Janssen Research & Development *

Clinical Protocol

A Double-Blind, Doubly-Randomized, Placebo-Controlled Study of Intranasal Esketamine in an Adaptive Treatment Protocol to Assess Safety and Efficacy in Treatment-Resistant Depression (SYNAPSE)

Protocol ESKETINTRD2003; Phase 2a
AMENDMENT INT-3

JNJ-54135419 (esketamine)

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This study will be conducted under US Food & Drug Administration IND regulations (21 CFR Part 312).

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Prepared by: Janssen Research & Development, LLC
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GCP Compliance: This study will be conducted in compliance with Good Clinical Practice, and applicable regulatory requirements.

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PROTOCOL AMENDMENTS

Original Protocol issued 8 Oct 2013.

Amendments are listed beginning with the most recent amendment.

Amendment INT-3 (28 April 2014)

This amendment is considered to be substantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the European Union.

The overall reason for the amendment is to add objective cognitive assessments and an additional time point for urinalysis and the BPIC-SS for Panel A.

Applicable Section(s)	Description of Change(s)
Rationale: Add Cogstate® computerized test battery and Hopkins Verbal Learning Test-Revised (HVLT-R) to formally assess cognition in Panel A subjects receiving esketamine in the extended 9-week optional open label treatment phase.	
Synopsis; Abbreviations; Time and Events Schedule; 2.1 Objectives; 3.1 Overview of Study Design; 3.2 Study Design Rationale; 9.1.2 Screening Phase; 9.1.3 Double-Blind Treatment Phase; 9.1.4 Optional Open-Label Treatment Phase; 9.7 Safety Evaluations; 11.8 Safety Analyses; 15. Study Specific Materials	A secondary objective ("i") was added to indicate that the effects of intranasal esketamine on cognition will be assessed using the Cogstate® computerized test battery and Hopkins Verbal Learning Test-Revised (HVLT-Revised). The Cogstate® computerized test battery and Hopkins Verbal Learning Test-Revised (HVLT-R) have been added at the following 5 time points for Panel A: Visit 2 (Day 1, predose), Visit 11 (Day 25, predose), Visit 14 (Day 46, predose), Visit 16 (Day 74, predose), and Visit 18 (2 weeks after the last dose). The order of administration has been provided: "Cogstate® computerized test battery and HVLT-R: HVLT-R , detection, identification, one card learning, one back, Groton maze learning test, HVLT-R-delayed." Text has been added to indicate that within 1 week prior to the planned first dose of study medication, Panel A subjects will have two mandatory practice sessions on the Cogstate® computerized test battery performed at the clinical site. Information regarding the rationale, components, and statistical analysis of the Cogstate® computerized cognitive tests and HVLT-R were added. Text was added to indicate equipment and materials for Cogstate® computerized test battery and HVLT-R will be provided.

References	The following references were added: Benedict, R.H.B., Schretlen, D., Groninger, L., & Brandt, J. (1998). Hopkins Verbal Learning Test-Revised: Normative data and analysis of inter-form and test-retest reliability. The Clinical Neuropsychologist, 12, 43-55. Maruff, P., Werth, J., Giordani, B., Caveney, A.F., Feltner, D., & Snyder, P.J. (2006). A statistical approach for classifying change in cognitive function in individuals following pharmacologic challenge: an example with alprazolam. Psychopharmacology, 186, 7-17. Maruff, P., Thomas, E., Cysique, L., Brew, B., Collie, A., Snyder, P., & Pietrzak, R.H. (2009). Validity of the CogState Brief Battery: Relationship to Standardized Tests and Sensitivity to Cognitive Impairment in Mild Traumatic Brain Injury, Schizophrenia, and AIDS Dementia Complex. Archives of Clinical Neuropsychology, 24, 165-178. Pietrzak, R.H., Maruff, P., Mayes, L.C., Roman, S.A., Sosad, J.A., & Snyder, P.J. (2008). An examination of the construct validity and factor structure of the Groton Maze Learning Test, a new measure of spatial working memory, learning efficiency, and error monitoring. Archives of Clinical Neuropsychology, 23, 433-445. Snyder, P.J., Werth, J., Giordani, B., Caveney, A.F., Feltner, D., & Maruff, P. (2005). A method for determining the magnitude of change across different cognitive functions in clinical trials: The effects of acute administration of two different doses alprazolam. Human Psychopharmacology: Clinical and Experimental, 20, 263-273.
Rationale: Include an additional BPIC-SS assessment and urinalysis on Day 46 for Panel A to monitor for symptoms of cystitis.	
Time and Events Schedule	A BPIC-SS assessment and urinalysis have been added on Day 46 for Panel A.
Rationale: Avoid potential impact on cognitive and/or efficacy assessments.	
4.3 Prohibitions and Restrictions; 8. Prestudy and Concomitant Therapy; Attachment 1	Benzodiazepines are now prohibited from 12 hours prior to the start of each study drug administration (previously was 8 hours). The following new prohibition and restrictions were added: • Benztropine is prohibited from 8 hours prior to the start of cognition testing (i.e., Cogstate® computerized test battery and HVLT-R). • Non-benzodiazepine sleep aids are prohibited from 12 hours prior to the start of cognition testing (i.e., Cogstate® computerized test battery and HVLT-R).
Attachment 1	Text added to state that Cough /Cold Preparations containing diphenhydramine or dextromethorphan used PRN are not permitted within 12 hours of study medication dosing.
Rationale: Minor errors noted.	

Abbreviations; Synopsis; 3.1 Overview of Study Design; 3.2 Study Design Rationale; 4.1 Inclusion Criteria;4.2 Exclusion Criteria; 9.1.2 Screening Phase;9.2.1 Evaluations; 9.8 Other Evaluations	In Inclusion Criterion #4, text was added to clarify that subjects must meet Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition-**Text Revised** (DSM-IV-**TR**) diagnostic criteria for MDD ((DSM-IV-**TR** 296.22, 296.23, 296.32, or 296.33). The text previously referred to DSM-IV only. In Exclusion Criteria #4 and #5, text was added to clarify that DSM-IV-TR diagnoses will be used: • "4. Subject has a current **DSM-IV-TR** diagnosis of bipolar or related disorders, intellectual disability, or cluster b personality disorder (e.g., borderline personality disorder, antisocial personality disorder, histrionic personality disorder, and narcissistic personality disorder)." • "5. Subject has a current **DSM-IV-TR** diagnosis of bipolar or related disorders, intellectual disability, or cluster b personality disorder (e.g., borderline personality disorder, antisocial personality disorder, histrionic personality disorder, and narcissistic personality disorder)." Text was also added to indicate the M.I.N.I. and IDS-C_{30} are for DSM-IV-**TR.**
Protocol Amendments (INT-2) Table	Bolded text was added to the "Protocol Amendments" Table for INT-2, within the description of the changes: "The Bladder Pain/Interstitial Cystitis Symptom Score (BPIC-SS), a patient-reported assessment, was added to the protocol at the following time points to evaluate for potential treatment-emergent cystitis: Day 1, 15, **Early Termination**, 25, **46**, 74, and the 2nd follow up visit (performed 2 weeks after the last dose)."
Time and Events Schedule	"25" has been added in Footnote 25 (relating to the PHQ-9) to be consistent with the Protocol Amendment (INT-2) description of changes. Footnote 25 now states: "Panel A: predose on Day 15, **25,** 39, 60, and 74. Panel B: predose on Day 15 and 25."
9.1 Study Procedures	Table 6 has been revised to only be applicable to Panel A. In "*Table 6. Approximate Volume of Blood to be Collected From Each Panel A Subject*," the additional pharmacokinetic sample at 6 hours postdose on Day 1 and 11 for Panel A that was added in INT-2 was missing from the table. For this reason, the table was updated as follows: • The number of pharmacokinetic samples was increased from "7" to "9", which also resulted in the following revisions: o an increase in the total blood volume for the pharmacokinetic samples from "28 mL" to "36 mL" o an increase in the approximate total blood volume for the double-blind treatment phase from "104.5 mL" to "112.5 mL" o an increase in the approximate total blood volume for the study will from "153 mL" to "161 mL"
9.1 Study Procedures	Table 7 was created to describe the blood samples being collected for Panel B ("*Table 7. Approximate Volume of Blood to be Collected From Each Panel B Subject*").

Amendment INT-2 (04 March 2014)

This amendment is considered to be substantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the European Union.

The overall reason for the amendment: The overall reason for the amendment is to extend the duration of the optional open label treatment phase for Panel A and to incorporate an additional safety evaluation to assess for potential treatment-emergent cystitis.

Applicable Section(s)	Description of Change(s)
Rationale: Extend the duration of the optional open label treatment phase for Panel A only to allow the exploration of the efficacy and safety of intranasal esketamine when tapered from twice-weekly dosing to once per week dosing and then dosing once every other week.	
Synopsis; Time and Events Schedule; 3.1 Overview of Study Design; 3.2 Study Design Rationale; 9.1.3 Double-Blind Treatment; 9.1.4 Optional Open-Label Treatment; 16.1 Study-Specific Design Considerations;	• An additional 5 study visits, which include dosing of intranasal esketamine, were added to the optional open label treatment phase on Day 32, 39, 46, 60, and 74. Therefore, Panel A subjects can receive up to a total of 9 doses of intranasal esketamine in the optional open label treatment phase (on Days 15, 18, 22, 25, 32, 39, 46, 60, and 74). • The total duration of the subject's participation in Panel A was revised from "11 to 16 weeks" to "14 to 23 weeks." • The study visits numbers for the optional open label treatment phase now range from Visit 8B to 16. As a result, the study visit numbers for the posttreatment phase have been changed to Visit 17 to 20. • The Panel A study design figure was updated to include the 5 additional study visits/doses. • The following text regarding the esketamine doses was modified to: "All subjects will start with intranasal esketamine 56 mg on Day 15. Subsequent doses on Days 18, 22, ~~and~~ 25, **32, 39, and 46** can be down-titrated to the next lower dose, or titrated up to the next higher dose ~~at any visit~~ if **clinically indicated** ~~desired~~ based on the Investigator's clinical judgment of efficacy and tolerability. **The two remaining doses of esketamine administered after Day 46 (i.e., Day 60 and 74) will remain stable. No further dose adjustment is permitted after Day 46; the same dose administered on Day 46 will be administered on Day 60 and 74.**"
Rationale: As a precautionary measure the study will exclude subjects meeting diagnostic criteria for current or prior history of post-traumatic stress disorder (PTSD) or obsessive-compulsive disorder (OCD) based on the diagnostic interview conducted at screening.	
Synopsis; 4.2 Exclusion Criteria	Exclusion Criterion # 5 has been revised to: "Subject has a current or prior diagnosis of a psychotic disorder, ~~or~~ MDD with psychosis, **post-traumatic stress disorder (PTSD), or obsessive compulsive disorder (OCD)**."
Rationale: As a precautionary measure, subjects will be closely monitored for symptoms of cystitis.	

