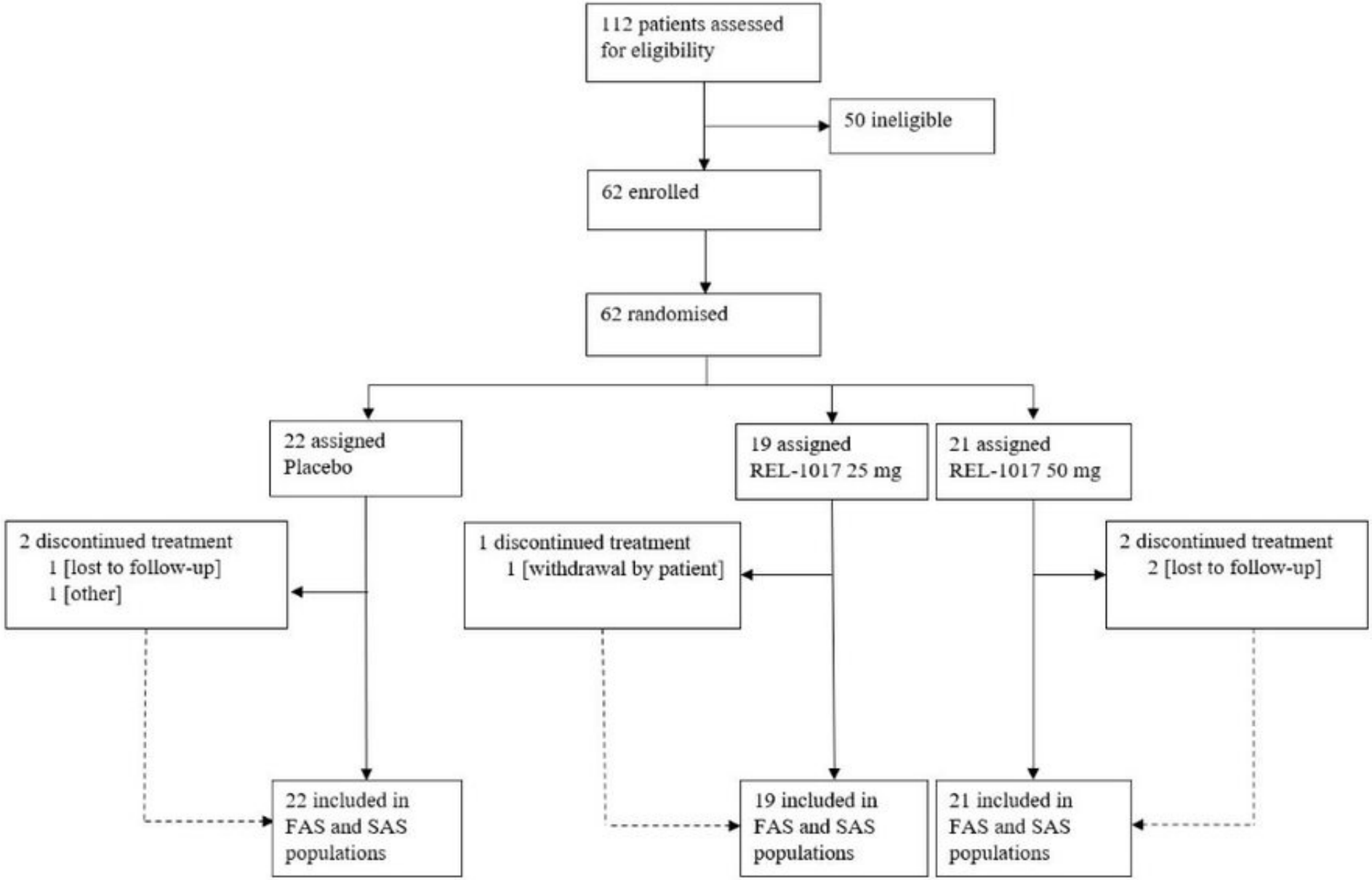
Data supplement for Fava et al., "REL-1017 (Esmethadone) as Adjunctive Treatment in Patients With Major Depressive Disorder: A Phase 2a Randomized Double-Blind Trial." Am J Psychiatry (doi: 10.1176/appi.ajp.2021.21020197)



Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Fava M, Stahl S, et al. REL-1017 (Esmethadone) as Adjunctive Treatment in Patients with Major Depressive Disorder: a Phase 2a Double-Blind Randomized Trial.

SUPPLEMENTARY FILES FOR:

REL-1017 (Esmethadone) as Adjunctive Treatment in Patients with Major Depressive Disorder: a Phase 2a Double-Blind Randomized Trial

Table of Contents:

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Protocol and Protocol Amendment History

Protocol Date: 13-Dec-2016 (Version 1.0)

- Original Protocol

Amendment 1: 19-Jan-2017 (Version 2.1)

- Misc. updates

Amendment 2: 29-Jan-2018 (Version 3.0)

- Sponsor contact updated
- CRO name updated
- QIDS-SR was replaced with SDQ
- Added data to support dosing justification
- Sample size increased to 120 patients screened to randomize 20 subjects per arm
- MGH CTNI added, entry review process updated
- Clarifying definition of current episode
- Loading dose of 75 mg REL-1017 added for initial dosing of Arm 1 REL-1017 25 mg. A dose of 100 mg REL-1017 added for initial dose of Arm 2, 50 mg REL-1017
- Inclusion Criterion #1: modification to allow enrollment of females >1 year postmenopausal
- Inclusion Criterion #3: duration of diagnosis of MDE requirements at study entry changed from 12 months to 36 months
- Inclusion Criterion #4: updated duration of current MDD drug treatment from 6 weeks to 8 weeks
- Inclusion Criterion #5: adjustment of inclusion criteria with the addition of SAFER
- Inclusion Criterion #6: adjustment of inclusion criteria for HAM-D-17
- Inclusion Criterion #10: modification to require ability to read and understand English

- Exclusion Criteria NEW: ECG exclusion added
- Exclusion Criterion #13: clarified depressive disorder
- Added measurements for evaluation of Safety Objectives
- Objectives: added measurements for evaluation of PK Objectives
- Objectives: added measurements for evaluation of Efficacy Objectives
- Prohibited Medications: allowance of chronic and use of hypnotics during the study
- Rater Qualifications: Section 4.6 added
- Section 6: study procedures and assessments, time 0
- Follow-up Assessments: added C-SSRS
- Central ECGs: added central ECG readings to the protocol
- ECG Clarification: clarification of ECG capture and reading
- Table 1 Section 6.2.5.8: pregnancy testing added
- Section 7.1.4: other adverse events of interest (additional text added)
- Appendices: rating scales added

Amendment 3: 05-Apr-2018 (Version 4.0)

- Exclusion Criterion #1: addition of safety criteria
- Exclusion Criterion #4: made synopsis and Sect. 4.2 consistent
- Inclusion Criterion #9: synopsis - removed reference to protocol section (synopsis is a standalone document)
- Exclusion Criteria #15 Section 4.3: made consistent with synopsis Exclusion Criteria #15
- Exclusion Criteria #19: revision of "washout period"
- Exclusion Criterion #23 and 24: Section 4.3: reversed order to be consistent with synopsis
- Section 4.4.1 Prohibited Medications: revision of benzodiazepine guidance

- Section 4.4.1 Prohibited Medications: addition of safety guidance to investigator
- Section 4.4.1: Table 3
- Section 4.5: patient discontinuation/withdrawal criteria
- Section 4.6: rater qualifications (language added)
- Section 6.2.5.1 Clinical Chemistry: addition of magnesium
- Section 6.6.3.2: CADSS correction
- Change of Scale: Positive Symptoms Subscale (BPRS) was replaced with the 4-Item PSRS in all sections referencing scales
- Appendix 11.7 Added scale: Antidepressant Treatment Response Questionnaire (ATRQ)
- Appendix 11.8: Symptoms of Depression Questionnaire (SDQ): all questions (1-44): scale timeframe changed
- SAFER interview administration: schedule modified
- Section 10 References: SIGMA (2011) scale added

Amendment 4: 19-Nov-2018 (Version 5.0)

- Inclusion Criterion #1 (Synopsis and Section 4.2): addition of women of childbearing potential
- Inclusion Criterion #9 (Synopsis and Section 4.2): addition of women of child-bearing potential
- Exclusion Criterion #5 (Synopsis and Section 4.2): revision of ECG criterion: QTc changed to QTcF
- Synopsis Table 1: time and events schedule (added urine pregnancy test at EOP and FU visits)
- Section 1.1.2 In Vivo: addition of pre-clinical toxicology data

- Section 4.4.2 Contraceptive Precautions: revision to precautions for male patients, and addition of contraceptive precautions for female patients
- Section 6.1.7 Outpatient Observation Period (Day 10 to Day 14): pregnancy test added
- 6.1.8 Follow-Up Period (Day 15 to Day 21): pregnancy test added
- Section 6.2.5.8 Pregnancy Testing: pregnancy tests added
- Section 7.3.3: pregnancy revised due to the inclusion of women of childbearing potential
- Section 10 References: addition of FDA guidance reference

Amendment 5: 07-Mar-2019 (Version 6.0)

- Page 2, Sponsor address: updated sponsor address
- Synopsis and Section 4.2: Inclusion Criteria #9 (revised "breastfeeding" to "lactating" for clarity)
- Contraceptive Precautions: two methods of contraception added, and abstinence removed from 1st
- Section 7.1.8 Serious Adverse Events and Serious Unexpected Adverse Events: safety reporting email address updated

Supplemental Trial Design and Oversight:

This was a Phase 2a, multicenter, randomized, double-blind, placebo-controlled, 3-arm study to assess the safety and tolerability of once daily oral doses of 25 mg and 50 mg REL-1017 compared with placebo and administered for 7 consecutive days as adjunctive treatment for patients diagnosed with MDD. The Screening Visit assessed patient eligibility to participate in the trial and occurred between Day -30 and Day -2. Patients were admitted to the CRU the day prior to receiving placebo or REL-1017 (Day -1) and remained in the CRU under clinical supervision for at least 48 hours after the last dose was received on Day 7. Patients were discharged on Day 9, but returned to the CRU 7 days after their last dose of REL-1017 or placebo for follow-up evaluations. The personnel telephoned each patient 14 days after the last dose of REL-1017 or placebo and conducted a follow-up interview to assess the patient's condition. An unblinded pharmacist assigned study treatment to patients according to randomized treatment codes provided by a statistician that did not participate in the study in any additional capacity. The study drug solution was prepared within 3 hours of administration in blinded containers, then was dispensed to blinded study staff for patient administration.

After providing written informed consent, patients were randomized 1:1:1 to receive either:

1. 75 mg REL-1017 (Day 1), 25 mg REL-1017 (Days 2-7)
2. 100 mg REL-1017 (Day 1), 50 mg REL-1017 (Days 2-7); or
3. placebo (Ocean Spray® Diet Cranberry Juice alone).

Trial Participants:

The study population included adult male and female patients, ages 18-65 years, with a diagnosis of MDD, and a Hamilton Rating Scale for Depression (HAM-D-17) total score of ≥19. Written informed consent was provided at screening and was required for enrollment. All patients were required to discontinue all prohibited medications prior to screening at the clinical research unit (CRU) to meet the protocol requirements. Benzodiazepines and nonbenzodiazepine sleep aids

taken as scheduled medications and at stable doses not higher than that in the FDA-approved label for at least 30 days prior to the screening visit were allowed in the study. Initiation of these medications during the study was not allowed. Any medication taken consistently by the patient for 30 days prior to Screening and that was not a prohibited medication may have been continued during the study if appropriately documented.

