CLINICAL STUDY PROTOCOL

Protocol Title: A Phase 3, Multicenter, Randomized, Double-Blind, Placebo-Controlled Study to Assess the Efficacy and Safety of REL-1017 for Major Depressive Disorder (The RELIANCE-II Study)

Protocol Number: **REL-1017-302**

IND Number:	133345
Name of Investigational Product:	REL-1017
Phase of Development:	3
Indication:	Major Depressive Disorder
Sponsor:	Paul Greene, PhD SVP Clinical Operations Relmada Therapeutics, Inc. 2222 Ponce de Leon Blvd, Floor 3 Coral Gables, FL 33134 Tel: 786-629-1376 Email: pgreene@relmada.com
Protocol Version:	9.0
Amendment Version:	8.0
Protocol Date:	11 Nov 2024

-CONFIDENTIAL-
This document and its contents are the property of and confidential to Relmada Therapeutics, Inc. Any unauthorized copying or use of this document is prohibited.

PROTOCOL APPROVAL SIGNATURES

Protocol Title:	A Phase 3, Multicenter, Randomized, Double-Blind, Placebo-Controlled Study to Assess the Efficacy and Safety of REL-1017 for Major Depressive Disorder (The RELIANCE-II Study)
Protocol Number:	REL-1017-302
Protocol Version	9.0
Amendment Version	8.0

This study will be conducted in compliance with the clinical study protocol (and amendments), International Council for Harmonisation (ICH) guidelines for current Good Clinical Practice (GCP), and applicable regulatory requirements.

Sponsor Signatory

Paul Greene, PhD
SVP Clinical Operations
Relmada Therapeutics

Signed by:
Paul Greene
Signer Name: Paul Greene
Signing Reason: I approve this document
Signing Time: 11/13/2024 | 4:12:48 AM PST
9C35547D6F0742E7B3966E375989A776
Signature

INVESTIGATOR SIGNATURE PAGE

Protocol Title: A Phase 3, Multicenter, Randomized, Double-Blind, Placebo-Controlled Study to Assess the Efficacy and Safety of REL-1017 for Major Depressive Disorder (The RELIANCE-II Study)

Protocol Number: REL-1017-302

Confidentiality and Current Good Clinical Practice (GCP)/E6(R2):

I agree, as an Investigator conducting this study:

- To assume responsibility for the proper conduct of the study at this site.
- To conduct the study in compliance with this protocol, with any future amendments, and with any other written study conduct procedures provided, reviewed, and approved by Relmada Therapeutics, Inc.
- Not to implement any deviations from or changes to this protocol without agreement from the Sponsor and prior review and written approval from the Institutional Review Board (IRB)/Independent Ethics Committee (IEC), except where necessary to eliminate an immediate hazard to the study participants or for administrative aspects of the study (where permitted by all applicable regulatory requirements).
- That I am thoroughly familiar with the appropriate use of the investigational drug, as described in this protocol, and any other information provided by the Sponsor including, but not limited to, the current Investigator's Brochure or equivalent document provided by Relmada Therapeutics, Inc.
- That I am aware of, and will comply with, GCP and all applicable regulatory requirements, including the regulations governing the use of controlled substances.
- To ensure that all persons assisting me with the study are adequately informed about the investigational drug and that they are qualified to perform their study-related duties and functions as described in this protocol.
- That I have been informed that certain regulatory authorities require the Sponsor to obtain and supply details about the qualified Investigator's ownership interest in the Sponsor or the study drug and more generally about his or her financial ties with the Sponsor. Relmada Therapeutics, Inc., will obtain and disclose any relevant information in this regard solely for complying with regulatory requirements.

Hence, I:

- Agree to supply Relmada Therapeutics, Inc., with all information regarding ownership interest and financial ties with Relmada Therapeutics, Inc. (including those of my spouse and dependent children);
- Agree to promptly update this information if any relevant changes occur during the study and for 1 year following completion of the study; and
- Agree that Relmada Therapeutics, Inc. may disclose this information about such ownership interests and financial ties to regulatory authorities.

<Name>
<Title>

_____
Investigator Signature

_____
Date (DD-Mmm-YYYY)

_____
Institution

1 SYNOPSIS

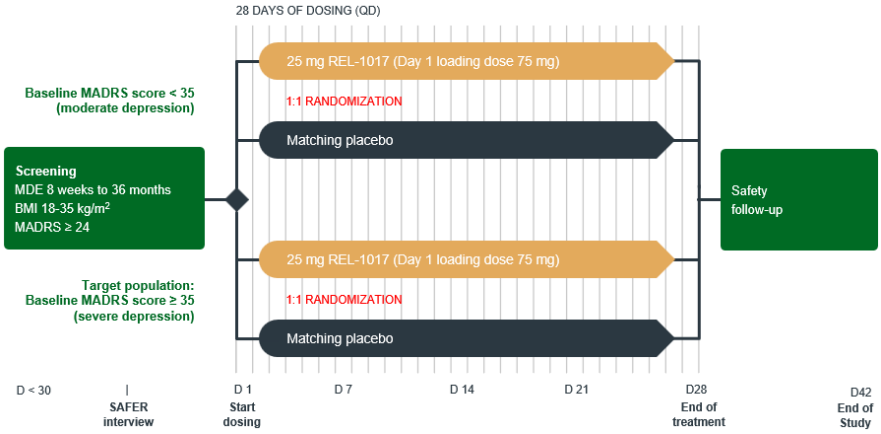
Title of Study	A Phase 3, Multicenter, Randomized, Double-Blind, Placebo-Controlled Study to Assess the Efficacy and Safety of REL-1017 for Major Depressive Disorder (The RELIANCE-II Study)
Sponsor	Relmada Therapeutics, Inc. (Relmada)
Investigators/Study Sites	Approximately 80 sites planned in North America and other US territories.
Phase of Development	3

Objectives	**Endpoints**
Primary	
To evaluate the therapeutic efficacy of REL-1017 compared to placebo in subjects with inadequate response to ongoing antidepressant therapy (ADT) at Day 28 on the Montgomery-Åsberg Depression Rating Scale (MADRS10) total score	Absolute change from Baseline to Day 28 of the MADRS10 total score for REL-1017 compared to placebo in subjects with inadequate response to ongoing ADT

Objectives	Endpoints
Secondary	
Secondary Efficacy Objectives To evaluate the therapeutic efficacy of REL-1017 compared to placebo in subjects with inadequate response to ongoing antidepressant therapy (ADT) in the following measurements: • MADRS10 response rate (improvement ≥50% compared with total Baseline score) at Day 28 • MADRS10 remission rate (total score ≤10) at Day 28 • MADRS10 score at Day 7 • Clinical Global Impression of Severity (CGI-S) score at Day 28 Secondary Efficacy Objectives in the Sub-Population with Baseline MADRS10 ≥ 35 To evaluate the therapeutic efficacy of REL-1017 compared to placebo in the Sub-Population with Baseline MADRS10 ≥ 35 in the following measurements: • MADRS10 response rate (improvement ≥50% compared with total Baseline MADRS10 score at Day 28 • MADRS10 remission rate (total score ≤10) at Day 28 • MADRS10 score at Day 7 • Clinical Global Impression of Severity (CGI-S) score at Day 28	Secondary Efficacy Endpoints • MADRS10 response rate (improvement ≥50% compared with total Baseline score) at Day 28 • MADRS10 remission rate (total score ≤10) at Day 28 • Absolute change from Baseline to Day 7 of the MADRS10 total score • Absolute change from Baseline to Day 28 of the CGI-S score Secondary Efficacy Endpoints in the Sub-Population with Baseline MADRS10 ≥ 35 • Absolute change from Baseline to Day 28 of the MADRS10 total score for REL-1017 • MADRS10 response rate (improvement ≥50% compared with total Baseline MADRS10 score) at Day 28 • MADRS10 remission rate (total score ≤10) at Day 28 • Absolute change from Baseline to MADRS10 score at Day 7 • Absolute change from Baseline Clinical Global Impression of Severity (CGI-S) score at Day 28
Exploratory	
To evaluate the impact of REL-1017 for 28 days compared to placebo in subjects with inadequate response to ongoing ADT in the Total and the Sub-Population with Baseline MADRS10 ≥ 35 on measures of depression, anxiety, sleep, sexual function, cognitive function, select biomarkers, and quality of life.	To evaluate the impact of REL-1017 for 28 days compared to placebo: • Absolute change from Screening to Day 28 of the Hamilton Anxiety Depression Scale (HAM-A) total score • Absolute change from Screening to Day 28 of the SDQ total and component scores • Absolute change from Baseline to Day 28 in the Sheehan Disability Scale (SDS) total score and domain scores • CGI-I score at Day 28 • Time course of MADRS10 response rate (defined as a ≥50% decrease from Baseline of MADRS10 total score) at all post-Baseline assessment time points • Time course in MADRS10 remission rate (defined as absolute MADRS10 score ≤10) at

Objectives	Endpoints
	all post-Baseline assessment time points • Absolute change from Baseline to Day 28 in MADRS10 component scores • MADRS10 total score as a function of the time since first diagnosis of Major Depressive Disorder (MDD), sex and age • Correlation between selected biomarkers with therapeutic efficacy as measured via MADRS10 total score until Day 28 • Time course of the changes from Baseline in MADRS10 total score at all post-Baseline assessment time points • Absolute change from Baseline in DSST • Absolute change from Baseline in PDQ-D-5 – cognition scale • Absolute change from Baseline in ASEX
Safety	
Safety and Tolerability Objectives • To evaluate safety and tolerability of REL-1017 • To evaluate the effect of REL-1017 on electrocardiographic parameters	Safety Endpoints • Treatment-emergent adverse events (TEAEs) • Evaluate the effects on the electrocardiogram (ECG), including heart rate, PR, QRS and QT interval with Fridericia's correction (QTcF) intervals • Vital signs and weight • Physical examination • Clinical laboratory parameters (chemistry, hematology, and urinalysis) • C-SSRS • Clinician-Administered Dissociative States Scale (CADSS)
Pharmacokinetic	
Pharmacokinetic Objectives • To evaluate pharmacokinetics (PK) of REL-1017 and potential metabolites	Pharmacokinetic Endpoints • Estimation of REL-1017 PK profile (maximum observed plasma concentration [C_{max}], trough concentration [C_{trough}], and apparent terminal elimination half-life at steady-state [$t_{1/2}$] based on sparse PK sampling

Study Design	This is an outpatient, 2-arm, Phase 3, multicenter, randomized, double-blind, placebo-controlled study to assess the efficacy and safety of REL-1017 at 25 mg once daily (QD) for MDD in subjects with inadequate response to ongoing ADT. During the study, subjects currently receiving ADT (i.e., a marketed ADT treatment course at the approved dosing regimen), who experience an inadequate therapeutic response, will be stratified according to MADRS10 total score (<35 or ≥ 35) at Baseline,

	then randomized in a 1:1 ratio to REL-1017 or placebo, and receive treatment for 28 days. The primary efficacy analysis will be based on the Day 28 assessment of the Total Population. 
Investigational Product/Reference Product	REL-1017 25 mg and matching placebo
Study Drug, Dose Schedule, and Mode of Administration	REL-1017 25 mg tablets for oral administration (PO) The following dose schedule will be used in the study: <u>Day 1 – loading dose:</u> • Three REL-1017 25 mg tablets (75 mg total) administered at the clinical study site • Three matching placebo tablets administered at the clinical study site <u>Day 2 to Day 28 – maintenance dose:</u> • One REL-1017 25 mg tablet QD • One matching placebo tablet QD Participants will be asked to stay on a stable dosing regimen of the current antidepressant therapy (ADT) between Day 1 and Day 28, while exposed to REL-1017 or matching placebo. Dosing with REL-1017 should take place once daily at the same time as the first-line ADT is taken.
Study Population and	Adult participants (aged 18 to 65 years, inclusive) diagnosed with MDD

Duration of Participation	(based on Structured Clinical Interview for DSM-5 [SCID-5] for MDD, matching Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition [DSM-5]), who are currently in a major depressive episode (MDE), with a Hamilton Depression Rating Scale (HAMD17) score ≥19 at Screening, and with a substantiated inadequate response to 1 to 3 courses of a valid course of an approved antidepressant in the current MDE, i.e., to an approved dose and duration (minimum 6 weeks) of a marketed ADT treatment course (as confirmed by assessment of an independent interview by a Massachusetts General Hospital-Clinical Trials Network and Institute [MGH-CTNI]-certified clinician using the Antidepressant Treatment Response Questionnaire [ATRQ]). The maximum duration of the MDE must not exceed 36 months. A total of approximately 280 participants, independently qualified by the Investigator and an independent MGH-CTNI-certified clinician, will be randomized in a 1:1 ratio to the active REL-1017 25 mg QD arm (n=140) or placebo arm (n=140). An unblinded interim analysis for sample size re-estimation (IA-SSR) is planned based on the calculation of the conditional power (Mehta, 2011) and adopting the Cui-Hung-Wang paradigm (Cui, 1999) when approximately 210 participants completed Day 28 assessments, at an information fraction of about 75%. This IA-SSR may result in an increase of total sample size by up to 460 participants. After signing of the informed consent form (ICF), each participant will undergo an up to 30-day Screening period and a 28-day randomized double-blind placebo-controlled period, for a total of approximately 58 days of study participation, plus a 14-day safety follow up.
Planned Sample Size	Approximately 280 subjects (140 per treatment group) are required to demonstrate efficacy for the primary endpoint in this study, the change from Baseline to Day 28 in the MADRS10 total score. The sample size was selected to detect a difference between means of the REL 1017 and placebo groups equal to Δ=3.8 points with a common standard deviation of σ = 11 (Cohen's d: $\Delta / \sigma = 0.35$). The difference in treatment group means for this study is anticipated to be larger than the prior REL-1017-301 study due to changes made in this trial's screening criteria to strengthen the selection of subjects with a true MDD diagnosis. The planned standard deviation for the primary endpoint is approximately the same as the one observed in REL 1017 301. An unblinded interim analysis is planned to take place when approximately 75% of the total number of subjects have completed their Day 28 visit or have prematurely dropped out of the study. The interim analysis will include sample size re-estimation based on conditional power (CP) with a 20% futility boundary, i.e., a CP below

	20% indicates the study is futile. Based on these assumptions, a two-sample, two-sided t-test achieves at least 80% power with 140 subjects per group (N=280 total) and a type 1 error rate of 5% (α=0.05). The number of additional subjects derived from the IA sample size re-estimation procedure will range from no additional subjects (N=280) to a maximum of 180 additional subjects (N=460). Sample size calculations and design of the interim analysis were performed with the software package East® v6.5.
Inclusion Criteria	To enroll in the clinical study, participants must meet the following inclusion criteria: 1. Must be able to read, speak, and understand English or Spanish and must provide written informed consent prior to the initiation of any protocol-specific procedures. 2. Male or female participant aged 18 to 65 years, inclusive. 3. Body mass index (BMI) between 18.0 and 35.0 kg/m^2, at Screening. 4. Participant understands the study requirements, is willing and able to commit to meet all study requirements, adhere to both approved ADT and study drug regimen, and complete all assessments and all scheduled visits, per Investigator judgment. 5. Women of childbearing potential (WOCBP) and men whose sexual partners are WOCBP must use at least 1 highly effective method of contraception from Screening and for at least 2 months after the last study drug administration. For WOCBP and men with female sexual partners of childbearing potential, examples of medically acceptable forms of contraception include vasectomy or male condom for participants, plus an additional method of contraception for their female partners. Highly effective methods of contraception are those which have a failure rate of <1% (when implemented consistently and correctly) and include: • Intrauterine device (IUD) • Bilateral tubal ligation, bilateral salpingectomy, or bilateral tubal occlusive procedure • Hormonal contraceptives (e.g., oral, patch, or injectable) • A double-barrier protection method (e.g., condom, sponge, or vaginal diaphragm with spermicide cream, foam, or gel) • Abstinence from heterosexual intercourse is accepted if this is the participant's usual lifestyle and must be continued until at least 2 months after the last dose of study drug. Women who are not of childbearing potential must be congenitally or surgically sterile (hysterectomy and/or bilateral oophorectomy/salpingo-oophorectomy, as determined by the participant's medical history) or must be post-menopausal. Post-menopausal is defined as being amenorrheic for at least 1 year without another cause and a follicle-stimulating hormone (FSH) level ≥40 mIU/mL as confirmation. 6. Diagnosed with MDD as defined by the Diagnostic and Statistical Manual, Fifth Edition (DSM-5), and confirmed by the SCID-5 MDD. The documentation of MDD diagnosis (medical records or alternative verified documentation) must be available and present in the source

	documents. Patient-reported MDD is not sufficient for inclusion in the study. 7. Inclusion criteria 7 removed in Protocol v6.0 (Amendment 5.0). 8. At Baseline, before definitive admission and randomization of the participant, the MADRS10 scale will be administered, and the participant must show a MADRS10 score of ≥24. 9. Diagnosed with a current MDE lasting from 8 weeks to 36 months as defined by the DSM-5 and confirmed by the SCID-5 MDD, as well as independent confirmation of HAMD17 score, SAFER/ATRQ, and contextual appropriateness to be a participant in this study, after evaluation by an MGH-CTNI clinician. Documentation of current MDE (medical records or alternative verified documentation) must be available and present in the source documents. Patient-reported MDE is not sufficient for inclusion in the study. 10. Treated and stabilized at same dose for at least 6 weeks prior to Screening,and experiencing <50% improvement on an approved dosing regimen of ADT (e.g., selective serotonin reuptake inhibitor [SSRI], serotonin and norepinephrine reuptake inhibitor [SNRI], bupropion [a norepinephrine–dopamine reuptake inhibitor {NDRI} and nicotinic receptor antagonist]) during the current MDE, and committed to remaining on the same stable dosing regimen for the Screening period and for the entire study, at or above the minimally adequate dose in the ATRQ. Documentation of stable and ongoing ADT (medical records or alternative verified documentation) must be available and present in the source documents. Maximal doses and recommended doses for each ADT are at the discretion of the Investigator and Medical Monitor, except for citalopram and escitalopram (See Section 16). Note: Participants taking trazodone and/or bupropion as secondary ADT are permitted. 11. An appropriate and valid participant in the study, after independent MGH-CTNI SAFER/ATRQ assessment of the participant's MDD condition to confirm the diagnosis of MDD, as well as the inadequate response to 1 to 3 valid courses of treatment with an antidepressant medication in the current MDE, defined as <50% improvement with an antidepressant medication at doses listed on the SAFER and ATRQ Interview Forms.
Exclusion Criteria	1. History or presence of clinically significant abnormality as assessed by physical examination, medical history, 12-lead ECG, vital signs, or laboratory values, which in the opinion of the Investigator would jeopardize the safety of the participant or the validity of the study results, including established QT prolongation, long QT syndrome, torsades de pointes, bradyarrhythmia, ventricular tachycardia, or uncompensated heart failure (greater than New York Heart Association [NYHA] Class 1 congestive heart failure [CHF]), uncontrolled hypokalemia, or uncontrolled hypomagnesemia 2. More than class 2 angina pectoris or a myocardial infarction (MI) or acute coronary syndrome within the past 3 months. 3. Any medical, psychiatric condition, or social context that, in the opinion of the investigator and the Medical Monitor, is likely to

	unfavorably alter the risk-benefit of the subject participation, to interfere with protocol compliance, or to confound safety or efficacy assessments. 4. Have any significant illness, of any nature, including possible Coronavirus-SARS-2 related fever and symptoms, requiring hospitalization, emergency treatment, or isolation (quarantine) within 4 weeks prior to Screening or during the Screening period, and as determined by the Investigator. 5. History or first degree relative with history of unexplained sudden death or long QT syndrome. 6. QTcF (QT interval with Fridericia's correction) ≥450 msec for males, or ≥470 msec for females, calculated as an average of triplicate 12-lead ECG at Screening. 7. Current or recent uncontrolled orthostasis or orthostatic hypotension necessitating treatment. 8. Poorly controlled diabetes as defined by a glycosylated hemoglobin (HbA1c) >7.5%, despite standard care. Subjects with HbA1c >7.5% may continue in the study if approved by the Relmada Medical Monitor after discussion with the Principal Investigator. Re-testing might be suggested. 9. Any long-term use (i.e., >120 days in a 6-month period) of prescribed opioids or controlled substances within 6 months, prior to Screening – as verified by appropriate medical records, Prescription Monitoring Program (PMP) or alternative verified documentation as requested by study Medical Monitor or delegate – or any recreational use of opioids or controlled substances. 10. More than 3 doses of opioids use within 30 days prior to Baseline. 11. Exclusion criteria 11 removed in Protocol v7.0 (Amendment 6.0). 12. Use of any anxiolytic, antipsychotic, anticonvulsant/antiepileptic, mood stabilizer, or stimulant medications, for psychiatric indications, within 30 days prior to Baseline: • Antipsychotics: o The stable dose of low dose antipsychotics (e.g. 50mg QHS Seroquel/Quetiapine) for the treatment of insomnia, secondary to MDD, may be allowed during the study o Antipsychotics being used on an ongoing basis for MDD/ADT augmentation (e.g. Abilify, Rexulti), at any dose, will not be considered • Stimulants/Amphetamines: o Use of stimulants/amphetamines are not permitted • Anxiolytics: o Use of benzodiazepines or buspirone will not be permitted during the study. • Mood Stabilizers: (e.g gabapentin , lamotrigine) will not be permitted during the study regardless the indication. Prescription/pharmacy records are required as documentation to support all medication use. Any taper of prohibited medications should be medically appropriate as determined by the investigator and the Medical Monitor and should result in no withdrawal symptoms. 13. As needed (PRN) use of St. John's Wort, [Hypericum Perforatum]

	within 30 days prior to Baseline. The stable use of St. John's Wort, [Hypericum Perforatum] may be permitted after discussion with and approval by the Medical Monitor. 14. Having received ketamine or esketamine within 60 days prior to Screening or planning to receive ketamine or esketamine at any time during participation in the study. 15. Having received memantine or dextromethorphan treatment within 30 days prior to Screening. 16. History of allergy or hypersensitivity to methadone or related drugs. 17. Receiving new-onset psychotherapy (individual, group, marriage, or family therapy) within 2 months prior to Screening, or planning to start psychotherapy at any time during participation in the study. 18. Any lifetime experience of electroconvulsive therapy (ECT) and/or vagus nerve stimulation (VNS), or any other type of physical brain stimulation. 19. Received repetitive transcranial magnetic stimulation (rTMS) less than 6-months prior to the Screening visit. 20. Any current and primary psychiatric disorder (ie, a condition that is the primary focus of distress and/or treatment other than MDD), as defined by the DSM-5, verified by appropriate medical records or alternative verified documentation as requested by study Medical Monitor or delegate, and confirmed by psychiatric history and/or examination by the Investigator. These disorders include, but are not limited to, any psychotic disorder, post-traumatic stress disorder, borderline personality disorder, antisocial personality disorder, obsessive-compulsive disorder, intellectual disability, or pervasive developmental disorder. 21. Participants who, in the Investigator's judgment, are at significant risk for suicide must be excluded. A participant who answers yes to question 4 and/or 5 in the suicidal ideation section of the C-SSRS within the last 6 months or a yes to suicidal behavior in the past 12 months at screening must be excluded. A subject with a yes to questions 4 and/or 5 or a yes to suicidal behavior at baseline, must be excluded. 22. Any lifetime history of bipolar I or II disorder, psychosis and/or mania as defined by the DSM-5 and confirmed by psychiatric history and/or examination by the Investigator. 23. Comorbid moderate to heavy alcohol or substance use disorder, as defined by DSM-5, at Screening or within the 12 months prior to Screening. Heavy drinking is defined as an average of 3 or more drinks per day, in the last month. 24. A positive result on the urine drug screen (UDS) or a positive breath alcohol test within 30 days prior to and at Baseline (Day 1). A positive result for a psychoactive drug not previously disclosed will result in screen failure. Retesting is permitted at the discretion of the Principal Investigator and the Medical Monitor. Please consult with the Medical Monitor in the case of a positive screening UDS result for benzodiazepines or other disclosed medications that have been approved by the Medical Monitor. Re-screening of participants who fail for a positive THC result is permitted after an appropriate washout period and if the participant does not meet the criteria for substance

	use disorder, as defined by DSM-5 . 25. HAMD17 score <19 at Screening and assessed by CTNI or an increase in absolute value of >40% or a decrease in absolute value of >20% on the HAMD17 score between Screening and time of assessment at the CTNI SAFER interview. 26. Exclusion criteria 26 removed in Protocol v5.0 (Amendment 4.0). 27. Evidence of clinically significant hepatic or renal impairment, including an estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2 (CKD-EPI 2009 calculation), alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >2.0 × upper limit of normal (ULN), bilirubin >1.5 × ULN (participants with history of Gilbert's syndrome diagnosis may be included if approved by Medical Monitor), Creatine Phosphokinase (CPK) >2.0 × ULN, or clinically significant abnormal endocrine laboratory values (including clinically significant abnormal thyroid parameters, i.e., thyroid stimulating hormone [TSH] <0.9 × LLN or >1.25 × ULN). Subjects with excursions from the above allowed normal ranges may continue in the study if approved by the Relmada Medical Monitor after discussion with the Principal Investigator. Re-testing might be suggested. 28. Diagnosed with and/or treated for any type of cancer (excluding basal cell carcinoma and in situ melanoma) within 4 years prior to Screening. 29. Any planned elective surgery requiring general anesthesia. 30. Participant has had gastric bypass surgery or has had any procedures or disorders that are likely to significantly interfere with gastrointestinal transit or absorption. 31. Enrolled in a clinical trial in the last 5 months prior to screening, or previously enrolled at any time in a clinical trial for an indication that conflicts with a primary diagnosis of MDD as determined by the Medical Monitor. These studies include, but are not limited to, those targeted at any psychotic disorder, post-traumatic stress disorder, borderline personality disorder, antisocial personality disorder, obsessive-compulsive disorder, intellectual disability, or pervasive developmental disorder. 32. Females who are currently lactating. 33. Any subject who, in the opinion of the sponsor, investigator, or medical monitor, should not participate in the trial.
Study Procedures	After signing the ICF, an identification number will be assigned to the participant, and an outpatient Screening visit will be conducted during which the participant will undergo an interview and medical assessments to determine eligibility for study participation. Participants who meet all the Screening entry criteria will have the Baseline visit (Day 1) scheduled. Participants who are deemed eligible by the Principal Investigator and confirmed by the Medical Monitor will undergo the SAFER/ATRQ interview by clinicians at the MGH-CTNI. Eligibility for enrollment will also be assessed by an independent medical adjudicator, who is a clinical psychiatrist, to ensure appropriate diagnosis and clinical presentation.