Synopsis; T&E; 1.1.2 Clinical Studies; Section 3.2 Study Design Rationale; 9.1.3 Double-Blind Treatment; 9.1.4 Optional Open Label Treatment; 9.7 Safety Evaluations; 11.8 Safety Evaluations; References	• The Bladder Pain/Interstitial Cystitis Symptom Score (BPIC-SS), a patient-reported assessment, was added to the protocol at the following time points to evaluate for potential treatment-emergent cystitis: Day 1, 15, 25, 74, and the 2nd follow up visit (performed 2 weeks after the last dose). • A secondary objective was added for this new safety evaluation. The following text was added as a subbullet to Secondary Objective #2 "*Potential treatment-emergent symptoms of cystitis using the Bladder Pain/ Interstitial Cystitis Symptom Score (BPIC-SS)*" • Text has been added to indicate that if a subject has a BPIC-SS score > 18, with no evidence of a urinary tract infection on urinalysis and microscopy, the subject will be referred to a specialist for evaluation. • The order of administration of the BPIC-SS in the double-blind and optional open-label treatment phases has been added. • Text was added to indicate the BPIC-SS will be analyzed similar to the CADSS, BPRS+, and MOAA/S. For the BPIC-SS, descriptive statistics of each of the scores and changes from predose will be summarized at each scheduled time point. • The following reference was added: "*Humphrey L, Arbuckle R, Moldwin R, Nordling J, van de Merwe J, Meunier J, Crook T, Abraham L. The Bladder Pain/Interstitial Cystitis Symptom Score: Development, Validation, and Identification of a Cut Score. European Urology 2012; 61:271 - 279.*"
Rationale: Without increasing the total number of clinical laboratory tests (blood chemistry, hematology, urinalysis) for a Panel A subject, modify the time points to ensure these tests are performed over the duration of the extended optional open label treatment phase.	
Synopsis	The time points for clinical laboratory tests (blood chemistry, hematology, urinalysis) for Panel A during the optional open-label treatment phase were revised from "Day 18 and Day 25" to "Day 25 and Day 74."
Rationale: Monitor respiratory rate at all scheduled vital sign assessment time points on all dosing days.	
Synopsis; Time and Events Schedule; 2.1 Objectives	In the Time and Events Schedules, the rows that stated "Vital Signs: BP and HR only" has been revised to "blood pressure, heart rate, and respiratory rate". All vital sign time points will now include blood pressure, heart rate, respiratory rate, and temperature.
Rationale: Add information regarding the practice intranasal devices being provided for each subject.	
Synopsis; 3.1 Overview of Study Design; 6. Dosage and Administration; 9.1.3 Double-Blind Treatment; 15. Study Specific Materials	Text was added to indicate that practice/demonstration intranasal devices will be provided. Prior to the first dose, subjects will practice spraying (into the air) the demonstration intranasal device that is filled with water.
Rationale: Specify the study visits that will require subjects to remain at the clinical site longer than the minimum duration of 2 hours.	

Synopsis; 3.1 Overview of Study Design; 4.3 Prohibitions and Restrictions; 6. Dosage and Administration; 9.1.3 Double-Blind Treatment; 9.1.4 Optional Open-Label	The following text revision was made: "On all dosing days, all subjects must remain at the clinical site until at least 2 hours postdose; ~~or~~ **a** longer **duration will be** ~~if~~ required **on Day 1 and 11 (up to 6 hours postdose for study procedures) and on Day 25 (3 hours postdose for study procedures**), and should be accompanied by a responsible adult when released from the clinical site."
Rationale: Permit enrollment of subjects with signs and symptoms of rhinitis at Screening, and provide guidance regarding how to manage a subject that develops nasal congestion during the study.	
Synopsis; 4.2 Exclusion Criteria	The text "*or signs and symptoms of rhinitis*" have been removed from Exclusion Criterion #10.
6. Dosage and Administration	The following text was added: "**If the subject has nasal congestion on the dosing day, it is recommended the dosing day be delayed (per the permitted visit window). If an intranasal decongestant is used to reduce congestion, it cannot be used within 12 hours prior to study medication dosing. If a delay is not feasible, the subject should be instructed to blow his/her nose prior to the first intranasal spray**."
Rationale: Add an exclusion criterion that defines clinically-significant ECG abnormalities at Screening that are exclusionary.	
Synopsis; 4.2 Exclusion Criteria	Exclusion criterion #25 was added: "Has clinically significant ECG abnormalities, defined as: – QTcB interval ≥470 msec at Screening or predose on Day 1 – Evidence of 2nd and 3rd degree AV block, or 1st degree AV block with PR interval >200ms, left bundle branch block (LBBB) or right bundle branch block (RBBB) at Screening or predose on Day 1. – At Screening, history of additional risk factors for torsades des pointes (e.g. heart failure, hypokalemia, family history of Long QT Syndrome, or the use of concomitant medications that prolong the QT/QTc interval)"
Rationale: Provide clarification of exclusion criteria.	

4.2 Exclusion Criteria	• Exclusion criterion #1: Fibromyalgia has been added. • The following revision was made to Exclusion criterion #3: "*Subject has alanine aminotransferase (ALT) ~~and~~ **or** aspartate aminotransferase (AST) values ≥2 x the upper limit of normal **at Screening***" • The following modifications were made to Exclusion criterion #13: "*Subject has a positive test result(s) for drugs of abuse (including barbiturates, methadone, opiates, cocaine, ~~cannabinoids~~, **phencyclidine**, and amphetamine/methamphetamine) at Screening **or predose on Day 1**. **In addition to the drugs of abuse previously mentioned, cannabinoids will also be tested on Day 1***." In addition, the following subbullet was added "**A positive test result for cannabinoids predose on Day 1 is exclusionary**." • The following addition was made to Exclusion criterion #14: "*Subject has a positive test result(s) for alcohol at Screening **or predose on Day 1***."
Rationale: Pharmacogenomic evaluations will only be used for research related to esketamine.	
Synopsis; 3.2 Study rationale; 9.6 Pharmacogenomic Evaluations	Text that indicated DNA samples would be used for research related to esketamine "or depression" has been been revised to indicate these samples would be used for research related to esketamine only.
Rationale: Incorporate a potential interim pharmacokinetic/pharmacodynamics analysis for Panel A.	
Synopsis; 9.4 Pharmacokinetic/Pharmacodynamic Evaluations; 11.5 Pharmacokinetic/Pharmacodynamic Analyses	The following text has been added: "**A pharmacokinetic/pharmacodynamics analysis may be performed after at least 80% of Panel A subjects have completed the double-blind treatment phase. Subject pharmacokinetic data, MADRS total score, and CADSS scores would be matched to the treatment assignment by an authorized external group who are not part of the study team. The data will be anonymized (i.e., identification numbers would be removed or changed). The results of the analysis would be used to assist in the evaluation of potential intranasal esketamine doses for future clinical studies**. The results of **the~~is~~ pharmacokinetic/pharmacodynamics evaluation** ~~analysis~~ would be presented in a separate report.""
Rationale: Evaluate subjects for potential withdraw symptoms following the last dose of study medication in the optional open-label treatment phase.	
Time and Events Schedule	The PWC-20 assessment was added to the 3 hour postdose time point at the last visit of the optional open label treatment phase.
Rationale: Provide guidance on permitted visit windows in optional open-label and posttreatment phases.	
Time and Events Schedule	Footnote 1 was revised to "Visit window: Day 15 (+/- 1 day), ~~all other visits~~ **Day 18, 22, and 25** can occur +/- 2 days. **The follow up visits can occur +/- 3 days. For Panel A only: Day 32, 39, and 46 can occur +/- 2 days, Day 60 and 74 can occur +/- 3 days.**"
Rationale: Provide clinical sites with guidance regarding staff and equipment requirements on dosing days.	
15. Study Specific Materials	"**Guidance for minimum requirements for site staff and equipment on dosing days**" has been added to the list of materials being provided.
Rationale: Revise study prohibitions and restrictions.	

4.3 Prohibitions and Restrictions; 8. Prestudy and Concomitant Therapy	The following revisions were made: – "Subjects must abstain from using alcohol within 24 hours before and after study drug administration. Subjects must abstain from prohibited drugs **or substances** from Screening through to the end of the treatment phase (double-blind and optional open-label treatment phase, if applicable)." – "Subjects should not ingest grapefruit juice, Seville oranges, or quinine ~~for 24 hours~~ **72 hours** before an intranasal dose of esketamine is to be administered." – "Potent CYP3A4 inhibitors are not permitted within 1 week, or within a period less than 5 times the drug's half-life, whichever is longer, before the first dose of study medication and **until at least 1 day after the last dose of study medication** ~~throughout the study~~." – "Potent CYP3A4 inducers are not permitted for 30 days prior to the first dose of study medication and ~~throughout the study~~ **until at least 1 day after the last dose of study medication**. Examples of potent CYP3A4 inducers are provided in Attachment 1." – "Any new psychotropic medication(s) started during screening, or any increase in the dose of a currently prescribed (allowed) psychotropic medication(s) during screening **until at least 1 day after the last dose of study medication** ~~and throughout the study.~~" – "Treatment with any MAO-inhibitor within the past 2 weeks prior to Day 1 dosing **until at least 1 day after the last dose of study medication** ~~and throughout the study~~." – The following text was copied from Section 8 into Section 4.3 "**Benzodiazepines are prohibited from 8 hours prior to each study drug administration."**
Rationale: To further characterize the pharmacokinetics of esketamine.	
Synopsis; Time and Events Schedule	A pharmacokinetic sample was added at 6 hours postdose on Day 1 and 11.
Rationale: The PHQ-9 was added to provide data on this measure in the event it may be used as the self-report depression rating scale in the phase 3 program.	