Inclusion Criteria:

To participate in the study, patients must have met the following criteria:

1. Males and females between 18 and 65 years of age, inclusive.
2. Diagnosed with recurrent MDD as defined by the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), and confirmed by the Mini International Neuropsychiatric Interview (MINI), Version 7.0.2.
3. Diagnosed with a current MDE lasting 8 weeks to 36 months as defined by the DSM-5 and confirmed by the MINI.
4. Treated with an adequate dosage of an SSRI, SNRI, or bupropion during the current MDE for at least 8 weeks prior to Screening with the same, adequate dosage for the last 4 weeks. Minimum adequate doses were defined in the ATRQ. The maximum dose allowed for paroxetine is 40 mg QD, for fluoxetine is 60 mg QD, and for sertraline is 200 mg QD.
5. Have experienced an inadequate response to 1 to 3 courses of treatment with an antidepressant medication in the current episode, as defined as <50% improvement with an antidepressant medication at doses listed on the SAFER Interview.
6. Hamilton Depression Rating Scale-17 (HAM-D-17) ≥19 at Screening and Check-in (Day -1).
7. Body mass index (BMI) between 18.0 and 35.0 kg/m^2, inclusive, and a minimum weight of 50.0 kg.
8. Per the Investigator's judgment, able to meet all study requirements, including the

confined/inpatient portion of the study, adherence with both approved ADT and study drug regimen, and completion of all assessments and all scheduled visits.

9. Male and female patients of reproductive potential must be using and willing to continue using medically acceptable contraception, from Screening and for at least 2 months after the last study drug administration. Female patients must have a negative pregnancy test and must not be lactating.
10. Must be able to read, speak, and understand English and must provide written informed consent prior to the initiation of any protocol-specific procedures.

Exclusion Criteria:

To participate in the study, patients must not have met any of the following 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 patient or the validity of the study results, including torsades de pointes, any bradyarrhythmias, or uncompensated heart failure.
2. Chronic use of prescribed opioids (i.e., >120 days in a 6-month period) up to 6 months prior to Screening or any recreational use of opioids.
3. Evidence of clinically significant hepatic or renal impairment, including alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >1.5 × upper limit of normal (ULN), bilirubin >1 × ULN, or endocrine laboratory values (including clinically significant thyroid parameters, i.e., thyroid-stimulating hormone [TSH], triiodothyronine [T3], and thyroxine [T4]).
4. History or family history of sudden unexplained death or long QT syndrome (measure of the time between the start of the Q wave and the end of the T wave in the heart's electrical cycle).
5. An average QTcF ≥450 msec or an average QRS interval ≥120 msec from the 12-lead ECGs

performed at Screening.

6. History of clinically diagnosed hypotension requiring treatment.
7. History or presence of any condition in which an opioid is contraindicated (e.g., significant respiratory depression, acute or severe bronchial asthma or hypercarbia, bronchitis, or has/is suspected of having paralytic ileus).
8. No more than 3 prescribed doses of an opioid within the 6 months prior to Screening and no use at all within the last month.
9. Use of an antipsychotic, anticonvulsant, or mood stabilizer, regardless of indication, within the 3 months prior to Screening.
10. History of allergy or hypersensitivity to methadone or related drugs (e.g., opioids).
11. Positive test for hepatitis B or human immunodeficiency virus (HIV). Patients with a positive hepatitis C test may be considered for inclusion with approval from the Medical Monitor.
12. Any current and primary psychiatric disorder, as defined as a condition that is the primary focus of distress and/or treatment, other than MDD.
13. Any lifetime history of bipolar I or II disorder, any psychotic disorder, posttraumatic stress disorder, borderline personality disorder, antisocial personality disorder, obsessive compulsive disorder, eating disorder, intellectual disability, or pervasive developmental disorder.
14. History in the past 12 months of a primary diagnosis of anxiety disorder or panic disorder not related to the current MDE.
15. Current diagnosis of alcohol or substance use disorder, as defined by the DSM-5, at Screening or within the 12 months prior to Screening. Patients with the following diagnoses within the past 12 months, however, may be included at the Investigator's discretion: mild alcohol use disorder, mild cannabis use disorder, and any severity tobacco use disorder.
16. A confirmed positive result on the urine drug/alcohol screen at Screening or Check-in. If the

urine drug/alcohol screen is positive at Screening, retesting or rescreening may be scheduled with prior approval from the Medical Monitor.

17. Patients who, in the Investigator's judgment, were at significant risk for suicide. A patient with a C-SSRS ideation score of 4 or 5 within the last 6 months or any suicide attempt within the past year must be excluded, as should a patient with an ideation score of 4 or 5 or any suicide attempt at the Check-in or Baseline Visit.
18. Patients with a 20% improvement between Screening and Check-in (Day -1) on the HAM-D-17.
19. Patients who did not safely discontinue medications prohibited in Section 9.4.7.
20. Patients receiving new onset psychotherapy (individual, group, marriage, or family therapy) within 2 months prior to Screening or plans to start at any time during participation in the study.
21. Patients who have received electroconvulsive therapy, repetitive transcranial magnetic stimulation, or vagus nerve stimulation or who have participated in a ketamine study within the last 6 months.
22. Patients with any clinically significant neurological, hepatic, renal, metabolic, hematological, immunological, cardiovascular, pulmonary, chronic pain, or gastrointestinal disorder. Medical conditions that are minor or well-controlled may be permitted if they will not increase the safety risk to the patient or compromise interpretation of the safety or efficacy endpoints.
23. Patients taking fluvoxamine or St. John's Wort.
24. Patients who have participated in a clinical study with an investigational medication in the past 6 months, or who have participated in more than 4 clinical studies with investigational medications in the past 2 years.

Other Criteria:

Aside from the above inclusion and exclusion criteria the patient must have agreed to abide by each of

the following restrictions for the specified time:

1. Patients were asked to abstain from alcohol for 24 hours prior to Screening, 24 hours prior to Check-in to the CRU, while in the CRU, and 24 hours prior to the Day 14 visit.
2. Patients were required to abstain from recreational drug use throughout the study, from Screening until after the Day 21 Follow-up telephone call. Abstinence from drug use was confirmed with urine drug screens at Screening and Check-in, and patients were reminded to adhere to restrictions throughout the study.
3. Dosing occurred in the morning after an overnight fast of at least 8 hours.
4. Patients were required to abstain from blood donation during the study and for 30 days after the last study visit. A copy of the ICF with the study restrictions were provided to each patient. Patients were reminded by phone calls prior to study visits to adhere to study restrictions as described in the ICF.
5. Patients were required to follow the ICF and the clinic code of conduct.

Patient Trial Discontinuation:

Any patient who voluntarily withdrew consent or was discontinued (e.g., because of an AE) from the trial prior to completion was considered as withdrawn from the trial. Patients may have discontinued from the trial for any reason including any of the following circumstances:

1. Occurrence of intolerable AE, as assessed by the Investigator or designee.
2. Clinically significant abnormality on vital signs, ECG, clinical laboratory, or physical examination evaluations, as assessed by the Investigator or designee.
3. Subjects with a QTcF ≥500 msec or ≥60 msec above the patient's baseline value were to be discontinued from study medication and re-assessed. The ECG readings were to be repeated until values returned below 500 msec, or ≤60 msec above baseline. These findings were to be reviewed by a cardiologist. Any additional intervention for the safety of the subject were to be

discussed with the Study Medical Monitor and Sponsor.

4. ALT or AST ≥2 x ULN.
5. Withdrawal of consent.
6. Lost to follow-up.
7. Administrative reasons (e.g., Sponsor decision).
8. Major violation of the protocol.
9. If in the opinion of the qualified Investigator, it is in the best interest of the patient.
10. Noncompliance with study requirements and restrictions.
11. Use of a concomitant medication that, in the opinion of the Investigator or designee, could interfere with the study procedures or data integrity or compromise the safety of the patient.
12. Positive breath alcohol test at Check-in to the CRU.
13. Termination of the study.

Trial Procedures:

The overall duration of the study will be up to 51 days per patient. All patients will be admitted to the clinical research unit (CRU) for a period of approximately 10 days during which they will receive daily doses of REL-1017 or Placebo (in the morning after an overnight fast) for 7 consecutive days. At the Screening Visit (between Day -30 and Day -2), an assessment was performed of patient eligibility to participate in the study. To promote appropriate patient enrollment and data quality, an external medical team reviewed the patient's eligibility based on key protocol inclusion and exclusion criteria, sites submitted specific screening information detailed in the Massachusetts General Hospital (MGH) Clinical Trials Network and Institute (CTNI) manual within 72 hours after the Screening Visit for review by the external medical team before proceeding to perform an independent review of eligibility. To ensure that appropriate patients were entered into the study, MGH CTNI raters conducted a remote interview. The assessments administered were the SAFER Interview, which included the HAM-D-17,

and the MGH ATRQ.[1] The interview was performed remotely by the MGH CTNI rater, and the patient was contacted at his or her home or other offsite location after the Screening visit, during which call the assessments were performed. Patients deemed eligible by the principal investigator and confirmed by the medical monitor underwent the SAFER (State versus trait; Accessibility; Face validity; Ecological validity; and Rule of 3 Ps [pervasive, persistent, and pathological]) interview by clinicians at the MGH CTNI. Sites were notified of the results within 24 hours of the interview. Only patients whose eligibility was confirmed by the SAFER Interview were allowed to enroll in the study.

During the inpatient period for the study, patients will receive a standard diet. No additional food or beverages were to be consumed while patients stayed in the CRU. Meals were served at specific times after the completion of any scheduled assessments. REL-1017 was provided as a powder and prepared as a solution with Ocean Spray® Diet Cranberry Juice (to mask the taste of dextromethadone) on site to obtain a final volume of 100 mL.

The study drugs consisted of the following items:

- Arm 1: REL-1017 (d-methadone) 75 mg of powder in 100 mL of Ocean Spray® Diet Cranberry Juice QD on Day 1, 25 mg of powder in 100 mL Ocean Spray® Diet Cranberry Juice QD on Day 2-7.
- Arm 2: REL-1017 (d-methadone) 100 mg of powder in 100 mL of Ocean Spray® Diet Cranberry Juice QD on Day 1, 50 mg of powder in 100 mL Ocean Spray® Diet Cranberry Juice QD on Day 2-7.
- Arm 3: Placebo = 100 mL Ocean Spray® Diet Cranberry Juice.

An unblinded pharmacist assigned study drug to patients according to randomized treatment codes provided by the Interactive Web Response System (IWRS). The randomization code used in the IWRS was prepared by a statistician who was not working as a statistician on the study in any other capacity. The study drug solution was prepared within 3 hours of administration in blinded containers, and then

was dispensed to blinded study staff for administration to the patients.

The administration of study drug was observed by a staff member. After drinking the 100 mL of study drug, 25 mL of noncarbonated water was added to the study drug container and gently swirled. The patient drank this rinse volume followed by 125 mL of noncarbonated water immediately thereafter. All dosing must have been completed within 5 minutes. Dosing took place QD in the morning after an overnight fast (at least 8 hours).

Patients were placed in a quiet, isolated room and under continuous monitoring to address potential adverse reactions and other effects that could require immediate medical attention. Patients remained in the CRU under clinical supervision for at least 48 hours after the last treatment dose was received on Day 7. Patients were discharged from the CRU on Day 9, but returned 7 days after their last dose of study drug for Follow-up evaluations. The study personnel telephoned each patient 14 days after the last dose of study drug and conducted a Follow-Up interview to assess the patient's condition.

Supplemental Endpoints:

The primary objective of this study was safety and tolerability: The following assessments were conducted to measure safety and tolerability of 7 days of daily dosing with REL-1017 25 mg or 50 mg through-out the study: frequency and severity of adverse events (AEs), treatment-emergent adverse events (TEAEs) serious adverse events (SAEs), serious unexpected adverse events (SUSARs), and adverse events of special interest (AESI). All AEs were recorded from Screening visit (Day -2) until the Follow-Up visit (Day 21). AEs that occur after the Screening visit (Day -2) and prior to the Baseline visit (Day 1) initial dosing of study drug were recorded as Baseline signs and symptoms. Other safety assessments included vital signs measurements; weight; physical examination; clinical laboratory parameters (chemistry, hematology, and urinalysis); electrocardiogram (ECG) parameters; Columbia-Suicide Severity Rating Scale (C-SSRS);

Clinician Administered Dissociative States Scale (CADSS); Clinical Opiate Withdrawal Scale (COWS); and 4-Item Positive Symptom Rating Scale (PSRS).

