minimal impact in past week □ Mild impact in past week □ Moderate impact in past week □ Severe impact in past week □ Very severe impact in past week
Feelings of guilt, shame or being a failure	In the past week how often have you **felt guilty about things that you have done, or shame, or that your life is a failure**? What has been the impact on your life?	□ Never □ Seldom □ Occasional □ Often □ Very often □ All the time	□ No impact in past week □ Minimal impact in past week □ Mild impact in past week □ Moderate impact in past week □ Severe impact in past week □ Very severe impact in past week

Symptom	**Questions**	**Frequency of symptom in PAST WEEK**	**Impact on <u>your emotions, activities, and your relationships with others</u> in the PAST WEEK**
Feeling stressed/ overwhelmed/oppressed	In the past week how often have you found that you are **easily stressed, overwhelmed, or oppressed by your responsibilities**? What has been the impact on your life?	□ Never □ Seldom □ Occasional □ Often □ Very often □ All the time	□ No impact in past week □ Minimal impact in past week □ Mild impact in past week □ Moderate impact in past week □ Severe impact in past week □ Very severe impact in past week
Sleeping too much	In the past week how often have you found that you **are sleeping more than usual and still not feeling fully rested and revived**? What has been the impact on your life?	□ Never □ Maybe a little □ One day □ Some days □ Most days □ Every day	□ No impact in past week □ Minimal impact in past week □ Mild impact in past week □ Moderate impact in past week □ Severe impact in past week □ Very severe impact in past week
Sleeping too little, difficulty getting to sleep, staying asleep or early-morning waking	In the past week how often have you found it difficult to get to sleep or stay asleep or wake up too early? What has been the impact on your life?	□ Never □ Maybe a little □ One day □ Some days □ Most days □ Every day	□ No impact in past week □ Minimal impact in past week □ Mild impact in past week □ Moderate impact in past week □ Severe impact in past week □ Very severe impact in past week

Symptom	**Questions**	**Frequency of symptom in PAST WEEK**	**Impact on <u>your emotions, activities, and your relationships with others</u> in the PAST WEEK**
Decreased appetite	In the past week how often have you found that your **appetite has decreased**? What has been the impact on your life?	□ Never □ Seldom □ Occasional □ Often □ Very often □ All the time	□ No impact in past week □ Minimal impact in past week □ Mild impact in past week □ Moderate impact in past week □ Severe impact in past week □ Very severe impact in past week
Overeating	In the past week how often have you found that **your appetite has increased**? What has been the impact on your life?	□ Never □ Seldom □ Occasional □ Often □ Very often □ All the time	□ No impact in past week □ Minimal impact in past week □ Mild impact in past week □ Moderate impact in past week □ Severe impact in past week □ Very severe impact in past week
Persistent physical symptoms that do not respond to treatment	In the past week how often have you had **physical pains like headaches, stomach aches, joint pains** that do not respond well to treatment? What has been the impact on your life?	□ Never □ Seldom □ Occasional □ Often □ Very often □ All the time	□ No impact in past week □ Minimal impact in past week □ Mild impact in past week □ Moderate impact in past week □ Severe impact in past week □ Very severe impact in past week

Symptom	**Questions**	**Frequency of symptom in PAST WEEK**	**Impact on <u>your emotions, activities, and your relationships with others</u> in the PAST WEEK**
Other problems	In the past week how often have you had **other problems** or concerns not mentioned that trouble you? If so, what are they? What has been the impact on your life?	□ Never □ Seldom □ Occasional □ Often □ Very often □ All the time	□ No impact in past week □ Minimal impact in past week □ not Mild impact in past week □ Moderate impact in past week □ Severe impact in past week □ Very severe impact in past week
OVERALL IMPACT OF SYMPTOMS ON FUNCTIONING	Overall, in the past week how much have the **whole group of symptoms** described above affected you, your work life, and your relationships with others?	□ Never □ Seldom □ Occasional □ Often □ Very often □ All the time	□ No impact in past week □ Minimal impact in past week □ Mild impact in past week □ Moderate impact in past week □ Severe impact in past week □ Very severe impact in past week
Treatment **To be used at baseline visit**	In the past week, how many times did you miss or forget to take your antidepressant medication?	□ Never missed □ Missed 1 time □ Missed 2-3 times □ Missed 4 or more times □ I don't take antidepressant medication	

Symptom	**Questions**	**Frequency of symptom in PAST WEEK**	**Impact on <u>your emotions, activities, and your relationships with others</u> in the PAST WEEK**
Treatment **To be used at all post-baseline visits**	In the past week, how many times did you miss or forget to take your 1 pill from bottle A and 1 pill from bottle B of your study medication?	□ Never missed □ Missed 1 time □ Missed 2-3 times □ Missed 4 or more times	