Synopsis; Time and Events Schedule; Abbreviations; 2.1 Objectives; 3.2 Study Design Rationale; 9.1.3 Double Blind Treatment; 9.1.4 Optional Open Label Treatment; 9.2.1 Efficacy Evaluations; References	• The PHQ-9 assessment was added at the following time points: – Panel A: Day 1 (predose); Day 15 of optional open label treatment phase (i.e. Visit 8B), Day 25, 39, 60 and 74. – Panel B: Day 1 (predose); Day 15 of optional open label treatment phase (i.e. Visit 8B) and Day 25. • An exploratory objective for the PHQ-9 was added. • The background and rationale for this assessment was added. • The order of administration of the PHQ-9 in the double-blind treatment phase and the optional open-label treatment phase is provided. • The following reference was added: "**Spitzer RL, Kroenke K, Williams JB. Validation and utility of a self report version of PRIME-MD: the PHQ primary care study. JAMA 1999; 282:1737– 44**."
Rationale: Refer to Instructions for Use document for dose administration.	
Synopsis; 6. Dosage and Administration	Sprays to each nostril should be delivered **per the Instructions for use document** ~~in rapid succession~~ at the scheduled time points ~~(i.e., there should be no waiting between sprays to the right and left nostrils at each time point)~~.
Rationale: Add abbreviations used in the text.	
Abbreviations	ASA = American Society of Anesthesiologists BPIC-SS = Bladder pain/interstitial cystitis symptom score PHQ-9 = Patient Health Questionnaire-9 PTSD = post-traumatic stress disorder OCD = obsessive compulsive disorder.
Rationale: Explain how the sleep and appetite items of the modified MADRS will be handled.	
3.2 Study Design Rationale	The following text has been added "**The modified MADRS with a 2-hour recall period assessment contains the same 10-items, but the sleep and appetite items will not be assessed. Instead, the predose MADRS scores for the sleep and appetite items recorded earlier on the same day will be carried forward.**"
Rationale: Clarification of text regarding serious adverse event reporting.	
12.3.1 All Adverse Events	The following text revision was made: "Serious adverse events, including those spontaneously reported to the investigator **from the time a signed and dated ICF is obtained until completion of the subject's last study-related procedure or within 30 days after the last dose of study drug (if last study-related procedure is less than 30 days after the last dose of study drug)**, must be reported using the Serious Adverse Event Form."
Rationale: Define "postdose".	
Time and Events Schedule	The definition of postdose was provided **"(Postdose time points are from the time of the first intranasal spray at t = 0)"**
Rationale: Specify "past psychiatric and family psychiatric history" will be collected at Screening.	
Time and Events Schedule	The following modification was made to the Medical History and Demographics row: "Medical history, ~~and~~ demographics, **past psychiatric and family psychiatric history**"

Rationale: Clarify protocol text, where required.	
Synopsis; 3.1 Overview of Study Design; 9.1.2 Screening Phase	Text was revised to indicate "The subject can discontinue the antidepressant within 1 week, or within a period ~~less than~~ **at least** 5 times the drug's half-life….."
1.1.1 Nonclinical Studies	Text was revised to "In rabbits, based on these results, 10 ~~and 100/50~~ mg/kg/day was~~ere~~ considered the no observed adverse effect level for maternal and developmental toxicity, respectively."
Synopsis; 6. Dosage and Administration	Text was revised to "Food will be restricted for at least 2 hours before each administration of study medication. Drinking ~~of water or any other permitted beverage~~ **of any fluids** will be restricted for at least 30 minutes before the first nasal spray."
Synopsis; 11.3 Efficacy Analyses	The following text revisions regarding the dose response analyses were made: "Details of the dose response analysis will be provided in the Statistical Analysis Plan. In addition to conducting the dose response analyses separately for Panel A and Panel B, the analysis ~~Additional dose response analyses~~ may be conducted by pooling data from Panel A and Panel B. ~~Further details of this dose response analysis will be presented in the Statistical Analysis Plan.~~
11.2 Sample Size Determination	The 2 paragraphs below were copied from other sections of the protocol and added here. "*For Panel A, if the placebo response or other assumptions at the end of Period 1 are not as expected, up to a maximum of 78 subjects (i.e., up to 18 additional subjects) may be enrolled to ensure that an adequate number of subjects are re-randomized in Period 2. The assessment will be based on a blinded IWRS review of the number of subjects who are re-randomized as well as dropout rate*s." "*For Panel B, if the placebo response or other assumptions at the end of Period 1 are not as expected, up to a maximum of 52 subjects (i.e., up to 12 additional subjects) may be enrolled to ensure that an adequate number of subjects are re-randomized in Period 2. The assessment will be based on a blinded IWRS review of the number of subjects who are re-randomized as well as dropout rates*."
10.2 Withdrawal From Study	The following text added: "Violation of protocol procedures **(determined on a case by case basis)"**
10.2 Withdrawal From Study	The following text revisions were made: "If a subject discontinues the optional open-label treatment phase prior to receiving all **9 (Panel A) or 4 (Panel B)** doses~~, for reasons other than withdrawal of consent,~~ an Early Termination visit is not required. The subject would continue into the posttreatment phase."

Time and Events Schedule	• The following was added to the "Screening and Double-Blind Treatment Phase" Time and Events Schedule: – Footnote 24: "*For inpatient subjects, telephone contact visits can be performed at the inpatient location*." – Footnote 23: "*The PWC-20 will not be performed in Visit 8A if the subject will be participating in the optional open label treatment phase*." – Footnote 11: "*For the MOAA/S, If a score of 5 is reached prior to 1 hour postdose, assessments are to still to continue through 1 hour postdose*" – An "X^{13}" was added to the Total Cholesterol row to align with the footnote text regarding this sample.
Time and Events Schedule	The following was added to the "Optional Open-Label Treatment and Posttreatment Phase" Time and Events Schedule: • Footnote 12: "~~*Day 15 only*~~. ***For inpatient subjects, telephone contact visits can be performed at the inpatient location***." • The following text was added to Footnote 8: "**If a score of 5 is reached prior to 1 hour postdose, assessments are to still to continue through 1 hour postdose**."
9. Study Evaluations; 16.1 Study Specific Design Considerations	The metabolomics blood volume changed from "5 mL" to" 6 mL", which increased the total volume to 153 mL (from 150 mL).
9.1.1 Overview	The following text was deleted: "~~Blood collections for pharmacokinetic assessments should be kept as close to the specified time as possible. Other measurements may be done earlier than specified time points, if needed~~"
9.7 Safety Evaluations	The following text was added: "*The MOAA/S scores range from 0 [No response to painful stimulus; corresponds to ASA continuum for general anesthesia] to 5 [Readily responds to name spoken in normal tone (awake); corresponds to ASA continuum for minimal sedation*." "*On each dosing day, if a score of 5 is reached prior to the 1 hour postdose time point, assessments are to still to continue through 1 hour postdose."*
Synopsis; 10.1 Completion	The following text was added: "A subject will be considered to have completed the optional open label treatment phase if he or she has received **all 9 doses (Panel A) or 4 doses (Panel B)** of optional open-label treatment."

9.7 Safety Evaluations	The following text was revised to the Urine Drug Screen and Alcohol test to clarify the handling of these samples at Screening: Urine Drug Screen: barbiturates, methadone, opiates, cocaine, cannabinoids **(not included at Screening, but included at all other time points)**, **phencyclidine**, and amphetamine/methamphetamine~~, and benzodiazepines~~: • **"*The central laboratory will analyze the urine drug screen performed at Screening. The local laboratory will be used for all other time points.*"** Alcohol test (urine or breath, as specified) • **"*The central laboratory will analyze the urine drug screen performed at Screening. The local laboratory will be used for all other time points.*"**
8. Prestudy and concomitant therapy	The following text was added to clarify the minimum amount of information to be captured as prestudy antidepressant therapy: "All antidepressant treatment(s), including adjunctive treatment for MDD, used prior to Screening either in the current or prior depressive episodes (known from the subject's psychiatric history or verbal report) **that will be used to support subject eligibility (per Inclusion Criterion #6 which requires an inadequate response to at least 2 antidepressant treatments, at least one of which is in the current episode of depression)**, but that are not continuing at the Screening visit, are to be recorded at Screening on the appropriate antidepressant therapy-specific case report form"
Rationale: Remove prohibited medications that do not have to be prohibited in this study.	
Attachment 1	PRN (as needed) and chronic use of the following medications/substances are now permitted: DHEA, Fish oils, Ginko, Ginseng, omega-3-fatty acids
Rationale: Update Investigator's Brochure references.	
References	The citation for Edition 2 (14 October 2013) and the Edition 2, IB addendum (In preparation) have been added.
Rationale: Minor errors were noted.	
Throughout the protocol	Minor grammatical, formatting, or spelling changes were made.

Amendment INT-1 (06 December 2013)

This amendment is considered to be substantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the European Union.

The overall reason for the amendment: The overall reason for the amendment is to add a new panel (Panel B) to evaluate the efficacy of intranasal esketamine in Japanese subjects with TRD.

Applicable Section(s)	Description of Change(s)
Rationale: To incorporate study design specifics for Panel B.	

Applicable Section(s)	Description of Change(s)
Throughout the protocol	1. The cohort described in the original protocol is now identified as Panel A. 2. Study design details, including statistical methods, for Panel B were added. 3. Rationale for study design elements of Panel B was added. 4. Text was added to indicate Panel A and B can be conducted in parallel, but that Panel B will only be initiated after completion of the ESKETINTRD1002 study. 5. A study design figure has been added.
Rationale: Describe how the number of re-randomized and dropout subjects (or other assumptions) at the end of Period 1 will be reviewed.	
Synopsis; 5. Treatment Allocation and Blinding	Text has been added to indicate this will be a blinded IWRS review.
Rationale: Provide additional guidance to clinical site regarding staff and equipment requirements for dosing.	
Synopsis; 6. Dosage and Administration	Text has been added to indicate that the clinical site must have access to a clinician experienced with ventilation management. In addition, equipment for supportive ventilation and resuscitation needs to be present at the site.
Rationale: Provide additional guidance on the frequency of pulse oximetry monitoring.	
Time and Events Schedule; Section 9.7 Safety Evaluations	Text has been added to indicate continuous pulse oximetry will be monitored from 5 minutes before the first spray, then after the first spray, every 15 minutes for approximately 1 hour postdose.
Rationale: Incorporate nasal tolerability questionnaire and clinical global assessment of alertness into the recommended order of assessments.	
9.1.3 Double-Blind Treatment Phase; 9.1.4 Optional Open-Label Treatment Phase	1. The nasal tolerability questionnaire has been added within the order of PRO assessments. 2. The clinical global assessment of alertness has been added to the order of clinician-administered assessments.
Rationale: The M.I.N.I. is currently being updated for DSM-5 and will not be available by the start of this study.	
Synopsis; 3.1 Overview of Study Design; 3.2 Study Design Rationale; 4.1 Inclusion Criteria; 4.2 Exclusion Criteria; 9.1.2 Screening Phase	1. DSM-IV-TR diagnostic criteria for MDD will be used instead of DSM-5 as the M.I.N.I. is currently being updated to align with DSM-5 but will not be available at the time of study start. 2. Exclusion criterion for history of substance abuse or dependence has been revised to remove reference to using DSM-5 criteria.
Rationale: Provide additional guidance on prestudy and concomitant therapies, including antidepressant therapies.	
8. Prestudy and concomitant therapy	Text was added to clarify the duration and how prestudy therapies (antidepressant and non-antidepressant treatments) are to be captured in the study.
Rationale: Align prohibitions and restrictions and exclusion criterion with the current esketamine risk language.	