The C-SSRS contains 6 questions addressing 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. The CADSS is a 23-item scale measurement of present-state dissociative symptoms with good interrater reliability and construct validity. A higher score indicates a higher likelihood of the presence of a dissociative state.[2] The COWS is a clinician-administered instrument that rates 11 common opiate withdrawal signs or symptoms. The summed score of the 11 items can be used to assess a patient's level of opiate withdrawal and to make inferences about their level of physical dependence on opioids.[3] The 4-Item PSRS was adapted from the Brief Psychiatric Rating Scale developed by Ventura and colleagues. The scale assesses paranoia and hallucinations as well as some specific issues related to difficulty thinking and having unusual thoughts. Scores range from 1 (not present) to 7 (extremely severe). Total score on the 4-Item PSRS can range from 4 to 28.[4,5] The primary efficacy endpoint was the change from Baseline (Day 1) to EDP (Day 7) on the Montgomery-Asberg Depression Rating Scale (MADRS) with measurements made pre dose on Day 1 and Day 2; post-dose on Day 4 and Day 7 within 3 hours of dosing; and on Day 14. The MADRS 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. A higher MADRS score indicates more severe depression, and each item yields a score of 0 to 6. The overall score ranges from 0 to 60 with scores above 34 indicating severe depression. MADRS was chosen as the

primary efficacy tool over the HAM-D-17 tool in assessing depressive symptoms as the MADRS provides higher reliability statistics for detecting initial symptoms of unipolar depression.[6]

The responses to each question on the MADRS were summarized using frequencies (numbers and percentages) by treatment and visits. The total score was summarized using descriptive statistics (number of patients, mean, SD, median, minimum, and maximum) as well as using frequencies (numbers and percentages) for the following categories: (a) <34; and ≥34 by treatment and visits; (b) ≤10; and >10 by treatment and visits (remitters). The change from baseline and percentage change from baseline of total score in MADRS were summarized using descriptive statistics (number of patients, mean, SD, median, minimum, and maximum).

A reduction of at least 50% from the baseline MADRS total score at EDP (Day 7) (responders) was summarized using frequencies (numbers and percentages). The change from baseline of total score in MADRS was analyzed and summarized. A line plot of mean change from Baseline of total MADRS score versus visit by treatment group was to be generated for the FAS and PPP populations.

The secondary efficacy endpoints were 1) change from Baseline (Day 1) to EDP (Day 7) on the Symptoms of Depression Questionnaire (SDQ) with measurements made pre-dose on Day 1 and Day 2; post-dose on Day 4 and Day 7 within 3 hours of dosing; and on Day 14; 2) change from Baseline (Day 1) to EDP (Day 7) on the Clinical Global Impressions of Severity Scale (CGI-S) with measurements made pre-dose on Day 1 and Day 2; post-dose on Day 4 and Day 7 within 3 hours of dosing; and on Day 14; 3) evaluation of Clinical Global Impressions Improvement Scale (CGI-I) total score on Day 7 (post-dose), as well as CGI-I scores on Days 2 (pre-dose), 4 (post-dose), and 14. The SDQ is a 44-item, self-report scale designed to measure the severity of symptoms across several subtypes of depression. The total SDQ consisted of 44 items subdivided

into 5 subscales of SDQ-1 (lassitude, mood, cognitive/social function) with 18 items, SDQ-2 (anxiety, agitation, anger, and irritability) with 13 items, SDQ-3 (desire to be dead) with 6 items, SDQ-4 (disruptions in sleep quality) with 3 items, and SDQ-5 (changes in appetite and weight) with 4 items. The SDQ was developed to more fully capture the heterogeneity of symptom presentations of depressive disorders than current, widely used scales for MDD. The CGI-I is a standard method used in clinical studies to quantify and track patient change over time; scores range from 1 to 7, and a lower CGI-I score indicates greater improvement in symptoms.

Supplemental Statistical Analysis:

The study analysis populations consisted of the following: 1) randomized population - all patients who were randomized; 2) the Per-Protocol (PP) population – those patients who had been randomized and had received and completed all treatment doses as expected by the 3 treatments randomization assignment (in addition, the patient had at least one efficacy measurement [MADRS, SDQ, CGI-I, or CGI-S] completed at the Baseline visit and at Day 7; 3) safety population (SAS) - all randomized patients who received any study treatment (REL 1017 or Placebo) (summaries or analysis on the SAF population were based on the treatment actually received, if different than the assigned treatment); pharmacokinetic population - all patients who received REL-1017, with at least 1 post-dose blood draw (to be reported where available) to determine plasma concentration of dextromethadone; and 4) FAS population - all randomized patients who received any study treatment (REL-1017 or Placebo) and had a Baseline and at least 1 post-Baseline efficacy measurement (MADRS, SDQ, CGI-S, or CGI-I). All patients randomized in the study were included in the SAS and FAS populations. The PK population consisted of 60 patients (96.8%) and the per protocol (PP) population consisted of 61 patients (98.4%). REL-1017 doses were selected based on the safety and tolerability profiles emerged from the Phase 1 studies and on PK/PD modeling to define AUCtau of dextromethadone in preclinical and clinical studies and on the AUCtau of ketamine at doses that provide antidepressant efficacy, which supported

the conclusion that REL-1017 25 mg and 50 mg QD once a day would both be efficacious and safe while providing a different exposure. Pharmacokinetic parameters were reported for this population where available.

The sample size of 20 patients in each of the 2 active treatment groups (REL-1017 25 mg and 50 mg) and 20 patients in the placebo group was chosen to obtain reasonable evidence of safety and efficacy without exposing undue numbers of patients to the investigative product at this phase of clinical development.

Demographics and Baseline characteristics (age, sex, race, ethnicity, body weight, height, oral body temperature, and BMI) were summarized using descriptive statistics (number of patients, mean, SD, median, minimum, and maximum for continuous variables and the proportion of patients for categorical variables) for the SAS population.

Supplemental Efficacy Results:

Patient Baseline Characteristics: Overall, the majority of patients (54; 87.1%) failed 1 prior treatment in the current MDE based on the Antidepressant Treatment Response Questionnaire (ATRQ). For each group, placebo had the most that failed 1 treatment (21; 95.5%), followed by REL-1017 25 mg (n=17; 89.5%), and REL-1017 50 mg (n=16; 76.2%). Seven patients (11.3%) failed 2 treatments, with 1 (4.5%), 2 (10.5%), 4 (19.0%) for the placebo, REL-1017 25 mg, and REL-1017 50 mg groups, respectively. One patient (4.8%) in the REL-1017 50 mg group failed 3 prior treatments. The most commonly failed treatment was with prior SSRIs (39 [62.9%]).

The mean baseline HAM-D-17 score was 25.6 in the placebo group, 25.1 in the REL-1017 25 mg dosing group, and 25.0 in the REL-1017 50 mg dosing group. No change in baseline HAM-D-17 analysis was made as this was not an efficacy measure.

Montgomery-Asberg Depression Rating Scale (MADRS): For the placebo group, the mean total MADRS score on Days 1,4, 7, and 14 were 33.8 (SD: 4.0), 27.8 (SD: 7.8), 25.1 (SD: 11.0), and 27.1

(SD: 10.0), respectively. For the REL-1017 25 mg dosing group mean MADRS score values on Days 1, 4, 7, and 14 were 32.9 (SD: 6.0), 19.5 (SD: 8.7), 16.2 (SD: 9.7), and 17.2 (SD: 10.7), respectively. For the REL-1017 50 mg dosing group, the mean value of the MADRS score was 35.2 [SD: 3.9] on Day 1 and 20.9 (SD: 9.8), 18.6 (SD: 10.1), and 16.8 (SD: 11.6), on Day 4, 7, and 14, respectively (see Table S-2).

Statistically significant differences in the MADRS change from Baseline were observed at Day 4 and Day 7 post-dose and at the end of the observation period on Day 14 for patients in both the REL-1017 25 mg and REL-1017 50 mg treatment groups compared with that of the placebo group in the FAS population (Supplemental Table S-2). For the FAS population, at Day 4 post-dose, the least squares mean difference was -7.9 (90% CI, -12.8, -3.1; p-value 0.0087; effect size 0.9) for the REL-1017 25 mg group and the least squares mean difference was -7.6 (90% CI, -12.4, -2.9; p-value 0.0096; effect size 0.8) for the REL-1017 50 mg group compared with that of the placebo group (Supplemental Table S-2). For the FAS population, at Day 7 post-dose, the least squares mean difference was -8.7 (90% CI, -14.3, -3.1; p-value 0.0122; effect size 0.8) for the REL-1017 25 mg group and the least squares mean difference was -7.2 (90% CI, -12.7, -1.8; p-value 0.0308; effect size 0.7) for the REL-1017 50 mg group compared with that of the placebo group (Table 3). For the FAS population, at the end of the observation period at Day 14, the least squares mean difference was -9.4 (90% CI, -15.4, -3.5; p-value 0.0103; effect size 0.9) for the REL-1017 25 mg group and the least squares mean difference was -10.4 (90% CI, -16.1, -4.6; p-value 0.0039; effect size 1.0) for the REL-1017 50 mg group compared with that of the placebo group (Table 3).

The number of remitters with a MADRS score ≤10 at Day 7 was 3 patients (14.3%) for the placebo group, 7 patients (36.8%) for the REL 1017 25 mg group and 5 patients (23.8%) for the REL-1017 50 mg group. The number of remitters with a MADRS score ≤10 at Day 14 was 1 patient (5.0%) for the placebo group, 5 patients (31.3%) for the REL-1017 25 mg group and 7 patients (38.9%) for the REL-

1017 50 mg group (Supplemental Table S-2).

The number of responders with MADRS score Percentage Change from Baseline (PCFB) ≤-50% in the FAS and PP population at Day 7 was 5 patients (23.8%) for the placebo group, 8 patients (42.1%) for the REL-1017 25 mg group and 12 patients (57.1%) for the REL-1017 50 mg group. At Day 14 the number of responders with MADRS score Percentage Change from Baseline (PCFB) ≤-50% was 4 patients (20.0%) for the placebo group, 8 patients (50.0%) for the REL-1017 25 mg group, and 8 patients (44.4%) for the REL-1017 50 mg group (Supplemental Table S-4).

Symptoms of Depressions Questionnaire (SDQ): The mean total SDQ score for the FAS population was 163.1 (SD: 26.0) on Day 1 pre-dose, 119.6 (SD: 29.4) on Day 4, 115.0 (SD: 31.0) on Day 7 at the EDP, and 115.1 (SD: 30.4) on Day 14 at EOP (Figure 2-B). The mean change from baseline and the mean percentage change from baseline in the SDQ-total scores for the FAS population showed improvement on Day 4 (-44.0 [30.0] and -26.302 [SD: 15.586] respectively). The mean change from baseline in the SDQ-total scores for the FAS population showed improvement on Day 7 (-48.7 [SD: 32.3] and (-29.174 [SD: 17.175], respectively). On Day 14, the improvement was sustained.

Patients treated with REL-1017 improved significantly as compared with placebo on three SDQ subscales including SDQ-1 (lassitude, mood, cognitive/social functioning), SDQ-2 (anxiety, agitation, anger and irritability), and SDQ-4 (disruptions in sleep quality). Statistically significant differences in the SDQ-1 change from baseline were observed on Day 14 for patients in the REL-1017 25 mg (least squares mean difference was -13.9 [90% CI, -21.3, -6.6; p-value 0.0025]) and REL-1017 50 mg (least squares mean difference was -15.0 [90% CI, -22.1, -7.8; p-value 0.0009]) treatment groups compared with that of the placebo group. Statistically significant differences in the SDQ-2 change from baseline were observed on Day 14 for patients in the REL-1017 25 mg (least squares mean difference was -4.6 [90% CI, -8.2, -0.9; p-value 0.0398]) and REL-1017 50 mg (least squares mean difference was -7.2

[90% CI, -10.7, -3.7; p-value 0.0012]) treatment groups compared with that of the placebo group. Statistically significant differences in the SDQ-4 change from baseline were observed on Day 14 for patients in the REL-1017 25 mg (least squares mean difference was -2.7 [90% CI, -4.3, -1.1; p-value 0.0055]) and REL-1017 50 mg (least squares mean difference was -2.8 [90% CI, -4.3, -1.3; p-value 0.0029]) treatment groups compared with that of the placebo group. There were no significant differences observed between the treated groups and placebo in two SDQ subscales including SDQ-3 (desire to be dead) and SDQ-5 (changes in appetite and weight) (Supplemental Table S-6).