APPENDIX 4. PLACEBO RESPONSE PROPENSITY SCALE (CLINICAL TRIAL VERSION)

Overview

The Placebo Response Propensity Scale (PRPS) was designed to assess the probability of placebo response within clinical trials and population-based studies. Within clinical trials the PRPS should be administered at the Screening and/or Baseline visit. In a clinical trial the term ***health condition(s)*** in the PRPS instructions should be changed to the health condition under study (eg, in a depression study the questions should ask about depression). The PRPS can be patient completed or administered by the site. Mode of administration should be included in the study data file. Any translations should be developed in collaboration with the developer following ISPOR guidelines and be certified.

Using the PRPS within trials allows assessment of results with and without those with a high propensity for PBO response allowing for more confidence when trying to establish a true signal of efficacy. Each study using the PRPS provides an opportunity for additional analysis of the data giving more confidence in study results and helps identify patients most likely to be responders.

For the first set of questions, please think about your beliefs and experiences in general. For each item below, please select one answer to indicate the extent to which you agree or disagree with the statement.

	Completely Disagree	**Mostly Disagree**	**Moderately Disagree**	**Slightly Disagree**	**Neutral**	**Slightly Agree**	**Moderately Agree**	**Mostly Agree**	**Completely Agree**
I forget to take my medications when I am supposed to									
I am responsible for my health									
Doctors have helped me a lot in the past									
I take my medications exactly as I am supposed to									
I find medications to be helpful									
I do not take my medications if I think I can do without them									
I think people in research studies should be paid money									

For each item below, please select one response to indicate the extent to which you agree or disagree with the statement relative to your **health condition(s)**.

	Completely Disagree	**Mostly Disagree**	**Moderately Disagree**	**Slightly Disagree**	**Neutral**	**Slightly Agree**	**Moderately Agree**	**Mostly Agree**	**Completely Agree**
Whether or not I get better depends on factors or things outside of my control									
I am ashamed of my condition									
My condition will probably get better on its own									
No one understands my condition									
My condition limits my ability to enjoy life									
I talk with other people about my condition									
Medications									

	Completely Disagree	**Mostly Disagree**	**Moderately Disagree**	**Slightly Disagree**	**Neutral**	**Slightly Agree**	**Moderately Agree**	**Mostly Agree**	**Completely Agree**
for my condition are not likely to help me									
I have struggled with this condition for a long time									
My condition is serious									
So far, I like the study doctor(s)									
Whether or not I get better depends on factors or things outside of my control									

For each item below, please select one response to indicate the extent to which you agree or disagree with the statement relative to your **health condition(s)**.

	Completely Disagree	**Mostly Disagree**	**Moderately Disagree**	**Slightly Disagree**	**Neutral**	**Slightly Agree**	**Moderately Agree**	**Mostly Agree**	**Completely Agree**
The clinic staff at study site listen carefully to what I say									
I think I will get placebo (sugar pill) in this study									
I think I will improve even if I get a placebo (sugar pill) in this study									
I think the study doctor spends, or will spend, a lot of time with me									
The study doctor is not concerned about my condition									
The study doctor is an expert in my condition									
The study doctor helps, or will help, me to get better									
The study doctor makes me feel comfortable									
The clinic staff at study site really know what they are doing									

For each item below, please select one response to indicate the extent to which you agree or disagree with the statement relative to your **health condition(s)**.

	Completely Disagree	**Mostly Disagree**	**Moderately Disagree**	**Slightly Disagree**	**Neutral**	**Slightly Agree**	**Moderately Agree**	**Mostly Agree**	**Completely Agree**
I really want to be treated for my condition									
I am not likely to get better with the study medicine									
I have, or expect I will, improve with the study medicine									
Seeking to participate in this study has made me feel better									
I like or will like coming to the study site clinic for my appointments									
I make, or will make, myself better with the study medication									
I have a feeling that the study medicine brings, or will bring, about good for me									

For the following question, please choose the response that applies to your **health condition(s)**.

	0	**1**	**2-3**	**4-5**	**More than 5**
Please indicate the number of research studies in which you have previously participated					

For the following question, please choose the response that applies to your **health condition(s)**.

	Very Much Better	**Much Better**	**A Little Better**	**Unchanged**	**A Little Worse**	**Much Worse**	**Very Much Worse**
Compared to how you feel now, how do you expect to feel at the end of the study?							

For the following question, please choose the response that applies to your **health condition(s)**.

	Very Much Better	**Much Better**	**A Little Better**	**Unchanged**	**A Little Worse**	**Much Worse**	**Very Much Worse**
How do you think the study medication will make you feel?							