Applicable Section(s)	Description of Change(s)
4.1 Inclusion Criteria; 4.3 Prohibitions and Restrictions	Section 4.1 Inclusion Criteria: • Text within Inclusion Criteria #12 was revised as follows: "A man who is sexually active with a woman of childbearing potential and has not had a vasectomy must agree to use a**n effective** ~~barrier~~ method of birth control (e.g., either condom with **spermicide)**~~/gel/film/cream/suppository or partner with occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/suppository~~ and all men must also not donate sperm during the study and for 3 months after receiving the last dose of study drug." Section 4.3 Prohibitions and Restrictions: • The restrictions regarding alcohol use were revised from "*Subjects must abstain from alcohol and prohibited drugs from Screening through to the end of the treatment phase (double-blind and optional open-label treatment phase, if applicable)*" to "*Subjects must abstain from using alcohol within 24 hours before and after study drug administration*" • The text regarding a man who is sexually active with a woman of childbearing potential has been revised from "*must use a double barrier method of birth control (i.e., male condom, female diaphragm or cervical cap, or condom")* to state that he must use "*an effective method of birth control (e.g., condom with spermicide)*" during the study and for 3 months after receiving the last dose of study drug.
Rationale: Clarification of study diary procedures.	
Time and Events Schedule; 9.1.2 Double-Blind Treatment Phase; 9.1.5 Posttreatment Phase	• Additional guidance for dispensing and review of diary. • In Time and Events Schedule: – an "x" was added to Day 15 for dispensing the subject diary. – The "x" at Visit 3 and 6 was deleted for review of subject diary.
Rationale: To further assess the subject's level of alertness after dosing.	
Synopsis; Time and Events Schedule; 2.1 Objectives; 3.2 Study Design Rationale; 9.1.3 Double Blind Treatment Phase ; 9.7 Safety Evaluations; 11.8 Safety Analyses	The "Clinical Global Assessment of Alertness" assessment has been added .
Rationale: Age range modified to align Panel A and B.	
Synopsis; 3.1 Overview of Study Design; 3.2 Study Design Rationale; 4.1 Inclusion Criteria	The lower end of the age range has been revised from 18 to 20 years of age.
Rationale: Add "rhinoplasty" to list of excluded anatomical or medical conditions that may impede delivery or absorption of study medication.	
Synopsis; Section 4.2 Exclusion Criteria	Rhinoplasty was added to Exclusion Criteria
Rationale: Define how a completer will be defined for the optional open-label treatment phase.	
10.1 Completion	Text was added to indicate that subject will be considered to have completed the optional open label treatment phase if he or she has received all 4 doses of optional open-label treatment.

Applicable Section(s)	Description of Change(s)
Rationale: Clarification of reasons for withdrawal.	
10.2 Withdrawal from Study	• The following text was deleted "Discontinuation of study treatment. A subject's study treatment will be discontinued if:" and all sub-bullets previously under this heading are now main bullets. • "Blind is broken (double blind treatment phase only)" was deleted.
Rationale: Clarification of requirements for Early Termination visit.	
Synopsis; 3.1 Overview of Study Design; 9.1.3 Double Blind Treatment Phase; 9.1.4 Optional Open Label Treatment Phase; 10.2 Withdrawal from Study	– Text was clarified to indicate that subjects that withdrawal from the study due to "withdrawal of consent" are not required to complete an early termination visit and posttreatment phase. – Study procedures for the ET visit were aligned with the Day 15 study visit procedures.
Rationale: Provide guidance on dose adjustment in optional open label treatment phase.	
Synopsis; 3.1 Overview of Study Design	Text was added to indicate that after the first dose, subsequent doses can be down-titrated to the next lower dose, or titrated up to the next higher dose, at any visit, if desired, based on the investigator's clinical judgment of efficacy and tolerability.
Rationale: Revision of the schedule of study procedures.	
	– Vital signs (BP/HR) were added at 10 minutes postdose and a 12-lead ECG was added at 1hour postdose on dosing days in the DB treatment phase. – – All efficacy assessments scheduled to occur at predose at Visit 8B (Day 15) for subjects that do not conduct visit 8A and 8B on the same day have been deleted.
Rationale: Clarification of statistical analysis methods for safety data.	
Synopsis; 3.1 Overview of Study Design; 3.2 Study Design Rationale; 11. 1 Subject information; 11.8 Safety Analyses	1. Text modified to indicate safety data from Panel A and Panel B will be pooled for analysis and analyzed separately. 2. Text describing the statistical analysis of the Physical Examination has been deleted.
Rationale: Clarify that interim analysis could be performed for each panel, if necessary.	
Synopsis; 11.9 Interim Analysis	Text was clarified to indicate "An interim analysis may be performed **for each panel** as needed. If ~~one is~~ required, further details will be provided in a separate charter and interim analysis plan."
Rationale: Provide additional details regarding Prohibited Therapies.	
Time and Events Schedule; 4.3 Prohibitions and Restrictions; Attachment 1	1. Examples of potent CYP3A4 inhibitors and inducers were provided in Attachment 1. 2. Text added to confirm intranasal steroids are not prohibited. 3. Text added to state ECT, deep brain stimulation (DBS), transcranial magnetic stimulation (TMS), and vagus nerve stimulation (VNS) are prohibited from Screening to the end of the study.
Rationale: Provide additional guidance on recording prestudy and concomitant therapies.	
Section 8. Prestudy and Concomitant Therapy	Text has been added to clarify the duration and how to record prestudy and concomitant therapies, including antidepressant therapies.

Applicable Section(s)	Description of Change(s)
Rationale: Indicate that tympanic temperature is recommended.	
Synopsis; Time and Events Schedule; 9.7 Safety Evaluations	Text has been added to indicate tympanic temperature is recommended.
Rationale: Indicate that a guidance document for the completion of the ATRQ will be provided to sites.	
15. Study Specific Materials	"Guidance document for ATRQ" was added.
Rationale: Minor errors were noted.	
Throughout the protocol	Minor grammatical, formatting, or spelling changes were made.

SYNOPSIS

A Double-Blind, Doubly-Randomized, Placebo-Controlled Study of Intranasal Esketamine in an Adaptive Treatment Protocol to Assess Safety and Efficacy in Treatment-Resistant Depression (SYNAPSE)

Esketamine (JNJ54135419) is the S-enantiomer of the ketamine racemate (1:1 racemic mixture of R-ketamine and esketamine). Ketamine and esketamine are approved medications in several countries for the induction of general anesthesia and for use as adjunct to other anesthetics.

The mechanism of action results from a noncompetitive binding to the N-methyl-D-aspartate (NMDA)-receptor (ligand-gated calcium channel) at the phencyclidine binding site. It also has additional binding sites (NMDA and non-NMDA glutamate receptors, nicotinic and muscarinic, cholinergic, and monoaminergic and opioid receptors, and voltage-dependent sodium and L-type calcium channels).

Esketamine has been shown to have a 3- to 4-times stronger binding affinity for the phencyclidine site of the NMDA receptor than the R-enantiomer, making it more feasible for intranasal delivery.

Intranasal esketamine is being developed for the treatment of treatment-resistant depression (TRD).

OBJECTIVES AND HYPOTHESIS

Primary Objective

To assess the efficacy and dose response of intranasal esketamine (Panel A: 28 mg, 56 mg, and 84 mg; Panel B: 14 mg and 56 mg) compared with placebo in improving depressive symptoms in subjects with treatment-resistant depression (TRD), as assessed by a change from baseline in the Montgomery-Asberg Depression Rating Scale (MADRS) total score for the combined periods in the double-blind treatment phase.

Secondary Objectives

1. To evaluate sustained response (≥50% reduction from baseline in MADRS total score) with onset by Day 2 through the end of the double-blind phase (Day 15).
2. To investigate the safety and tolerability of intranasal esketamine in TRD subjects, with special attention to:
    a. Local nasal tolerability, using a nasal tolerability questionnaire and nasal examinations;
    b. Effects on heart rate, blood pressure, respiratory rate, and blood oxygen saturation (SpO_2);
    c. Effects on suicidal ideation/behavior measured by the Columbia Suicide Severity Rating Scale (C-SSRS);
    d. Effects on alertness and sedation measured by the Modified Observer's Assessment of Alertness/Sedation (MOAA/S) and the Clinical Global Assessment of Alertness;
    e. Psychosis-like side effects by using a four-item positive symptom subscale of the Brief Psychiatric Rating Scale (BPRS+) consisting of: suspiciousness, hallucinations, unusual thought content, and conceptual disorganization;
    f. Effects on dissociative symptoms using the Clinician Administered Dissociative States Scale (CADSS);
    g. Potential withdrawal symptoms following cessation of intranasal esketamine treatment, as measured by the clinician-administered 20-item Physician Withdrawal Checklist (PWC-20).
    h. Potential treatment-emergent symptoms of cystitis using the Bladder Pain/ Interstitial Cystitis Symptom Score (BPIC-SS)

   i. Cognition, using the Cogstate® computerized test battery and the Hopkins Verbal Learning Test-Revised (HVLT-R) (Panel A only)

3. To assess the effect of intranasal esketamine compared to intranasal placebo on:
   a. Depressive symptoms, as assessed by the 16-item Quick Inventory of Depressive Symptomatology- Self Report ($QIDS\text{-}SR_{16}$);
   b. Remission, defined as a MADRS total score ≤10;
   c. Response, defined as a ≥50% reduction from baseline in MADRS total score;
   d. The severity of illness using the Clinical Global Impression - Severity (CGI-S) and the Patient Global Impression - Severity (PGI-S);
   e. Symptoms of anxiety as assessed by the Generalized Anxiety Disorder 7-item Scale (GAD-7)
4. To evaluate the pharmacokinetics (PK) of intranasal esketamine in subjects with TRD

Exploratory Objectives

1. Subject perspective of global change in major depressive disorder (MDD) from baseline, as measured by the Patient Global Impression of Change (PGI-C)
2. To assess the effect of intranasal esketamine compared to intranasal placebo on depressive symptoms, as assessed by the 9-item Patient Health Questionnaire (PHQ-9)
3. Impact on health status as assessed using the EuroQol-5D, 5-level version (EQ-5D-5L)
4. To evaluate whether pretreatment concentrations of inflammatory and neurotrophic markers and plasma glycine correlate with the magnitude of clinical change, as measured by the MADRS, following intranasal administration of esketamine
5. To assess the impact of intranasal esketamine on plasma inflammatory and neurotrophic markers and glutamatergic pathway metabolic markers

Hypothesis

The primary hypothesis for each panel is that intranasal esketamine (Panel A: 28 mg, 56 mg, 84 mg; Panel B: 14 mg and 56 mg) is superior to intranasal placebo in improving depressive symptoms in adult subjects with TRD, as assessed by the change from baseline in the MADRS total score for the combined periods in the double-blind treatment phase.

OVERVIEW OF STUDY DESIGN

This is a 2-panel, doubly-randomized, double-blind, placebo-controlled, multicenter study in approximately 100 male and female adult subjects with TRD.

Panel A will be conducted in approximately 60 subjects in the United States and Belgium. Panel B will be conducted in approximately 40 Japanese subjects in Japan.