Clinical Global Impressions of Severity Questionnaire (CGI-S): On the CGI-S scale, 16 patients (25.8%) in the FAS population were categorized as moderately ill on Day 1 pre-dose, compared with 18 patients (29.5%) on Day 4, 10 patients (16.4%) on Day 7 post dose, and 10 patients (18.5%) on Day 14, end of the evaluation period. A total of 45 patients (72.6%) were categorized as markedly ill on Day 1 pre-dose, compared with 18 patients (29.5%) on Day 4 and Day 7 post dose, and 16 patients (29.6%) on Day 14, end of the evaluation period post dose. One patient each (1.9%) was categorized as severely ill on Day 1, pre-dose and on Day 14, end of the evaluation period post dose. No patient was categorized as severely ill on Day 4 and on Day 7 post dose (Figure 2-C).

Clinical Global Impressions of Improvement Questionnaire (CGI-I): On the CGI-I scale, 20 patients (32.8%) in the FAS population were categorized as minimally improved and 16 patients (26.2%) as much improved on Day 4. On Day 7 post dose, 13 patients (21.3%) were categorized as minimally improved and 21 patients (34.4%) as much improved. On Day 14 end of the evaluation period, 11 patients (20.4%) were categorized as minimally improved and 13 patients (24.1%) as much improved (Figure 2-D).

Supplemental Safety Results:

Adverse Events: For the SAS Population, the most common TEAEs that occurred in at least 5% of patients were headache, 8 (12.9%) (3 [13.6%] placebo group, 2 [10.5%] REL-1017 25 mg group,

3 [14.3%] REL-1017 50 mg group), constipation 7 (11.3%) (3 [13.6%] placebo group, 1 [5.3%] REL-1017 25 mg group, 3 [14.3%] REL-1017 50 mg group) and nausea 5 (8.1%) (2 [9.1%] placebo group, 1 [5.3%] REL-1017 25 mg group, 2 [9.5%] REL-1017 50 mg group); Table 4.

The intensity of the TEAEs observed for all patients was similar across treatment groups, and most events were mild (46.8%). No severe events were reported. When analyzed by relationship to study drug and maximum intensity, the TEAEs that were considered possibly, probably, or definitely related to study drug were similar across treatment groups. For all patients, the maximum intensity of possibly, probably, or definitely related to study drug was observed in the SOCs of gastrointestinal disorders and nervous system disorders. For gastrointestinal disorders, 5 (8.1%) were possibly, 2 (3.2%) were probably, and 3 (4.8%) were considered definitely related to study drug. Constipation was the most commonly reported event considered possibly, probably, or definitely related to study drug. For nervous system disorders, 4 (6.5%) were possibly, 5 (8.1%) were probably, and 1 (1.6%) was considered definitely related to study drug.

For nervous system disorder events, similar numbers of treatment-related events were observed across treatment groups. The most common TEAEs in the gastrointestinal disorders SOC were constipation, nausea, and abdominal discomfort. The most common TEAEs in the nervous system disorders SOC were headache, somnolence, dizziness, and sedation. Across all treatment groups with REL-1017 and for placebo, the most common treatment-related TEAE overall was in the gastrointestinal disorders SOC and was constipation. Similar numbers were observed across treatment groups with no evidence of increased frequency of specific organ class TEAEs in the REL-1017 groups compared to placebo.

There were no occurrences of clinically significant QTcF prolongation, defined as a value ≥500 msec or an increase ≥60 msec above the patient's baseline value that continuously occurred over a period of time. For 2 patients in the REL-1017 50 mg dose group a change from baseline of >30 msec in the

QTcF was observed. At the postbaseline measurement, QTcF was >450 msec for 1 subject in the REL-1017 25 mg dose group and 3 subjects in the REL-1017 50 mg dose group. There were no patients with QTcF >450 and a change from baseline of >30 msec. These changes compared to placebo were not statistically significant. None of the abnormal ECG readings were considered clinically significant.

Columbia-Suicide Severity Rating Scale (C-SSRS): The C-SSRS has 4 constructs relevant to recent suicidal ideation: (1) severity (1=wish to be dead, 2=nonspecific active suicidal thoughts, 3=suicidal thoughts with methods, 4=suicidal intent, and 5=suicidal intent with plan); (2) intensity (sum across 6 items each rated 0 to 5: most severe ideation, frequency, duration, controllability, deterrents, and reason); (3) behavior; and (4) lethality. Suicide risk was assessed with the C-SSRS.

One subject in each treatment group reported any suicidal ideation on Day 14. These patients responded Yes at Day 14 for "Wish to be dead." The patient in the placebo group (002-001) also reported Yes for "Wish to be dead" at Check-in, Day 1, Day 9, Day 14, and Follow-up. The patient in the REL-1017 25 mg group (005-009) also reported Yes for "Wish to be dead" at Check-in and Day 14. The patient in the REL-1017 50 mg group (007-009) also reported Yes for "Wish to be dead" at Day 8, Day 9, Day 14, and Follow-up. These assessment results were not reported as TEAEs. The patients had no suicidal behavior.

Clinical Opiate Withdrawal Scale (COWS): The total COWS score was derived by adding the score for each item of the 11 items of each assessment. Total COWS scores by treatment group and visit are displayed in Supplemental Table S-12. All mean scores were ≤1 across placebo and treatment group, which indicates that opioid withdrawal symptoms were infrequent and unlikely. The most commonly identified symptoms of opioid withdrawal assessed with the COWS tool include elevated resting pulse rate, sweating, increased pupil size, bone or joint aches, runny nose or tearing (not from cold), yawning, anxiety or irritability, restlessness, and gooseflesh skin; none of these complaints

were reported as TEAEs.

The mean COWS scores from Day 8 to Day 14 were consistent for placebo and the REL-1017 groups and were similar across treatment groups. Overall, on Day 8, Day 9, and Day 14 the mean COWS scores were 0.6 (SD:0.9), 0.7 (SD:1.1), and 0.6 (1.0), respectively.

Further assessment of potential opioid withdrawal was conducted in evaluating common symptoms of withdrawal TEAEs in the SAS population. Restlessness on Day 1 post-dose was evaluated by the Investigator as definitely related to study drug, and tremor on Day 9 was evaluated by the Investigator as possibly related to study drug, and were each reported by 1 patient each in the REL-1017 50-mg group. These events were mild and each resolved within 1 day without treatment. Gastrointestinal events were the most common TEAEs. By dosing group, 8 patients (36.4%) in the placebo group, 5 patients (26.3%) in the REL-1017 25-mg group, and 5 patients (23.8%) in the REL-1017 50-mg group experienced gastrointestinal events and 4 patients (18.2%) in the placebo group, 2 patients (10.5%) in the REL-1017 25-mg group, and 4 patients (19.0%) in the REL-1017 50-mg group were the most common reported treatment-related TEAEs. These events were reported during the dosing period and therefore unlikely to be related to opioid withdrawal symptoms.

Clinician-Administered Dissociative States Scale (CADSS): The CADSS total score of 23 items as well as total scores for subscales were summarized by treatment group and by using frequencies for the categories: ≤4; and >4 by treatment and visits. The CADSS total score of ≤4 is considered normal. The change from baseline of total score in CADSS was summarized using descriptive statistics and change from baseline using frequencies for the categories: ≤0; and >0 by treatment and visits. A higher score indicates a higher likelihood of the presence of a dissociative state. On Day 1 pre-dose, the CADSS overall score was 1.2 (SD 5.3). For placebo and the REL-1017 dosing groups, the results were similar (placebo 0.5 [SD 1.4]), REL-1017 25 mg 1.6 [SD 5.1]), and REL-1017 50 mg 1.7 [SD 7.6]). One patient in each dosing group had a CADSS score >4. On Day 1, 2 hours after loading doses

the scores were 0.3 [SD 1.3], 0.2 [SD 0.5], and 1.8 [SD 4.3] for placebo, REL-1017 25 mg and REL-1017 50mg, respectively. On Day 9, the mean CADSS score was 1.1 (SD 5.0) for the placebo group, 0.0 (SD 0.0) for the REL-1017 25 mg dosing group, and 0.0 (SD 0.0) for the REL-1017 50 mg dosing group. The overall CADSS score on Day 9 was 0.4 (SD 2.9). One patient in the placebo had a CADSS score >4. No dose-related trend was observed in CADSS scores.

Positive Symptom Rating Scale (PSRS): On Day 1, the mean 4-Item PSRS for each dosing group was 4.0 (SD:0.0) for each group. The mean values remained stable at 2 hours post-dose on Day 1 and on Day 7, and on Day 9 at discharge across all groups. No difference between placebo and REL-1017 groups was observed.

Supplemental Tables and Figures:

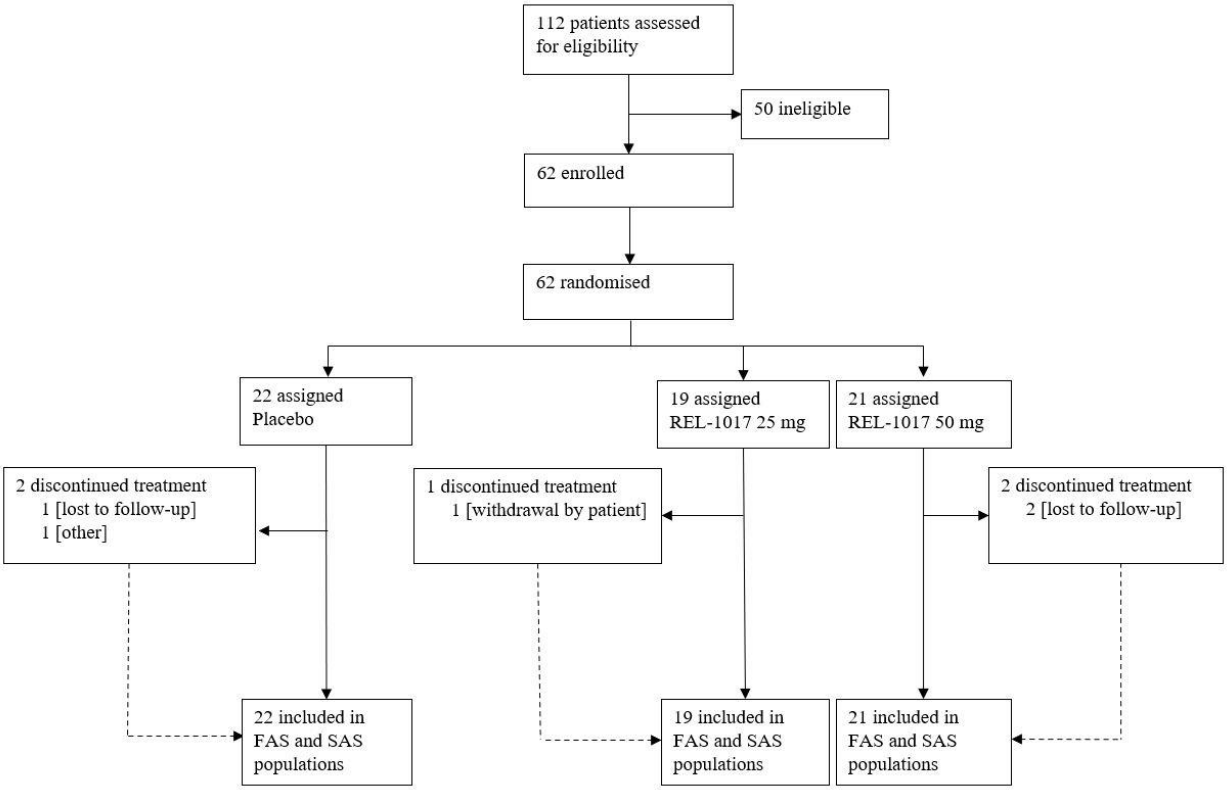
Supplemental Table S-1. Disposition of Patients (FAS Population) REL-1017 Trial

	Placebo (N=22)	**REL-1017 25 mg (N=19)**	**REL-1017 50 mg (N=21)**	**All Patients (N=62)**
Patients Randomized	22	19	21	62
Patients who completed the study	20 (90.9%)	18 (94.7%)	19 (90.5%)	57 (91.9%)
Patients who discontinued early	2 (9.1%)	1 (5.3%)	2 (9.5%)	5 (8.1%)
Reasons for early discontinuation				
Withdrawal by subject	0	1 (5.3%)	0	1 (1.6%)
Lost to follow-up	1 (4.5%)	0	2 (9.5%)	3 (4.8%)
Other: Subject does not meet Inclusion Criterion #1	1 (4.5%)	0	0	1 (1.6%)

Abbreviations: N=number of patients.