	To ensure that appropriate participants are entered into the study, MGH-CTNI-certified clinician will independently assess each participant using the SAFER interview, in addition to the HAMD17, and the MGH-ATRQ. The interview will be performed remotely; an MGH-CTNI clinician will contact the participant at his or her home or another off-site location after receiving notification from the study site. Sites will then be notified of the results of the interview. Only participants whose eligibility is confirmed by the SAFER/ATRQ interview, and the independent clinical adjudicator/Medical Monitor will be allowed to proceed in the study.
	The primary statistical analysis of the main estimand will be performed on the total population using a mixed-effect model repeated measures (MMRM) analysis. The absolute change from Baseline in the MADRS10 total score is the primary endpoint to be analyzed by MMRM. The MMRM model will include the post-baseline primary endpoints as the dependent variables, and treatment, visit, the interaction of treatment-by-visit and the MADRS10 total score at Baseline as independent variables. An unstructured covariance matrix will be used in the MMRM to account for the correlated nature of the multiple MADRS10 total scores collected across visits for each subject. The MMRM results for Day 28 (Visit 7) will be considered as the primary efficacy comparison of REL-1017 versus placebo. Adjustments will be made to the point estimates and inferential statistics if the sample size for the study is increased following the DMC's recommendation from the sample size re-estimation interim analysis. The analysis of the secondary efficacy endpoints will be performed in a similar way unless stated otherwise. Details of all analyses are provided in the Statistical Analysis Plan (SAP) for this study.

2 TABLE OF CONTENTS

1 SYNOPSIS 5
2 TABLE OF CONTENTS 17
2.1 List of In-text Tables 21
2.2 List of In-text Figures 21
3 LIST OF ABBREVIATIONS 22
4 INTRODUCTION 26
4.1 Background on Major Depressive Disorder 26
4.2 Background on Investigational Product 28
4.3 Preclinical Pharmacology/Toxicology Studies 29
4.3.1 Clinical Studies 30
4.3.2 Results from Clinical Study REL-1017-111 30
4.3.3 Results from Clinical Study REL-1017-112 31
4.3.4 Results from REL-1017-202 32
4.3.5 Results from Phase 3 Study REL-1017-301 33
4.3.6 Results from Phase 3 Study REL-1017-303 34
4.4 Study Rationale for Conducting REL-1017-302 Study 34
4.5 Study Objectives and Endpoints 35
4.6 Pharmacokinetics Evaluation 38
4.7 Biomarker, Pharmacogenomic (DNA), and Expression (RNA) Evaluations 38
5 INVESTIGATIONAL PLAN 39
5.1 Description of Overall Study Design and Plan 39
5.2 Discussion of Study Design 41
5.3 End of Study Treatment 41
6 SELECTION OF STUDY POPULATION 42
6.1 Inclusion Criteria 42
6.2 Exclusion Criteria 43
6.3 Rescreening 47
6.4 Study Withdrawal and Removal of Participants 47
7 TREATMENT 49
7.1 Study Drug Administration 49
7.1.1 Required Antidepressant Therapy 49
7.2 Study Drug Identification 49
7.2.1 Packaging and Labeling 49

7.2.2 Handling, Storage, and Accountability 50
7.2.3 Dispensing 50
7.3 Method of Assigning Participants to Treatment Groups 50
7.4 Selection of Dosing Regimen 51
7.5 Treatment Period 52
7.6 Code Breaking 52
7.7 Dosage Modification 52
7.8 Accountability and Compliance 52
7.9 Prior and Concomitant Medications and Therapies 53
7.10 Prohibited and/or Restricted Comedications and Therapies 54
7.11 Contraceptive Precautions 55
7.12 Rater Qualification 56
8 STUDY PROCEDURES 57
8.1 Informed Consent 62
8.2 Study Procedures 62
8.2.1 Screening (up to Day -30) 62
8.2.2 Baseline (Day 1) 62
8.2.3 Dosing/Treatment Period 62
8.2.4 Placebo-Control Reminder Script (PCRS) 63
8.2.5 End of Treatment 63
8.2.6 End of Study 63
8.3 Appropriateness of Measures 64
8.4 Entry Criteria Measurements 64
8.4.1 Structured Clinical Interview for DSM-5 64
8.4.2 SAFER Interview 64
8.4.3 Antidepressant Treatment Response Questionnaire (ATRQ) 64
8.4.4 Hamilton Depression Rating Scale (HAMD17) 64
9 EFFICACY ASSESSMENTS 66
9.1 Primary Efficacy Assessment 66
9.1.1 Montgomery-Åsberg Depression Rating Scale (MADRS10) 66
9.2 Key Secondary Efficacy Assessment 66
9.2.1 Clinical Global Impression of Severity (CGI-S) 66
9.2.2 Absolute Change from Baseline of the MADRS10 Total Score at Day 7 66
9.2.3 Remission Rate at Day 28 visit 66
9.2.4 Response Rate at Day 28 visit 67

9.3 Exploratory Assessments....67
9.3.1 Hamilton Anxiety Depression Scale (HAM-A)....67
9.3.2 Symptoms of Depression Questionnaire (SDQ)....67
9.3.3 Sheehan Disability Scale (SDS)....68
9.3.4 Clinical Global Impression of Improvement (CGI-I)....68
9.3.5 Digit Symbol Substitution Test (DSST)....68
9.3.6 Perceived Deficits Questionnaire – Depression (PDQ-D-5)....68
9.3.7 Arizona Sexual Experience Scale (ASEX)....69
10 SAFETY ASSESSMENTS....70
10.1 Medical and Psychiatric History....70
10.2 Vital Signs....70
10.3 Physical Examination....71
10.4 Electrocardiograms....71
10.5 Laboratory Assessments....72
10.5.1 Viral Screen....74
10.5.2 Breath Alcohol Test....74
10.6 Adverse Events....74
10.6.1 Adverse Event Definition....74
10.6.2 Serious Adverse Events and Serious Unexpected Adverse Events....75
10.6.3 Clinical Laboratory Abnormalities and Other Abnormal Assessments....75
10.7 Evaluation of Adverse Events and Serious Adverse Events....75
10.7.1 Classification of Adverse Event Intensity....76
10.7.1.1 Classification of Adverse Event Causality....76
10.7.2 Classification of Adverse Event Outcome....78
10.8 Reporting Procedures....78
10.8.1 Serious Adverse Events and Serious Unexpected Adverse Events....78
10.8.2 Any Adverse Event....79
10.8.3 Pregnancy....79
10.9 Follow-Up of Adverse Events and Serious Adverse Events....79
10.10 Other Measurements/Assessments....80
10.10.1 Columbia-Suicide Severity Rating Scale (C-SSRS)....80
10.10.2 Clinician-Administered Dissociative States Scale (CADSS)....81
10.11 Pharmacokinetic Assessments....81
11 PHARMACOKINETICS....82

11.1 Pharmacokinetic Sampling 82
11.2 Pharmacogenetic Analysis 82
12 STATISTICAL ANALYSIS 83
12.1 Statistical and Analytical Plans 83
12.2 Analysis Populations 83
12.3 Determination of Sample Size 83
12.4 Missing Data 84
12.5 Primary Endpoint Estimand ....**Error! Bookmark not defined.**
12.6 Efficacy Estimand Analysis ....**Error! Bookmark not defined.**
12.6.1 Correlation Between Biomarkers and Therapeutic Efficacy**Error! Bookmark not defined.**
12.7 Subgroup Analyses ....**Error! Bookmark not defined.**
12.8 Analysis of Safety Assessments 84
12.8.1 Adverse Events 84
12.8.2 Other Safety Analyses 85
12.9 Demographics and Other Baseline Characteristics 85
12.10 Interim Analysis 86
12.11 Data Monitoring Committee 86
12.12 Analysis of Pharmacokinetics 86
13 STUDY MANAGEMENT 87
13.1 Regulatory Guidelines 87
13.2 Institutional Review Board/Independent Ethics Committee 87
13.2.1 Informed Consent 87
13.3 Principal Investigator Reporting Requirements 88
13.4 Privacy 88
13.5 Participant Identifiers 88
13.6 Purpose of Collecting Personal Information and Use 88
13.6.1 Access and Disclosure of Personal Information 88
13.6.2 Release of Participant Information 89
13.6.3 Consent for Collection, Access, Use, and Disclosure of Participant Information89
13.7 Study Monitoring 89
13.7.1 Quality Control 89
13.7.2 Study Monitoring by Sponsor and/or Third-Party 90
13.7.3 Principal Investigator's Data Responsibility 91
13.8 Data Quality Assurance 91
13.9 Data Handling 91

13.10 Source Documents....92
13.11 Study and Site Closure....92
13.12 Records Retention....93
13.13 Protocol Amendment and Protocol Deviation....93
13.13.1 Protocol Amendment....93
13.13.2 Protocol Deviations....94
13.14 Ethical Considerations....94
13.15 Financing and Insurance....94
13.16 Publication Policy/Disclosure of Data....94
14 REFERENCES....95
15 COMEDICATIONS TO BE USED WITH CAUTION....99
15.1 QT....99
15.2 Drug-Drug Interactions....99
16 ALLOWED BACKGROUND ANTIDEPRESSANT THERAPY....101

2.1 List of In-text Tables

Table 1. Time from Discontinuation of Prohibited Medications, Supplements, and Other Substances or Therapies....54
Table 2. Schedule of Assessments....58
Table 3 List of Laboratory Assessments....73
Table 4. Classification of Adverse Event Causality....77
Table 5. Classification of Adverse Event Outcomes....78

2.2 List of In-text Figures

Figure 1. Study Design....40

3 LIST OF ABBREVIATIONS

Abbreviation	Definition
ADT	antidepressant therapy
AE	adverse event
ALT	alanine aminotransferase
ASEX	Arizona Sexual Experience Scale
AST	aspartate aminotransferase
ATRQ	Antidepressant Treatment Response Questionnaire
$AUC_{0\text{-}inf}$	area under the plasma concentration-time curve from time 0 to infinity
AUC_{tau}	area under the plasma concentration-time curve from time 0 until the dosing interval of 24 hours ($AUC_{0\text{-}24}$)
BDNF	brain-derived neurotrophic factor
b-HCG	beta-human chorionic gonadotropin
BID	twice daily
BMI	body mass index
BP	blood pressure (systolic and diastolic)
CADSS	Clinician-Administered Dissociative States Scale
CFR	Code of Federal Regulations
CGI-I	Clinical Global Impression of Improvement
CGI-S	Clinical Global Impression of Severity
CHF	congestive heart failure
CHW	Cui-Hung-Wang
CI	confidence interval
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CL/F	apparent clearance/bioavailable fraction absorbed
C_{max}	maximum observed plasma concentration
CNS	central nervous system
COWS	Clinical Opiate Withdrawal Scale
COVID-19	novel coronavirus 2019
CRO	contract research organization
C_{ss}	steady-state concentration
C-SSRS	Columbia Suicide Severity Rating Scale
CTCAE	common terminology for adverse events
CTNI	Clinical Trials Network and Institute
C_{trough}	trough concentration
CYP	cytochrome
DEA	US Drug Enforcement Administration
DMC	Data Monitoring Committee
DNA	deoxyribonucleic acid
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
DSST	Digit Symbol Substitution Test
ECG	electrocardiogram
eCRF	electronic case report form
ECT	electroconvulsive therapy
EDC	electronic data capture
EDP	end of dosing period

EDTA	ethylene diamine tetra-acetic acid
eGFR	estimated glomerular filtration rate
EOS	end of study
EOT	end of treatment
ePRO	electronic patient reported outcomes
ER	emergency room
FAS	full analysis set
FDA	United States Food and Drug Administration
FSH	follicle-stimulating hormone
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
HAM-A	Hamilton Anxiety Rating Scale
HAMD17	Hamilton Depression Rating Scale
HbA1c	glycosylated hemoglobin
HCl	hydrochloride
HIV	human immunodeficiency virus
HR	heart rate
IA	interim analysis
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Council for Harmonisation
ID	identification
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IRT	Interactive Response Technology
ITT	intent-to-treat
IUD	intrauterine device
LLN	lower limited of normal
LS	least square means
MAD	multiple ascending dose
MADRS10	Montgomery-Åsberg Depression Rating Scale 10 item-questionnaire
MDD	major depressive disorder
MDE	major depressive episode
MedDRA	Medical Dictionary for Regulatory Activities
MGH	Massachusetts General Hospital
MI	myocardial infarction
MMRM	mixed-effect model repeated measure
mTORC1	mammalian target of rapamycin complex 1
NIH	National Institutes of Health
NIMH	National Institute of Mental Health
NMDAR	N-methyl-D-aspartate receptor
NSDUH	National Survey on Drug Use and Health

NYHA	New York Heart Association
PCP	phencyclidine
PCRS	Placebo-Control Reminder Script
PCS	Physical Componet scale
PDQ-D-5	Perceived Deficits Questionnaire - Depression
PK	pharmacokinetic(s)
PO	by mouth
POC	proof of concept
PRN	pro re nata (when necessary)
PSRS	4-Item Positive Symptom Rating Scale
QD	quaque die (once daily)
QHS	quaque hora somni (every night at bedtime)
QIDS	Quick Inventory of Depression Symptomatology
QIDS-SR16	Quick Inventory of Depressive Symptomatology (16-Item)
QT	measure of the time between the start of the Q wave and the end of the T wave in the heart's electrical cycle
QTc	corrected QT
QTcF	QT interval with Fridericia's correction
REL-1017	esmethadone hydrochloride
Relmada	Relmada Therapeutics, Inc.
RR	respiratory rate
RNA	ribonucleic acid
rTMS	repetitive transcranial magnetic stimulation
SAD	single ascending dose
SAE	serious adverse event
SAFER	State versus Trait; Assessability; Face Validity; Ecological Validity; and Rule of Three Ps (Pervasive, Persistent, and Pathological)
SAP	Statistical Analysis Plan
SCID-5	Structured Clinical Interview for DSM-5 Disorders
SDQ	Symptoms of Depression Questionnaire
SDS	Sheehan Disability Scale
SIGH-A	Structured Interview Guide for the HAMA
SIGH-D	Structured Interview Guide for the HAMD17
SIGMA	Structured Interview Guide for the MADRS10
SNRI	serotonin and norepinephrine reuptake inhibitor
SOC	system organ class
SOP	standard operating procedure
SSR	sample size reestimation
SSRI	selective serotonin reuptake inhibitor
STAR*D	Sequenced Treatment Alternatives to Relieve Depression
$t_{½}$	apparent terminal elimination half-life
TEAE	treatment-emergent adverse event

T_{max}	time to maximum observed plasma concentration
TdP	torsade de pointes
TSH	thyroid stimulating hormone
ULN	upper limit of normal
US	United States
VAST-D	US Department of Veterans Affairs Augmentation and Switching Treatments for Improving Depression Outcomes
VNS	vagus nerve stimulation
WHODrug	World Health Organization Drug Dictionary
WOCBP	women of childbearing potential

4 INTRODUCTION

4.1 Background on Major Depressive Disorder

Major Depressive Disorder (MDD) is a widely prevalent, serious, and life-threatening health condition. It is the most common mental disorder and is a major cause of disability across the world. In 1994, it was estimated that approximately 1.5 million disability-adjusted life years were lost in developed countries as a result of depression.(British Psychological Society & The Royal College of Psychiatrists 2010)

Prevalence data from the 2017 National Survey on Drug Use and Health (NSDUH) show that an estimated 17.3 million adults in the United States (US) had at least one major depressive episode (MDE) in their lifetime (7.1% of all US adults). An estimated 11 million US adults aged 18 years or older experienced at least one MDE with severe functional impairment (4.5% of all US adults). Of adults with a MDE, 63.8% had severe functional impairment.(National Institute of Mental Health 2017) Approximately 70% of patients do not fully remit symptomatically after a full valid course of antidepressant monotherapy,(Trivedi 2006), and up to 85% of patients will relapse at least once.(Hardeveld 2010).

The rates of response and remission are shown to decrease, and the odds of relapse increase with each successive switch or augmentation of currently marketed antidepressants. This observation is documented in the reports from the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) and the US Department of Veterans Affairs Augmentation and Switching Treatments for Improving Depression Outcomes (VAST-D) studies.(Gaynes 2012; Gaynes 2009; Rush 2006; Rush 2012; Sinyor 2010) John Rush reports that in the STAR*D study, Quick Inventory of Depressive Symptomatology (16-Item) (QIDS-SR16) remission rates were 36.8%, 30.6%, 13.7%, and 13.0% for the first, second, third, and fourth acute treatment steps, respectively. The overall cumulative remission rate was 67%. Overall, those who required more treatment steps had higher relapse rates during the naturalistic follow-up period in the STAR*D study. In addition, lower relapse rates were found among participants who were in remission at follow-up entry than for those who were not after the first 3 treatment steps.(Gaynes 2012; Rush 2006; Rush 2012; Sinyor 2010; Trivedi 2006)

Patients are diagnosed with depression based on Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-5) criteria. Once the diagnosis of MDD is confirmed, it is crucial to assess and monitor the severity of MDD. The Montgomery-Åsberg Depression Rating Scale (MADRS10) is a highly reliable and sensitive measure of depression severity (Montgomery 1979), with a total score ranging from 0 to 60. The grading of depression severity on the MADRS10, based on the total score, is generally accepted as follows: Normal: 0-6; Mild: 7-19; Moderate: 20-34; Severe: 35-60. Approximately 46% of people with mild depressive symptoms and 88% of people with severe depressive symptoms reported difficulty with work, home or social activities related to their symptoms (Pratt 2014). Severe MDD is associated with a higher risk of suicidal ideation and attempts compared to moderate depression (Beck 1989). Two-thirds

of suicides occur in people with depression, and the risk of suicide in the most severely ill is up to 20 times that in the general population (Isometsa 2014). Compared to moderate MDD, severe MDD is more likely to interfere with the ability to function in everyday life, including work, social activities, and relationships (Kessler 2006), and interferes with functional outcomes (Herrmann 1998). Individuals with severe depression might experience longer depressive episodes and have a higher risk of recurrent episodes compared to those with moderate depression (Monroe 2011). Severe depression carries a higher level of social withdrawal, isolation, and stigma, which can further exacerbate the condition (Paykel 2003). Severe MDD may be less responsive to standard first-line treatments (Rush 2006). The biological underpinnings of depression might differ based on severity; neuroimaging studies have shown distinct patterns of brain activity and structure in patients with more severe depression (Frodl 2013; Javaheripour 2021). While no biomarker consistently improves the accuracy of MDD diagnosis, DSM-5 includes symptom severity as a diagnostic criterion. As mentioned earlier, MADRS10 score of 35 or more indicates severe depression, enhancing diagnostic accuracy. Patients with severe depression are likely to be a more uniform group, and uniformity may be a desirable feature for evaluating novel MDD therapies, such as uncompetitive N-methyl-D-aspartate receptor (NMDAR) antagonists. In trials of SSRIs, drug–placebo differences in antidepressant efficacy are relatively small even for severely depressed patients and the relationship between initial severity and antidepressant efficacy is attributable to decreased responsiveness to placebo among very severely depressed patients, rather than to increased responsiveness to medication (Kirsch 2008). In contrast, the uncompetitive NMDAR antagonist ketamine showed significant efficacy in patients with more severe depression, but not in patients with milder depression (Su 2017). Using positron emission tomography (PET) and a radioligand specific for the synaptic vesicle protein 2A (SV2A), this index of axon terminal density was found to be inversely associated with depression severity: lower SV2A was seen only in patients with severe depression (Holmes 2019). Consistently, patients with lower SV2A density and severe depression exhibited increased SV2A density and reduction in depression severity 24 hours after ketamine treatment (Holmes 2022).

Antidepressant treatment responses for uncompetitive NMDAR antagonists increase with the severity of baseline MDD (Su 2017; Fava 2023). In contrast, in trials of serotonergic drugs, while response in the placebo arm was decreased in patients with severe depression, as expected, response in the treatment arm was not increased (Kirsch 2008). Therefore, the increased response in the treatment arm in patients with severe depression may be a unique feature of NMDAR antagonist antidepressants, not shared by serotonergic drugs.

The noted differences in responses to NMDAR uncompetitive antagonists in patients with severe depression signal a distinct MOA compared to the MOA of serotonergic drugs.