In both panels, each subject will participate in up to 4 phases:

- A screening phase of up to 4 weeks,
- A double-blind treatment phase (Day 1 to Day 15) which includes two 1-week treatment periods (Period 1 and Period 2),
- An optional open-label treatment phase (Panel A: Day 15 to 74 ; Panel B: Day 15 to 25), and
- An 8-week posttreatment (follow up) phase

The duration of the subject's participation will be approximately 14 to 23 weeks for Panel A and 14 to 16 weeks for Panel B. The end of study will occur when the last subject in the trial completes his/her last study assessment.

Panel A and B may be conducted in parallel; but Panel B will only be initiated after completion of the planned Phase 1 study (ESKETINTRD1002) to assess the pharmacokinetics, safety, and tolerability of intranasal esketamine in Japanese subjects.

Screening Phase (Day -28 to Day -1)

The screening phase for Panel A and B is the same.

After giving informed consent, subjects who are 20 to 64 years of age (inclusive), will be screened within 4 weeks of Day 1 (i.e., Day -28 to Day -1) to determine eligibility for study participation.

Subjects must meet Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition-Text Revised (DSM-IV-TR) diagnostic criteria for MDD, without psychotic features, based upon clinical assessment (DSM-IV-TR 296.22, 296.23, 296.32, or 296.33) and confirmed by the Mini International Neuropsychiatric Interview (MINI). Subjects must have an Inventory of Depressive Symptomatology 30-item Clinician-rated (IDS-C_{30}) total score ≥34. In addition, the major depressive episode must be deemed valid using the "State vs. Trait, Assessibility, Face Validity, Ecological Validity, Rule of Three P's" (SAFER) criteria interview, administered by remote, independent raters.

Subjects must have had an inadequate response to at least 2 antidepressants, at least one of which is in the current episode of depression, assessed using the Massachusetts General Hospital – Antidepressant Treatment Response Questionnaire (MGH-ATRQ) and prior medication history.

Note: Subjects who are not currently taking an antidepressant at Screening are eligible to participate in this study (i.e., subjects may participate in the study whether or not they are taking an antidepressant).

- Subjects who are taking an antidepressant at the Screening visit must have been receiving a stable dose (i.e., dose has been unchanged) for at least 2 weeks before the Screening visit.
    - With the exception of monoamine oxidase (MAO) inhibitors, which are prohibited, the subject may continue the antidepressant at the same dose during the double-blind treatment phase, or
    - The subject can discontinue the antidepressant within 1 week, or within a period at least 5 times the drug's half-life [exception: At least 4 weeks for fluoxetine and 2 weeks for MAO inhibitors], whichever is longer, before the planned first dose of study drug.
        - The decision to continue or discontinue the current antidepressant will be made by the subject and investigator, based on their clinical judgment. Antidepressant medication will not be discontinued for the sole purpose of allowing subjects to participate in the study.

Disallowed therapies, including psychotropic medications, are described in the exclusion criteria, Section 8 (Prohibitions and Restrictions), and Attachment 1.

Within 1 week prior to the planned first dose of study medication, Panel A subjects will have two mandatory practice sessions on the Cogstate® computerized test battery performed at the clinical site.

Other screening evaluations (e.g., biomarker, safety) will be performed as per the Time and Events Schedule.

All adverse events and special reporting situations, whether serious or non-serious, will be reported from the time a signed and dated informed consent form (ICF) is obtained until completion of the subject's last study-related procedure (which may include contact for follow-up of safety).

Double-Blind Treatment Phase

In both panels, all subjects will participate in two treatment periods (Period 1 and Period 2) during the double-blind treatment phase. Efficacy, safety, PK, biomarker, and/or pharmacogenomic evaluations performed at each visit are described in the Time and Events Schedule. If a subject withdraws before the end of the double-blind treatment phase for reasons other than withdrawal of consent, the Early Termination visit should be conducted at the time of discontinuation, followed by completion of the posttreatment phase.

Prior to the first dose, subjects will practice spraying (into the air) a demonstration intranasal device that is filled with water.

All subjects will self-administer the study medication in Period 1 and Period 2 under the direct supervision of the investigator or designee (Refer to Section 6, Dosage and Administration). On all dosing days, all subjects must remain at the clinical site until at least 2 hours postdose; a longer duration will be required on Day 1 and 11 (up to 6 hours postdose for study procedures) and on Day 25 (3 hours postdose for study procedures), and should be accompanied by a responsible adult when released from the clinical site. In addition, subjects must not drive a car or work with machines for 24 hours after study medication dosing.

Panel A

Period 1

On Day 1, subjects (n = 60) will be randomized using a 3:1:1:1 ratio to 1 of the following 4 treatment groups: Intranasal placebo (n = 30), intranasal esketamine 28 mg (n = 10), intranasal esketamine 56 mg (n = 10), or intranasal esketamine 84 mg (n = 10) administered on Day 1 and Day 4.

Period 2

Subjects that received intranasal esketamine 28, 56, or 84 mg in Period 1 will receive the same treatment in Period 2 on Day 8 and Day 11.

Period 2 treatment for subjects that received placebo in Period 1 will be determined by the QIDS-SR_{16} score on Day 8 (predose):

- Subjects that received intranasal placebo in Period 1 and have a QIDS-SR_{16} score < 11 (mild or no symptoms) will receive intranasal placebo again in Period 2 on Day 8 and Day 11.
- Subjects that received intranasal placebo in Period 1 and have a QIDS-SR_{16} score ≥11 (moderate to severe) will be re-randomized to receive intranasal placebo or intranasal esketamine 28 mg, 56 mg, or 84 mg in a 1:1:1:1 ratio on Day 8 and Day 11. The randomization will be stratified by the QIDS-SR_{16} score [moderate (QIDS-SR_{16} score 11 to 16) or severe (QIDS-SR_{16} score > 16)].

Panel B

Period 1

On Day 1, subjects (n = 40) will be randomized using a 2:1:1 ratio to 1 of the following 3 treatment groups: Intranasal placebo (n = 20), intranasal esketamine 14 mg (n = 10), or intranasal esketamine 56 mg (n = 10) administered on Day 1 and Day 4.

Period 2

Subjects that received intranasal esketamine 14 or 56 mg in Period 1 will receive the same treatment in Period 2 on Day 8 and Day 11.

Period 2 treatment for subjects that received placebo in Period 1 will be determined by the QIDS-SR_{16} score on Day 8 (predose):

- Subjects that received intranasal placebo in Period 1 and have a QIDS-SR_{16} score < 11 (mild or no symptoms) will receive intranasal placebo again in Period 2 on Day 8 and Day 11.
- Subjects that received intranasal placebo in Period 1 and have a QIDS-SR_{16} score ≥11 (moderate to severe) will be re-randomized to receive intranasal placebo, intranasal esketamine 14 mg, or intranasal esketamine 56 mg in a 1:1:1 ratio on Day 8 and Day 11. The randomization will be stratified by the QIDS-SR_{16} score [moderate (QIDS-SR_{16} score 11 to 16) or severe (QIDS-SR_{16} score > 16)].

Optional **Open-Label Treatment Phase**

In both panels, on Day 15, following completion of the double-blind treatment phase assessments, subjects may participate in an optional open-label treatment phase. Subjects that discontinue early from the double-blind treatment phase are not eligible to participate in the optional open-label treatment phase. The Investigator must agree and confirm the subject is eligible to participate based on his/her clinical judgment.

The efficacy, safety, and biomarker evaluations that will be performed at each visit during the optional open-label treatment phase are described in the Time and Event Schedule. Where specified in the Time and Events Schedule, study procedures performed on the last day of the double-blind treatment phase (Day 15) that are also required predose on Day 15 of the optional open-label treatment phase will only be performed once.

During the optional open-label treatment phase, Panel A subjects can receive up to 9 doses of intranasal esketamine on Days 15, 18, 22, 25, 32, 39, 46, 60, and 74. Panel B subjects can receive up to 4 doses of intranasal esketamine on Days 15, 18, 22, and 25. On all dosing days, all subjects must remain at the clinical site until at least 2 hours postdose; a longer duration will be required on Day 1 and 11 (up to 6 hours postdose for study procedures) and on Day 25 (3 hours postdose for study procedures), and should be accompanied by a responsible adult when released from the clinical site. In addition, subjects must not drive a car or work with machines for 24 hours after study medication dosing.

If a subject discontinues the optional open-label treatment phase prior to receiving all 9 (Panel A) or 4 (Panel B) doses, for reasons other than withdrawal of consent, the subject will continue into the posttreatment phase (see "Posttreatment Phase" below).

Panel A

The doses for the optional open label treatment phase include 28 mg, 56 mg, and/or 84 mg.

All subjects will start with intranasal esketamine 56 mg on Day 15. Subsequent doses on Days 18, 22, 25, 32, 39, and 46 can be down-titrated to the next lower dose, or titrated up to the next higher dose if clinically indicated based on the Investigator's clinical judgment of efficacy and tolerability. The two remaining doses of esketamine administered after Day 46 (i.e., Day 60 and 74) will remain stable. No further dose adjustment is permitted after Day 46; the same dose administered on Day 46 will be administered on Day 60 and 74.

Panel B

The doses for the optional open label treatment phase include 14 mg, 28 mg, and/or 56 mg.

All subjects will start with intranasal esketamine 56 mg on Day 15. Subsequent doses on Days 18, 22, and 25 can be down-titrated to the next lower dose, or titrated up to the next higher dose, at any visit if desired based on the Investigator's clinical judgment of efficacy and tolerability.

Posttreatment Phase

The posttreatment phase for Panel A and B is the same.

The efficacy and safety evaluations that will be performed at each visit during the posttreatment phase are described in the Time and Event Schedule.