Note: Percentages were calculated on the basis of the number of patients in the randomized population per treatment arm and overall.

Supplemental Figure S-1: CONSORT Diagram



Abbreviations: FAS=Full Analysis Set; SAS=Safety Analysis Set.

Supplemental Table S-2. Total Score in MADRS by Visit (FAS Population) REL-1017 Trial

Visit; Time Point	Total Score	Placebo (N=22)	REL-1017 25 mg (N=19)	REL-1017 50 mg (N=21)	All Patients (N=62)
Day 1, Pre-dose		22	19	21	62
	Mean (SD)	33.8 (4.0)	32.9 (6.0)	35.2 (3.9)	34.0 (4.7)
	Median	33.5	34.0	35.0	34.0
	Min, Max	27, 43	18, 44	26, 43	18, 44
	<34	11 (50.0%)	9 (47.4%)	6 (28.6%)	26 (41.9%)
	≥34	11 (50.0%)	10 (52.6%)	15 (71.4%)	36 (58.1%)
	≤10	0	0	0	0
	>10	22 (100%)	19 (100%)	21 (100%)	62 (100%)
Day 2, Pre-dose	N	22	19	21	62
	Mean (SD)	28.5 (6.4)	26.2 (10.0)	28.8 (6.8)	27.9 (7.8)
	Median	30.5	27.0	27.0	29.0
	Min, Max	15, 40	3, 43	16, 41	3, 43
	<34	17 (77.3%)	14 (73.7%)	16 (76.2%)	47 (75.8%)
	≥34	5 (22.7%)	5 (26.3%)	5 (23.8%)	15 (24.2%)
	≤10	0	1 (5.3%)	0	1 (1.6%)
	>10	22 (100%)	18 (94.7%)	21 (100%)	61 (98.4%)
Day 4, Post-dose	N	21	19	21	61
	Mean (SD)	27.8 (7.8)	19.5 (8.7)	20.9 (9.8)	22.8 (9.4)
	Median	29.0	20.0	21.0	22.0
	Min, Max	5, 40	4, 40	2, 37	2, 40
	<34	15 (71.4%)	18 (94.7%)	18 (85.7%)	51 (83.6%)
	≥34	6 (28.6%)	1 (5.3%)	3 (14.3%)	10 (16.4%)
	≤10	1 (4.8%)	3 (15.8%)	3 (14.3%)	7 (11.5%)
	>10	20 (95.2%)	16 (84.2%)	18 (85.7%)	54 (88.5%)
Day 7 (EDP), Post-dose	N	21	19	21	61
	Mean (SD)	25.1 (11.0)	16.2 (9.7)	18.6 (10.1)	20.1 (10.8)
	Median	28.0	17.0	18.0	20.0

Visit; Time Point	Total Score	Placebo (N=22)	REL-1017 25 mg (N=19)	REL-1017 50 mg (N=21)	All Patients (N=62)
	Min, Max	4, 40	1, 38	1, 34	1, 40
	<34	15 (71.4%)	18 (94.7%)	17 (81.0%)	50 (82.0%)
	≥34	6 (28.6%)	1 (5.3%)	4 (19.0%)	11 (18.0%)
	≤10	3 (14.3%)	7 (36.8%)	5 (23.8%)	15 (24.6%)
	>10	18 (85.7%)	12 (63.2%)	16 (76.2%)	46 (75.4%)
Day 14 (EOP)	N	20	16	18	54
	Mean (SD)	27.1 (10.0)	17.2 (10.7)	16.8 (11.6)	20.7 (11.6)
	Median	30.0	16.0	17.5	22.5
	Min, Max	4, 41	2, 37	2, 37	2, 41
	<34	14 (70.0%)	15 (93.8%)	17 (94.4%)	46 (85.2%)
	≥34	6 (30.0%)	1 (6.3%)	1 (5.6%)	8 (14.8%)
	≤10	1 (5.0%)	5 (31.3%)	7 (38.9%)	13 (24.1%)
	>10	19 (95.0%)	11 (68.8%)	11 (61.1%)	41 (75.9%)

Abbreviations: EDP=End of Dosing Period; EOP=End of the Observation Period; FAS=Full Analysis Set; MADRS=Montgomery-Asberg Depression Rating Scale; Max=maximum; Min=minimum; N=number of patients; SD=standard deviation.

Note: Percentages were calculated on the basis of the number of patients in the FAS population within the treatment arm at each visit

Supplemental Table S-3. MADRS: Summary by Visit and Change from Baseline (FAS Population) REL-1017 Trial

Visit, Time point	Statistics	Placebo (N=22) Observed Value	CFB	REL-1017 25 mg (N=19) Observed Value	CFB	REL-1017 50 mg (N=21) Observed Value	CFB	All Patients (N=62) Observed Value	CFB
Baseline [a]	**n**	22	NA	19	NA	21	NA	62	NA
	Mean (SD)	33.8 (4.0)		32.9 (6.0)		35.2 (3.9)		34.0 (4.7)	
	Median	33.5		34.0		35.0		34.0	
	Min, Max	27, 43		18, 44		26, 43		18, 44	
Day 2, Predose	**n**	22	22	19	19	21	21	62	62
	Mean (SD)	28.5, (6.4)	-5.3 (7.8)	26.2 (10.0)	-6.7 (8.4)	28.8 (6.8)	-6.4 (6.2)	27.9 (7.8)	-6.1 (7.4)
	Median	30.5	-2.5	27.0	-4.0	27.0	-6.0	29.0	-4.0
	Min, Max	15, 40	-28, 3	3, 43	-31, 1	16, 41	-20, 3	3, 43	-31, 3
Day 4, Postdose	**n**	21	21	19	19	21	21	61	61
	Mean (SD)	27.8 (7.8)	-6.1 (9.6)	19.5, (8.7)	-13.4 (9.9)	20.9 (9.8)	-14.3 (10.6)	22.8 (9.4)	-11.2 (10.6)
	Median	29.0	-3.0	20.0	-13.0	21.0	-13.0	22.0	-9.0
	Min, Max	5, 40	-38, 6	4, 40	-38, 1	2, 37	-35, 4	2, 40	-38, 6
Day 7 (EDP), Postdose	**n**	21	21	19	19	21	21	61	61
	Mean (SD)	25.1, (11.0)	-8.8 (12.5)	16.2 (9.7)	-16.8 (11.3)	18.6 (10.1)	-16.6 (9.8)	20.1 (10.8)	-14.0 (11.7)
	Median	28.0	-5.0	17.0	-15.0	18.0	-17.0	20.0	-13.0
	Min, Max	4, 40	-39, 6	1, 38	-42, -1	1, 34	-34, 1	1, 40	-42, 6

Day 14 (EOP)	**n**	20	20	16	16	18	18	54	54
	Mean (SD)	27.1, (10.0)	-7.2 (12.1)	17.2 (10.7)	-16.3 (9.4)	16.8 (11.6)	-18.4 (12.0)	20.7 (11.6)	-13.6 (12.2)
	Median	30.0	-3.5	16.0	-17.5	17.5	-15.0	22.5	-10.0
	Min, Max	4, 41	-39, 4	2, 37	-32, -3	2, 37	-36, 4	2, 41	-39, 4

Abbreviations: CFB, change from baseline; EDP, End of Dosing Period; EOP, End of Observation Period; FAS, Full Analysis Set; MADRS, Montgomery-Asberg Depression

Rating Scale; Max, maximum; Min, minimum; N, number of subjects; NA, not applicable; SD, standard deviation.

[a] **Baseline was defined as the last available, valid, non-missing assessment prior to first study drug administration.**

Supplemental Table S-4. MADRS Summary by Visit and Percentage Change from Baseline (FAS Population) REL-1017 Trial

Visit, Time point	Statistics	Placebo Observed Value (N=22)	PCFB	REL-1017 25 mg Observed Value (N=19)	PCFB	REL-1017 50 mg Observed Value (N=21)	PCFB	All Patients Observed Value (N=62)	PCFB
Baseline[a]	n	22	NA	19	NA	21	NA	62	NA
	Mean (SD)	33.8 (4.0)		32.9 (6.0)		35.2 (3.9)		34.0 (4.7)	
	Median	33.5		34.0		35.0		34.0	
	Min, Max	27, 43		18, 44		26, 43		18, 44	
Day 2, Predose	N	22	22	19	19	21	21	62	62
	Mean (SD)	28.5 (6.4)	-14.735 (19.703)	26.2 (10.0)	-20.749 (23.841)	28.8 (6.8)	-17.911 (17.489)	27.9 (7.8)	-17.654 (20.192)
	Median	30.5	-6.978	27.0	-16.667	27.0	-16.216	29.0	-11.775
	Min, Max	15, 40	-65.116, 8.108	3, 43	-91.176, 2.941	16, 41	-55.556, 9.091	3, 43	-91.176, 9.091
	PCFB≤-50%		1 (4.5%)		2 (10.5%)		2 (9.5%)		5 (8.1%)
	PCFB >-50%		21 (95.5%)		17 (89.5%)		19 (90.5%)		57 (91.9%)
Day 4, Postdose	N	21	21	19	19	21	21	61	61
	Mean (SD)	27.8 (7.8)	-16.695 (23.925)	19.5 (8.7)	-39.115 (26.074)	20.9 (9.8)	-39.900 (28.227)	22.8 (9.4)	-31.667 (27.930)
	Median	29.0	-8.824	20.0	-39.394	21.0	-33.333	22.0	-29.032
	Min, Max	5, 40	-88.372,17.647	4, 40	-88.235, 5.556	2, 37	-94, 595, 12.121	2, 40	-94.595, 17.647
			2 (9.5%)		6 (31.6%)		8 (38.1%)		16 (26.2%)
			19 (90.5%)		13 (68.4%)		13 (61.9%)		45 (73.8%)
	PCFB≤-50%		2 (9.5%)		6 (31.6%)		8 (38.1%)		16 (26.2%)

	PCFB >-50%		19 (90.5%)		13 (68.4%)		13 (61.9%)		45 (73.8%)
Day 7 (EDP), Postdose	N	21	21	19	19	21	21	61	61
	Mean (SD)	25.1 (11.0)	-24.608 (32.892)	16.2 (9.7)	-49.169 (29.511)	18.6 (10.1)	-47.255 (28.049)	20.1 (10.8)	-40.055 (31.813)
	Median	28.0	-15.152	17.0	-45.455	18.0	-51.351	20.0	-39.394
	Min, Max	4, 40	-90.698, 17.647	1, 38	-97.059, -5.000	1, 34	-97.059, 3.030	1, 40	-97.059, 17.647
	PCFB≤-50%		5 (23.8%)		8 (42.1%)		12 (57.1%)		25 (41.0%)
	PCFB >-50%		16 (76.2%)		11 (57.9%)		9 (42.9%)		36 (59.0%)
Day 14 (EOP)	N	20	20	16	16	18	18	54	54
	Mean (SD)	27.1 (10.0)	-19.095 (30.531)	17.2 (10.7)	-49.834 (29.174)	16.8 (11.6)	-51.755 (32.863)	20.7 (11.6)	-39.089 (34.083)
	Median	30.0	-10.102	16.0	-47.899	17.5	-40.760	22.5	-32.308
	Min, Max	4, 41	-90.698, 10.811	2, 37	-94.118, -7.500	2, 37	-94.595, 12.121	2, 41	-94.595, 12.121
	PCFB≤-50%		4 (20.0%)		8 (50.0%)		8 (44.4%)		20 (37.0%)
	PCFB >-50%		16 (80.0%)		8 (50.0%)		10 (55.6%)		34 (63.0%)

Abbreviations: EDP=End of Dosing Period; EOP=End of Observation Period; FAS=Full Analysis Set; MADRS=Montgomery-Asberg Depression Rating Scale; Max=maximum; Min=minimum; N=number of patients; NA=not applicable; PCFB=percentage change from baseline; SD=standard deviation.

a. Baseline was defined as the last available, valid, non-missing assessment prior to first study drug administration.