While post-hoc analyses carry the risk of Type 1 statistical error, the meaningful and statistically significant results achieved with REL-1017 in the population of patients with severe depression are aligned with results of studies with other NMDAR antagonists (Su 2017), suggesting class-

specific efficacy in the severe population for uncompetitive NMDAR antagonist antidepressants, in contrast with results seen with serotonergic drugs (Kirsch 2008). Patients with severe depression may suffer from a disorder with a different pathophysiology compared to patients with milder symptomatology (Frodl 2013; Su 2017; Javaheripour 2021; Holmes 2022) and therefore may uniquely respond to drugs targeting restoration of impaired neural plasticity as a specific pathophysiological mechanism for MDD (Stahl 2022).

4.2 Background on Investigational Product

Relmada Therapeutics, Inc. (Relmada) is developing REL-1017, an orally available formulation of esmethadone hydrochloride (HCl), for the treatment of MDD.

REL-1017 is a novel N-methyl-D-aspartate receptor (NMDAR) antagonist being investigated as treatment for MDD. REL-1017 is believed to work via pathways that are markedly different from most currently approved drugs.

NMDARs are known to have a role in depression, as well as in normal physiological functions. REL-1017 shows preferential blocking of specific NMDAR subtypes (GluN2D) that may be of particular importance in MDD. With this mechanism, REL-1017 is being evaluated to determine whether it can provide antidepressant effects without psychotomimetic effects or other cognitive side effects seen with esketamine and without metabolic and neurological side effects seen with monoaminergic antidepressants.

REL-1017 is designated as esmethadone (USAN). It is the dextro-(the dextrorotary) isomer of its racemic methadone. As described by the US Drug Enforcement Administration (DEA), the opioid effects of racemic methadone are due to its levorotary-isomer, while the dextrorotary-isomer lacks significant respiratory depressant action and addiction liability (Drug Enforcement Administration. Diversion Control Division. Drug & Chemical Evaluation Section. Methadone. https://www.deadiversion.usdoj.gov/drug_chem_info/methadone/methadone.pdf [published December 2019]). In addition to having a preference for specific NMDAR subtypes, REL-1017 has been shown to increase plasma levels of brain-derived neurotrophic factor (BDNF) in humans(De Martin S 2018) and potentially induce neural plasticity in experimental models of depression.(Fogaça 2019)

Two Phase 1 studies demonstrated that single doses of REL-1017 up to 150 mg/day and repeated dosing up to 75 mg a day are well-tolerated and without clinically significant opioid effects or psychotomimetic AEs and the nausea was the dose limiting toxicity.(Bernstein 2019)

Nonclinical studies have demonstrated antidepressant-like activity of REL-1017 in animal models of depressed behavior, including the forced swim test, the novelty suppressed feeding test, the female urine sniffing test, and the chronic unpredictable stress paradigm with equivalent efficacy to ketamine (Investigator's Brochure [IB], Section 4.1.1.1).

The 7-day Phase 2 proof of concept (POC) study, REL-1017-202, demonstrated rapid onset of action, and an off-treatment robust and durable antidepressant response to REL-1017 as an adjunctive treatment in individuals with MDD who experienced an inadequate response in 1 to 3 adequate ADTs during their current MDE.

In this Phase 2 POC study, participants in both the REL-1017 25 mg and 50 mg treatment groups experienced rapid, robust, and durable statistically significant improvement of their depression, assessed on the Montgomery-Åsberg Depression Rating Scale (MADRS10), in comparison to participants on placebo. Consistent improvements were observed on the Clinical Global Impression of Severity (CGI-S), Clinical Global Impression of Improvement (CGI-I), and Symptoms of Depression Questionnaire (SDQ) scales. The improvement assessed on the MADRS10 score was observed as early as on Day 4 of exposure to REL-1017 in both dose groups and continued through Day 7 and Day 14. In an off-treatment follow-up period from Day 8 to Day 14, a persistent response was observed with p-values ≤0.03 and effect sizes ranging from 0.7 to 1.0 on the respective endpoint scales, including MADRS10, SDQ, CGI-S, and CGI-I. The findings of this Phase 2 POC study also confirmed the favorable safety and tolerability profile observed in the Phase 1 single-dose and multiple-dose studies.

At therapeutic doses for MDD, REL-1017 did not induce the opioid effects typically observed with opioid agonist drugs. In addition, in both, Phase 1 and Phase 2 studies, upon abrupt discontinuation of the study drug REL-1017 on Day 10 and on Day 7, respectively, there were no signs and symptoms of withdrawal in the REL-1017 group compared with the placebo groups.

Also, the completed Phase 1 and 2 studies did not show psychotomimetic symptoms, such as dissociative symptoms, hallucinations, paranoid delusions, confusion, difficulty concentrating, agitation, alterations in mood, nightmares, catatonia, ataxia, anesthesia, learning and memory deficits typically observed with other potent NMDAR antagonists (e.g., ketamine, esketamine, etoxadrol, phencyclidine [also known as PCP], tenocyclidine, which are well-known high-potency NMDAR antagonists).

4.3 Preclinical Pharmacology/Toxicology Studies

Preclinical *in vitro* and *in vivo* data for REL-1017 are presented in the IB.

Based on *in vitro* tests with racemic methadone, positive results have been seen in multiple *in vivo* assays, e.g., the mouse dominant lethal assay and the mammalian spermatogonial chromosome aberration tests.

The *in vivo* studies of methadone have mainly involved the racemic mixture, which includes d- and l-methadone acting as an NMDAR-antagonist and l-methadone acting as an opioid receptor agonist. For safety and tolerance, the expected side effects of REL-1017 alone were expected to be of the same nature or less compared to racemic methadone, especially in terms of opioid-

related effects. Some NMDAR antagonists have been shown to produce neurotoxic effects in animals.

The following pharmacology/toxicology studies have been recently completed with results summarized below. These results will be summarized in next version of the IB.

Consistent with ICH guidance, a complete battery of *in vitro* and *in vivo* GLP-compliant genotoxicity studies have been performed with REL-1017. REL-1017 was negative in the bacterial mutagenicity (Ames assay), *in vitro* micronucleus, and *in vivo* micronucleus and DNA fragmentation (Comet assay). These results show REL-1017 to have a low probability of genotoxicity.

REL-1017 did not produce Olney lesions in male or female rats; neither did racemic methadone. Additionally, the methadone racemate did not produce Olney lesions.

In vitro studies using recombinant CYP enzyme testing indicate that REL-1017 is metabolized by multiple enzymes. However, the only clinically relevant inhibition was observed with CYP2D6. Additionally, REL-1017 was found to be a pan-cytochrome inducer in human hepatocytes (Relmada data on file).

4.3.1 Clinical Studies

Relmada has completed two Phase 1 studies, a single-ascending dose study (SAD) (REL-1017-111) and a multiple-ascending dose study (REL-1017-112, multiple ascending doses (MAD)), to investigate the safety, tolerability, and pharmacokinetics (PK) of REL-1017 in healthy volunteers. Relmada has also completed a Phase 2 study (REL-1017-202) to investigate the dose-exposure, safety, tolerability, and efficacy of REL-1017-202 in adjunctive therapy of participants with MDD in an MDE experiencing an inadequate response to a first-line SSRI or SNRI antidepressant. Results of these 3 studies are presented below; further details are presented in the IB.

4.3.2 Results from Clinical Study REL-1017-111

This was a Phase 1a SAD double-blind, randomized, placebo-controlled, safety, and PK study in healthy participants with sequential cohorts of 7 subjects (2 sentinel subjects, followed by exposure to an additional 5 subjects at each dose level of REL-1017 (oral administration) with ascending dose arms from 5 mg to 200 mg. PK parameters were calculated for all dose levels, although some individual results had to be excluded due to emesis or statistical analysis criteria.(Bernstein 2019)

Pharmacogenomics analysis was performed for participants exhibiting either fast or slow metabolism of REL-1017. Overall, there were minimal subjective pharmacodynamics (PD) effects of REL-1017 up to 150 mg, as assessed by the Bond-Lader visual analog scale (VAS).

The overall conclusions from the REL-1017-111 study are as follows:

- The maximum tolerated dose for oral REL-1017 in healthy opiate-naïve participants was determined to be 150 mg, and the dose limiting toxicity was nausea and vomiting.
- PK analysis revealed high variability of the REL-1017 parameters, including maximum observed plasma concentration (C_{max}), time to maximum observed plasma concentration (T_{max}), area under the plasma concentration-time curve from time 0 to infinity ($AUC_{0\text{-}inf}$), apparent terminal elimination half-life ($t_{½}$), and apparent clearance/bioavailable fraction absorbed (CL/F). Statistical models demonstrated linear proportionality of C_{max} and $AUC_{0\text{-}inf}$ versus dose, with no statistical significance. In general, there were no specific trends observed for T_{max} or $t_{½}$ of REL-1017.
- The objective measure of PD effects, pupillometry, showed a dose-response with a modest increase in pupil constriction in higher dose groups.
- The study drug appeared to be safe. No indication of respiratory depression or clinically significant QTcF prolongation was reported.
- For tolerability, nausea and vomiting became potential AEs with higher doses.

4.3.3 Results from Clinical Study REL-1017-112

This was a Phase 1b MAD double-blind, randomized, placebo-controlled study safety, tolerability, and PK study in healthy participants. Eight participants were randomized into 3 arms: 25 mg, 50 mg, and 75 mg REL-1017. In each arm, 2 participants were dosed with placebo in cranberry juice and 6 participants with a powder formulation of REL-1017 in cranberry juice with an identical appearance and taste. Doses were administered from Day 1 to Day 10.(Bernstein 2019)

Overall conclusions from REL-1017-112 study were:

- C_{max} demonstrated a progressive increase from one dose to the next due to drug accumulation. Area under the plasma concentration-time curve from time 0 until the dosing interval of 24 hours (AUC_{tau}) values also demonstrated about a 3-fold accumulation over the 10-day dosing period. No dose dependency was seen in this parameter.
- Steady-state was achieved by Day 7 for the 25 mg and 50 mg REL-1017 dose levels and by Day 6 for the 75 mg dose level.
- Dose proportionality between the 25 mg, 50 mg, and 75 mg dose levels was demonstrated for the single-dose parameters C_{max} and AUC_{tau} and for steady-state parameters C_{max}, AUC_{tau}, and steady-state concentration (C_{ss}).
- Despite the confirmed overall dose proportionality in the 2 completed PK studies, the comparison of concentration and exposure between the 50 mg (n=6) and 75 mg REL-1017 (n=5) treatment groups demonstrated only slight differences.

- The subjective PD effects of REL-1017, as assessed by the Bond-Lader VAS, were minimal. The objective measure of PD effects, pupillometry, showed a dose-response for the 75 mg REL-1017 group.
- The multiple-dose of REL-1017 appeared to be safe, with no indication of respiratory depression or opioid withdrawal. Dose proportional QTcF prolongation was observed, with a placebo-adjusted increase from baseline of 10-12 msec with steady-state 25 mg once daily (QD) dosing.

4.3.4 Results from REL-1017-202

Study REL-1017-202 was a Phase 2a, inpatient, multicenter, randomized, double-blind, placebo-controlled, 3-arm study to assess the safety, tolerability, PK profile, and symptom response of a 7-day dosing with REL-1017 25 mg QD and 50 mg QD as adjunctive therapy in the treatment of participants diagnosed with MDD. The participants were adults with MDD diagnosed with a current MDE who had experienced an inadequate response to 1 to 3 courses of treatment with an adequate dose and duration of antidepressant medication, as defined by the Antidepressant Treatment Response Questionnaire (ATRQ). The study was conducted inpatient at 10 study sites in the United States. A total of 62 participants were randomized in the study: 19 participants to the REL-1017 25 mg treatment group, 21 participants to the REL-1017 50 mg treatment group, and 22 participants to the placebo group.

The overall conclusions from clinical Study REL-1017-202 were the following:

- The mean change from Baseline in the total MADRS10 score for the Intent-to-Treat (ITT) population showed a clinically and statistically significant improvement from Baseline to the end of the dosing period (EDP, on Day 7) in both active REL-1017 25 mg and 50 mg arms (-16.8 [standard deviation (SD): 11.3] and -16.6 [SD: 9.8], respectively) versus the placebo control arm (-8.8 [SD: 12.5]), while taking a constant dose of first-line antidepressant treatment. The statistically significant improvement on MADRS10 was sustained at Day 14, last MADRS10 measurement day.
- The mean change from Baseline in the SDQ total scores for the ITT population showed improvement. The mean change from Baseline and the mean percentage change from Baseline in the SDQ total scores for the ITT population showed improvement on Day 7 (-48.7 [SD: 32.3] and (-29.174 [SD: 17.2], respectively). The improvement persisted until Day 14. The least square (LS) means difference was statistically significant for the REL-1017 25 mg group and the REL-1017 50 mg group at Day 14.
- The mean change from Baseline to Day 7 of the CGI-S total scores in the ITT population was clinically and statistically significant. On Day 7, 13 participants (21.3%) were categorized as minimally improved and 21 participants (34.4%) as much improved. A mean of 3.2 (SD: 1.1) on the CGI-I scale was reported for the placebo group compared

with a mean of 2.4 (SD: 1.0) for the REL-1017 25 mg group and 2.3 (SD: 1.0) for the REL-1017 50 mg group at Day 7 post-dose at the EDP.

- The plasma REL-1017 exposure was dose-dependent, and the 50 mg dose level demonstrated a consistently higher exposure compared with the 25 mg dose in MDD patients.
- Both the 25 mg and 50 mg dose levels showed a similar elimination half-life after single as well as multiple doses in MDD patients along with other elimination PK parameters.
- The plasma REL-1017 levels reached a plateau (steady-state) after an initial loading dose of 75 mg REL-1017 in the 25 mg arm, and a loading dose of 100 mg in the 50 mg arm, on Day 2 for the REL-1017 25 mg dose group and on Day 3 for the REL-1017 50 mg group.
- No events were serious or severe, and no participants experienced treatment-emergent AEs (TEAEs) on the loading- or maintenance doses. None led to study drug discontinuation or discontinuation from the study. No participant died during the study.
- TEAEs counts were in the low single digits in all treatment arms. The TEAEs in the gastrointestinal disorders system organ class (SOC) were constipation, nausea, and diarrhea. The TEAEs in the nervous system disorders SOC were headache, somnolence, and dizziness.
- No TEAEs related to respiratory function were reported. The respiratory rate did not vary between treatment arms.
- No treatment-related effect on vital signs was observed.
- There were no clinically meaningful instances of QTcF prolongation.
- The Clinical Opiate Withdrawal Scale (COWS) mean scores from Day 8 to Day 14 were indistinguishable between placebo and the REL-1017 treatment arms, indicating an absence of opioid-type withdrawal symptoms.
- There was no distinction between placebo control and the REL-1017 treatment arms in the Clinician-Administered Dissociative States Scale (CADSS) score at any time point. No psychotomimetic or dissociative symptoms were observed in any participant.
- The mean 4-Item Positive Symptom Rating Scale (PSRS) showed no difference between placebo and REL-1017 treatment arms at any time point.

4.3.5 Results from Phase 3 Study REL-1017-301

Study REL-1017-301 was a Phase 3, outpatient, multicenter, randomized, double-blind, placebo-controlled, 2-arm study to assess the efficacy and safety of REL-1017 as adjunctive therapy in adult subjects with MDD experiencing inadequate response to ongoing ADT treatment. A total of 227 subjects were randomized in the study: 113 subjects to the REL-1017 25 mg treatment group, 114 to the placebo group.

REL 1017 did not meet the primary efficacy endpoint in the ITT analysis, however, the statistically significant difference in response rate and the prespecified supportive PP analysis suggest meaningful treatment effects. Post-hoc analyses in patients with severe MDD showed statistically significant efficacy with robust effect size.

In the ITT population analysis (n=227) the raw mean CFB was -15.1 [standard deviation (SD) 11.3] for esmethadone (n=113) and -12.9 (SD 10.4) for placebo (n=114), with a raw mean difference (MD) of 2.3, which was not statistically significant (p=0.154; Cohen's effect size (ES) 0.21). In the PP population analysis (n=198) the raw mean CFB was -15.6 (SD 11.2) for esmethadone (n=101) and -12.5 (SD 9.9) for placebo (n=97), with a MD of 3.1, (p=0.051, ES: 0.29). Post-hoc analyses in patients with baseline MADRS10 ≥35: ITT (n=112): MMRM mean difference 6.0; p=0.0109, ES=0.51; PP (n=98): MMRM mean difference7.3; p=0.0024, ES=0.64). Adverse events (AEs) were predominantly mild or moderate and transient, with no significant differences between groups (Fava 2023).

REL-1017 was safe and generally well-tolerated with no opioid-like effects, no withdrawal effects, and no psychotomimetic effects.

4.3.6 Results from Phase 3 Study REL-1017-303

Study REL-1017-303 was a Phase 3, outpatient, multicenter, randomized, double-blind, placebo-controlled, 2-arm study to assess the efficacy and safety of REL-1017 as a monotherapy in the treatment of MDD. Subjects were adults with a diagnosis of MDD currently experiencing a MDE. A total of 232 subjects were randomized in the study: 116 subjects to the REL-1017 25 mg treatment group, 116 to the placebo group.

REL-1017 demonstrated a non-significant improvement compared to placebo on the primary efficacy endpoint of change in the MADRS10 total score from Baseline at Week 4; -14.8 points for REL-1017 and –13.9 points for placebo; p=0.85).

REL-1017 was safe and generally well-tolerated with no opioid-like effects, no withdrawal effects, and no psychotomimetic effects.

4.4 Study Rationale for Conducting REL-1017-302 Study

Relmada proposes to evaluate REL-1017 for the treatment of MDD in Phase 3 studies to confirm and expand upon the efficacy and safety evidence observed in the initial Phase 2 study.

Preclinical and clinical data suggest that NMDAR antagonists such as ketamine and esmethadone modulate neural plasticity and increase prefrontal connectivity, thereby potentially reversing the effects of chronic stress and depression. The antidepressant-like effects of REL-1017 were mediated by mammalian target of rapamycin complex 1 (mTORC1) and BDNF.(Fogaça 2019) Hanania 2020) The elucidation of these processes, however, has only been

derived from animal research. There remains a need for research with depressed human participants to determine if these putative mechanisms of action involving the NMDAR and downstream pathways can successfully mediate and extend REL-1017's antidepressant-like effects in depressed participants.(Hanania 2020)

The preclinical and previous clinical experience with REL-1017 provided the basis for the initiation of the Phase 2 REL-1017-202 study which established the evaluation of the efficacy and safety of REL-1017 at 25 mg and 50 mg to MDD patients in the clinic setting. In this study, participants with MDD, diagnosed with a current MDE and experiencing an inadequate response to 1 to 3 courses of ADT, were randomized 1:1:1 to treatment with ADT plus placebo, or ADT plus REL-1017 at 25 mg or 50 mg for 7 days of daily dosing. The results of this study demonstrated robust, rapid and sustained statistically significant antidepressant effects of REL-1017 when administered together with standard ADT that resulted in an improvement in the MADRS10 score by more than 16 points at Day 7 and as early as Day 4 and sustained to Day 14. The demonstrated PD effect did not appear to be enhanced on 50 mg QD. Therefore, the proposed dose selected for the planned REL-1017-302 study is 25 mg daily.

The better response to NMDAR uncompetitive antagonists in patients with severe depression signal a distinct MOA compared to the MOA of serotonergic drugs and indicate that patients with severe depression may uniquely respond to REL-1017 (Su, 2017).

This study, REL-1017-302, will extend the findings from the Phase 2 study in a larger study population of outpatients, to evaluate the effect of REL-1017 during 28 days of daily dosing. Demonstration of the sustained efficacy of treatment for 4 weeks of therapy is clinically representative of long-term management of MDD as a chronic condition. Because REL-1017 is proposed for the treatment of patients diagnosed with MDD, the participants for the REL-1017-302 study will be adults with MDD who are diagnosed with a current MDE who have experienced an inadequate response to 1 to 3 courses of treatment with an adequate dose and duration (as defined by the ATRQ). These participants are stratified according to the severity of the disorder and then are randomized in a 1:1 ratio to the active REL-1017 in the 25-mg/day arm or placebo arm. This population will provide the opportunity to compare the safety and efficacy effects of 4 weeks of treatment with REL-1017 compared with placebo in participants with MDD and ongoing ADT with inadequate response.

4.5 Study Objectives and Endpoints

Objectives	Endpoints
Primary	
To evaluate the therapeutic efficacy of REL-1017 compared to placebo in subjects with inadequate response to ongoing ADT at Day 28 on the Montgomery-Åsberg Depression Rating	Absolute change from Baseline to Day 28 of the MADRS10 total score in REL-1017 compared to placebo in subjects with inadequate response to ongoing ADT

Objectives	Endpoints
Scale (MADRS10) total score	
Secondary	
Secondary Efficacy Objectives To evaluate the therapeutic efficacy of REL-1017 compared to placebo in subjects with inadequate response to ongoing ADT in the following measurements: • MADRS10 response rate (improvement ≥50% compared with total Baseline score) at Day 28 • MADRS10 remission rate (total score ≤10) at Day 28 • MADRS10 score at Day 7 • Clinical Global Impression of Severity (CGI-S) score at Day 28	Secondary Efficacy Endpoints • MADRS10 response rate (improvement ≥50% compared with total Baseline score) at Day 28 • MADRS10 remission rate (total score ≤10) at Day 28 • Absolute change from Baseline to Day 7 of the MADRS10 total score • Absolute change from Baseline to Day 28 of the CGI-S score
Secondary Efficacy Objectives To evaluate the therapeutic efficacy of REL-1017 compared to placebo in the Sub-Population with Baseline MADRS10 ≥ 35 in the following measurements: • MADRS10 response rate (improvement ≥50% compared with total Baseline score) at Day 28 • MADRS10 remission rate (total score ≤10) at Day 28 • MADRS10 score at Day 7 • Clinical Global Impression of Severity (CGI-S) score at Day 28	Secondary Efficacy Endpoints in the Sub-Population with Baseline MADRS10 ≥ 35 • Absolute change from Baseline to Day 28 of the MADRS10 total score for REL-1017 • MADRS10 response rate (improvement ≥50% compared with total Baseline score) at Day 28 • MADRS10 remission rate (total score ≤10) at Day 28 • Absolute change from Baseline MADRS10 score at Day 7 • Absolute change from Baseline Clinical Global Impression of Severity (CGI-S) score at Day 28
Exploratory	
To evaluate the impact of REL-1017 for 28 days compared to placebo in subjects with inadequate response to ongoing ADT in the Total Population and in the Sub-Population with Baseline MADRS10 ≥ 35 on measures of depression, anxiety, sleep, sexual function, cognitive function, select biomarkers, and quality of life.	To evaluate the impact of REL-1017 for 28 days compared to placebo in the Total Population and the Sub-Population with Baseline MADRS10 ≥ 35 for the following analyses: • Absolute change from Screening to Day 28 of the HAM-A total score • Absolute change from Screening to Day 28

Objectives	Endpoints
	of the SDQ total and component scores • Absolute change from Baseline to Day 28 in SDS total score and each domain score • CGI-I score at Day 28 • Time course of MADRS10 response rate (defined as a ≥50% decrease from Baseline of MADRS10 total score) at all post-Baseline assessment time points • Time course in MADRS10 remission rate (defined as absolute MADRS10 score ≤10) at all post-Baseline assessment time points • Absolute change from Baseline to Day 28 in MADRS10 component scores • MADRS10 total score as a function of the time since first diagnosis of MDD • Correlation between selected biomarkers with therapeutic efficacy as measured via MADRS10 total score until Day 28 • Time course of the changes from Baseline in MADRS10 total score at all post-Baseline assessment time points • Absolute change from Baseline in DSST • Absolute change from Baseline in PDQ-D-5 – cognition scale • Absolute change from Baseline in ASEX
Safety	
<u>Safety and Tolerability Objectives</u> • To evaluate safety and tolerability of REL-1017 • To evaluate the effect of REL-1017 on electrocardiographic parameters	<u>Safety Endpoints</u> • Treatment-emergent adverse events (TEAEs) • Evaluate the effects on the ECG, including heart rate, PR, QRS and QTcF intervals • Vital signs and weight • Physical examination • Clinical laboratory parameters (chemistry, hematology, and urinalysis) • C-SSRS • Clinician-Administered Dissociative States Scale (CADSS)

Pharmacokinetic	
Pharmacokinetic Objectives • To evaluate pharmacokinetics (PK) of REL-1017 and potential metabolites	Pharmacokinetic Endpoints Estimation of REL-1017 PK profile (maximum observed plasma concentration [C_{max}], trough concentration [C_{trough}], and apparent terminal elimination half-life at steady-state [$t_{1/2}$] based on sparse PK sampling

4.6 Pharmacokinetics Evaluation

- The PK of REL-1017 and potential metabolites will be evaluated on Day 1, 7, 14 and 28.
- The timing of each assessment is detailed in Section 11 and the Schedule of Assessments (Table 2).