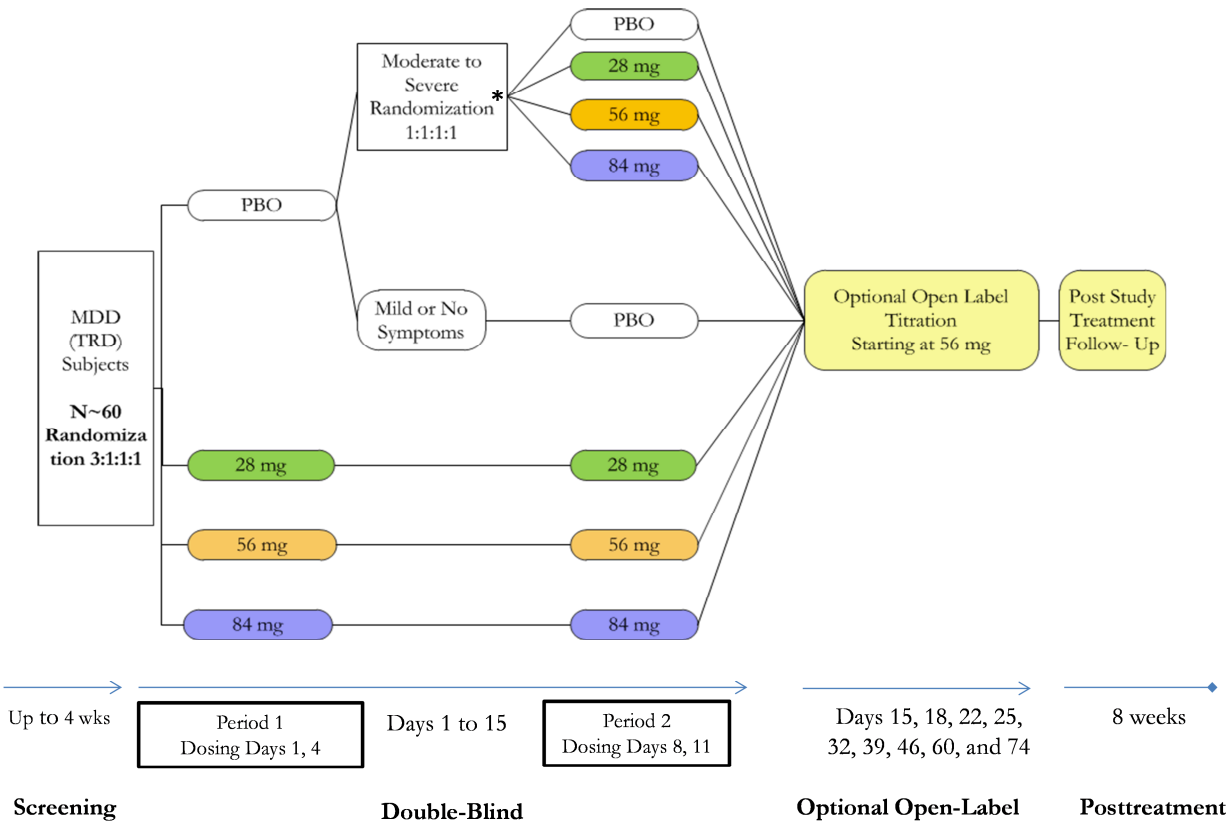
All subjects who receive at least 1 dose of study medication should have 4 follow up visits conducted at 1, 2, 4, and 8 weeks after the last dose of study medication. The follow up visits at 1, 4, and 8 weeks after the last dose of study medication will be telephone contacts. The follow up visit at 2 weeks after the last dose of study medication is a clinic visit.

Any clinically significant abnormalities persisting at the end of the study will be followed by the investigator until resolution or until a clinically stable endpoint is reached.

All adverse events and special reporting situations, whether serious or non-serious, will be reported until completion of the subject's last study-related procedure (which may include contact for follow-up of safety).

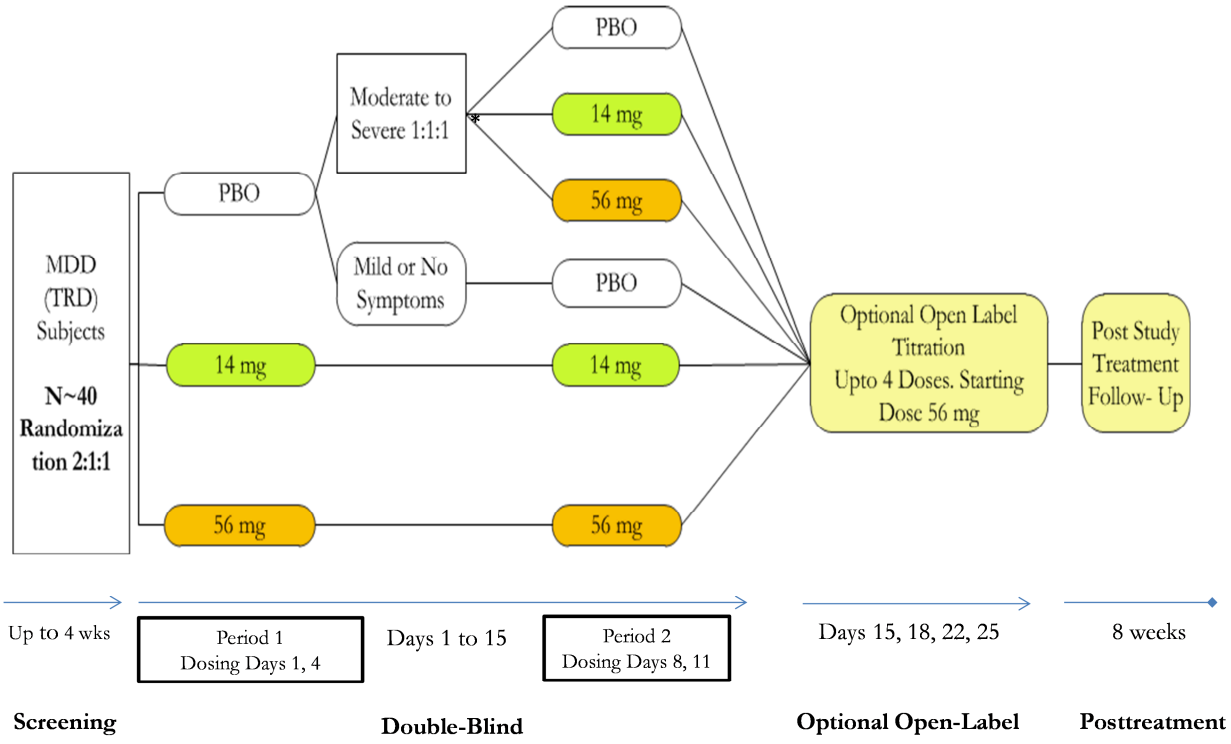
Diagrams of the study design for Panel A and Panel B are provided below.

Study Design: Panel A



* Randomization stratified by QIDS–SR_{16} total score [Moderate = 11 to 16; Severe > 16]

Study Design: Panel B



* Randomization stratified by QIDS–SR_{16} total score [Moderate = 11 to 16; Severe > 16]

SUBJECT POPULATION

The inclusion and exclusion criteria for Panel A and Panel B are the same. Panel A will be conducted in approximately 60 subjects in the United States and Belgium. Panel B will be conducted in approximately 40 Japanese subjects in Japan.

Key Inclusion Criteria

- Man or woman, 20 to 64 years of age, inclusive.
- Subject must meet Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition-Text Revised (DSM-IV-TR) diagnostic criteria for MDD, without psychotic features, based upon clinical assessment (DSM-IV-TR 296.22, 296.23, 296.32, or 296.33), and confirmed by the Mini International Neuropsychiatric Interview (MINI).

- The subject's major depressive episode and treatment response must be deemed "valid" using the SAFER criteria interview (which includes the MADRS, a review of the MGH-ATRQ performed at Screening, and SAFER Criteria Inventory) administered by remote, independent raters.
- Subject must have had an inadequate response to at least 2 antidepressants, at least one of which is in the current episode of depression. The ATRQ will be used to assess antidepressant treatment response during the current episode. Prior medication history will be used to determine antidepressant treatment response in prior episode(s).
- Have an IDS-C_{30} total score ≥34 at Screening and predose at Day 1.

Key Exclusion Criteria

- Subject has a current diagnosis of bipolar and related disorders, intellectual disability, or cluster b personality disorder (e.g., borderline personality disorder, antisocial personality disorder, histrionic personality disorder, and narcissistic personality disorder).
- Subject has a current or prior diagnosis of a psychotic disorder, MDD with psychosis, post-traumatic stress disorder (PTSD), or obsessive compulsive disorder (OCD).
- Anatomical or medical conditions that may impede delivery or absorption of study medication (e.g., undergone facial reconstruction, rhinoplasty, significant structural or functional abnormalities of the nose or upper airway; obstructions or mucosal lesions of the nostrils or nasal passages; undergone sinus surgery in the previous 2 years).
- Has an abnormal or deviated nasal septum with any 1 or more of the following symptoms: blockage of 1 or both nostrils, nasal congestion (especially 1-sided), frequent nosebleeds, frequent sinus infections, and at times has facial pain, headaches, and postnasal drip.
- Has a history of substance abuse (drug or alcohol) or dependence (except nicotine or caffeine) within the previous 1 year of screening visit.
- Subject has known allergies, hypersensitivity, intolerance, or contraindication to esketamine/ketamine or its excipients.

DOSAGE AND ADMINISTRATION

All doses of study medication will be self-administered under the direct supervision of the investigator or designee. Instructions for use of the intranasal device will be provided as a separate document.

The clinical site must have access to a clinician experienced with ventilation management. In addition, equipment for supportive ventilation and resuscitation needs to be present at the site.

Prior to the first dose, subjects will practice spraying (into the air) a demonstration intranasal device that is filled with water.

[redacted]. It is provided in a nasal spray pump, which delivers 16.14 mg esketamine (14 mg esketamine base) per 100 mcl spray. Each individual device contains 28 mg (i.e., 2 sprays).

The placebo solution will be provided as a clear, colorless intranasal solution of water for injection with a bittering agent (denatonium benzoate [Bitrex®] at a final concentration of 0.001 mg/mL) added to simulate the taste of the intranasal solution with active drug. Benzalkonium chloride is added as a preservative at a concentration of 0.3 mg/mL.

Food will be restricted for at least 2 hours before each administration of study medication. Drinking of any fluids will be restricted for at least 30 minutes before the first nasal spray.

Subjects will be instructed to refrain from blowing their nose for at least 1 hour after the last intranasal spray.

On all dosing days, all subjects must remain at the clinical site until at least 2 hours postdose; a longer duration will be required on Day 1 and 11 (up to 6 hours postdose for study procedures) and on Day 25 (3 hours postdose for study procedures), and should be accompanied by a responsible adult when released from the clinical site. Subjects must not drive a car or work with machines for 24 hours after study medication dosing.

Double-blind treatment phase

Refer to the "*Overview of Study Design- Double-Blind Treatment Phase*" section above for a description of the study design for Panel A and Panel B.

Subjects in Panel A and Panel B will self-administer intranasal esketamine or intranasal placebo on Days 1, 4, 8, and 11.

Panel A

On each dosing day, all subjects will self-administer 1 spray into each nostril at t = 0, 5, and 10 minutes. Time 0 is defined as the time of the first 100-mcl spray. Each subject will use 2 sprays per device per time point (t = 0, 5, and 10 minutes) on each dosing day. Sprays to each nostril should be delivered per the Instructions for Use document at the scheduled time points. The table below describes how each treatment will be administered in the double-blind treatment phase.