Supplemental Table S-5. MADRS Change from Baseline to EDP (Day 7) and to EOP (Day 14) (FAS Population) REL-1017 Trial

Table 3. MADRS: Change from Baseline to EDP (Day 7) and to EOP (Day 14) (FAS Population)						
Visit, Time Point	**Treatment Group**	**N**	**LS Mean (SE)[a]**	**Difference of LS Means (90% CI)[b]**	**P-value[c]**	**Effect Size[d]**
Day 2, Predose	REL-1017 25 mg	19	-7.3 (1.9)	-1.9 (-5.8, 2.1)	0.4340	0.3
	Placebo	22	-5.5 (1.6)			
	REL-1017 50 mg	21	-5.7 (1.7)	-0.3 (-4.1, 3.6)	0.9092	0.0
	Placebo	22	-5.5 (1.6)			
Day 4, Postdose	REL-1017 25 mg	19	-14.0 (2.2)	-7.9 (-12.8, -3.1)	0.0087	0.9
	Placebo	21	-6.1 (2.0)			
	REL-1017 50 mg	21	-13.7 (2.1)	-7.6 (-12.4, -2.9)	0.0096	0.8
	Placebo	21	-6.1 (2.0)			
Day 7 (EDP), Postdose	REL-1017 25 mg	19	-17.4 (2.5)	-8.7 (-14.3, -3.1)	0.0122	0.8
	Placebo	21	-8.7 (2.3)			
	REL-1017 50 mg	21	-15.9 (2.4)	-7.2 (-12.7, -1.8)	0.0308	0.7
	Placebo	21	-8.7 (2.3)			
Day 14 (EOP)	REL-1017 25 mg	16	-16.8 (2.7)	-9.4 (-15.4, -3.5)	0.0103	0.9
	Placebo	20	-7.4 (2.4)			
	REL-1017 50 mg	18	-17.8 (2.6)	-10.4 (-16.1, -4.6)	0.0039	1.0
	Placebo	20	-7.4 (2.4)			

Abbreviations: CI=confidence interval; EDP=End of Dosing Period; EOP=End of Observation Period; FAS=Full Analysis Set; LS=least squares; MADRS=Montgomery-Asberg Depression Rating Scale; N=number of patients.

Notes: Baseline was defined as the last available, valid, non-missing assessment prior to first study drug administration.

a. The LS means, LS mean difference, and the associated 90% CI and p-value for change from baseline are based on a mixed model of repeated measures with fixed effect terms for treatment, stratification (concomitant antidepressant drug) factor, visit (Day 2, Day 4, Day 7, Day 14), interaction between treatment and visit, and baseline endpoint as a covariate.

b. Difference between REL-1017 and placebo in the LS means.

c. p-value for testing the null hypothesis of no difference.

d. Cohen's effect size was calculated on the basis of the LS mean differences and pooled standard deviations.

Supplemental Table S-6. Response and Remission Rates, REL-1017 Trial

		Placebo	REL-1017 25 mg	REL-1017 50 mg	NNT (25 mg)	NNT (50 mg)
Day 7	**Response Rate**	0.238	0.421	0.571	6	3
Day 14	**Response Rate**	0.2	0.5	0.444	4	5
Day 7	**Remission Rate**	0.143	0.368	0.238	5	11
Day 14	**Remission Rate**	0.05	0.313	0.389	4	3

Abbreviations: NNT, number needed to treat.

Supplemental Table S-7. Analysis of SDQ 5 subscales from Baseline to EOP (Day 14) (FAS Population) REL-1017 Trial

SDQ Subscale	Treatment Group	N	LS Mean (SE)	Difference of LS Means Drug vs Placebo (90% CI)	P-Value
Lassitude, mood, cognitive /social functioning	Placebo	20	-14.6 (3.0)		
	REL-1017 25 mg	16	-28.6 (3.5)	-13.9 (-21.3, -6.6)	0.0025
	REL-1017 50 mg	18	-29.6 (3.2)	-15.0 (-22.1, -7.8)	0.0009
Anxiety, agitation, anger, and irritability	Placebo	20	-8.9 (1.5)		
	REL-1017 25 mg	16	-13.4 (1.7)	-4.6 (-8.2, -0.9)	0.0398
	REL-1017 50 mg	18	-16.0 (1.6)	-7.2 (-10.7, -3.7)	0.0012
Desire to be dead	Placebo	20	-4.2 (0.8)		
	REL-1017 25 mg	16	-6.2 (1.0)	-2.0 (-4.1, 0.1)	0.1088
	REL-1017 50 mg	18	-5.8 (0.9)	-1.7 (-3.7, 0.3)	0.1725
Disruptions in sleep quality	Placebo	20	-2.5 (0.6)		
	REL-1017 25 mg	16	-5.3 (0.7)	-2.7 (-4.3, -1.1)	0.0055
	REL-1017 50 mg	18	-5.3 (0.7)	-2.8 (-4.3, -1.3)	0.0029
Changes in appetite and weight	Placebo	20	-1.9 (0.4)		
	REL-1017 25 mg	16	-1.7 (0.5)	0.2 (-0.8, 1.2)	0.7272
	REL-1017 50 mg	18	-1.7 (0.4)	0.2 (-0.8, 1.2)	0.7472

Abbreviations: SDQ=Symptoms of Depression Questionnaire; CI=confidence interval; EOP=End of Observation Period; FAS=Full Analysis Set; LS=least squares; N=number of subjects; SE=standard error.

Note: Baseline was defined as the last available, valid, non-missing assessment before first study drug administration.

Supplemental Table S-8. Analysis of SDQ Change from Baseline to EDP (Day 7) and to EOP (Day 14) by Subscales (ITT Population) REL-1017 Trial

Visit, Time Point	Treatment Group	N	LS Mean (SE)[a]	Difference of LS Means Drug vs Placebo (90% CI)[b]	P-Value[c]	Effect Size (90% CI)[d]
Subscale: SDQ-1						
Day 2, Predose	REL-1017 25 mg	19	-19.2 (3.1)	-4.6 (-11.2, 2.0)	0.2511	0.4 (0.0, 0.9)
	Placebo	22	-14.6 (2.8)			
	REL-1017 50 mg	21	-15.0 (2.9)	-0.4 (-6.9, 6.0)	0.9122	0.0 (0.0, 0.5)
	Placebo	22	-14.6 (2.8)			
Day 4, Postdose	REL-1017 25 MG	19	-23.0 (3.5)	-5.8 (-13.4, 1.8)	0.2050	0.4 (0.0, 0.9)
	Placebo	21	-17.2 (3.2)			
	REL-1017 50 MG	21	-22.7 (3.3)	-5.5 (-12.9, 1.9)	0.2160	0.4 (0.0, 0.9)
	Placebo	21	-17.2 (3.2)			
Day 7 (EDP), Postdose	REL-1017 25 MG	19	-23.0 (3.5)	-5.8 (-13.4, 1.8)	0.2050	0.4 (0.0, 0.9)
	Placebo	21	-17.2 (3.2)			
	REL-1017 50 MG	21	-22.7 (3.3)	-5.5 (-12.9, 1.9)	0.2160	0.4 (0.0, 0.9)
	Placebo	21	-17.2 (3.2)			
Day 14 (EOP)	REL-1017 25 MG	16	-28.6 (3.5)	-13.9 (-21.3, -6.6)	0.0025	1.0 (0.5, 1.6)
	Placebo	20	-14.6 (3.0)			
	REL-1017 50 MG	18	-29.6 (3.2)	-15.0 (-22.1, -7.8)	0.0009	1.1 (0.6, 1.7)
	Placebo	20	-14.6 (3.0)			
Subscale: SDQ-2						
Day 2, Predose	REL-1017 25 MG	19	-9.0 (1.5)	0.1 (-3.0, 3.3)	0.9487	0.0 (0.0, 0.5)
	Placebo	22	-9.1 (1.3)			
	REL-1017 50 MG	21	-10.1 (1.4)	-1.0 (-4.1, 2.0)	0.5745	0.2 (0.0, 0.7)
	Placebo	22	-9.1 (1.3)			
Day 4, Postdose	REL-1017 25 MG	19	-12.2 (1.8)	-2.2 (-6.1, 1.7)	0.3559	0.3 (0.0, 0.8)
	Placebo	21	-10.0 (1.6)			
	REL-1017 50 MG	21	-11.9 (1.7)	-1.9 (-5.7, 1.9)	0.4093	0.3 (0.0, 0.8)
	Placebo	21	-10.0 (1.6)			
Day 7 (EDP), Postdose	REL-1017 25 MG	19	-12.8 (1.9)	-2.2 (-6.3, 1.9)	0.3641	0.3 (0.0, 0.8)

	Placebo	21	-10.6 (1.7)			
	REL-1017 50 MG	21	-13.5 (1.7)	-2.9 (-6.9, 1.1)	0.2289	0.4 (0.0, 0.9)
	Placebo	21	-10.6 (1.7)			
Day 14 (EOP)	REL-1017 25 MG	16	-13.4 (1.7)	-4.6 (-8.2, -0.9)	0.0398	0.7 (0.1, 1.2)
	Placebo	20	-8.9 (1.5)			
	REL-1017 50 MG	18	-16.0 (1.6)	-7.2 (-10.7, -3.7)	0.0012	1.1 (0.6, 1.6)
	Placebo	20	-8.9 (1.5)			
Subscale: SDQ-3						
Day 2, Predose	REL-1017 25 MG	19	-3.8 (0.8)	-0.6 (-2.2, 0.9)	0.4963	0.2 (0.0, 0.7)
	Placebo	22	-3.1 (0.7)			
	REL-1017 50 MG	21	-3.1 (0.7)	0.1 (-1.5, 1.6)	0.9346	0.0 (0.0, 0.5)
	Placebo	22	-3.1 (0.7)			
Day 4, Postdose	REL-1017 25 MG	19	-5.9 (0.9)	-2.2 (-4.2, -0.3)	0.0636	0.6 (0.1, 1.1)
	Placebo	21	-3.7 (0.8)			
	REL-1017 50 MG	21	-4.9 (0.8)	-1.2 (-3.1, 0.7)	0.2798	0.3 (0.0, 0.9)
	Placebo	21	-3.7 (0.8)			
Day 7 (EDP), Postdose	REL-1017 25 MG	19	-6.3 (1.0)	-2.0 (-4.2, 0.2)	0.1319	0.5 (0.0, 1.0)
	Placebo	21	-4.2 (0.9)			
	REL-1017 50 MG	21	-6.0 (0.9)	-1.7 (-3.8, 0.4)	0.1841	0.4 (0.0, 0.9)
	Placebo	21	-4.2 (0.9)			
Day 14 (EOP)	REL-1017 25 MG	16	-6.2 (1.0)	-2.0 (-4.1, 0.1)	0.1088	0.5 (0.0, 1.1)
	Placebo	20	-4.2 (0.8)			
	REL-1017 50 MG	18	-5.8 (0.9)	-1.7 (-3.7, 0.3)	0.1725	0.4 (0.0, 1.0)
	Placebo	20	-4.2 (0.8)			
Subscale: SDQ-4						
Day 2, Predose	REL-1017 25 MG	19	-2.8 (0.9)	0.4 (-1.6, 2.5)	0.7272	0.1 (0.0, 0.6)
	Placebo	22	-3.2 (0.9)			
	REL-1017 50 MG	21	-3.1 (0.9)	0.1 (-1.9, 2.1)	0.9320	0.0 (0.0, 0.5)
	Placebo	22	-3.2 (0.9)			
Day 4, Postdose	REL-1017 25 MG	19	-4.2 (0.9)	-0.5 (-2.6, 1.6)	0.6912	0.1 (0.0, 0.7)
	Placebo	21	-3.7 (0.9)			
	REL-1017 50 MG	21	-4.7 (0.9)	-1.0 (-3.0, 1.0)	0.4195	0.3 (0.0, 0.8)
	Placebo	21	-3.7 (0.9)			