4.7 Biomarker, Pharmacogenomic (DNA), and Expression (RNA) Evaluations

Assessment of biomarkers and their potential relationship to treatment groups and therapeutic efficacy will be explored. Blood samples will be collected to measure genetic and protein markers, including, but not limited to, BDNF allelic variants and plasma levels, as well as exploratory metabolite characterization. The timing of each assessment is detailed in the Schedule of Assessments (Table 2).

5 INVESTIGATIONAL PLAN

5.1 Description of Overall Study Design and Plan

The REL-1017-302 study is an outpatient, 2-arm, Phase 3, multicenter, randomized, double-blind, placebo-controlled study to assess the efficacy and safety of REL-1017 at 25 mg QD for MDD in subjects with inadequate response to ADT. During the study, subjects currently receiving ADT (i.e., a marketed ADT treatment course at the approved dosing regimen), who experience an inadequate therapeutic response, will be stratified according to MADRS10 score at Baseline, then randomized in a 1:1 ratio to REL-1017 or placebo, and receive treatment for 28 days.

The primary efficacy analysis will be based on the Day 28 assessment of the Total Population.

At Baseline subjects will be stratified according to depression severity:

- Baseline MADRS10 score <35 (moderate depression)
- Baseline MADRS10 score ≥ 35 (severe depression)

Subjects in each group will subsequently be randomized 1:1 to REL-1017 or placebo.

The overall duration of the study, including Screening and intervention period, will be approximately 58 days per participant. If a participant discontinues from the study prior to completing the Day 28/End of Treatment visit, the participant will be asked to complete an End of Study visit 14 days after the last dose of study drug, extending their participation up to 72 days.

Participants who are deemed eligible by the Principal Investigator will undergo the SAFER/ATRQ interview by clinicians at the Massachusetts General Hospital Clinical Trials Network and Institute (MGH-CTNI). Eligibility for enrollment will also be assessed by an independent medical adjudicator, who is a clinical psychiatrist, to ensure appropriate diagnosis and clinical presentation.

To ensure that appropriate participants are entered into the study, MGH-CTNI-certified clinician will independently assess each participant using the SAFER interview, in addition to the HAMD17, and the MGH-ATRQ. The interview will be performed remotely; an MGH-CTNI clinician will contact the participant at his or her home or another off-site location after receiving notification from the study site. Sites will then be notified of the results of the interview. Only participants whose eligibility is confirmed by the SAFER/ATRQ interview and the independent clinical adjudicator/Medical Monitor will be allowed to proceed in the study.

A total of approximately 280 participants, independently qualified by the Investigator and an independent MGH-CTNI-certified clinician, will be randomized in a 1:1 ratio to the active REL-1017 25 mg QD arm (n=140) or matching placebo arm (n=140).

An unblinded interim analysis for sample size re-estimation (IA-SSR) based on the calculation of the conditional power (Mehta 2011) and adopting the Cui-Hung-Wang (CHW) paradigm, (Cui 1999) could result in an increase of total sample size of up to 64%.

The Screening visit will assess eligibility to participate in the study and will occur for approximately 4 weeks (up to Day -30).

After signing the Informed Consent Form (ICF), an identification number will be assigned to the participant, and a Screening visit will be conducted during which the participant will undergo an interview and medical assessments to determine eligibility for study participation. Participants who meet all the Screening entry criteria will have the Baseline visit (Day 1) scheduled.

Active REL-1017 and matching placebo treatment arms will take the investigational therapeutic intervention according to the following schedule:

Day 1 – loading dose:

- Three REL-1017 25 mg tablets (75 mg total) administered at the clinical study site
- Three matching placebo tablets administered at the clinical study site

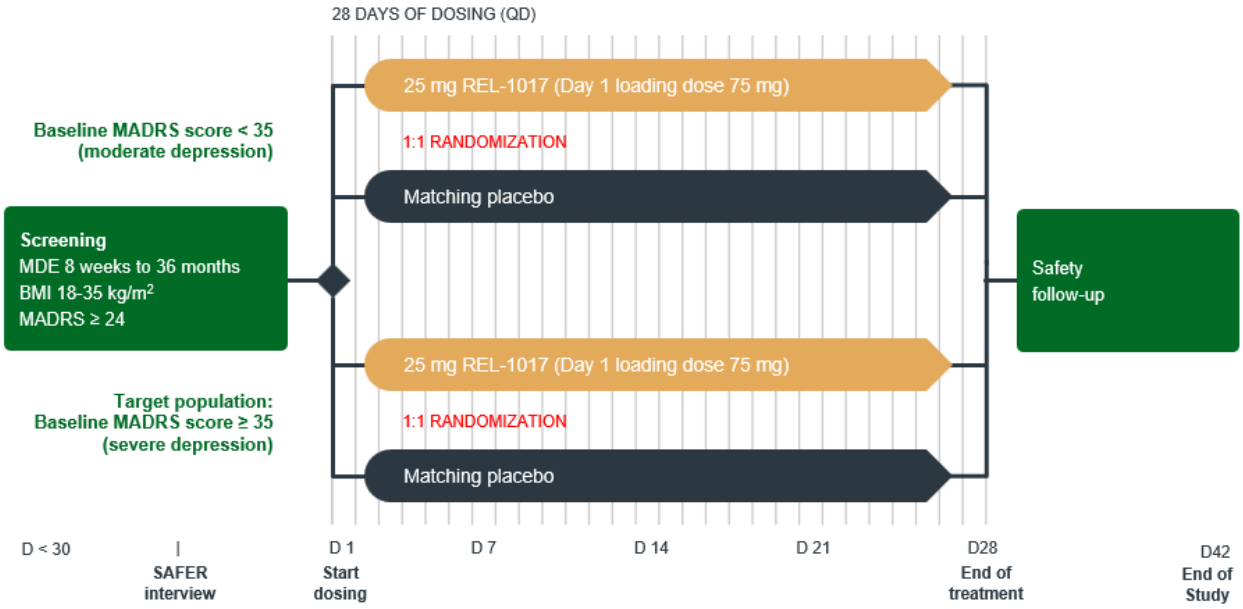
Day 2 to Day 28 – maintenance dose:

- One REL-1017 25 mg tablet QD
- One matching placebo tablet QD

Participants will be asked to stay on a stable dosing regimen of the same, current ADT during the 30-day Screening period, between Day 1 and Day 28, while exposed to REL-1017 or matching placebo. The investigational medication should be taken QD, and at the same time the participant takes her/his first-line ADT.

The study design is presented in Figure 1.

Figure 1. Study Design



On Day 1, a loading dose of 75 mg REL-1017 or matching placebo is administered

Abbreviations: BMI=body mass index; D=day; MADRS10=Montgomery-Åsberg Depression Rating Scale 10 item-questionnaire; MDE=major depressive episode; QD=once daily in the morning; SAFER=State versus Trait; Assessability; Face Validity; Ecological Validity; and Rule of Three Ps (Pervasive, Persistent, and Pathological)

5.2 Discussion of Study Design

This is an outpatient study to be conducted at approximately 80 sites in the United States.

Enrolling in the study will be adults diagnosed with MDD (based on Structured Clinical Interview for DSM-5 Disorders [SCID-5] for MDD, matching Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition [DSM-5]), who are currently in an MDE, with a HAMD17 score ≥19 at Screening and independently confirmed by SAFER assessment, and with a substantiated inadequate response to 1 to 3 courses of treatment of an ADT course during the current MDE for at least 6 weeks prior to Screening and stabilized 6 weeks prior to Baseline, and commitment to remain on the same stable dosing regimen (as confirmed by assessment of an independent clinician using the ATRQ).

5.3 End of Study Treatment

A participant will have fulfilled the requirements for study completion if/when the participant has completed all study periods, including the last scheduled visit as indicated in the Schedule of Assessments (Table 2).

6 SELECTION OF STUDY POPULATION

Approximately 280 participants, who are qualified by the Investigator, an independent MGH-CTNI-certified clinician, and an independent medical adjudicator will be randomized in a 1:1 ratio to an active REL-1017 at 25 mg/day tablets QD arm (n=140) or matching placebo tablets QD arm (n=140). A potential increase of the total sample size by up to 460 subjects may be decided after an interim analysis (IA) conducted by an independent, firewalled Data Monitoring Committee (DMC).

After signing an ICF, each participant will undergo an up to 30-day Screening period and a 28-day randomized double-blind placebo-controlled period, for a total of approximately 58 days of study participation.

6.1 Inclusion Criteria

To enroll in the clinical study, participants must meet the following inclusion criteria:

1. Must be able to read, speak, and understand English or Spanish and must provide written informed consent prior to the initiation of any protocol-specific procedures.
2. Male or female participant aged 18 to 65 years, inclusive.
3. Body mass index (BMI) between 18.0 and 35.0 kg/m^2, at Screening.
4. Participant understands the study requirements, is willing and able to commit to meet all study requirements, adhere to both approved ADT and study drug regimen, and complete all assessments and all scheduled visits, per Investigator judgment.
5. Women of childbearing potential (WOCBP) and men whose sexual partners are WOCBP must use at least 1 highly effective method of contraception from Screening and for at least 2 months after the last study drug administration. For WOCBP and men with female sexual partners of childbearing potential, examples of medically acceptable forms of contraception include vasectomy or male condom for participants, plus an additional method of contraception for their female partners. Highly effective methods of contraception are those which have a failure rate of <1% (when implemented consistently and correctly) and include:
    - Intrauterine device (IUD)
    - Bilateral tubal ligation, bilateral salpingectomy, or bilateral tubal occlusive procedure
    - Hormonal contraceptives (e.g., oral, patch, or injectable)
    - A double-barrier protection method (e.g., condom, sponge, or vaginal diaphragm with spermicide cream, foam, or gel)
    - Abstinence from heterosexual intercourse is accepted if this is the participant's usual lifestyle and must be continued until at least 2 months after the last dose of study drug.

Women who are not of childbearing potential must be congenitally or surgically sterile (hysterectomy and/or bilateral oophorectomy/salpingo-oophorectomy, as determined by the participant's medical history) or must be post-menopausal. Post-menopausal is defined as being amenorrheic for at least 1 year without another cause and a follicle-stimulating hormone (FSH) level ≥40 mIU/mL as confirmation.

6. Diagnosed with MDD as defined by the Diagnostic and Statistical Manual, Fifth Edition (DSM-5), and confirmed by the SCID-5 MDD. The documentation of MDD diagnosis (medical records or alternative verified documentation) must be available and present in the source documents. Patient-reported MDD is not sufficient for inclusion in the study.
7. Inclusion criteria 7 removed in Protocol v6.0 (Amendment 5.0).
8. At Baseline, before definitive admission and randomization of the participant, the MADRS10 scale will be administered, and the participant must show a MADRS10 score of ≥24.
9. Diagnosed with a current MDE lasting from 8 weeks to 36 months as defined by the DSM-5 and confirmed by the SCID-5 MDD, as well as independent confirmation of HAMD17 score, SAFER/ATRQ, and contextual appropriateness to be a participant in this study, after evaluation by an MGH-CTNI clinician. Documentation of current MDE (medical records or alternative verified documentation) must be available and present in the source documents. Patient-reported MDE is not sufficient for inclusion in the study.
10. Treated and stabilized at same dose for at least 6 weeks prior to Screening, and experiencing <50% improvement on an approved dosing regimen of ADT (e.g., SSRI, SNRI, or bupropion [a NDRI and nicotinic receptor antagonist]) during the current MDE, and committed to remaining on the same stable dosing regimen for the Screening period and for the entire study, at or above the minimally adequate dose in the ATRQ. Documentation of stable and ongoing ADT (medical records or alternative verified documentation) must be available and present in the source documents. Maximal doses and recommended doses for each ADT are at the discretion of the Investigator and Medical Monitor, except for citalopram and escitalopram (See Section 16).
    Note: Participants taking trazodone and/or bupropion as secondary ADT are permitted.
11. An appropriate and valid participant in the study, after independent MGH-CTNI SAFER/ATRQ assessment of the participant's MDD condition to confirm the diagnosis of MDD, as well as the inadequate response to 1 to 3 valid courses of treatment with an antidepressant medication in the current MDE, defined as <50% improvement with an antidepressant medication at doses listed on the SAFER and ATRQ Interview Forms.

6.2 Exclusion Criteria

Individuals meeting any of the following criteria at Screening or Baseline are ineligible to participate in this study:

1. History or presence of clinically significant abnormality as assessed by physical examination, medical history, 12-lead ECG, vital signs, or laboratory values, which in the opinion of the Investigator would jeopardize the safety of the participant or the validity of the study results, including established QT prolongation, long QT syndrome, torsades de pointes, bradyarrhythmia, ventricular tachycardia, uncompensated heart failure (greater than NYHA Class 1 CHF), uncontrolled hypokalemia, or uncontrolled hypomagnesemia.
2. More than class 2 angina pectoris or a MI or acute coronary syndrome within the past 3 months.
3. Any medical, psychiatric condition, or social context that, in the opinion of the investigator and the Medical Monitor, is likely to unfavorably alter the risk-benefit of subject participation, to interfere with protocol compliance, or to confound safety or efficacy assessments.
4. Have any significant illness, of any nature, including possible Coronavirus-SARS-2 related fever and symptoms, requiring hospitalization, emergency treatment, or isolation (quarantine) within 4 weeks prior to Screening or during the Screening period, and as determined by the Investigator.
5. History or first degree relative with history of unexplained sudden death or long QT syndrome.
6. QTcF (QT interval with Fridericia's correction) ≥ 450 msec for males, or > 470 msec for females, calculated as an average of triplicate 12- lead ECG at Screening.
7. Current or recent uncontrolled orthostasis or orthostatic hypotension necessitating treatment.
8. Poorly controlled diabetes as defined by a glycosylated hemoglobin (HbA1c) >7.5%, despite standard care. Subjects with HbA1c >7.5% may continue in the study if approved by the Relmada Medical Monitor after discussion with the Principal Investigator. Re-testing might be suggested.
9. Any long-term use (i.e., >120 days in a 6-month period) of prescribed opioids or controlled substances within 6 months, prior to Screening – as verified by appropriate medical records, Prescription Monitoring Program (PMP) or alternative verified documentation as requested by study Medical Monitor or delegate - or any recreational use of opioids or controlled substances.
10. More than 3 doses of opioids use within 30 days prior to Baseline.
11. Exclusion criteria 11 removed in Protocol v7.0 (Amendment 6.0).
12. Use of any anxiolytic, antipsychotic, anticonvulsant/antiepileptic, mood stabilizer, or stimulant medications, for psychiatric indications, within 30 days prior to Baseline:
    - Antipsychotics:
        - The stable dose of low dose antipsychotics (e.g. 50mg QHS Seroquel/Quetiapine) for the treatment of insomnia, secondary to MDD, may be allowed during the study

    - Antipsychotics being used on an ongoing basis for MDD/ADT augmentation (e.g. Abilify, Rexulti), at any dose, will not be considered
  - Stimulants/Amphetamines will not be permitted during the study, regardless of indication.
  - Anxiolytics:
    - Use of benzodiazepines or buspirone will not be permitted during the study
  - Mood stabilizers (e.g. gabapentin, lamotrigine) will not be permitted during the study, regardless of indication.
  - Prescription/pharmacy records are required as documentation to support all medication use. Any taper of prohibited medications should be medically appropriate as determined by the investigator and the Medical Monitor and should result in no withdrawal symptoms.
13. As needed (PRN) use of St. John's Wort [Hypericum Perforatum] within 30 days prior to Baseline. The stable use of St. John's Wort [Hypericum Perforatum] may be permitted after discussion with and approval by the Medical Monitor.
14. Having received ketamine or esketamine within 60 days prior to Screening or planning to receive ketamine or esketamine at any time during participation in the study.
15. Having received memantine or dextromethorphan treatment within 30 days prior to Screening.
16. History of allergy or hypersensitivity to methadone or related drugs.
17. Receiving new-onset psychotherapy (individual, group, marriage, or family therapy) within 2 months prior to Screening, or planning to start psychotherapy at any time during participation in the study.
18. Any lifetime experience of electroconvulsive therapy (ECT) and/or vagus nerve stimulation (VNS) or any other type of physical brain stimulation.
19. Received repetitive transcranial magnetic stimulation (rTMS) less than 6 months prior to the Screening visit.
20. Any current and primary psychiatric disorder (i.e., a condition that is the primary focus of distress and/or treatment other than MDD), as defined by the DSM-5, verified by appropriate medical records or alternative verified documentation as requested by study Medical Monitor or delegate, and confirmed by psychiatric history and/or examination by the Investigator. These disorders include, but are not limited to, any psychotic disorder, post-traumatic stress disorder, borderline personality disorder, antisocial personality disorder, obsessive-compulsive disorder, intellectual disability, or pervasive developmental disorder.
21. Participants who, in the Investigator's judgment, are at significant risk for suicide must be excluded. A participant who answers yes to question 4 and/or 5 in the suicidal ideation section of the C-SSRS within the last 6 months or a yes to suicidal behavior in the past 12

months at screening must be excluded. A subject with a yes to questions 4 and/or 5 or a yes to suicidal behavior at baseline, must be excluded..

22. Any lifetime history of bipolar I or II disorder, psychosis and/or mania as defined by the DSM-5 and confirmed by psychiatric history and/or examination by the Investigator.
23. Comorbid moderate to heavy alcohol or substance use disorder, as defined by DSM-5, at Screening or within the 12 months prior to Screening. Heavy drinking is defined as an average of 3 or more drinks per day, in the last month.
24. A positive result on the urine drug screen (UDS) or a positive breath alcohol test within 30 days prior to and at Baseline (Day 1). A positive result for a psychoactive drug not previously disclosed will result in screen failure. Retesting is permitted at the discretion of the Principal Investigator and the Medical Monitor. Please consult with the Medical Monitor in the case of a positive screening UDS result for benzodiazepines or other disclosed medications that have been approved by the Medical Monitor. Re- screening of participants who fail for a positive THC result is permitted after an appropriate washout period and if the participant does not meet the criteria for substance use disorder, as defined by DSM-5.
25. HAMD17 score <19 at Screening and assessed by CTNI or an increase in absolute value of >40% or a decrease in absolute value of >20% on the HAMD17 score between Screening and time of assessment at the CTNI SAFER interview.
26. Exclusion criteria 26 removed in Protocol v5.0 (Amendment 4.0).
27. Evidence of clinically significant hepatic or renal impairment, including an estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2 (CKD-EPI 2009 calculation), alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >2.0 × upper limit of normal (ULN), bilirubin >1.5 × ULN (participants with history of Gilbert's syndrome diagnosis may be included if approved by Medical Monitor), creatine phosphokinase (CPK) >2.0 × ULN, or clinically significant abnormal endocrine laboratory values (including clinically significant abnormal thyroid parameters, i.e., thyroid stimulating hormone [TSH] < 0.9 × LLN or > 1.25 × ULN. Subjects with excursions from the above allowed normal ranges may continue in the study if approved by the Relmada Medical Monitor after discussion with the Principal Investigator. Re-testing might be suggested.
28. Diagnosed with and/or treated for any type of cancer (excluding basal cell carcinoma and in situ melanoma) within 4 years prior to Screening.
29. Any planned elective surgery requiring general anesthesia.
30. Participant has had gastric bypass surgery or has had any procedures or disorders that are likely to significantly interfere with gastrointestinal transit or absorption.
31. Enrolled in a clinical trial in the last 5 months prior to screening, or previously enrolled at any time in a clinical trial for an indication that conflicts with a primary diagnosis of MDD as determined by the Medical Monitor. These studies include, but are not limited to, those targeted at any psychotic disorder, post-traumatic stress disorder, borderline

personality disorder, antisocial personality disorder, obsessive-compulsive disorder, intellectual disability, or pervasive developmental disorder.

32. Females who are currently lactating.
33. Any subject who, in the opinion of the sponsor, investigator, or medical monitor, should not participate in the trial.

6.3 Rescreening

Individuals who sign the ICF to participate in the study but who do not subsequently meet all the requirements as outlined in the inclusion and exclusion criteria and therefore are not randomized (screen failures), may be rescreened once, at the discretion of and with written permission from, the Medical Monitor , with the exception of participants presenting with alcohol or drug abuse or previously undisclosed psychoactive drugs; participants excluded because of QTc prolongation, unless they were receiving a QT-prolonging drug which has subsequently been discontinued; those who had hypokalemia or hypomagnesemia which has been corrected for a minimum of 14 days; or those screen failed by the independent medical adjudicator.

6.4 Study Withdrawal and Removal of Participants

If a participant discontinues study treatment and/or is withdrawn from the study for any reason, the study site must immediately notify the Medical Monitor, and the date and the reason for study treatment discontinuation or study withdrawal must be recorded on the electronic case report form (eCRF).

Participants may be discontinued from the study for any reason. Participants who are discontinued from study drug treatment (e.g., because of an AE) prior to study completion (i.e. Day 28) will be followed up until Day 42 (End of Study (EOS)) to complete the required assessments as indicated in the Schedule of Assessments (Table 2). Study drug discontinuation reasons may include:

- Occurrence of intolerable AE, as assessed by the Investigator or designee.
- Clinically significant abnormality on vital signs, ECGs, clinical laboratory tests, or physical examination evaluations, as assessed by the Investigator or designee.
- QTcF > 500 msec or an increase from Baseline > 60 msec (including on the local ECG machine interpretation). Triplicate ECGs will be conducted. If the triplicate ECG, as assessed by an appropriately trained expert (the ECG Core Lab or a cardiac electrophysiologist) verifies that the QTcF interval is > 500 msec or that the increase from Baseline is > 60 msec from Baseline (Day 1 pre-dose), the study drug will be stopped, and causality will be investigated.
- Non-compliance with protocol study requirements and restrictions.
- Administrative reasons (e.g., Sponsor decision).

- Major violation of the protocol.
- Investigator decision it is in the best interest of the participant.
- Use of a concomitant medication that, in the opinion of the Investigator or designee, would interfere with the study procedures or data integrity or compromise the safety of the participant.