Panel A: Dose Administration in the Double-Blind Treatment Phase

Intranasal Treatment	Time of Administration (Time 0 is defined as the time of the first 100-mcl spray)		
	0	**5 minutes**	**10 minutes**
Placebo	1 spray of placebo to each nostril	1 spray of placebo to each nostril	1 spray of placebo to each nostril
Esketamine 28 mg	1 spray of esketamine to each nostril	1 spray of placebo to each nostril	1 spray of placebo to each nostril
Esketamine 56 mg	1 spray of esketamine to each nostril	1 spray of esketamine to each nostril	1 spray of placebo to each nostril
Esketamine 84 mg	1 spray of esketamine to each nostril	1 spray of esketamine to each nostril	1 spray of esketamine to each nostril

Panel B

On each dosing day, all subjects will self-administer 1 spray into each nostril at t = 0 and 5 minutes. Time 0 is defined as the time of the first 100-mcl spray. Each subject will use 1 spray per device (i.e., 2 devices per time point at t = 0 and 5 minutes) on each dosing day. Sprays to each nostril should be delivered per the Instructions for Use document at the scheduled time points. The table below describes how each treatment will be administered in the double-blind treatment phase.

Panel B: Dose Administration in the Double-Blind Treatment Phase

Intranasal Treatment	Time of Administration (Time 0 is defined as the time of the first 100-mcl spray)			
	0		**5 minutes**	
	Device 1	**Device 2**	**Device 3**	**Device 4**

Placebo	1 spray of placebo into one nostril	1 spray of placebo into the other nostril	1 spray of placebo into one nostril	1 spray of placebo into the other nostril
Esketamine 14 mg	1 spray of esketamine into one nostril	1 spray of placebo into the other nostril	1 spray of placebo into one nostril	1 spray of placebo into the other nostril
Esketamine 56 mg	1 spray of esketamine into one nostril	1 spray of esketamine into other nostril	1 spray of esketamine into one nostril	1 spray of esketamine into other nostril

Optional open-label treatment phase

Refer to the "*Overview of Study Design- Optional Open Label Treatment Phase*" section above for a description of the study design for Panel A and Panel B.

Subjects in Panel A will self-administer intranasal esketamine on Days 15, 18, 22, 25, 32, 39, 46, 60, and 74. Time 0 is defined as the time of the first 100-mcl spray. Sprays to each nostril should be delivered per the Instructions for Use document at the scheduled time points. The table below describes how each treatment will be administered in the optional open-label treatment phase.

Panel A: Dose Administration in Optional Open-Label Treatment Phase

Intranasal Treatment	Time of Administration (Time 0 is defined as the time of the first 100-mcl spray)		
	0	**5 minutes**	**10 minutes**
Esketamine 28 mg	1 spray of esketamine to each nostril	-	-
Esketamine 56 mg	1 spray of esketamine to each nostril	1 spray of esketamine to each nostril	-
Esketamine 84 mg	1 spray of esketamine to each nostril	1 spray of esketamine to each nostril	1 spray of esketamine to each nostril

Subjects in Panel B will self-administer intranasal esketamine on Days 15, 18, 22, and 25. Time 0 is defined as the time of the first 100-mcl spray. For the esketamine 28 mg and 56 mg doses, sprays to each nostril should be delivered per the Instructions for Use document at the scheduled time points. The table below describes how each treatment will be administered in the optional open-label treatment phase.

Panel B: Dose Administration in Optional Open-Label Treatment Phase

Intranasal Treatment	Time of Administration (Time 0 is defined as the time of the first 100-mcl spray)	
	0	**5 minutes**
Esketamine 14 mg	1 spray of esketamine to one nostril	-
Esketamine 28 mg	1 spray of esketamine to each nostril	-
Esketamine 56 mg	1 spray of esketamine to each nostril	1 spray of esketamine to each nostril

EFFICACY EVALUATIONS

The primary efficacy evaluation will be the MADRS total score.

Other secondary and exploratory efficacy evaluations include the QIDS-SR_{16}, PHQ-9, CGI-S, PGI-S, PGI-C, GAD-7, and the EQ-5D-5L.

PHARMACOKINETIC EVALUATIONS

Venous blood samples will be collected for determination of the plasma concentrations of esketamine, noresketamine, and other metabolites (if warranted) at the time points specified in the Time and Events Schedule. The exact dates and times of study medication dosing and PK blood sampling must be recorded.

The plasma concentrations of 4β-hydroxycholesterol will be measured in select PK samples as a probe to assess the potential for repeated administration of intranasal esketamine to induce hepatic cytochrome P450 3A4 enzyme activity. Total cholesterol will be measured in a separate blood sample at these same time points to ensure that changes in 4β-hydroxycholesterol are not attributable to changes in total cholesterol.

BIOMARKER EVALUATIONS

Human Inflammation Multi-Analyte Panel (MAP)

Blood (serum) samples will be collected to allow for an exploratory pharmacodynamic evaluation using the Human Inflammation MAP, a panel of 45 proteins involved in key biologically inflammatory and neurotrophic pathways.

Metabolomics

Blood (plasma) samples will be collected to allow for the exploratory analysis of novel predictive and drug exposure biomarkers.

PHARMACOGENOMIC (DNA) EVALUATIONS

A pharmacogenomic blood sample will be collected from all enrolled subjects on Day 1. DNA samples will be analyzed for the *CYP2B6* gene. Additional analyses may also be conducted if it is hypothesized that this may help resolve issues with the clinical data.

DNA samples will be used for research related to esketamine. They may also be used to develop tests/assays related to esketamine. Pharmacogenomic research may consist of the analysis of one or more candidate genes or of the analysis of genetic markers throughout the genome (as appropriate) in relation to esketamine.

SAFETY EVALUATIONS

Safety evaluations will be performed at the time points specified in the Time and Events Schedule.

Safety evaluations include adverse events, clinical laboratory tests, electrocardiograms (ECG), vital signs (blood pressure, heart rate, respiratory rate, temperature), pulse oximetry, physical examination, nasal examination, and a nasal tolerability questionnaire.

In addition, the C-SSRS will be performed to assess suicidal ideation and behavior, the CADSS will be administered to assess treatment-emergent dissociative symptoms, the BPRS+ will be administered to assess treatment-emergent psychotic symptoms, the MOAA/S will be used to measure treatment-emergent sedation, the Clinical Global Assessment of Alertness will be used to measure the subject's level of alertness, the BPIC-SS will be conducted to assess for potential treatment-emergent

cystitis, and the PWC-20 will be administered to assess potential withdrawal symptoms following cessation of intranasal esketamine treatment.

In Panel A only, the Cogstate® computerized test battery and HVLT-R will be administered to assess effects on cognition during the 9-week optional open label treatment phase with intranasal esketamine.

STATISTICAL METHODS

Statistical analysis will be done by the sponsor or under the authority of the sponsor. A general description of the statistical methods to be used to analyze the efficacy and safety data is outlined below. Specific details will be provided in the Statistical Analysis Plan. Each panel will be analyzed upon completion.

Sample Size Determination

Panel A

The sample size for Panel A is determined based on the following treatment differences between intranasal esketamine and placebo for the mean change from baseline in MADRS total score: a 9 point treatment difference was assumed for Period 1 (Day 8), a 7 point treatment difference for Period 2 (Day 15) was assumed for subjects with a moderate QIDS-SR_{16} score (QIDS-SR_{16} score = 11 to 16) and a 9 point treatment difference for Period 2 (Day 15) was assumed for subjects with a severe QIDS-SR_{16} score (QIDS-SR_{16} score > 16). Based on results of a previous esketamine IV study (ESKETIVTRD2001), it is estimated that 40% of placebo subjects at the end of Period 1 (Day 8 predose) will have a moderate QIDS-SR_{16} score and 55% will have a severe QIDS-SR_{16} score. Additional assumptions for the sample size calculation included a standard deviation of 10, 92.5% power for the combined data from both Day 8 and Day 15, an overall 1-sided significance level of 0.05, and a 5% drop-out rate for Period 1. It is calculated that this panel of the doubly-randomized design will require 60 subjects to be randomly assigned to treatment on Day 1 in a 3:1:1:1 ratio (30 subjects on placebo and 10 subjects per intranasal esketamine dose group).

For Panel A, if the placebo response or other assumptions at the end of Period 1 are not as expected, up to a maximum of 78 subjects (i.e., up to 18 additional subjects) may be enrolled to ensure that an adequate number of subjects are re-randomized in Period 2. The assessment will be based on a blinded IWRS review of the number of subjects who are re-randomized as well as dropout rates.

Panel B

The sample size for Panel B is determined using the same assumptions for MADRS total score, QIDS-SR_{16}, and drop-out rate as was used for Panel A. Additional assumptions for this panel for the sample size calculation included 90% power for the combined data from both Day 8 and Day 15, and an overall 1-sided significance level of 0.1. It is calculated that this panel of the doubly-randomized, outcome based design will require 40 subjects to be randomly assigned to treatment on Day 1 in a 2:1:1 ratio (20 subjects on placebo and 10 subjects per intranasal esketamine dose group).

For Panel B, if the placebo response or other assumptions at the end of Period 1 are not as expected, up to a maximum of 52 subjects (i.e., up to 12 additional subjects) may be enrolled to ensure that an adequate number of subjects are re-randomized in Period 2. The assessment will be based on a blinded IWRS review of the number of subjects who are re-randomized as well as dropout rates.

Efficacy Analysis

Panel A and Panel B will be analyzed separately for efficacy. The same statistical methodology applies to both panels. The efficacy analyses will be based on the combination of efficacy data from the two periods within each panel of the double-blind treatment phase, unless specified otherwise.

For each period in the double-blind treatment phase, an intent-to-treat (ITT) analysis set will be defined to include all randomized subjects who receive at least 1 dose of study drug and have both the baseline and at least one post-baseline MADRS total score within that period. The efficacy analyses of data in Period 1 and Period 2 will be based on each respective ITT analysis set.

For the primary efficacy analysis, change from baseline (Day 1 predose) in MADRS total score to Day 8 predose assessment of the double-blind treatment phase will be analyzed using an analysis of covariance (ANCOVA) model, with factors for treatment, center, and Period 1 baseline MADRS total score as the continuous covariate. Data from all randomized, treated subjects with change values during Period 1 will be included in the analysis of Period 1. Change from baseline in MADRS total score in Period 2 (Day 8, predose to Day 15) will be analyzed using an ANCOVA model with factors for treatment, center, Period 2 baseline QIDS-SR_{16} score (moderate or severe), and Period 2 baseline MADRS total score as the continuous covariate. Only data from Period 1 placebo subjects who are re-randomized (those with moderate and severe QIDS-SR_{16} scores on predose Day 8) who continue into Period 2 and have a change value during Period 2 will be included in the analysis of Period 2. The comparison of intranasal esketamine dose groups with placebo will be carried out by a combined test on the weighted test statistics for each period in the double-blind treatment phase. The 'adaptive' weight will be based on the actual sample sizes for the final analysis (Liu 2012).

Descriptive statistics for values and changes from baseline will be provided at each time point within each period of the double-blind treatment phase.

Details of the dose response analysis will be provided in the Statistical Analysis Plan. In addition to conducting the dose response analyses separately for Panel A and Panel B, the analysis may be conducted by pooling data from Panel A and Panel B.