Day 7 (EDP), Postdose	REL-1017 25 MG	19	-4.3 (1.0)	-0.3 (-2.4, 1.8)	0.8096	0.1 (0.0, 0.6)
	Placebo	21	-4.0 (0.9)			
	REL-1017 50 MG	21	-4.7 (0.9)	-0.7 (-2.8, 1.3)	0.5502	0.2 (0.0, 0.7)
	Placebo	21	-4.0 (0.9)			
Day 14 (EOP)	REL-1017 25 MG	16	-5.3 (0.7)	-2.7 (-4.3, -1.1)	0.0055	1.0 (0.4, 1.6)
	Placebo	20	-2.5 (0.6)			
	REL-1017 50 MG	18	-5.3 (0.7)	-2.8 (-4.3, -1.3)	0.0029	1.0 (0.5, 1.6)
	Placebo	20	-2.5 (0.6)			
Subscale: SDQ-5						
Day 2, Predose	REL-1017 25 MG	19	-1.7 (0.5)	0.1 (-0.9, 1.0)	0.9097	0.0 (0.0, 0.6)
	Placebo	22	-1.8 (0.4)			
	REL-1017 50 MG	21	-1.4 (0.4)	0.3 (-0.6, 1.3)	0.5509	0.2 (0.0, 0.7)
	Placebo	22	-1.8 (0.4)			
Day 4, Postdose	REL-1017 25 MG	19	-1.7 (0.4)	0.0 (-1.0, 0.9)	0.9422	0.0 (0.0, 0.6)
	Placebo	21	-1.7 (0.4)			
	REL-1017 50 MG	21	-1.8 (0.4)	-0.1 (-1.1, 0.8)	0.7856	0.1 (0.0, 0.6)
	Placebo	21	-1.7 (0.4)			
Day 7 (EDP), Postdose	REL-1017 25 MG	19	-2.0 (0.5)	-0.3 (-1.4, 0.8)	0.6512	0.1 (0.0, 0.7)
	Placebo	21	-1.7 (0.5)			
	REL-1017 50 MG	21	-1.7 (0.5)	0.0 (-1.1, 1.1)	0.9948	0.0 (0.0, 0.5)
	Placebo	21	-1.7 (0.5)			
Day 14 (EOP)	REL-1017 25 MG	16	-1.7 (0.5)	0.2 (-0.8, 1.2)	0.7272	0.1 (0.0, 0.7)
	Placebo	20	-1.9 (0.4)			
	REL-1017 50 MG	18	-1.7 (0.4)	0.2 (-0.8, 1.2)	0.7472	0.1 (0.0, 0.6)
	Placebo	20	-1.9 (0.4)			

Abbreviations: SDQ=Symptoms of Depression Questionnaire; CI=confidence interval; EDP=End of Dosing Period; EOP=End of Observation Period; ITT=Intent-To-Treat; LS=least squares; N=number of subjects; SE=standard error.

Note: Baseline was defined as the last available, valid, non-missing assessment before first study drug administration.

a. The LS means, LS mean difference, and the associated 90% CI and p-value for change from baseline are based on a mixed model of repeated measures with fixed effect terms for treatment, stratification (concomitant antidepressant drug) factor, visit (Day 2, Day 4, and Day 7, Day 14), interaction between treatment and visit, and baseline endpoint as a covariate.

b. Difference between REL-1017 and placebo in the LS means.

c. p-value for testing the null hypothesis of no difference.

d. Cohen's effect size was calculated on the basis of the LS mean differences and pooled standard deviations.

Supplemental Table S-9. Analysis of SDQ Change from Baseline to EDP (Day 7) and to EOP (Day 14) by Cognition (ITT Population) REL-1017 Trial

Visit, Time Point	Treatment Group	N	LS Mean (SE)[a]	Difference of LS Means Drug vs Placebo (90% CI)[b]	P-Value[c]	Effect Size (90% CI)[d]
Day 2, Predose	REL-1017 25 mg	19	-5.9 (1.0)	-1.2 (-3.3, 0.9)	0.3579	0.3 (0.0, 0.8)
	Placebo	22	-4.8 (0.9)			
	REL-1017 50 mg	21	-4.4 (0.9)	0.3 (-1.7, 2.4)	0.7812	0.1 (0.0, 0.6)
	Placebo	22	-4.8 (0.9)			
Day 4, Postdose	REL-1017 25 mg	19	-6.2 (1.1)	-0.6 (-3.0, 1.8)	0.6691	0.1 (0.0, 0.7)
	Placebo	21	-5.6 (1.0)			
	REL-1017 50 mg	21	-5.9 (1.0)	-0.4 (-2.7, 2.0)	0.7869	0.1 (0.0, 0.6)
	Placebo	21	-5.6 (1.0)			
Day 7 (EDP), Postdose	REL-1017 25 mg	19	-7.6 (1.2)	-2.8 (-5.4, -0.2)	0.0752	0.6 (0.0, 1.1)
	Placebo	21	-4.8 (1.1)			
	REL-1017 50 mg	21	-7.5 (1.1)	-2.7 (-5.2, -0.2)	0.0757	0.6 (0.0, 1.1)
	Placebo	21	-4.8 (1.1)			
Day 14 (EOP)	REL-1017 25 mg	16	-6.7 (1.0)	-3.1 (-5.2, -0.9)	0.0816	0.8 (0.2, 1.3)
	Placebo	20	-3.6 (0.9)			
	REL-1017 50 mg	18	-8.0 (0.9)	-4.3 (-6.4, -2.3)	0.0008	1.1 (0.6, 1.7)
	Placebo	20	-3.6 (0.9)			

Abbreviations: SDQ=Symptoms of Depression Questionnaire; CI=confidence interval; EDP=End of Dosing Period; EOP=End of Observation Period; ITT=Intent-To-Treat; LS=least squares; N=number of subjects; SE=standard error.

Note: Baseline was defined as the last available, valid, non-missing assessment before first study drug administration.

a. The LS means, LS mean difference, and the associated 90% CI and p-value for change from baseline are based on a mixed model of repeated measures with fixed effect terms for treatment, stratification (concomitant antidepressant drug) factor, visit (Day 2, Day 4, and Day 7, Day 14), interaction between treatment and visit, and baseline endpoint as a covariate.

b. Difference between REL-1017 and placebo in the LS means.

c. p-value for testing the null hypothesis of no difference.

d. Cohen's effect size was calculated on the basis of the LS mean differences and pooled standard deviations.

Supplemental Table S-10. Analysis of CGI-S at EDP (Day 7) and EOP (Day 14) (FAS Population) REL-1017 Trial

Visit, Time Point	Treatment Group	N	LS Mean (SE)[a]	Difference of LS Means Drug vs Placebo (90% CI)[b]	P-Value[c]	Effect Size[d]
Day 2, Predose	Placebo	22	-0.3 (0.2)			
	REL-1017 25 mg	19	-0.6 (0.2)	-0.2 (-0.6, 0.2)	0.3499	0.3
	REL-1017 50 mg	21	-0.5 (0.2)	-0.2 (-0.5, 0.2)	0.4936	0.2
Day 4, Postdose	Placebo	21	-0.6 (0.2)			
	REL-1017 25 mg	19	-1.1 (0.3)	-0.6 (-1.1, 0.0)	0.0951	0.5
	REL-1017 50 mg	21	-1.3 (0.2)	-0.7 (-1.3, -0.2)	0.0272	0.7
Day 7 (EDP), Postdose	Placebo	21	-0.8 (0.3)			
	REL-1017 25 mg	19	-1.7 (0.3)	-1.0 (-1.6, -0.3)	0.0245	0.7
	REL-1017 50 mg	21	-1.7 (0.3)	-0.9 (-1.6, -0.2)	0.0253	0.7
Day 14 (EDP)	Placebo	20	-0.7 (0.3)			
	REL-1017 25 mg	16	-1.6 (0.3)	-0.9 (-1.7, -0.2)	0.0454	0.7
	REL-1017 50 mg	18	-2.0 (0.3)	-1.3 (-2.0, -0.6)	0.0043	0.9

Abbreviations: CGI-S=Clinical Global Impressions of Severity; CI=confidence interval; EDP=End of Dosing Period; EOP=End of Observation Period; FAS=Full Analysis Set; LS=least squares; N=number of subjects; SE=standard error.

Note: Baseline was defined as the last available, valid, non-missing assessment before first study drug administration.

a. The LS means, LS mean difference, and the associated 90% CI and p-value for change from baseline are based on a mixed model of repeated measures with fixed effect terms for treatment, stratification concomitant antidepressant drug factor, visit (Day 2, Day 4, and Day 7, Day 14), interaction between treatment and visit, and baseline endpoint as a covariate.

b. Difference between REL-1017 and placebo in the LS means.

c. p-value for testing the null hypothesis of no difference.

d. Cohen's effect size was calculated on the basis of the LS mean differences and pooled standard deviations.

Supplemental Table S-11. Analysis of CGI-I at EDP (Day 7) and EOP (Day 14) (FAS Population) REL-1017 Trial

Visit, Time Point	Treatment Group	N	LS Mean (SE)[a]	Difference of LS Means Drug vs Placebo (90% CI)[b]	P-Value[c]	Effect Size[d]
Day 2, Predose	Placebo	22	3.6 (0.2)			
	REL-1017 25 mg	19	3.4 (0.2)	-0.2 (-0.6, 0.2)	0.3184	0.3
	REL-1017 50 mg	21	3.3 (0.2)	-0.3 (-0.6, 0.1)	0.2424	0.4
Day 4, Postdose	Placebo	21	3.4 (0.2)			
	REL-1017 25 mg	19	2.7 (0.2)	-0.7 (-1.2, -0.2)	0.0176	0.8
	REL-1017 50 mg	21	2.6 (0.2)	-0.8 (-1.2, -0.3)	0.0081	0.8
Day 7 (EDP), Postdose	Placebo	21	3.2 (0.2)			
	REL-1017 25 mg	19	2.4 (0.2)	-0.8 (-1.3, -0.2)	0.0177	0.8
	REL-1017 50 mg	21	2.3 (0.2)	-0.9 (-1.4, -0.3)	0.0072	0.9
Day 14 (EDP)	Placebo	20	3.3 (0.3)			
	REL-1017 25 mg	16	2.6 (0.3)	-0.7 (-1.3, 0.0)	0.0895	0.8
	REL-1017 50 mg	18	2.3 (0.3)	-1.0 (-1.6, -0.4)	0.0109	0.8

Abbreviations: CGI-I, Clinical Global Impressions of Improvement; CI, confidence interval; EDP, end of dosing period; EOP, End of Observation Period; FAS, Full Analysis Set; LS, least squares; SE, standard error.

Note: The baseline value of the CGI-S scores was used as the baseline value of the CGI-I score.

a. The LS means, LS mean difference, and the associated 90% CI and p-value for CGI-I scale are based on a mixed model of repeated measures with fixed effect terms for treatment, stratification (concomitant antidepressant drug) factor, visit (Day 2, Day 4, Day 7, Day 14), interaction between treatment and visit, and CGI-S baseline endpoint as a covariate.

b. Difference between REL-1017 and placebo in the LS means.

c. p-value for testing the null hypothesis of no difference.

d. Cohen's effect size was calculated on the basis of the LS mean differences and pooled standard deviations.