Participants will be considered terminated from the study for the following:

- Withdrawal of consent
- Lost to follow-up
- Death

Additionally, the Sponsor may stop the study at any time for safety, regulatory, legal, or other reasons aligned with GCP. This study may be terminated at the discretion of the Sponsor or any regulatory agency. An Investigator may elect to discontinue or stop the study at his or her study site for any reason, including safety or low enrollment.

7 TREATMENT

7.1 Study Drug Administration

Active REL-1017 in 25 mg tablets or matching placebo tablets will be provided at the clinical study site in bottles containing a supply of 7 tablets with an overage of 8 tablets to account for holidays, travel, and rescheduled or missed visits. The investigational medication will be dispensed at Baseline (Day 1), on Day 7, Day 14, and Day 21, for QD oral administration by mouth (PO) according to the following schedule:

Day 1 (Baseline) − loading dose:

- REL-1017 arm: Three (3) 25 mg tablets (75 mg total) administered at the clinical study site
- Placebo arm: Three (3) matching tablets administered at the clinical study site

Day 2 to Day 28:

- REL-1017 arm: One (1) 25 mg tablet QD
- Placebo arm: One (1) matching tablet QD

Dosing should take place QD at the same time as the first-line ADT is taken. On clinic days, this would be approximately 2 hours prior to in-clinic visit.

7.1.1 Required Antidepressant Therapy

Participants will continue to take the same stable dose of the approved ADT medication from Screening throughout the course of the study.

7.2 Study Drug Identification

7.2.1 Packaging and Labeling

Each bottle of study drug will be clearly labeled with study-specific information meeting all the applicable regulatory requirements.

Clinical study drug supply labels will bear the following information:

- Statement indicating that the drug is an investigational drug
- Recommended storage conditions of the drug
- Bottle number
- Lot number of the drug
- Name and address of the Sponsor
- Protocol code or study number
- Quantity of drug per bottle

7.2.2 Handling, Storage, and Accountability

All study drugs will be transported, received, stored, and handled strictly in accordance with the container or product label, the instructions supplied to the research site and its designated pharmacy, the site's standard operating procedures (SOPs), and applicable regulations (*Guidelines for Temperature Control of Drug Products during Storage and Transportation, GUIDE-0069*). Appropriate storage temperature and transportation conditions will be maintained for the study drug from the point of manufacture to the location of delivery of the study drug.

Upon receipt by the study site, the study drug will be promptly transferred to the appropriate environmentally controlled storage area. The research pharmacy staff will examine the shipment and temperature monitoring devices (if applicable) to verify that the study drug was received in acceptable condition. Once inspected, the study drug will be stored in a restricted access, secured area with access limited to authorized research site staff, under physical conditions consistent with the investigational product's specific requirements. After study drug is dispensed to participants, the remaining study drug will be returned to storage in a restricted access, secured area under physical conditions consistent with the investigational product's specific requirements, in full compliance with US DEA regulations on storage and inventory control, dispensing and drug accounting.

The research site's pharmacist or delegate is responsible for ensuring that all study drug received at the site is inventoried and accounted for throughout the study, according to applicable regulations. All bottles of dispensed study drug that have been provided to the participants will be returned to the pharmacy, and any unused study drug will be recorded. Dispensed study drug tablets that have not been used will not be provided to participants again (even to the same participant), nor relabeled or reassigned for use by other participants. Study drug that has been dispensed, but not used, will be stored and disposed of according to the Sponsor's instructions.

The contents of dispensed study drug bottles will not be combined. Unused study drug and dispensed study drug will be available for verification by the Sponsor's site monitor.

7.2.3 Dispensing

Details regarding the administration of the study drug will be outlined in the pharmacy manual. Only eligible participants will receive the study drug. Only authorized research site staff may supply or administer the study drug.

7.3 Method of Assigning Participants to Treatment Groups

As of protocol version 8.0, Subjects will be stratified at Baseline based on Baseline MADRS10 score < 35 (moderate depression) and Baseline MADRS10 score ≥ 35 (severe depression).

Within each treatment group and stratum, blocked randomization will be used to avoid bias of temporal changes in the treatment effect, to increase the likelihood that known and unknown participant attributes (e.g., demographics and Baseline characteristics) are evenly balanced between treatment groups, and to enhance the validity of statistical comparisons between treatment groups.

Each potential participant will be assigned a unique identification number during the Screening process after signing the ICF. This number will be used to identify the participant throughout the study. Once an identification number is assigned, it cannot be reassigned to any other participant.

The random treatment assignment code used in the Interactive Response Technology (IRT) platform will be prepared by an unblinded randomization statistician.

7.4 Selection of Dosing Regimen

Since extensive clinical experience is available for racemic methadone and some clinical experience is available for REL-1017,(Fraser 1962; Isbell 1948; Moryl 2016; Olsen 1977) the 25-mg dose was selected based on the comparative characteristics of racemic methadone and available data with REL-1017 in healthy participants(Bernstein 2019) as well as the evidence from the Phase 2 study.

In a study of PK and pharmacodynamics of methadone enantiomers with a single oral dose of racemate in healthy participants, significant differences between the 2 enantiomers were noted.(Boulton 2001) Moreover, in the study of REL-1017 alone in opiate-naive participants, a dose as high as 150 mg was tested with no severe adverse reactions.(Bernstein 2019) Due to differences in the pharmacologic activity of the methadone enantiomers, opioid-related AEs observed with racemic methadone are not anticipated to occur with the administration of esmethadone. As described by the US DEA, the opioid effects of racemic methadone are due to its levo-isomer, while the dextro-isomer lacks significant respiratory depressant action and addiction liability.(2019)

Based on the safety data from Protocol REL-1017-111, single doses of 5 mg, 20 mg, 60 mg, 100 mg, and 150 mg of REL-1017 or placebo were well-tolerated. The results of Protocol REL-1017-112 evaluated 10 days of dosing with as high as 75 mg, and no impact on safety was observed. Regardless of the confirmed dose proportionality, the comparison of concentration and exposure between the 50 mg and 75 mg REL-1017 treatment groups demonstrated only slight differences. Consequently, 25 mg and 50 mg doses were chosen for administration in Protocol REL-1017-202 as daily doses over a period of 7 days.

To reach steady-state quickly for REL-1017 25 mg and REL-1017 50 mg treatment arms in Study REL-1017-202, a loading dose on Day 1 was administered. In the 25 mg REL-1017 tablet arm, a loading dose of 75 mg was taken at the study site on Day 1, followed by a 25 mg REL-1017 tablet QD on Days 2 to 7. In the 50 mg REL-1017 tablet arm, a loading dose of

100 mg REL-1017 was taken at the study site on Day 1, followed by a 50 mg REL-1017 tablet QD on Days 2 to 7.

PK/PD modeling, based on the demonstrated AUC_{tau} of REL-1017 in preclinical and clinical studies and on the AUC_{tau} of ketamine at doses that provide antidepressant efficacy, supports the conclusion that REL-1017 at 25 mg and 50 mg QD were predicted to be the optimal safe, well-tolerated and equally effective doses. In the current study, a dose of 25 mg was selected on the basis of the equivalent PD observed in the REL-1017-202 study at the 25 mg and 50 mg dosing levels.

7.5 Treatment Period

Participants will take a loading dose of REL-1017 75 mg (3 tablets) or matching placebo at Baseline (Day 1) and a maintenance dose of REL-1017 25 mg (1 tablet QD) or matching placebo thereafter during the 28-day treatment period.

7.6 Code Breaking

The compliance auditor(s) who verify conformity to study procedures and the unblinded statisticians who generate the randomization code and perform the interim analysis will have access to the treatment codes. Under normal circumstances, the blind will not be broken until all participants have completed treatment. Blinded dosing information will be broken only in an emergency where knowledge of the dosing information could impact further treatment decisions or aid in the emergency treatment of a participant. Individual code breaks will result in the withdrawal of the participant from the study. The date, time, and reason for the unblinding will be documented in the appropriate section of the eCRF and in the source documents.

7.7 Dosage Modification

Not applicable.

7.8 Accountability and Compliance

Study drug will be dispensed under the supervision of study personnel at scheduled site visits, and treatment compliance will be verified accordingly.

The pharmacist or other designated individual will maintain records of study treatment delivered to the study site, the inventory at the study site, the distribution to and use by each participant, and the return of materials to the Sponsor for storage or disposal. These records should include

dates, quantities, batch/serial numbers, expiration dates, in-clinic temperature log, and unique code numbers assigned to the product and study participants.

At each visit after initiation of treatment, study site personnel will record compliance of the participant with the assigned regimen. Participants will be instructed to bring their bottles containing unused tablets back for inspection at each study visit. Participants are to be reminded of the importance of compliance, with an emphasis on taking their study drug on schedule and maintaining the prescribed interval between doses.

Investigators will maintain records that adequately document that the participants were provided with the correct bottle of tablets and reconcile the products received from the drug dispensing center. Investigational product will not be returned to the Sponsor until accountability and reconciliation have been fully conducted by the site monitor.

Study drug bottles must be returned at each visit, and tablet counts will be recorded to assess compliance. Non-compliance is defined as taking less than 80% or more than 120% of study drug during any outpatient evaluation period (visit to visit). Discontinuation for non-compliance is at the Investigator's discretion and is to be recorded on the eCRF.

The site should confirm with the subject at each visit that there are no changes to the current ADT and that the subject has been compliant with the prescribed dosing regimen. This will be recorded in the source.

7.9 Prior and Concomitant Medications and Therapies

All medications taken and therapies received within 6 months prior to Screening must be noted on the eCRF. Medications taken and therapies received related to the primary indication (MDD) within the 5 years prior to Screening will also be recorded on the eCRF. The reported concomitant medications and/or therapies will be reviewed and evaluated by the Investigator to determine if they affect a participant's eligibility or continued participation in the study.

Any medication or therapy that is taken by or administered to the participant during the course of the study must be recorded in the eCRF. The entry must include the dose, regimen, route, indication, and dates of use.

All medications and other treatments taken by the participant during the study, including those treatments initiated before the start of the study, must be recorded on the eCRF.

After the Baseline visit, medication to treat minor treatment-emergent illness(es) is generally permitted; however, therapies discussed in the next section are expressly prohibited throughout the study.

7.10 Prohibited and/or Restricted Comedications and Therapies

Prohibited and/or restricted medications or therapies are provided in Exclusion Criteria, Section 6.2 and in Section 15 on comedication management. Table 1 provides the required duration of time to discontinue selected prohibited medications and therapies. Restricted comedication management is the responsibility of the Investigator, exercising good clinical judgment in the interest of the participant's health and contextual considerations.

Decisions regarding comedication must be made by the Investigator or prescribing physician in accordance with the guidance provided in comedication management (Section 15) and to the best clinical judgment of the Investigator, in consultation with the Medical Monitor. Initiation of any new comedication during the study is not permitted unless a medical necessity requires a therapeutic decision made according to the best clinical judgment of the Investigator, prescribing physician, and Medical Monitor. Specifically, a QT-prolonging drug should not be started by a participant during the study, except with the Medical Monitor's agreement.

The Investigators and their designees are obliged to ensure the well-being of all participants during this study. If medical intervention is required during the study, no medication or treatment will be withheld from a participant; and the Investigator will ensure proper documentation is kept in the eCRF. Due to each participant's clinical condition being unique, there is no specific rescue plan or rescue therapy recommended. If a participant experiences a clinical condition, the Investigator should use clinical judgment and knowledge of the participant's specific condition to institute the best treatment for that participant.

Table 1. Time from Discontinuation of Prohibited Medications, Supplements, and Other Substances or Therapies

Prohibited Medications, Supplements, and Other Substances or Therapies	Minimum Time from Discontinuation to Screening[a]
Electroconvulsive therapy (ECT) and/or vagus nerve stimulation (VNS) or any other type of physical brain stimulation	Lifetime
Ketamine and/or esketamine	60 days
Repetitive transcranial magnetic stimulation (rTMS)	180 days
Long-term opiate use (i.e. >120 days)	180 days
New-onset psychotherapy	60 days
Memantine and/or dextromethorphan	30 days

[a] The Medical Monitor should be contacted for any questions regarding the potential for pharmacological interactions with concomitant medications used by participants during the study. These include off-label use of medications for depression.

PRN use of Prohibited Medications, Supplements, and Other Substances or Therapies	Minimum Time from Discontinuation to Baseline[a]
Anxiolytic drugs	5 half-lives or 5 days, whichever is longer
Antipsychotic drugs	30 days
Anticonvulsants/Antiepileptic drugs	30 days
Mood stabilizers	30 days
Stimulants	30 days
More than 3 doses of opioids	30 days
St. John's Wort	30 days

[a] The Medical Monitor should be contacted for any questions regarding the potential for pharmacological interactions with concomitant medications used by participants during the study. These include off-label use of medications for depression.

Use of sleeping aids and hypnotics (as needed or stable dose) may be permitted after discussion with and approval by the Medical Monitor.

7.11 Contraceptive Precautions

For men with female sexual partners of childbearing potential, examples of medically acceptable forms of contraception include vasectomy or male condom for participants, plus an additional method of contraception for their female partners. For vasectomized men, original documents obtained after vasectomy or a note from the family physician is required to confirm sterility. In the absence of such documents, it will be assumed that the participant has childbearing potential, and the participant will be advised to use the medically acceptable birth control methods. Abstinence from heterosexual intercourse is accepted if this is the participant's usual lifestyle and must be continued until at least 2 months after the last dose of study drug.

Men must also abstain from sperm donation from Screening until at least 2 months after the last study drug administration.

WOCBP (not surgically sterilized and between menarche and 1 year post-menopause) must agree to use at least 1 highly effective method of contraception from Screening through at least 2 months after last study drug administration. Women who are not of childbearing potential must be congenitally or surgically sterile (hysterectomy and/or bilateral oophorectomy/salpingo-oophorectomy, as determined by the participant's medical history) or must be post-menopausal. Post-menopausal is defined as being amenorrheic for at least 1 year without another cause and a confirmatory FSH level ≥40 mIU/mL.

Highly effective methods of contraception are those which have a failure rate of <1% (when implemented consistently and correctly) and include:

- Intrauterine device (IUD)
- Bilateral tubal ligation, bilateral salpingectomy, or bilateral tubal occlusive procedure
- Hormonal contraceptives (e.g., oral, patch, or injectable)
- A double-barrier protection method (e.g., condom, sponge, or vaginal diaphragm with spermicide cream, foam, or gel)
- Abstinence from heterosexual intercourse is accepted if this is the participant's usual lifestyle and must be continued until at least 2 months after the last dose of study drug.

Women who are lactating are prohibited from participating in the study.

Women must also abstain from egg donation from Screening until at least 2 months after the last study drug administration.

7.12 Rater Qualification

The raters must demonstrate sufficient assessment experience as well as appropriate educational background and indication experience.

Raters performing the clinical assessments will require training and approval by the Sponsor designated vendor prior to rating in this study.

The vendors designated by the Sponsor will conduct rater qualification and will provide documentation about each rater's certification and/or training.

If possible, each participant will be interviewed and assessed throughout the study by the same rater.

8 STUDY PROCEDURES

Table 2 outlines the timing of procedures and assessments to be performed throughout the study. Sections 9 and 10 provide details regarding efficacy assessments and safety assessments, respectively, and Section 10.5 specifies laboratory assessment samples to be obtained.

The study plans to enroll participants through 2023 when coronavirus 2019 (COVID-19) is likely to be continuing to pose a significant public health risk in the United States. This study aligns with the US Food and Drug Administration (FDA) Guidance related to COVID-19 and allows Investigators maximum flexibility to conduct study procedures on-site or remotely, based on the participant's or site's needs, taking into consideration community and individual risk.

Potential study participants will have this possibility thoroughly explained and documented in the ICF prior to the conduct of any study procedures. Participants would be trained and receive a mobile application through which study sites can conduct assessments, including clinician-rated scales via telehealth. Electronic patient-reported outcomes (ePRO) assessments may also be collected remotely via a mobile application or on-site capturing source into the database.

Technology will provide the flexibility to allow for site discretion as well for potential laboratory draws, ECGs, or study drug distribution and/or collection to occur outside the site when necessary.

Table 2. Schedule of Assessments

Visit	1	2	4[a]	5	6	7	8
	Screening	Baseline				EOT	EOS[b]
Week	Up to -4	0	1	2	3	4	6
Assessment Day (± 2)	Up to -30	1	7	14	21	28	42
Informed Consent	X						
Medical History	X						
Psychiatric History	X						
Medication History	X						
Demographics and Height	X						
Inclusion/Exclusion Review	X	X					
Physical Examination	X	X[c]				X[c]	
Weight and BMI	X	X				X	X
Concomitant Medications/Therapies	X	X	X	X	X	X	X
Vital Signs (including body temperature) and Pulse Oximetry[d]	X	X	X	X	X	X	X
ECG[e]	X	X	X	X		X	X
LABORATORY TESTING							
Hematology	X	X				X	
Biochemistry[f]	X	X[f]				X[f]	X
Urinalysis (Dipstick)	X	X				X	
Hepatitis B, Hepatitis C, and HIV	X						
TSH	X						
Drug Screen (urine)[g]	X	X[g]		X		X	X
Breath Alcohol[h]	X	X					
Serum FSH Test for Females	X						
Urine Pregnancy Test for WOCBP [i]	X	X	X	X	X	X	

Visit	1	2	4[a]	5	6	7	8
	Screening	Baseline				EOT	EOS[b]
Week	Up to -4	0	1	2	3	4	6
Assessment Day (± 2)	Up to -30	1	7	14	21	28	42
Serum Pregnancy Test (B-HCG) for WOCBP [i]	X						
Blood Sample for DNA Extraction[J]		X					
PK Blood Sampling[k]		X	X	X		X	
SCALE ASSESSMENTS							
SAFER Interview	X[l]						
ATRQ	X[l]						
PCRS[m]		X	X	X	X	X	
MADRS10		X	X	X	X	X	X
SCID-5 MDD	X						
HAMD17	X						
CGI-S[n]		X	X	X	X	X	
CGI-I[n]			X	X	X	X	
HAM-A	X					X	
C-SSRS[o]	X	X	X	X	X	X	X
CADSS		X	X	X	X	X	X
PATIENT REPORTED OUTCOMES							
SDQ	X					X	
SDS	X	X				X	
DSST		X				X	
PDQ-D-5		X				X	
ASEX		X				X	
DOSING							

Visit	1	2	4[a]	5	6	7	8
	Screening	Baseline				EOT	EOS[b]
Week	Up to -4	0	1	2	3	4	6
Assessment Day (± 2)	Up to -30	1	7	14	21	28	42
Randomization		X					
Study Drug Dispensing		X	X	X	X		
Study Drug Return/Compliance/Accountability[p]			X	X	X	X	
AEs	X	X	X	X	X	X	X

Abbreviations: ADT: antidepressant therapy; AE = adverse event; ASEX = Arizona Sexual Experience Scale; ATRQ = Antidepressant Treatment Response Questionnaire; b-HCG = beta-human chorionic gonadotropin; BMI = body mass index; BP = blood pressure; CADSS = Clinician-Administered Dissociative States Scale; CGI-I = Clinical Global Impression of Improvement; CGI-S: Clinical Global Impression of Severity; C-SSRS = Columbia Suicide Severity Rating Scale; CTNI = Clinical Trials Network and Institute; DNA = deoxyribonucleic acid; DSST = Digit Symbol Substitution Test; ECG = electrocardiogram; EOS = end of study; EOT = end of treatment; FSH = follicle-stimulating hormone; HAM-A = Hamilton Anxiety Rating Scale; HAMD17 = Hamilton Depression Rating Scale; HIV = human immunodeficiency virus; HR = heart rate; MADRS10 = Montgomery-Åsberg Depression Rating Scale 10; MDD = major depressive disorder; MGH = Massachusetts General Hospital; PCRS = Placebo-Control Reminder Script; PDQ-D-5 = Perceived Deficits Questionnaire – Depression; PK = pharmacokinetics; RR = respiratory rate; SAFER = State versus trait; Assessability; Face validity; Ecological validity; and Rule of three Ps [pervasive, persistent, and pathological]); SCID-5-MDD = Structured Clinical Interview for DSM-5 Disorders; SDQ = Symptoms of Depression Questionnaire; SDS = Sheehan Disability Scale; SOP = Standard Operating Procedure; TSH = thyroid stimulating hormone; WOCBP = Women of Childbearing Potential

Note: Procedures performed after the assessment scales should follow from least invasive to more invasive. The following order is recommended: all assessment scales, vital signs, ECGs and lastly PK blood draw.

a. Visit 3 removed from Protocol v6.0 (Amendment 5.0).
b. Participants who discontinue or withdraw from study treatment prior to Day 28, provided informed consent has not been withdrawn, must complete an EOS visit.
c. Focused physical examination at Baseline (Day 1) and EOT (Day 28) will be limited to the following body systems: general appearance, abdomen, heart, respiratory/lungs, neurological, musculoskeletal, and any other assessments required to evaluate AEs. Additional physical examinations can be performed at the discretion of the Investigator.
d. Vital signs include body temperature (taken via aural, orally, or forehead), systolic and diastolic BP, HR, pulse oximetry, and RR measured after the participant has been resting in a supine or semi-supine position for at least 3 minutes. Measurement of orthostatic BP should follow the measurement of supine or semi-supine BP with participants asked to stand for 3 minutes before the measurement of orthostatic BP.
e. Triplicate ECG will be done at Screening. Day 42 ECGs will be collected singularly. Two sets of triplicate ECGs will be taken on Baseline (Day 1) pre-dose, 15 min apart (±5 minutes). For Days 7, 14, and 28, participants will have post-dose one set of triplicate ECG performed, after completed efficacy

assessments. All study ECGs should be conducted in a supine position (resting for at least 10 minutes).

f. Testosterone levels will be assayed for male participants on Baseline (Day 1) and EOT (Day 28).

g. Urine drug screen tests will be for tetrahydrocannabinol, opiates (including oxycodone), amphetamines, cocaine, and benzodiazepines. The UDS will be collected and sent to the central lab at the Screening Visit (including any screening retests), Day 14, Day 28 (EOT), and Day 42 (EOS). At Baseline, urine drug screening will be performed locally on-site. **At Baseline, urine drug screening will be performed locally on-site.**

h. Participants should abstain from alcohol for 24 hours prior to each study visit. Breath alcohol testing will be performed at Screening and Baseline (Day 1). If there is any doubt or concern regarding alcohol use, research site staff may request a breath alcohol test at subsequent visits during the study.

i. An on-site urine pregnancy test will be performed at each study visit for WOCBP. A serum pregnancy test (b-HCG) will be performed at Screening in addition to an onsite urine pregnancy test. During the study, a serum pregnancy test will be performed if a subject's urine pregnancy test result is positive.

j. A whole blood sample for DNA isolation will be collected from participants who consented for pharmacogenetic testing for potential pharmacogenetic analysis of genes that may affect the PK of REL-1017. This analysis will be restricted to the evaluation of genes that may be involved in the PK of REL-1017 (e.g., drug metabolism, disposition, elimination). One 4 mL whole blood sample for DNA isolation will be collected on Baseline (Day 1) from each participant. Samples will be collected and shipped according to the site's SOPs and instructions from the Sponsor and/or pharmacogenetic laboratory. The samples will be stored in a secure space with adequate measures to protect confidentiality. The samples will be retained while research on REL-1017 continues.

k. PK blood samples will be taken on Days 1 (immediately following the second set of ECG recording and before dosing) and 7, 14, and 28, where participants will have PK sample taken post-dose and within 10 minutes of ECG.

l. Sites should submit specific Screening information (detailed in the MGH-CTNI manual) at least 5 to 7 days prior to Baseline (Day 1) after the Medical Monitor has confirmed the participant has met all entry criteria. MGH-CTNI will administer the HAMD17 and ATRQ as independent confirmation of inclusion criteria.

m. The PCRS is read by the MADRS10 Rater to each participant immediately before administering the MADRS10 at Days 1, 7, 14, 21 and 28.

n. CGI-S is performed at Baseline prior to first administration of study drug; both the CGI-S and the CGI-I are performed at subsequent visits.

o. At Screening, the C-SSRS Baseline/Screening version is administered to participants. At subsequent visits, the C-SSRS Since Last Visit version is administered to participants.

p. Site personnel will record compliance of the participant's assigned regimen. Study drug bottles containing unused tablets must be returned at each visit and tablet counts will be recorded. See Section 7.8 for further details.

8.1 Informed Consent

Before performing any study-related procedures, the Investigator (or designee) will obtain signed informed consent from the participant.

In the event that rescreening occurs, the individual is required to sign a new ICF and must be assigned a new identification number.

8.2 Study Procedures

Logistical considerations (e.g., sequence of events, assessment windows) will be outlined in study-specific procedures. Post Screening psychiatric scales will be administered using the timeframes as outlined in the scales. Medical and psychiatric history will be recorded at Screening. Documentation of psychiatric history, including MDD diagnosis, and medical history (medical and pharmacy records or alternative verified documentation as requested by study Medical Monitor or delegate) must be available and present in the source documents.

8.2.1 Screening (up to Day -30)

After the ICF is signed, an identification number will be assigned to the participant, and an outpatient Screening visit will be conducted during which the participant will undergo an interview and medical assessments to determine eligibility for study participation.

8.2.2 Baseline (Day 1)

Participants meeting all Screening criteria will undergo further medical and psychiatric evaluations according to the Schedule of Assessments (Table 2) and inclusion and exclusion criteria (Section 6.1 and Section 6.2) to ensure they continue to qualify for participation in the study.

The Baseline criteria checklist must be completed on Day 1, prior to randomization, to determine if the participant is still eligible for participation in the study. Any participant who does not meet full Screening and Baseline entry criteria must be discontinued from the study and will be considered a screen failure. Participants who meet full Screening and Baseline entry criteria will be randomized into the treatment phase of the study.

8.2.3 Dosing/Treatment Period

The dosing period will begin on Day 1 and continue through the last dose of study drug. Study drug on Day 1 should preferably be administered in the morning. Assessments during the dosing

period will include measures of safety and tolerability, PK, and efficacy. For participants who discontinue study drug prior to Day 28, all end of treatment (EOT (Day 28)) assessments at the time of discontinuation and the EOS assessments 14 days after their last dose of study drug should be completed according to the Schedule of Assessments (Table 2), provided informed consent has not been withdrawn.

8.2.4 Placebo-Control Reminder Script (PCRS)

The Placebo-Control Reminder Script (PCRS) © (Hassman and Cohen, 2019, Version 5.0) educates clinical study participants of key causes of the placebo and nocebo effects, namely the tempering of participant study expectations, reminding participants what a placebo is and how that relates to their reporting of symptoms and potential side effects, and explaining how interactions with research site staff differ from their experience with previous providers. To do this, the PCRS informs participants that they are to be honest about their symptoms, site staff have no expectations of symptom improvement or worsening and will not be disappointed if they feel better, worse or the same, and asks participants to explain in their own words its content to ensure comprehension. Per the instructions on the PCRS, the MADRS10 rater will read the script verbatim immediately before administering this scale. The PCRS is read to EACH participant at EACH Visit (time point) outlined in Table 2, typically taking about 3 minutes to read. The PCRS has been empirically found to significantly manage (reduce) the placebo and nocebo effects.(Cohen 2021)

8.2.5 End of Treatment

For participants who discontinued or withdrew from study treatment prior to Day 28 because of a TEAE or serious TEAE, follow-up will continue until resolution of the event (return to normal or Baseline values) or until the AE is judged by the Investigator to no longer be clinically significant. Participants who discontinue prematurely will be asked to complete the End of Treatment assessments. See also Section 6.4.

8.2.6 End of Study

Participants who discontinue or withdraw from study treatment prior to Day 28, provided informed consent has not been withdrawn, must complete an EOS visit. If the End of Treatment visit and End of Study visit occur within 7 days of one another, the assessments will not have to be repeated, except at Investigator discretion.

All EOS assessments should be completed according to the Schedule of Assessments (Table 2).

8.3 Appropriateness of Measures

All safety and efficacy assessments have been well documented, validated, and applied as reliable, accurate, and relevant in this population.

Scales and measurements used in this study for efficacy and safety are described further in Section 9 and Section 10, respectively.

8.4 Entry Criteria Measurements

8.4.1 Structured Clinical Interview for DSM-5

The SCID-5 for MDD is a semi-structured interview guide to ensure consistency with the DSM-5 diagnostic classification. It is administered by a clinician or trained mental health professional who is familiar with the DSM-5 classification and diagnostic criteria.

8.4.2 SAFER Interview

A SAFER interview will be conducted remotely during the Screening period and eligibility will be assessed based on the inclusion/exclusion criteria by the Investigator and verified by an MGH-CTNI-certified clinician who will interview the participants using the HAMD17, SAFER/ATRQ, and eligibility criteria per the study protocol.

The SAFER interview assesses depression in a real-world setting and confirms that the participant's illness is a specific state and excludes participants with any symptoms that are nonspecific or not readily assessable.

8.4.3 Antidepressant Treatment Response Questionnaire (ATRQ)

The ATRQ is administered by the MGH-CTNI-certified clinician as part of the SAFER Interview. The ATRQ examines the efficacy and adequacy of any antidepressant treatment in a step-by-step procedure. This widely accepted questionnaire evaluates improvement (0% to 100%) and adequacy (adequate duration and dose).(Chandler 2010)

8.4.4 Hamilton Depression Rating Scale (HAMD17)

The HAMD17 has proven useful for many years as a way of determining a participant's level of depression before, during, and after treatment. Many clinicians consider the HAMD17 to be the gold standard for evaluating the severity of depression. The HAMD17 must be administered using the Structured Interview Guide for the Hamilton Depression Rating Scale (SIGH-D).(Williams 1988) The HAMD17 will be conducted at Screening by a certified site rater, and

prior to Baseline by an MGH-CTNI independent clinician, to confirm eligible score (≥19) for enrollment and that there has not been an increase in absolute value of >40% or a decrease in absolute value of >20% on the HAMD17 score since Screening(see also Section 8.4.2).

9 EFFICACY ASSESSMENTS

The Schedule of Assessments (Table 2) outlines the efficacy assessments to be performed throughout the study and their timing.

9.1 Primary Efficacy Assessment

9.1.1 Montgomery-Åsberg Depression Rating Scale (MADRS10)

The participant's depression level will be measured using MADRS10 as administered by a certified site rater at Baseline (Day 1, pre-dose) and post-dose for all subsequent visits as outlined in Table 2.

The MADRS10 is a 10-item questionnaire with each item scored between 0 and 6, resulting in a total score between 0 to 60. Higher MADRS10 scores indicate worsening depression, with scores at or above 35 indicating severe depression. The questionnaire is most frequently used in clinical studies to measure outcomes in antidepressant efficacy studies. This scale exhibits construct validity (internal homogeneity) and concurrent validity relative to the HAMD17 and the concepts of endogenous and non-endogenous depression. The questionnaire includes questions on the following symptoms: (1) apparent sadness; (2) reported sadness; (3) inner tension; (4) reduced sleep; (5) reduced appetite; (6) concentration difficulties; (7) lassitude; (8) inability to feel; (9) pessimistic thoughts; (10) suicidal thoughts. The MADRS10 must be administered using the Structured Interview Guide for the MADRS10 (SIGMA).(Montgomery 1979; Williams 2008)

9.2 Secondary Efficacy Assessment

9.2.1 Clinical Global Impression of Severity (CGI-S)

The CGI-S measures the severity of the disease using a 7-point Likert scale ranging from 1=normal, not at all ill to 7=among the most extremely ill participants (Busner 2007).

CGI-S will be measured at pre-dose at Baseline (Day 1), and at post-dose at all subsequent visit time points outlined in Table 2.

9.2.2 Absolute Change from Baseline of the MADRS10 Total Score at Day 7

See above: this is the absolute change from Baseline of the MADRS10 total score at Day 7.

9.2.3 Remission Rate at Day 28 visit

Remission is defined as an absolute value of the MADRS10 total score ≤10 at Day 28.

9.2.4 Response Rate at Day 28 visit

Response is defined as the improvement ≥50% reflected in the absolute change from Baseline to Day 28 of the MADRS10 total score.

9.3 Exploratory Assessments

9.3.1 Hamilton Anxiety Depression Scale (HAM-A)

The HAM-A will be measured at Screening and at end of study outlined in Table 2 by a certified site rater using the Structured Interview Guide for the Hamilton Anxiety Scale (SIGH-A).

The HAM-A was one of the first rating scales developed to measure the severity of anxiety symptoms and is still widely used today in both clinical and research settings. The scale consists of 14 items, each defined by a series of symptoms, and measures both psychic anxiety (mental agitation and psychological distress) and somatic anxiety (physical complaints related to anxiety). Each of the 14 items are scored on a scale of 0 (not present) to 4 (severe), with a total score range of 0 to 56, where <17 total points indicates mild severity, 18 to 24 total points indicates mild to moderate severity, and 25 to 30 total points indicates moderate to severe.

9.3.2 Symptoms of Depression Questionnaire (SDQ)

The SDQ will be measured at Screening and on Day 28 as outlined in Table 2.

The SDQ is a 44-item, self-report scale designed to measure the severity of symptoms across several subtypes of depression. The SDQ was developed to more fully capture the heterogeneity of symptom presentations of depressive disorders than current, widely used scales for MDD. The SDQ includes items that inquire about an extensive number of depressive symptoms beyond the ones included in other commonly used scales such as the Quick Inventory of Depression Symptomatology (QIDS).

The 44 items are rated on a 6-point scale. Each item is rated based on a participant's perception of what is normal for the individual (score = 2), what is better than normal (score = 1), and what is worse than normal (scores = 3 to 6).

Items reflect a broad and heterogeneous collection of depression related symptom features. For example, Factor 1 measures common dimensions of depressive symptoms including lassitude, energy, mood, and cognitive and social functioning (component 1). Factor 3 includes items on outlook on life, pessimism, suicide, self-harm, and worthlessness (component 3). Factors 4 and 5 measure physiological features of depression, namely sleep difficulties and changes in appetite/weight, respectively. Moreover, the SDQ includes several items that inquire about anxiety symptoms often present among depressed participants.(Pedrelli 2014)

9.3.3 Sheehan Disability Scale (SDS)

The SDS will be measured at Screening, pre-dose at Baseline (Day 1), and at post-dose on Day 28.

The SDS is a brief, 3-domain self-report tool that assesses functional impairment in work/school, social life, and family life.

Work/school, social life, and family life/home responsibilities are each scored, individually, on a scale of 0 to 10.

Higher scores are associated with significant functional impairment (e.g., scores ≥5 on any of the 3 domains). (Sheehan DV 1983)

9.3.4 Clinical Global Impression of Improvement (CGI-I)

The CGI-I measures the improvement since the start of study treatment of the participants using a 7-point Likert scale ranging from 1=very much improved to 7= very much worse.

CGI-I will be measured at post-dose at all visit time points outlined in Table 2.

9.3.5 Digit Symbol Substitution Test (DSST)

The DSST will be measured at pre-dose at Baseline (Day 1), and at post-dose on Day 28. The DSST will be conducted via paper version only and completed at the site visit. The DSST evaluation may not be performed if participants are unable to attend the site visit. The DSST was developed as an experimental tool to assess cognitive dysfunction. It measures processing speed and attention. The DSST, a symbol-digit pair exercise, requires participants to match numbers with symbols within 90 seconds. Previous studies reported patients with MDD were associated with levels of executive dysfunction and impairment.(Jaeger 2018)

9.3.6 Perceived Deficits Questionnaire – Depression (PDQ-D-5)

The PDQ-D-5 will be measured at pre-dose at Baseline (Day 1), and at post-dose on Day 28. PDQ-D-5, a short version of PDQ-D-20, is a patient-reported measure of cognitive symptoms.(Fehnel 2016) The PDQ-D-5 measures 4 domains of cognition, namely, attention/concentration, retrospective memory, prospective memory, and planning/organization.

9.3.7 Arizona Sexual Experience Scale (ASEX)

The ASEX will be measured at pre-dose at Baseline (Day 1), and at post-dose on Day 28. ASEX assesses 5 global aspects of sexual dysfunction which includes: sex drive, arousal, penile/erection/vaginal lubrication, ability to reach orgasm, and satisfaction from orgasm. High Cronbach's alpha and test-retest reliability were reported, 0.91 and 0.801, respectively. Using the Gold Standard Clinician Rating Scale as the gold standard, ASEX has moderately high sensitivity (0.82) and high specificity (0.90). Each item is scored on a 6-point Likert scale (1 to 6). Total score ranges from 5 to 30, with higher scores reflecting more sexual dysfunction. Sexual dysfunction is defined by any one of the following conditions: ASEX total score ≥19, or any one item with a score of ≥5, or any 3 items with a score of ≥4.(McGahuey 2000)

10 SAFETY ASSESSMENTS

Safety assessments (vital signs, physical examinations, ECG recording, AEs, SAEs, and clinical laboratory results [routine hematology and biochemistry]) are to be performed at protocol-specified visits, as specified in the Schedule of Assessments (Table 2).

10.1 Medical and Psychiatric History

Medical and psychiatric history will be recorded at Screening. Documentation of psychiatric history, including MDD diagnosis, and medical history (medical and pharmacy records or alternative verified documentation as requested by study Medical Monitor or delegate) must be available and present in the source documents. Investigators should document the occurrence, signs, and symptoms of the participant's preexisting conditions, including all prior significant illnesses, up to and including 5 years before Screening. Additional preexisting conditions present at the time when informed consent is given and up to the time of first dosing (Day 1) are to be regarded as concomitant. Medical history will include alcohol consumption, substance use, and smoking history, if applicable.

Illnesses first occurring or detected during the study and/or worsening of a concomitant illness during the study are to be documented as AEs on the eCRF in accordance with Section 10.6. All changes not present at Baseline or described in the past medical history and identified as clinically noteworthy must be recorded as AEs.

Additionally, demographic data will be collected for all participants and include date of birth, sex at birth, race, and ethnicity, height, weight, and BMI.

10.2 Vital Signs

Vital signs (body temperature, pulse oximetry, respiratory rate, heart rate, and systolic and diastolic blood pressure (BP) measurements) will be evaluated at the visits outlined in Table 2. All vital signs will be measured after the participant has been resting in a supine or semi-supine position for at least 3 minutes. BP measurements are to be taken in the same arm for the duration of the study. Measurement of orthostatic BP/HR should follow the measurement of supine or semi-supine BP with participants asked to stand for 3 minutes before the measurement of orthostatic BP/HR.

Vital sign measurements will be repeated if clinically significant or machine/equipment errors occur. Out-of-range BP, RR, or HR measurements will be repeated at the Investigator's discretion. Any confirmed, clinically significant vital sign measurements must be recorded as AEs.

10.3 Physical Examination

A complete physical examination (general appearance; head, eyes, ears, nose and throat; heart; respiratory/lungs; abdomen; skin; cervical and axillary lymph nodes; and neurological and musculoskeletal systems) will be performed at Screening as outlined in Table 2. At subsequent visits, a focused physical examination will be conducted, limited to include the following body systems: general appearance, abdomen, heart, respiratory/lungs, neurological, musculoskeletal, and any other assessments required to evaluate AEs. Physical exams will be performed by a physician or an appropriately qualified practitioner as delegated at the discretion of the investigator.

Body weight (without shoes) and BMI will be recorded at Screening, Day 1, EOT (Day 28), and at EOS. Height (without shoes) will be recorded at Screening only.

Clinically significant findings prior to dosing will be recorded on the medical history eCRF. During the study, any clinically significant change from Screening will be documented as an AE on the AE eCRF. Clinically significant is defined as any variation in physical examination finding that has medical relevance and may result in an alteration in medical care.

10.4 Electrocardiograms

At Screening, one set of triplicate 12-lead, resting ECGs will be obtained (Table 2).

At Baseline (Day 1), pre-dose, but after Scale Assessments and Patient Reported Outcomes (PROs), two sets of triplicate ECGs will be taken, each ECG at least 30 seconds apart, and each set 15 min apart (+/- 5min).

On Days 7, 14 and 28, participants will have only one set of triplicate ECGs performed after efficacy assessments have been completed. Note: The post-dose ECGs will be aligned with the PK time point. Procedures performed after the assessment scales should follow from least invasive to more invasive. The following order is recommended: all assessment scales, vital signs, ECGs and lastly PK blood draw. The time of the subject's last reported dose prior to ECG should be captured in the source documents and eCRF. Participants will be required to lie quietly in a supine position with minimal movement and minimal exposure to noise and other environmental stimuli (e.g., TV, loud radio, interactions with other participants) for at least 10 minutes prior to each ECG collection.

At Day 42 (EOS), a single 12-lead, resting ECG will be obtained.

ECGs will be performed by the site team member and data interpretation will be done by a Core ECG Laboratory. The core laboratory analysis will constitute the primary ECG dataset for the study. The QTcF analysis will be based on the mean values of the triplicate ECG.

At Screening, the central reader will examine the ECG tracings for signs of cardiac disease that could exclude the participant from the study, as defined in the exclusion criteria. An assessment

of normal or abnormal will be recorded; if any ECG is considered abnormal, the abnormality will be documented in the eCRF. ECGs will be repeated if clinically significant abnormalities are observed or artifacts are present.

If a participant has a QTcF >500 msec or an increase from Baseline >60 msec (including on the local ECG machine interpretation), triplicate ECGs will be conducted after approximately 30 minutes. If the triplicate ECG, as assessed by an appropriately trained expert (the ECG Core Lab or a cardiac electrophysiologist) verifies that the QTcF interval is >500 msec or that the increase from Baseline is >60 msec from Baseline (Day 1 pre-dose) that is not due to a significant increase in the QRS duration, the study drug will be stopped and causality will be investigated.

10.5 Laboratory Assessments

Clinical laboratory tests (chemistry, hematology, and urinalysis) will be conducted at Screening, Baseline (Day 1), and at subsequent visits as shown in the Schedule of Assessments (Table 2) and below Table 3, unless otherwise noted. Blood and urine samples will be collected, processed, and shipped according to the research site's SOPs and instructions from the safety laboratory. All laboratory safety data will be reviewed by the Investigator or designee for clinical significance.

Additional laboratory samples may be taken at the discretion of the Investigator or designee (e.g., if the results of any tests fall outside reference ranges or clinical symptoms necessitate testing to ensure safety).

Table 3 List of Laboratory Assessments

Hematology	**Serum Chemistry**	**Urinalysis (Dipstick)***
hemoglobin hematocrit erythrocytes platelets leukocytes with differential (including eosinophils, neutrophils, basophils, lymphocytes and monocytes)	eGFR (Screening only) HbA1c (Screening only) creatinine total bilirubin (and fractionation of bilirubin >ULN) albumin blood urea nitrogen total protein alkaline phosphatase creatine phosphokinase aspartate aminotransferase alanine aminotransferase glucose sodium potassium magnesium calcium chloride phosphate bicarbonate TSH (Screening only) testosterone (men) (*at Baseline and Day 28*)	pH specific gravity protein glucose ketones bilirubin occult blood nitrite urobilinogen leukocyte esterase * Reflex microscopy when indicated
Pregnancy test: A serum FSH test will be conducted for all females at Screening. A urine pregnancy test and serum pregnancy test (b-HCG) will be conducted on all WOCBP at Screening. Negative pregnancy test results are required for enrollment in the study; a negative urine pregnancy test will be required at Baseline (Day 1) for confirmation. Urine pregnancy tests will be administered at all subsequent study visits for WOCBP as shown in the assessment schedule. During the study, a serum pregnancy test (b-HCG) will be performed if a participant's urine pregnancy test result is positive.		
Urine drug and alcohol screen: Drug and alcohol abuse testing will be performed according to the assessment schedule. Screening for drugs will include: tetrahydrocannabinol, opiates (including oxycodone), amphetamines, cocaine, and benzodiazepines. **NOTE**: At Baseline, urine drug screening will be performed locally on-site and the following drugs will be tested: amphetamine, barbiturates, buprenorphine, benzodiazepines, cocaine, methadone metabolite, methamphetamine, ecstasy, methadone, opiates, oxycodone, phencyclidine, tricyclic antidepressants, tetrahydrocannabinol.		
Serologic testing for hepatitis B surface antigens, hepatitis C antibodies, and HIV antibody testing will be performed at the Screening visit.		

10.5.1 Viral Screen

Serology tests will be used to screen for hepatitis B, hepatitis C, and HIV at Screening. Participants positive for hepatitis B, hepatitis C, and/or HIV will be eligible for the study based on Investigator discretion. Positive results will be managed according to local regulatory requirements and the site's SOPs.

10.5.2 Breath Alcohol Test

Breath alcohol testing will be performed according to the site's SOPs at Screening, Baseline (Day 1), and at subsequent visit time points outlined in Table 2. If there is any doubt or concern regarding alcohol use, research site staff may request a breath test for alcohol measures at subsequent visits during the study. Participants should abstain from alcohol for 24 hours prior to all site visits.

10.6 Adverse Events

The Investigator or designee and research site staff are responsible for the detection, documentation, classification, reporting, and follow-up of events meeting the definition of an AE or SAE. Procedures for managing AEs and SAEs are detailed in the research site's SOPs.

Spontaneously reported or observed AEs will be recorded from signing of the informed consent and throughout the study except in the case of screen failed subjects in which only Serious Adverse Events will be collected. AEs will be elicited using a non-leading question at every visit from Screening through EOS (Day 42).

All measures required for the management of AEs will be recorded in the source documentation.

10.6.1 Adverse Event Definition

An AE is any untoward medical occurrence in a study participant administered a pharmaceutical product and may not necessarily have a causal relationship with the administered treatment. An AE can therefore be any unfavorable and unintended sign (including a clinically significant laboratory abnormality, for example), symptom, or disease temporally associated with the use of a medicinal (investigational) product, regardless of relationship to the medicinal (investigational) product. During the study, an AE can also occur outside the time that the investigational product(s) was given (e.g., during the time from discontinuation of prohibited medications).

Preexisting conditions, diseases, or disorders are not considered AEs unless there is a change in intensity, frequency, or quality.

10.6.2 Serious Adverse Events and Serious Unexpected Adverse Events

An SAE is any untoward medical occurrence that at any dose

- Results in death,
- Is life-threatening (at the time of the event),
- Requires inpatient hospitalization or prolongation of existing hospitalization,
- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the participant 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 ER or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

A serious and unexpected AE is an SAE that is not identified in nature, intensity, or frequency in the risk information set out in the IB or on the label of the drug.

10.6.3 Clinical Laboratory Abnormalities and Other Abnormal Assessments

Abnormal laboratory findings (e.g., from chemistry, hematology, or urinalysis) or other abnormal assessments (e.g., from vital signs, ECGs, C-SSRS, CADSS) judged as clinically significant by the Investigator or designee will be recorded as AEs or SAEs if they meet the definitions provided in Section 10.6.1 and Section 10.6.2. Furthermore, abnormal laboratory findings or other abnormal assessments present at Baseline that significantly worsen following the start of the study (i.e., become clinically significant) will be reported as AEs or SAEs. However, abnormal laboratory findings present at the start of the study that do **not** worsen will not be reported as AEs or SAEs.