Supplemental Table S-12. Number and Percentage of CGI-I Scales by Visit (FAS Population)

REL-1017 Trial

Visit, Time point	CGI-I Scales	Placebo (N=22)	REL-1017 25 mg (N=19)	REL-1017 50 mg (N=21)	All Patients (N=62)
Day 2, Predose	1=Very Much Improved	0	0	0	0
	2=Much Improved	2 (9.1%)	3 (15.8%)	2 (9.5%)	7 (11.3%)
	3=Minimally Improved	5 (22.7%)	6 (31.6%)	10 (47.6%)	21 (33.9%)
	4=No Change	15 (68.2%)	10 (52.6%)	9 (42.9%)	34 (54.8%0
	5=Minimally Worse	0	0	0	0
	6=Much Worse	0	0	0	0
	7=Very Much Worse	0	0	0	0
Day 4	1=Very Much Improved	1 (4.8%)	1 (5.3%)	3 (14.3%)	5 (8.2%)
	2=Much Improved	2 (9.5%)	8 (42.1%)	6 (28.6%)	16 (26.2%)
	3=Minimally Improved	6 (28.6%)	6 (31.6%)	8 (38.1%)	20 (32.8%)
	4=No Change	12 (57.1%)	4 (21.1%)	4 (19.0%)	20 (32.8%)
	5=Minimally Worse	0	0	0	0
	6=Much Worse	0	0	0	0
	7=Very Much Worse	0	0	0	0
Day 7 (EDP), Postdose	1=Very Much Improved	2 (9.5%)	3 (15.8%)	4 (19.0%)	9 (14.8%)
	2=Much Improved	4 (19.0%)	8 (42.1%)	9 (42.9%)	21 (34.4%)
	3=Minimally Improved	3 (14.3%)	5 (26.3%)	5 (23.8%)	13 (21.3%)
	4=No Change	12 (57.1%)	3 (15.8%)	3 (14.3%)	18 (29.5%)
	5=Minimally Worse	0	0	0	0
	6=Much Worse	0	0	0	0
	7=Very Much Worse	0	0	0	0
Day 14 (EOP), Postdose	1=Very Much Improved	2 (10.0%)	2 (12.5%)	7 (38.9%)	11 (20.4%)
	2=Much Improved	4 (20.0%)	7 (43.8%)	2 (11.1%)	13 (24.1%)
	3=Minimally Improved	1 (5.0%)	3 (18.8%)	7 (38.9%)	11 (20.4%)
	4=No Change	12 (60.0%)	4 (25.0%)	1 (5.6%)	17 (31.5%)

	5=Minimally Worse	1 (5.0%)	0	1 (5.6%)	2 (3.7%)
	6=Much Worse	0	0	0	0
	7=Very Much Worse	0	0	0	0

Abbreviations: CGI-I=Clinical Global Impressions of Improvement; EDP=End of Dosing Period; EOP=End of Observation Period; FAS=Full Analysis Set; N=number of subjects

Percentages were calculated on the basis of the number of subjects in the FAS population within the treatment arm at each visit.

Supplemental Table S-13. Treatment-Emergent Adverse Events with Incidence in Any Treatment Group >5% by System Organ Class and Preferred Term (SAS Population) REL-1017 Trial

System Organ Class/ Preferred Term	Placebo (N=22)	REL-1017 25 mg (N=19)	REL-1017 50 mg (N=21)	All Patients (N=62)
Subjects with any TEAE[a]	12 (54.5%)	9 (47.4%)	15 (71.4%)	36 (58.1%)
Gastrointestinal disorders	8 (36.4%)	5 (26.3%)	5 (23.8%)	18 (29.0%)
Constipation	3 (13.6%)	1 (5.3%)	3 (14.3%)	7 (11.3%)
Nausea	2 (9.1%)	1 (5.3%)	2 (9.5%)	5 (8.1%)
Diarrhoea	3 (13.6%)	0	0	3 (4.8%)
Abdominal discomfort	2 (9.1%)	0	0	2 (3.2%)
Dyspepsia	0	2 (10.5%)	0	2 (3.2%)
Flatulence	0	1 (5.3%)	0	1 (1.6%)
Vomiting	0	1 (5.3%)	0	1 (1.6%)
Nervous system disorders	6 (27.3%)	4 (21.1%)	6 (28.6%)	16 (25.8%)
Headache	3 (13.6%)	2 (10.5%)	3 (14.3%)	8 (12.9%)
Somnolence	2 (9.1%)	1 (5.3%)	1 (4.8%)	4 (6.5%)
Dizziness	1 (4.5%)	1 (5.3%)	1 (4.8%)	3 (4.8%)
Sedation	1 (4.5%)	1 (5.3%)	0	2 (3.2%)
Infections and Infestations	2 (9.1%)	1 (5.3%)	1 (4.8%)	4 (6.5%)
Upper respiratory tract infection	2 (9.1%)	0	0	2 (3.2%)
Urinary tract infection	0	1 (5.3%)	0	1 (1.6%)
Musculoskeletal and connective tissue disorders	0	1 (5.3%)	3 (14.3%)	4 (6.5%)
Back pain	0	1 (5.3%)	2 (9.5%)	3 (4.8%)
General disorders and administration site conditions	0	1 (5.3%)	2 (9.5%)	3 (4.8%)
Fatigue	0	1 (5.3%)	0	1 (1.6%)
Investigations	0	0	3 (14.3%)	3 (4.8%)
Weight decreased	0	0	2 (9.5%)	2 (3.2%)
Cardiac disorders	0	1 (5.3%)	0	1 (1.6%)
Palpitations	0	1 (5.3%)	0	1 (1.6%)
Renal and urinary disorders	0	1 (5.3%)	0	1 (1.6%)

System Organ Class/ Preferred Term	**Placebo** **(N=22)**	**REL-1017 25 mg** **(N=19)**	**REL-1017 50 mg** **(N=21)**	**All Patients** **(N=62)**
Pollakiuria	0	1 (5.3%)	0	1 (1.6%)
Skin and subcutaneous tissue disorders	0	1 (5.3%)	0	1 (1.6%)
Pruritus	0	1 (5.3%)	0	1 (1.6%)

Abbreviations: AE=adverse event; MedDRA=Medical Dictionary for Regulatory Activities; PT=preferred term; SOC=system organ class, TEAE=treatment-emergent adverse event.

Notes: A subject who experienced multiple events within an SOC or PT was counted once for that SOC or PT. System organ class and preferred terms are sorted in decreasing frequency on the basis of the total number of subjects. Adverse events were coded with MedDRA Version 21.0. Percentages are calculated using N as the denominator.

[a] TEAE is an AE which starts or worsens after treatment with the study drug.

Supplemental Table S-14. Most Commonly Reported Concomitant Medications (SAS Population) REL-1017 Trial

ATC Class/ Preferred Medication Name	Placebo (N=22)	REL-1017 25 mg (N=19)	REL-1017 50 mg (N=21)	All Subjects (N=62)
Subjects with any concomitant medication	22	19	21	62
ACE Inhibitors, plain	4 (18.2%)	3 (15.8%)	2 (9.5%)	9 (14.5%)
Lisinopril	3 (13.6%)	3 (15.8%)	2 (9.5%)	8 (12.9%)
Benazepril	1 (4.5%)	0	0	1 (1.6%)
Dihydropyridine derivatives	2 (9.1%)	3 (15.8%)	3 (14.3%)	8 (12.9%)
Amlodipine	1 (4.5%)	2 (10.5%)	3 (14.3%)	6 (9.7%)
Amlodipine besilate	1 (4.5%)	1 (5.3%)	0	2 (3.2%)
HMG CoA reductase inhibitors	6 (27.3%)	6 (31.6%)	1 (4.8%)	13 (21.0%)
Atorvastatin	2 (9.1%)	4 (21.1%)	1 (4.8%)	7 (11.3%)
Rosuvastatin	0	2 (10.5%)	0	2 (3.2%)
Lovastatin	1 (4.5%)	0	0	1 (1.6%)
Pravastatin	1 (4.5%)	0	0	1 (1.6%)
Rosuvastatin calcium	1 (4.5%)	0	0	1 (1.6%)
Simvastatin	1 (4.5%)	0	0	1 (1.6%)
Other Antidepressants	8 (36.4%)	8 (42.1%)	8 (38.1%)	24 (38.7%)
Bupropion hydrochloride	4 (18.2%)	4 (21.1%)	2 (9.5%)	10 (16.1%)
Bupropion	1 (4.5%)	2 (10.5%)	1 (4.8%)	4 (6.5%)
Venlafaxine	1 (4.5%)	1 (5.3%)	2 (9.5%)	4 (6.5%)
Duloxetine hydrochloride	0	0	3 (14.3%)	3 (4.8%)
Duloxetine	1 (4.5%)	1 (5.3%)	0	2 (3.2%)
Vortioxetine hydrobromide	1 (4.5%)	0	1 (4.8%)	2 (3.2%)
Propionic acid derivatives	7 (31.8%)	3 (15.3%)	4 (19.0%)	14 (22.6%)
Ibuprofen	7 (31.8%)	2 (10.5%)	4 (19.0%)	13 (21.0%)
Naproxen	0	1 (5.3%)	0	1 (1.6%)
Selective serotonin reuptake inhibitors	16 (72.7%)	11 (57.9%)	13 (61.9%)	40 (64.5%)
Citalopram	5 (22.7%)	4 (21.1%)	2 (9.5%)	11 (17.7%)
Sertraline	3 (13.6%)	2 (10.5%)	4 (19.0%)	9 (14.5%)

ATC Class/ Preferred Medication Name	Placebo (N=22)	REL-1017 25 mg (N=19)	REL-1017 50 mg (N=21)	All Subjects (N=62)
Sertraline hydrochloride	4 (18.2%)	0	2 (9.5%)	6 (9.7%)
Escitalopram	1 (4.5%)	1 (5.3%)	1 (4.8%)	3 (4.8%)
Citalopram hydrobromide	0	2 (10.5%)	1 (4.8%)	3 (4.8%)
Escitalopram oxalate	1 (4.5%)	0	1 (4.8%)	2 (3.2%)
Fluoxetine	0	1 (5.3%)	1 (4.8%)	2 (3.2%)
Fluoxetine hydrochloride	1 (4.5%)	0	1 (4.8%)	2 (3.2%)
Paroxetine hydrochloride	1 (4.5%)	1 (5.3%)	0	2 (3.2%)
Thiazides, plain	1 (4.5%)	3 (15.8%)	5 (23.8%)	9 (14.5%)
Hydrochlorothiazide	1 (4.5%)	3 (15.8%)	5 (23.8%)	9 (14.5%)

Abbreviations: ACE=angiotensin-converting enzyme; ATC=Anatomical Therapeutic Chemical; HMG-CoA=β-Hydroxy β-methylglutaryl-CoA; N=number of subjects; WHO-DD=World Health Organization Drug Dictionary.

Note: Concomitant medications were defined as any medication other than the study drug that a patient received on or after the study drug administered during study participation. Medications were coded using WHO-DD version March 2016. Percentages were calculated using N as the denominator. ATC classes are sorted by alphabetical order and PTs are sorted in decreasing frequency within each ATC class on the basis of the total number of subjects. A subject was counted only once if the subject reported the same medications more than once within ATC class. There were 62 subjects taking 65 antidepressant drugs. Three patients were recorded as taking two antidepressant drugs each, while all others were taking only one antidepressant drug. Two patients in the placebo group were taking two antidepressant drugs each: sertraline + bupropion and citalopram + bupropion, respectively. One patient in the 50 mg treatment group was taking duloxetine + bupropion.

Supplemental Table S-15. COWS Total Score by Visit, REL-1017 Trial

	COWS Total Score, Mean (SD)		
Treatment Group	**Day 8**	**Day 9**	**Day 14**
Placebo	0.7 (1.1)	0.6 (1.0)	0.7 (1.1)
REL-1017 25 mg	0.7 (0.9)	0.4 (0.6)	0.5 (0.8)
REL-1017 50 mg	0.4 (0.7)	1.0 (1.5)	0.5 (1.2)
Overall	0.6 (0.9)	0.7 (1.1)	0.6 (1.0)

Abbreviations: COWS=Clinical Opiate Withdrawal Scale; SD=standard deviation.

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