The Investigator will exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

10.7 Evaluation of Adverse Events and Serious Adverse Events

The Investigator or designee is responsible for making an assessment as to the seriousness, intensity, causality, and outcome of an AE. Severity will be assessed using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events version 5.0 (CTCAE v5.0) accessible at: https://evs.nci.nih.gov/ftp1/CTCAE/About.html.

10.7.1 Classification of Adverse Event Intensity

Classification of AE intensity will be performed using the CTCAE v5.0. If there is insufficient information to determine intensity, the AE must still be reported.

Grade refers to the severity of the AE. The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on this general guideline:

- Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL*.
- Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
- Grade 4: Life-threatening consequences; urgent intervention indicated.
- Grade 5: Death related to AE.

*Instrumental Activities of Daily Living (ADL) refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

**Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

10.7.1.1 Classification of Adverse Event Causality

For each recorded AE or SAE, the Investigator or designee must assess the causality based on the following criteria (Table 4) to determine the relationship between the AE and study drug.

Table 4. Classification of Adverse Event Causality

Classification	Definition
Unrelated	The AE or SAE is judged to be *clearly and incontrovertibly due only to extraneous causes* (e.g., disease, environment) and does not meet the criteria for study drug relationship listed under probable, possible, or unlikely.
Unlikely	The AE or SAE is *unlikely related* to the study drug, when the AE or SAE: • Does not follow a reasonable temporal sequence from administration of the study drug. • May readily have been produced by the participant's clinical state, environmental or toxic factors, or other modes of therapy administered to the participant. • Does not follow a known pattern of response to the study drug. • Does not reappear or worsen when the study drug is re-administered.
Possible	The AE or SAE is *possibly related* to the study drug when the connection to the study drug appears unlikely but cannot be ruled out with certainty. This causal relationship is assigned when the AE or SAE: • Follows a reasonable temporal sequence from administration of the study drug. • May have been produced by the participant's clinical state, environmental or toxic factors, or other modes of therapy administered to the participant. • Follows a pattern of response to the suspected study drug.
Probable	The AE or SAE is *probably related* to the study drug when the connection to study drug can be made with a high degree of certainty. This causal relationship is assigned when the AE or SAE: • Follows a reasonable temporal sequence from administration of the study drug. • Cannot be reasonably explained by the known characteristics of the participant's clinical state, environmental or toxic factors, or other modes of therapy administered to the participant. • Disappears or decreases upon cessation or reduction in dose (note that there are important exceptions when an AE or SAE does not disappear upon discontinuation of the study drug, yet drug relatedness clearly exists e.g., bone marrow depression or tardive dyskinesia). • Follows a known pattern of response to the suspected study drug. • Reappears upon re-challenge.

Classification	Definition
Definite	The AE or SAE is *definitely related* to the study drug when the event: • Follows a reasonable temporal sequence from administration of the study drug. • Follows a known pattern of response to the suspected study drug. • Is confirmed by improvement on stopping and reappearance of the event on repeated exposure to the study drug. • Cannot be reasonably explained by the known characteristics of the participant's clinical state.

10.7.2 Classification of Adverse Event Outcome

For each recorded AE or SAE, the Investigator or designee must assess the outcome at the time of the last observation. The outcome of AEs or SAEs will be documented as outlined in Table 5.

Table 5. Classification of Adverse Event Outcomes

Classification	Definition
Fatal	The study participant died.
Resolved	The AE or SAE has ended.
Resolved with Sequelae	The AE or SAE has ended but changes are noted from Baseline.
Unresolved	The AE has not ended. An AE outcome can only be categorized as unresolved, if the AE is: • *Ongoing* at the end of the reporting period (i.e., 30 days after the last dose of study drug), and the Investigator deems that further follow-up is not medically required. • *Lost to follow-up* after repeated unsuccessful attempts to contact the participant. • *Ongoing and referred* to the participant's physician or a specialist for follow-up.

10.8 Reporting Procedures

10.8.1 Serious Adverse Events and Serious Unexpected Adverse Events

Any SAE, expected or unexpected, irrespective of relationship to study treatments, including death due to any cause, experienced by a study participant will be reported to Sponsor, by the Investigator or designee within **24 hours** of learning of the event.

The contact information for the pharmacovigilance team is as follows:

Email:	relmadapv@primevigilance.com; PVsafety@relmada.com; medical@relmada.com

Relmada assumes responsibility for the appropriate reporting of AEs to the regulatory authorities. All SAEs that are unlisted and associated with the use of the study drug will also be reported to all Investigators. The Investigator (or Sponsor, where required) must report these events to the appropriate IRB/IEC that approved the protocol (unless otherwise required and documented by the IRB/IEC).

All additional follow-up evaluations for SAEs will be reported to Sponsor within 24 hours of knowledge using the contact information above.

10.8.2 Any Adverse Event

Regardless of seriousness, intensity, or presumed relationship to study drug, all AEs will be recorded in the source documentation and assessed using CTCAE v5.0.

When the signs and symptoms are due to a common etiology, the diagnoses will be recorded whenever possible. In addition, the Investigator must record his or her opinion as to the intensity of the AE and whether the AE is related to the study drug. All measures required for management of the AE will be recorded in the source documentation.

10.8.3 Pregnancy

Given that the effects of the study drug on egg and sperm have not been fully elucidated, any pregnancy in the participant or participant's partner will be reported to the Sponsor within **24 hours** of learning of the event.

Follow-up information will be obtained where possible (i.e., with the consent of the participant or participant's partner) regarding the course and outcome of the pregnancy, including any post-natal sequelae in the infant.

10.9 Follow-Up of Adverse Events and Serious Adverse Events

All unresolved AEs will be followed for up to a minimum of 30 days after the participant's final dose of study drug on Day 28 unless the Investigator's judgment dictates otherwise, the event has resolved or stabilized before Day 28, or the participant is lost to follow-up.

All AEs that result in discontinuation and all SAEs will be followed until one of the following occurs:

- The event resolves.

- The event stabilizes.
- The event returns to a Baseline value if a Baseline value is available.
- The event can be attributed to agent(s) other than the study drug or to factors unrelated to study conduct.

When it becomes unlikely that any additional information can be obtained (e.g., participant or health care practitioner refuses to provide additional information, the participant is lost to follow-up), the Investigator or designee will ensure that the follow-up includes any pertinent supplemental investigations (e.g., laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals) to elucidate the nature and/or causality of the AE or SAE.

Investigators are not obligated to actively seek AEs or SAEs in former study participants that occur after the follow-up period. However, if the Investigator or designee learns of any AE or SAE at any time after a participant has been discharged from the study and the event is considered as reasonably related to the study drug, the Investigator will notify Sponsor.

10.10 Other Measurements/Assessments

10.10.1 Columbia-Suicide Severity Rating Scale (C-SSRS)

The C-SSRS Baseline/Screening version will be assessed at Screening, and the C-SSRS Since Last Visit version will be administered at Baseline (Day 1) and at subsequent visits as shown in Table 2.

The C-SSRS is routinely used to quantify the severity of suicidal ideation and behavior. Both the ideation and behavior components are sensitive to change over time.

The scale identifies behaviors that may be indicative of an individual's intent to commit suicide. This measure contains 6 "yes" or "no" questions in which respondents are asked to indicate whether they have experienced several thoughts or feelings relating to suicide over the past month. Each question addresses a different component of the respondent's suicide ideation severity: (1) desire to be dead; (2) suicidal thoughts; (3) consideration of suicide methods; (4) formed intent to commit suicide; (5) completed suicide plan; and (6) initiated suicide plan. A higher score indicates a higher intensity of suicidal ideation.(Posner 2011)

The C-SSRS will be administered to study participants during the course of the study and results reviewed throughout the conduct of the study by the Principal Investigator/delegated Investigator at each site, Medical Monitor, and the Independent Data Monitoring Committee (DMC). The DMC will monitor the trend in relative risk of suicide in the active group versus placebo and will make recommendations to the Sponsor on appropriate course of actions to be taken regarding individual participants and/or the clinical trial.

Site staff are trained and certified on the administration of the C-SSRS, which includes safety follow-up and action when suicidal ideation is communicated by participant to site staff as per local site policy (this may include a 24-hour emergency contact number for participants or their family members/friends, calling 911 or a suicide hotline, or referring the participant to the emergency room). If an upward trend in suicide score for any participant is observed during the course of treatment, the PI or delegated Investigator will assess and take appropriate measures, which may include withdrawing from the study, further evaluation or clinical management, and/or hospitalization as appropriate.

10.10.2 Clinician-Administered Dissociative States Scale (CADSS)

The CADSS will be measured at Baseline (Day 1), and at all subsequent visits as shown in Table 2.

The CADSS is an instrument for the measurement of present-state dissociative symptoms with good interrater reliability and construct validity. The CADSS can discriminate participants with dissociative disorders from healthy participants, as well as participants with other psychiatric disorders. The CADSS is a 23-item scale with each item scaled from 0 to 4 corresponding to the dissociative states of: Not at all, mild, moderate, severe, and extreme, thus resulting in a total score between 0 to 92. A higher total score indicates a higher likelihood of the presence of a dissociative state. Components of the CADSS were developed to identify amnesia (Items 14 and 15), depersonalization (Items 3 to 7), and derealization (Items 1, 2, 8 to 13, and 16 to 19).(Bremner 1998)

10.11 Pharmacokinetic Assessments

Standard PK assessments, appropriate for an early Phase 3 study, have been included as outlined in Section 11.1.

11 PHARMACOKINETICS

11.1 Pharmacokinetic Sampling

Venous blood samples will be collected at Baseline (Day 1) and at each subsequent study visit (Table 2) at the following time points to determine the plasma concentrations of REL-1017:

- On Day 1, PK samples will be obtained at pre-dose.
- On Days 7, 14, and 28, PK samples post-dose will be obtained.
- Note: PK blood samples will be taken on Days 1 (immediately following the second set of ECG recording and before dosing) and 7, 14, and 28, where participants will have PK sample taken post-dose and within 10 minutes of ECG.

Plasma samples will be collected, processed, and shipped according to the site's SOPs and instructions from the Sponsor or the bioanalytical laboratory.

The plasma samples will be analyzed by the bioanalytical laboratory using validated methods. Plasma samples may also be used for exploratory bioanalytical method development and/or metabolite(s) characterization.

Plasma samples will be shipped frozen on dry ice from the research site to the central lab and then shipped to the bioanalytical laboratory. Samples will not be shipped without prior arrangement with the bioanalytical laboratory and notification of the Sponsor.

Details of PK blood sample collection, processing, storage, and shipping procedures are provided in a separate laboratory manual.

11.2 Pharmacogenetic Analysis

At Baseline (Day 1) of dosing, a blood sample for DNA isolation will be collected from all participants in this study who signed the pharmacogenetic ICF for potential pharmacogenetic analysis of genes that may affect the PK of REL-1017. This analysis would be restricted to the evaluation of genes that may be involved in the PK of REL-1017 (e.g.,, drug metabolism, disposition, elimination).

One 4 mL whole blood sample for DNA isolation will be collected on Day 1 from each participant into an appropriately labeled ethylene diamine tetra-acetic acid (EDTA) tube. Samples will be collected and shipped according to the site's SOPs and instructions from the Sponsor and/or pharmacogenetic laboratory.

The samples will be stored in a secure space with adequate measures to protect confidentiality. The samples will be retained while research on REL-1017 continues, but not longer than 20 years.

12 STATISTICAL ANALYSIS

12.1 Statistical and Analytical Plans

This section provides general approaches for statistical analysis. They may be changed such as due to regulatory agencies feedback. Complete details of the statistical analyses to be performed will be documented in a Statistical Analysis Plan (SAP) that will be finalized prior to unblinding the study data. The SAP will include more details of the analysis populations, summary strategies, and any amendments to the proposed analyses, if necessary. Any changes to the final SAP will be outlined in the final clinical study report.

12.2 Primary Efficacy Analysis

The primary analysis of efficacy will be performed using an MMRM and main estimand. The MMRM will include the four post-baseline (Visits 4-7) MADRS10 total scores for each subject as (correlated) dependent outcomes. Treatment, visit, the interaction of treatment and visit, and the baseline total MADRS10 score will be independent variables in the MMRM. The main estimand approach to handling of missing MADRS10 total scores and occurrences of ICEs is described in the SAP. A number of sensitivity analyses including MNAR multiple imputations and a tipping point analysis will be performed on the Primary Endpoint. The SAP provides details of the primary efficacy analysis.

12.3 Analysis of Secondary Efficacy Endpoints

All secondary endpoints will be analyzed per the estimands described in the SAP.

12.4 Determination of Sample Size

Approximately 280 subjects (140 per treatment group) are required to demonstrate efficacy for the primary endpoint in this study, the change from Baseline to Day 28 in the MADRS10 total score.

The sample size was selected to detect a difference between means of the REL 1017 and placebo groups equal to Δ=3.8 points with a common standard deviation of σ = 11 (Cohen's d: $\Delta / \sigma = 0.35$). The difference in treatment group means for this study is anticipated to be larger than the prior REL-1017-301 study due to changes made in this trial's screening criteria to strengthen the selection of subjects with a true MDD diagnosis. The planned standard deviation for the primary endpoint is approximately the same as the one observed in REL 1017 301.

A planned interim analysis will take place when approximately 75% (210) of the initial N=280 subjects have completed their Day 28 visit or have prematurely dropped out of the study. The interim analysis will include a variety of analyses and a sample size re-estimation procedure that allows the final sample size to increase to a maximum N=460 subjects.

12.5 Missing Data

A mixed-effect model repeated measure (MMRM) will be used for the primary efficacy endpoint analysis, and also for the analysis of several secondary and exploratory endpoints. The main estimand will consider all missing data to be missing at random as required for valid inference in the MMRM model (direct likelihood with no imputation). However, a number of sensitivity analyses, including reference-based multiple imputation and a tipping point analysis, will be performed to investigate the robustness of the primary results with regards to missing values. Details on the handling of missing data and missing values imputation for other endpoints will be provided in the SAP.

12.6 Analysis of Safety Assessments

Safety and tolerability parameters will be listed by treatment and participant and displayed in summary tables using descriptive statistics.

12.6.1 Adverse Events

The number and percentage of participants with TEAEs, SAEs, and AEs leading to study treatment discontinuation, and additionally TEAEs by maximum severity and relationship to study treatment will be summarized by SOC, preferred term, and treatment, using CTCAE version 5.0.

Summary tables of AEs during and after the treatment with time-adjusted count- and incidence rates will be presented.

AEs involving potential abuse will be categorized in tabular form and will include time of onset and duration of the event, dose of drug taken, severity and outcome. If available, PK values for each individual participant who experienced these AEs will be provided to understand if there is a temporal correlation between drug plasma levels and AEs.

12.6.2 Other Safety Analyses

Descriptive statistics for vital signs, weight, and body temperature will be calculated and presented for each time point by treatment (absolute values and change from Baseline). Summary tables will present actual values and the change from Baseline.

The ECG analysis, including possible evaluation of a potential exposure-response relationship or a population PK/QTc model will be detailed in a separate ECG Analysis Statistical Analysis Plan.

Laboratory data will be summarized by the type of laboratory test and scheduled visit. Descriptive statistics will be calculated and presented for each time point by treatment (absolute values and change from Baseline). Descriptive statistics and the number of participants with laboratory test results below, within, and above normal ranges will be tabulated by visit. Biomarkers and genetic markers will also be summarized by treatment group. Pregnancy test results, drug abuse screen results, breath alcohol data, Hepatitis B, Hepatitis C, HIV assessments, viral screen data, and TSH will be listed only.

The C-SSRS data which is a validated standard assessment instrument of suicidal ideation severity, including suicidal ideation intensity, suicidal behavior, non-suicidal self-injuries behavior, and lethality, will be summarized by treatment group and visit. Additionally, a summary of participants answering Yes to questions of C-SSRS form grouped by any question, combinations of questions, and by each individual question will be provided by treatment group.

The CADSS total score and component scores will be summarized by treatment group and visit. Actual values and changes from Baseline will be presented.

12.7 Demographics and Other Baseline Characteristics

Demographics and Baseline characteristics (age, sex, race, ethnicity, body weight, height, body temperature, and BMI) will be summarized using descriptive statistics (number of participants, mean, SD, median, minimum, and maximum for continuous variables and the proportion of participants for categorical variables) for the Safety population. No formal statistical comparison between the groups will be performed.

The number of participants in each of the treatment groups will be presented, in addition to the number of participants who complete each visit. The reasons for all post-randomization study drug discontinuations or study terminations will be tabulated and grouped by treatment group and all participants combined. All deviations related to study inclusion or exclusion criteria, conduct of the study, participant management, or participant assessment will be described.

Medical history and psychiatric history will be coded using MedDRA. Medical history and psychiatric history will be listed including onset of MDD and onset of current MDE. Physical examination abnormalities will be listed.

Prior and concomitant medications will be coded using WHODrug Dictionary. Prior and concomitant medications will be listed. Concomitant medication will be summarized by treatment. Comorbidities and comedications will be summarized by treatment.

All depression-associated comedication before, during, and after the randomized exposure period will be listed.

12.8 Interim Analysis

An IA-SSR adopting the Cui-Hung-Wang (CHW) paradigm (Cui 1999) is planned for assessing the conditional power of the primary efficacy analysis and to reassess the sample size in accordance with the decision rules specified in the DMC Charter.

The IA will be performed by an independent, unblinded, firewalled DMC when approximately 210 participants (75%) of the initially planned 280 participants in this group have completed their 28-day assessments in the study or discontinued early.

12.9 Data Monitoring Committee

The Medical Monitor or designee and the DMC will be responsible for the ongoing review and evaluation of safety throughout the study (including all clinical laboratory and ECG data, as well as all AEs). The DMC will meet at regular prespecified times to review accumulating safety and tolerability data and make recommendations to Sponsor on the conduct of the study. The operation of the DMC will be governed by a charter that will describe the groups' frequency of meeting, procedures (including, but not limited to, cohort safety monitoring), and requirements for reporting its observations to the Sponsor.

12.10 Analysis of Pharmacokinetics

PK analyses will be described in detail by an expert PK vendor in a separate SAP and results will be provided in a separate PK report.

13 STUDY MANAGEMENT

13.1 Regulatory Guidelines

This study will be conducted in accordance with the accepted version of the Declaration of Helsinki and/or all relevant regulations, as set forth in Parts 50, 56, 312, Subpart D, of Title 21 of the US Code of Federal Regulations (CFR), in compliance with International Council for Harmonisation (ICH) and Good Clinical Practice (GCP) guidelines and according to the appropriate regulatory requirements in the countries where the study was conducted.

13.2 Institutional Review Board/Independent Ethics Committee

The Investigator will ensure that this protocol is reviewed and approved by the IRB/IEC. The IRB/IEC will also review and approve the site's ICF and any other written information provided to the participant, including any advertisements used for participant recruitment. Prior to the enrollment of participants, the Investigator or designee will forward copies of the IRB/IEC approval and approved study documentation to the Sponsor.

If, during the study, it is necessary to amend study documentation (e.g., protocol, ICF), the Investigator or designee will be responsible for ensuring that the IRB/IEC reviews and approves these amended documents. IRB/IEC approval of an amended ICF must be obtained before new participants consent to take part in the study using the amended form. Copies of the IRB/IEC approval of the amended study documentation and the approved materials will be forwarded to the Sponsor as soon as available.

13.2.1 Informed Consent

At Screening, the Investigator or designee will inform the participant of all aspects pertaining to the individual's participation in the study.

The process for obtaining the participant's informed consent will be in accordance with all applicable regulatory requirements. The Investigator or designee and the participant will both sign and date the ICF before participation in the study. The participant will receive a copy of the consent form, and the original form will be retained in the site study records. The participant's decision regarding participation in the study will be entirely voluntary. The Investigator or designee will emphasize to the participant that consent regarding study participation may be withdrawn at any time without penalty or loss of benefits to which the participant is otherwise entitled.

If the ICF is amended during the study, the Investigator will follow all applicable regulatory requirements pertaining to the implementation and use of the amended ICF, including use for previously consented participants.

13.3 Principal Investigator Reporting Requirements

In accordance with applicable local regulatory requirements, the Investigator may be obligated to provide periodic safety updates on the conduct of the study at his or her research site and notification of study closure to the IRB/IEC. Such periodic safety updates and notifications are the responsibility of the Investigator and not of the Sponsor. The Sponsor will be provided with copies of all notifications sent to the IRB/IEC.

13.4 Privacy

The research site's Privacy Officer will provide guidance on privacy issues to the Investigator. The Investigator or designee is responsible for complying with applicable privacy regulations per his or her jurisdiction. Only information identified in this protocol will be collected. The information collected will only be used for the purposes identified in this protocol.

13.5 Participant Identifiers

To ensure participant anonymity and to limit disclosure, participants will be assigned a unique identification number after signing the ICF at their first assessment. This identifier will be cross-referenced in the participant's chart. The identifier will not contain any potentially identifiable information.

A participant identifier log will be maintained, linking each participant's name to the corresponding identifier. This log will be stored at the research site in a secure location.

13.6 Purpose of Collecting Personal Information and Use

The purpose of collecting personal information from participants (including health and medical information) during this study is for scientific research and/or as supportive evidence for drug-related submissions to regulatory authorities, in compliance with legal or regulatory requirements.

13.6.1 Access and Disclosure of Personal Information

The knowledge gained through this study is the property of Relmada Therapeutics, Inc. The Sponsor, representatives, and affiliated companies of the Sponsor; the IRB/IEC; the FDA, and other regulatory agencies may inspect participant medical records related to the study to check the validity and accuracy of the data gathered in this study. Medical records (with participant

initials and/or date of birth only) may be copied. The confidentiality of participant records will be maintained except where the release of information is required by law.

With the agreement of the participant, the Investigator may inform the participant's primary care physician about his or her participation in the study and will forward any clinically significant findings from clinical tests such as the ECG and laboratory tests. The primary care physician may contact the Investigator for any further information regarding the individual's participation in the study. The participant has the right to request access to and request corrections of his or her medical record.

The Sponsor ensures that any authorized third-party that performs services on behalf of the Sponsor acts in a manner consistent with the confidentiality information outlined in this Protocol and ICF. While every effort will be made to protect the privacy of participant information, absolute confidentiality cannot be guaranteed. This does not limit the duty of the researchers and others to protect participant privacy.

13.6.2 Release of Participant Information

The results of this study will be reported in such a manner that participants will not be identifiable in any way. Published reports or presentations will refer to grouped data or coded individual data and not to any identifiable individuals. Study reports sent to the Sponsor or drug regulatory agencies (e.g., the FDA) will not include participant names.

13.6.3 Consent for Collection, Access, Use, and Disclosure of Participant Information

By signing the ICF, the participant consents to the collection, access, use, and disclosure of his or her information as described in the ICF document. If a participant withdraws consent, some of the participant's information may still be collected, used, and disclosed by those involved in this study per applicable laws.

13.7 Study Monitoring

13.7.1 Quality Control

Relmada performs quality control at intervals to ensure that the clinical study is conducted, and data are generated, documented (recorded), and reported in compliance with this study protocol, CRO's SOPs, the FDA, the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) GCP E6 consolidated guidelines, and other applicable regulations. The extent, nature, and frequency of quality control reviews will be based on CRO's SOPs and such considerations as the study objectives and/or endpoints, purpose of the study, study design complexity, and enrollment rate.

13.7.2 Study Monitoring by Sponsor and/or Third-Party

If applicable and in accordance with the applicable regulations, GCP, and the Sponsor's procedures, Sponsor and/or third-party monitors will periodically contact or visit the research site, as required. The extent, nature, and frequency of visits will be based on such considerations as the study objectives and/or endpoints, the purpose of the study, study design complexity, and enrollment rate.

During these contacts, the monitor may:

- Check and assess the progress of the study.
- Review study data that have been collected.
- Conduct source document verification (for those measures that have separate source documents and assuming the source documents are compared with the eCRF).
- Identify any issues and address their resolution.
- Prepare a monitoring report.

This will be done to verify that:

- The data are authentic, accurate, and complete;
- The safety and rights of participants are being protected; and,
- The study is conducted in accordance with the currently approved protocol (and any amendments), GCP, and all applicable regulatory requirements.

The Investigator agrees to allow the monitor direct access to all relevant documents and to allocate the Investigator's time and/or the time of research site staff to the monitor to discuss findings and any relevant issues.

At a minimum, source documentation will be available to substantiate the participant identification number, eligibility, and participation; proper informed consent procedures; dates of visits; adherence to protocol procedures; records of safety and efficacy parameters; adequate reporting and follow-up of AEs; administration of concomitant medication; and dates of participant's completion, discontinuation from treatment, or withdrawal from the study, including the reason, if appropriate.

As study drug is blinded when received at the site, no monitor that visits the site to review the pharmacy records will be unblinded to treatment allocations.

In addition to contact during the study, the monitor will also contact the site prior to the start of the study to discuss the protocol and data collection procedures with research site personnel.

At study closure, monitors will also conduct all activities as indicated in Section 13.12.

13.7.3 Principal Investigator's Data Responsibility

The completed eCRFs will be reviewed against source documents by the monitor at each monitoring visit. If any data, signatures, or forms are missing or incorrect, the Investigator or designee will be informed and corrections will be made. Electronic CRFs will be collected from the study site at the termination of the study after all monitoring visits and data queries are completed.

13.8 Data Quality Assurance

Actions to ensure the accuracy and reliability of data include the selection of qualified Investigators and appropriate study centers; the review of protocol procedures with the Investigator and associated study personnel prior to study start; the design of suitable source documents with appropriate instructions for use; the internal audit of source data according to GCP and internal procedures to ensure their accuracy, completeness, and verifiability; as well as the periodic site monitoring by the Sponsor. Written instructions will be provided for the collection, preparation, and shipment of blood, plasma, and urine samples. The Sponsor or designee will review source documents for accuracy and completeness during monitoring visits and after their return to the Sponsor; any discrepancies will be resolved with the Investigator or designee, as appropriate. The data will be entered into the clinical study database and verified for accuracy using the study-specific data verification plan.

13.9 Data Handling

Any data to be recorded directly on the eCRFs/eSource (to be considered as source data) will be identified at the start of the study. Data reported on the eCRF/eSource that are derived from source documents should be consistent with the source documents, or the discrepancies must be explained. See also Section 13.10.

Clinical data will be entered by site personnel on eCRFs/eSource for transmission to the Sponsor. Data on eCRFs/eSource transmitted via the web-based data system must correspond to and be supported by source documentation maintained at the study site, unless the study site makes direct data entry to the databases for which no other original or source documentation is maintained. In such cases, the study site should document which data are subject to direct data entry and should have in place procedures to obtain and retain copies of the information submitted by direct data entry. All study forms and records transmitted to the Sponsor must only include coded identifiers such that directly identifying personal information is not transmitted. The primary method of data transmittal is via the secure, internet-based electronic data capture (EDC)/eSource system. Access to the database is available to only authorized users via the study's internet web site, where a user's unique assigned username and password are required for access.

Any changes made to data after collection will be made through the use of the database and will be captured in the audit trail Electronic CRFs/eSource will be considered complete when all appropriate data has been entered, cleaned, and frozen.

13.10 Source Documents

Source documents are considered to be all information in original records and certified copies of original records of clinical findings, observations, data, or other activities in a clinical study necessary for the reconstruction and evaluation of the study. The Investigator will provide direct access to source documents and/or source data in the facilitation of study-related monitoring, audits, review by IRB/IECs, and regulatory inspections.

The Investigator/institution should maintain adequate and accurate source documents and study records that include all pertinent observations on each of the site's participants. Source data should be attributable, legible, contemporaneous, original, accurate, and complete. Changes to source data should be traceable, not obscure the original entry, and be explained if necessary.

13.11 Study and Site Closure

Upon completion of the study, the following activities, when applicable, will be conducted by the monitor in conjunction with the Investigator or designee, as appropriate:

- Return of all study data to the Sponsor
- Data clarifications or resolutions
- Accounting, reconciliation, and final disposition of used and unused study drugs
- Review of site study records for completeness
- Return of treatment codes to the Sponsor

In addition, the Sponsor reserves the right to suspend or prematurely discontinue this study at any time and for any reason. If such action is taken, the Sponsor will discuss this with the Investigator (including the reasons for taking such action) at that time. The Sponsor will promptly inform any other Investigators and/or institutions conducting the study if the study is suspended or terminated for safety reasons and will inform the regulatory authorities of the suspension or termination of the study and the reason(s) for the action. If required by applicable regulations, the Investigator will inform the IRB/IEC promptly and provide the study participants with the reason for the suspension or termination.

If the study is prematurely discontinued, all study data will be returned to the Sponsor. In addition, the site will conduct final disposition of all unused study drugs in accordance with the Sponsor's procedures for the study.

Financial compensation to the Investigators and/or institutions will be in accordance with the agreement established between the Investigator and the Sponsor.

13.12 Records Retention

Following closure of the study, the Investigator will maintain a copy of all site study records in their original format in a safe and secure location in accordance with the applicable FDA regulatory requirements. Records shall be maintained for a period of 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and the FDA is notified.

Essential documents include:

- Signed informed consent documents for all participants
- Identification number code list, Screening log (if applicable), and enrollment log
- Record of all communications between the Investigator and the IRB/IEC
- Copies of eCRFs and of documentation of corrections for all participants
- Investigational product accountability records
- All other source documents (e.g., medical records, hospital records, laboratory records)
- All other documents as listed in Section 8 of the ICH E6 guideline for GCP (essential documents for the conduct of a clinical study)

Normally, these records will be held in the Investigator's archives. If the Investigator is unable to meet this obligation, he or she will ask the Sponsor for permission to make alternative arrangements. Details of these arrangements will be documented.

13.13 Protocol Amendment and Protocol Deviation

13.13.1 Protocol Amendment

Amendments to the protocol that entail corrections of typographical errors, clarifications of confusing wording, changes in study personnel, and minor modifications that have no effect on the safety of participants or the conduct of the study will be classed as administrative amendments and will be submitted to the IRB/IEC for information only. The Sponsor will ensure that acknowledgement is received and filed. Amendments that are classed as substantial amendments must be submitted to the appropriate regulatory authorities and the IRBs/IECs for approval and will not be implemented at sites until such approvals are received other than in the case of an urgent safety measure.

13.13.2 Protocol Deviations

Should a protocol deviation impacting protocol assessments or procedures, subject safety or dosing occur, the Sponsor must be informed as soon as possible. Protocol deviations and/or violations and the reasons they occurred will be included in the clinical study report. Reporting of protocol deviations to the IRB/IEC will be conducted in accordance with mandates of applicable regulatory authorities.

13.14 Ethical Considerations

The Investigator will conduct the study in accordance with GCP and all applicable regulations, including, where applicable, the principles that have their origin in the Declaration of Helsinki. The study will also be carried out in keeping with applicable national and local laws and regulations. This may include an inspection by the Sponsor's representatives and/or regulatory authority's representatives at any time.

IRBs/IECs will review and approve this protocol and the ICF. All participants are required to give written informed consent before participation in the study.

13.15 Financing and Insurance

Before the study commences, the Sponsor (or its designee) and the Investigator (or the institution, as applicable) will agree on costs necessary to perform the study. This agreement will be documented in a financial agreement that will be signed by the Investigator (or the institution signatory) and the Sponsor (or its designee).

The Investigator is required to have adequate current insurance to cover claims for negligence and/or malpractice. The Sponsor will provide no-exclusion insurance coverage for the clinical study as required by national regulations.

13.16 Publication Policy/Disclosure of Data

Both the use of data and the publication policy are detailed within the clinical study agreement. Intellectual property rights (and related matters) generated by the Investigator and others performing the clinical study will be subject to the terms of a clinical study agreement that will be agreed between the institution and the Sponsor or their designee. With respect to such rights, the Sponsor or its designee will solely own all rights and interests in any materials, data, and intellectual property rights developed by Investigators and others performing the clinical study described in this protocol, subject to the terms of any such agreement. In order to facilitate such ownership, Investigators will be required to assign all such inventions either to their institution or directly to the Sponsor or its designee, as will be set forth in the clinical study agreement.

14 REFERENCES

Bernstein G, Davis K, Mills C, Wang L, McDonnell M, Oldenhof J, et al. Characterization of the Safety and Pharmacokinetic Profile of D-Methadone, a Novel N-Methyl-D-Aspartate Receptor Antagonist in Healthy, Opioid-Naive Subjects: Results of Two Phase 1 Studies. J Clin Psychopharmacol. 2019;39(3):226-37.

Boulton DW, Arnaud P, DeVane CL. Pharmacokinetics and pharmacodynamics of methadone enantiomers after a single oral dose of racemate. Clin Pharmacol Ther. 2001;70(1):48-57.

Bremner JD, Krystal JH, Putnam FW, Southwick SM, Marmar C, Charney DS, et al. Measurement of dissociative states with the Clinician-Administered Dissociative States Scale (CADSS). J Trauma Stress. 1998;11(1):125-36.

British Psychological Society & The Royal College of Psychiatrists. Depression: The Treatment and Management of Depression in Adults (Updated Edition). National Institute for Health and Clinical Excellence: Guidance. Leicester (UK)2010.

Busner J, Targum SD. The clinical global impressions scale: applying a research tool in clinical practice. Psychiatry. 2007;4(7):28-37.

Chandler GM, Iosifescu DV, Pollack MH, Targum SD, Fava M. RESEARCH: Validation of the Massachusetts General Hospital Antidepressant Treatment History Questionnaire (ATRQ). CNS Neurosci Ther. 2010;16(5):322-5.

Cohen EA, Hassman HH, Ereshefsky L, Walling DP, Grindell VM, Keefe RSE, et al. Placebo response mitigation with a participant-focused psychoeducational procedure: a randomized, single-blind, all placebo study in major depressive and psychotic disorders. Neuropsychopharmacology. 2021;46(4):844-50.

Cui L, Hung HM, Wang SJ. Modification of sample size in group sequential clinical trials. Biometrics. 1999;55(3):853-7.

De Martin S VO, Bernstein G, Alimonti A, et al. . The NMDAR Antagonist Dextromethadone Increases Plasma BDNF Levels in Healthy Volunteers Undergoing a 14-Day In-Patient Phase 1 Study. ACNP 57th Annual Meeting: Poster Session II: Neuropsychopharmacol; 2018. p. 228-382.

DEA. Methadone Diversion Control Division. Drug & Chemical Evaluation Section: Drug Enforcement Administration; 2019 [Available from: https://www.deadiversion.usdoj.gov/drug_chem_info/methadone/methadone.pdf.

Fava M, Stahl S, Pani L, et al. Efficacy and Safety of Esmethadone (REL-1017) in Patients With Major Depressive Disorder and Inadequate Response to Standard Antidepressants: A Phase 3 Randomized Placebo-Controlled Trial. American College of Neuropsychopharmacology (ACNP) December 2-6, 2023; Tampa FL2023.

Fehnel SE, Forsyth BH, DiBenedetti DB, Danchenko N, Francois C, Brevig T. Patient-centered assessment of cognitive symptoms of depression. CNS Spectr. 2016;21(1):43-52.

Fogaça MV, Fukumoto K, Franklin T, Liu RJ, Duman CH, Vitolo OV, et al. N-Methyl-D-aspartate receptor antagonist d-methadone produces rapid, mTORC1-dependent antidepressant effects. Neuropsychopharmacology. 2019;44(13):2230-8.

Fraser HF, Isbell H. Human pharmacology and addictiveness of certain dextroisomers of synthetic analgesics: I. d-3-Hydroxy-N-phenethylmorphinan, II. d-3-Methoxy-N-phenethylmorphinan,III. d-Methadone. Bulletin on Narcotics. 1962;14:25-35.

Frodl T, O'Keane V. How does the brain deal with cumulative stress? A review with focus on developmental stress, HPA axis function and hippocampal structure in humans. Neurobiol Dis. 2013;52:24-37.

Gaynes BN, Dusetzina SB, Ellis AR, Hansen RA, Farley JF, Miller WC, et al. Treating depression after initial treatment failure: directly comparing switch and augmenting strategies in STAR*D. J Clin Psychopharmacol. 2012;32(1):114-9.

Gaynes BN, Warden D, Trivedi MH, Wisniewski SR, Fava M, Rush AJ. What did STAR*D teach us? Results from a large-scale, practical, clinical trial for patients with depression. Psychiatr Serv. 2009;60(11):1439-45.

Hanania T, Manfredi P, Inturrisi C, Vitolo OV. The N-methyl-D-aspartate receptor antagonist d-methadone acutely improves depressive-like behavior in the forced swim test performance of rats. Exp Clin Psychopharmacol. 2020;28(2):196-201.

Hardeveld F, Spijker J, De Graaf R, Nolen WA, Beekman AT. Prevalence and predictors of recurrence of major depressive disorder in the adult population. Acta Psychiatr Scand. 2010;122(3):184-91.

Holmes SE, Finnema SJ, Naganawa M, DellaGioia N, Holden D, Fowles K, et al. Imaging the effect of ketamine on synaptic density (SV2A) in the living brain. Mol Psychiatry. 2022;27(4):2273-81.

Holmes SE, Scheinost D, Finnema SJ, Naganawa M, Davis MT, DellaGioia N, et al. Lower synaptic density is associated with depression severity and network alterations. Nat Commun. 2019;10(1):1529.

Isbell H, Eisenman AJ. The addiction liability of some drugs of the methadon series. J Pharmacol Exp Ther. 1948;93(3):305-13.

Isometsa E. Suicidal behaviour in mood disorders--who, when, and why? Can J Psychiatry. 2014;59(3):120-30.
Jaeger J. Digit Symbol Substitution Test: The Case for Sensitivity Over Specificity in Neuropsychological Testing. J Clin Psychopharmacol. 2018;38(5):513-9.

Javaheripour N, Li M, Chand T, Krug A, Kircher T, Dannlowski U, et al. Altered resting-state functional connectome in major depressive disorder: a mega-analysis from the PsyMRI consortium. Transl Psychiatry. 2021;11(1):511.

Kessler RC, Akiskal HS, Ames M, Birnbaum H, Greenberg P, Hirschfeld RM, et al. Prevalence and effects of mood disorders on work performance in a nationally representative sample of U.S. workers. Am J Psychiatry. 2006;163(9):1561-8.

Kirsch I, Deacon BJ, Huedo-Medina TB, Scoboria A, Moore TJ, Johnson BT. Initial severity and antidepressant benefits: a meta-analysis of data submitted to the Food and Drug Administration. PLoS Med. 2008;5(2):e45.

Lawrence JaH, HJ. Estimation and Confidence Intervals after Adjusting the Maximum Information. 2003:143-52.

McGahuey CA, Gelenberg AJ, Laukes CA, Moreno FA, Delgado PL, McKnight KM, et al. The Arizona Sexual Experience Scale (ASEX): reliability and validity. J Sex Marital Ther. 2000;26(1):25-40.

Mehta CR, Pocock SJ. Adaptive increase in sample size when interim results are promising: a practical guide with examples. Stat Med. 2011;30(28):3267-84.

Monroe SM, Harkness KL. Recurrence in major depression: a conceptual analysis. Psychol Rev. 2011;118(4):655-74.

Montgomery SA, Asberg M. A new depression scale designed to be sensitive to change. Br J Psychiatry. 1979;134:382-9.
Moryl N, Tamasdan C, Tarcatu D, Thaler HT, Correa D, Steingart R, et al. A phase I study of D-methadone in patients with chronic pain. J Opioid Manag. 2016;12(1):47-55.

National Institute of Mental Health. Data based on the National Survey on Drug Use and Health (NSDUH) 2017 [Available from: https://www.nimh.nih.gov/health/statistics/major-depression.

Olsen GD, Wendel HA, Livermore JD, Leger RM, Lynn RK, Gerber N. Clinical effects and pharmacokinetics of racemic methadone and its optical isomers. Clin Pharmacol Ther. 1977;21(2):147-57.

Paykel ES. Life events and affective disorders. Acta Psychiatr Scand Suppl. 2003(418):61-6.

Pedrelli P, Blais MA, Alpert JE, Shelton RC, Walker RS, Fava M. Reliability and validity of the Symptoms of Depression Questionnaire (SDQ). CNS Spectr. 2014;19(6):535-46.

Posner K, Brown GK, Stanley B, Brent DA, Yershova KV, Oquendo MA, et al. The Columbia-Suicide Severity Rating Scale: initial validity and internal consistency findings from three multisite studies with adolescents and adults. Am J Psychiatry. 2011;168(12):1266-77.

Rush AJ, Trivedi MH, Wisniewski SR, Nierenberg AA, Stewart JW, Warden D, et al. Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. Am J Psychiatry. 2006;163(11):1905-17.

Rush AJ, Wisniewski SR, Zisook S, Fava M, Sung SC, Haley CL, et al. Is prior course of illness relevant to acute or longer-term outcomes in depressed out-patients? A STAR*D report. Psychol Med. 2012;42(6):1131-49.

Sheehan DV. The Sheehan Disability Scales. In The Anxiety Disease and How to Overcome It. Charles Scribner and Sons1983. p. 151.

Sinyor M, Schaffer A, Levitt A. The sequenced treatment alternatives to relieve depression (STAR*D) trial: a review. Can J Psychiatry. 2010;55(3):126-35.

Stahl SM, De Martin S, Mattarei A, Bettini E, Pani L, Guidetti C, et al. Esmethadone (REL-1017) and Other Uncompetitive NMDAR Channel Blockers May Improve Mood Disorders via Modulation of Synaptic Kinase-Mediated Signaling. Int J Mol Sci. 2022;23(20).

Su TP, Chen MH, Li CT, Lin WC, Hong CJ, Gueorguieva R, et al. Dose-Related Effects of Adjunctive Ketamine in Taiwanese Patients with Treatment-Resistant Depression. Neuropsychopharmacology. 2017;42(13):2482-92.

Trivedi MH, Fava M, Wisniewski SR, Thase ME, Quitkin F, Warden D, et al. Medication augmentation after the failure of SSRIs for depression. N Engl J Med. 2006;354(12):1243-52.

Williams JB. A structured interview guide for the Hamilton Depression Rating Scale. Arch Gen Psychiatry. 1988;45(8):742-7.

Williams JB, Kobak KA. Development and reliability of a structured interview guide for the Montgomery Asberg Depression Rating Scale (SIGMA). Br J Psychiatry. 2008;192(1):52-8.

15 COMEDICATIONS TO BE USED WITH CAUTION

15.1 QT

Based on clinical data from Phase 1 and Phase 2 studies, multiple doses up to 10 days of REL-1017 appeared to be safe. These same studies have shown dose-dependent but not clinically meaningful QTcF prolongation. Concomitant use of drugs that cause QTcF prolongation but are not known to cause torsade de pointes (TdP) is permitted with caution at the discretion of the Investigator and with approval of the Medical Monitor if participants are not excluded based on QTcF at Screening. Concomitant medications known to cause TdP are excluded. In REL-1017-302, frequent ECG safety monitoring will be conducted as outlined in the Schedule of Assessments (Table 2).

Please refer to the following comprehensive online database for comedications' QT/TdP risk assessment.

QTcF and TdP risk assessment database: https://www.crediblemeds.org/

A QT prolongation and TdP risk assessment is provided interactively in the medication field on the website.

Citation: Woosley RL, Heise CW, Gallo T, Tate J, Woosley D, Romero KA. Website: www.CredibleMeds.org, QTdrugs List, Accessed 21 Apr 2020. AZCERT, Inc. 1822 Innovation Park Dr., Oro Valley, AZ 85755

15.2 Drug-Drug Interactions

Clinically significant CYP 450 drug-drug interaction data.

In vitro studies using recombinant CYP enzyme testing indicate that REL-1017 is metabolized by multiple enzymes. However, the only clinically relevant inhibition was observed with CYP2D6. Additionally, REL-1017 was found to be a pan-cytochrome inducer in human hepatocytes (Relmada data on file).

Concomitant use of CYP2D6 substrates with a narrow therapeutic window is permitted with caution. Participants who are using CYP2D6 substrates that may have narrow therapeutic window should be monitored closely as some participants may experience a greater exposure in the presence of REL-1017 (perpetrator effect).

Concomitant use of strong clinical inducers of P450-mediated metabolism is permitted with caution. Participants who are using substrates of for P450-mediated metabolism that may have a narrow therapeutic window should be monitored closely as some participants may experience a lower exposure of the concomitant medication(s) medications in the presence of REL-1017

(perpetrator effect). REL-1017 is metabolized via multiple metabolic pathways, so concomitant use of REL-1017 with other medications is less likely to cause a clinically relevant effect on REL-1017 concentration (victim effect).

Decisions regarding comedication must be made by the Investigator or prescribing physician in accordance with the guidance provided in comedication management in this protocol, and to the best clinical judgment of the Investigator and Study Medical Monitor.

Websites with comprehensive and up-to-date information include:

FDA: https://www.fda.gov/drugs/drug-interactions-labeling/drug-development-and-drug-interactions-table-substrates-inhibitors-and-inducers

Indiana University: https://drug-interactions.medicine.iu.edu/MainTable.aspx
All entries in the on-line table are color-coded for the degree of sensitivity to the specific CYPs.

Citation: Flockhart DA. Drug Interactions: Cytochrome P450 Drug Interaction Table. Indiana University School of Medicine (2007).

16 ALLOWED BACKGROUND ANTIDEPRESSANT THERAPY

SSRI

SSRI	Minimally adequate dose
paroxetine	20 mg QD
fluoxetine	20 mg QD
sertraline	50 mg QD
citalopram	20 mg QD (Maximum allowed dose 40 mg QD)
escitalopram	10 mg QD (Maximum allowed dose 20 mg QD)
vilazodone	20 mg QD
vortioxetine	10 mg QD

SNRI

SNRI	Minimally adequate dose
venlafaxine/venlafaxine XR	75 mg QD
desvenlafaxine	50 mg QD
duloxetine	60 mg QD or 30 mg BID
levomilnacipran	40 mg QD

Tricyclics and Tetracyclics

Tricyclics and Tetracyclics	Minimally adequate dose
amitriptyline	150 mg QD
amoxapine	150 mg QD
clomipramine	150 mg QD
doxepin	150 mg QD
desipramine	150 mg QD
imipramine	150 mg QD

Tricyclics and Tetracyclics	**Minimally adequate dose**
maprotiline	150 mg QD
nortriptyline	75 mg QD
protriptyline	30 mg QD

Other Antidepressant Therapy

Other Antidepressant Therapy	**Minimally adequate dose**
bupropion	300 mg QD
mirtazapine	15 mg QD
trazodone	300 mg QD