For all continuous secondary endpoints, descriptive statistics of actual values and changes from baseline by treatment group within each period will be provided. The change from baseline for QIDS-SR_{16} total score, CGI-S, PGI-S, and GAD-7 will be analyzed in the same way as for the MADRS total score. There will be no adjustments for multiplicity in the evaluation of these other efficacy endpoints.

A frequency table for the number and percentage of subjects meeting criteria for sustained response ≥50% reduction from baseline in MADRS total score with onset by Day 2 through the end of the double-blind phase (Day 15) will be provided for subjects who remain on the same treatment for the duration of the double-blind phase. Frequency tables for the number and percentage of subjects meeting criteria for response (≥50% reduction from baseline in MADRS total score) and remission (MADRS total score of ≤10) will be provided at each time point.

Descriptive statistics for values and changes from baseline for the MADRS total score will be provided for the group of subjects who are randomized at Period 1 to either intranasal esketamine or placebo and remain on the same treatment for the duration of the double-blind treatment phase (Day 15 or early withdrawal). Placebo subjects who are re-randomized in Period 2 and receive esketamine will not be included in these summaries.

Efficacy data from the optional open-label treatment phase will be summarized descriptively.

Details of the exploratory efficacy analyses will be provided in the Statistical Analysis Plan.

Pharmacokinetic Analysis

Plasma esketamine, noresketamine, 4β-hydroxycholesterol, and total cholesterol concentrations will be listed for all subjects by esketamine treatment and study day. All concentrations below the lowest quantifiable concentration in a sample or missing data will be labeled as such in the concentration data presentations. Concentrations below the lower quantifiable concentration will be treated as zero in the summary statistics.

Subjects will be excluded from the pharmacokinetic analysis if their data do not allow for accurate assessment (e.g., incomplete administration of the study drug; missing information of dosing and sampling times). All subjects and samples excluded from the analysis will be clearly documented in the study report.

Descriptive statistics will be used to summarize esketamine, noresketamine, 4β-hydroycholesterol, and total cholesterol concentrations at each sampling time point. For each esketamine treatment and day, descriptive statistics, including arithmetic mean, standard deviation (SD), coefficient of variation, median, minimum, and maximum will be calculated for each analyte at each sampling time.

Population PK analysis of plasma concentration-time data of esketamine will be performed using nonlinear mixed-effects modeling. Data may be combined with those of other selected studies to support a relevant structural model. Available baseline subject characteristics (demographics, laboratory variables, genotypes, race, etc.) will be tested as potential covariates affecting PK parameters. Details will be given in a population PK analysis plan and results of the population analyses will be presented in a separate report.

Pharmacokinetic and Pharmacodynamic Analysis

The relationship between MADRS total score (and possibly selected adverse events as additional pharmacodynamic parameters) and PK metrics of esketamine may be evaluated. If there is any visual trend in graphical analysis, suitable models will be applied to describe the exposure-effect relationships.

A pharmacokinetic/pharmacodynamics analysis may be performed after at least 80% of Panel A subjects have completed the double-blind treatment phase. Subject pharmacokinetic data, MADRS total score, and CADSS scores would be matched to the treatment assignment by an authorized external group who are not part of the study team. The data will be anonymized (i.e., identification numbers would be removed or changed). The results of the analysis would be used to assist in the evaluation of potential intranasal esketamine doses for future clinical studies.

The results of the pharmacokinetic/pharmacodynamics evaluation would be presented in a separate report.

Biomarker Analysis

Metabolomics (if analyzed)

Spearman rank correlation coefficients between pretreatment glycine concentrations and the MADRS total score percentage change from Day 1 (baseline) at all scheduled time points will be calculated to investigate whether pretreatment concentrations of glycine correlate with the magnitude of clinical change following the administration of intranasal esketamine or placebo. Changes in glutamate metabolic pathway markers induced by esketamine or placebo will be investigated using a pattern classifier algorithm. Samples from different studies on ketamine and esketamine in treatment resistant depression will be pooled for analysis.

Human Inflammation MAP (if analyzed)

Statistical analyses of the markers from the Human Inflammation MAP will use both a univariate and a multivariate approach to identify the least number of markers which yield the highest accuracy in

separating responders from non-responders to intranasal esketamine. A similar approach will be used to identify the pharmacodynamic effects of esketamine and placebo on inflammatory and neurotrophic markers.

Results of Human Inflammation MAP and metabolomics will be presented in a separate report.

Pharmacogenomic Analyses

A composite genotype and predicted phenotype will be derived from the raw genotyping data for *CYP2B6*. Allele and genotype frequencies will be tabulated. No formal statistical tests will be performed. Genetic results from other analyzed genes will be pooled together with data from other suitable studies for a meta-analysis.

Results of the pharmacogenomic analysis will be listed and summarized with other clinical studies in a separate pharmacogenomics report.

Safety Analyses

Safety data from Panels A and B will be pooled for analysis as well as analyzed separately for the safety summaries.

The primary safety analysis set will be defined to include all randomized subjects who receive at least 1 dose of double-blind study drug. For the optional open-label treatment phase, the safety analysis set will be defined to include all subjects who receive at least 1 dose of study drug during that phase. The same analyses of safety and tolerability will be conducted for the double-blind phase and the optional open-label treatment phase. Select safety summaries may be provided by Period 1 and Period 2 separately.

Adverse Events

The verbatim terms used in the CRF by investigators to identify adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). All reported adverse events with onset during the treatment phase (i.e., treatment-emergent adverse events, and adverse events that have worsened since baseline) will be included in the analysis. For each adverse event, the percentage of subjects who experience at least 1 occurrence of the given event will be summarized by treatment group.

Clinical Laboratory Tests

Laboratory data will be summarized by type of laboratory test. Reference ranges and markedly abnormal results (specified in the SAP) will be used in the summary of laboratory data. Descriptive statistics will be calculated for each laboratory analyte at baseline and at each scheduled time point.

ECG

The ECGs will be summarized with descriptive statistics on heart rate, RR, PR interval, QRS interval, QT interval, and corrected QT (QTc) interval using the following correction methods: QT corrected according to Bazett's formula (QTcB) and QT corrected according to Fridericia's formula (QTcF).

The frequency and percentage of subjects with QTc interval >450 milliseconds (ms), >480 ms, or >500 ms will be summarized, as will the percentage of subjects with QTc interval increases from baseline >30 ms or >60 ms.

All important abnormalities in ECG waveform that are changes from the baseline readings will be reported (e.g., occurrence of U waves).

Vital Signs

Descriptive statistics of temperature, pulse/heart rate, respiratory rate, pulse oximetry, and blood pressure (systolic and diastolic) values and changes from baseline will be provided at each scheduled time point. The percentage of subjects with values beyond clinically important limits will be summarized.

Nasal Exam and Nasal Tolerability Questionnaire

Changes in findings from the baseline nasal examination (including the upper respiratory tract/throat) will be listed by treatment group and period. Examinations will provide ratings (none, mild, moderate, or severe) that are based on a visual inspection of the nostrils, nasal mucosa, and throat for nasal erythema, rhinorrhea, rhinitis, capillary/blood vessel disruption and epistaxis. A shift table for changes in rating for each examination will be presented by treatment group.

In addition, scoring from the nasal tolerability questionnaire will be summarized descriptively by treatment group.

C-SSRS

Suicide-related thoughts and behaviors based on the C-SSRS will be summarized by treatment group in incidence and shift tables. Separate endpoints for suicidal ideation and suicidal behavior will be defined and summarized descriptively by treatment group. Missing scores will not be imputed.

CADSS, BPRS+, BPIC-SS, and MOAA/S

Descriptive statistics of each of the scores and changes from predose will be summarized at each scheduled time point.

Clinical Global Assessment of Alertness

The Clinical Global Assessment of Alertness will be analyzed descriptively.

PWC-20

The PWC-20 rating scale will be analyzed descriptively.

Cogstate® cognitive test battery and HVLT-R

Descriptive statistics of each of the cognitive domain scores and changes from baseline will be summarized at each scheduled time point.

Interim Analysis

An interim analysis may be performed for each panel as needed. If required, further details will be provided in a separate charter and interim analysis plan.

TIME AND EVENTS SCHEDULE 1 (OF 2): SCREENING AND DOUBLE-BLIND TREATMENT PHASES FOR PANEL A AND PANEL B

Phase	**Screening**	**Double-Blind Treatment [1]**													
Visit Number	**1**	***Period 1*: Visit 2 and Visit 4**										**3**	**8 A**	**-**
		***Period 2*: Visit 5 and Visit 7**										**6**		
Clinic Visit (C) or Telephone Contact (TC)	**C**	**C**										**TC [24]**	**C**	**C**
Day	**-28 to -1**	***Period 1*: Day 1 and Day 4**										**2**	**15**	**ET[2]**
		***Period 2*: Day 8 and Day 11**										**9**		
Time (*Postdose time points are from the time of the first intranasal spray at t = 0)*	**-**	**Predose**	**0**	**5 min**	**10 min**	**40 min**	**1 hr**	**1.5 hr**	**2 hr**	**3 hr**	**6 hr**	**-**	**-**	
Study Procedures														
Screening/Administrative														
Informed consent	X													
Inclusion/exclusion criteria	X													
Medical history,demographics, past psychiatric and family psychiatric history	X													
Prestudy therapy	X													
SAFER Interview [3]	X													
MINI [4]	X													
IDS-C_{30} [4]	X	X[6]												
MGH-ATRQ [4]	X													
Modified Berlin Questionnaire [5]	X													
Dispense subject diary [19]									X[7]				X	
Review of completed subject diary by study staff		X[18]												X
Intranasal Study Drug Administration														
Randomization		X[7]												
Panel A: Placebo or esketamine 28 mg, 56 mg, or 84 mg [8]			X	X	X									
Panel B: Placebo or esketamine 14 mg or 56 mg [8]			X	X										
Safety Assessments														
Physical examination	X	X[7]											X	X
Nasal examination	X	X[7]							X[7]				X	X
Nasal tolerability questionnaire [5]		X							X					
Height	X													
Body weight	X	X[7]											X	X
Vital signs [9]	X	X											X	X
Vital signs: BP, HR, and respiratory rate only					X	X	X		X					
12-lead ECG	X	X					X						X	X
Pulse oximetry		*Continuous[10]*												
C-SSRS (Screening/Baseline version) [4]	X													
C-SSRS (Since Last Visit version) [4]		X											X	X
MOAA/S [4]		*Every 5 minutes [11]*												
BPRS+ [4]		X				X			X					
CADSS[4]		X				X			X					
PWC-20 [4]													X[23]	X
Clinical Global Assessment of Alertness								X	X[21]					
BPIC-SS [5, 25]		X[6]											X	X

Phase	**Screening**	**Double-Blind Treatment [1]**												
Visit Number	**1**	***Period 1*: Visit 2 and Visit 4**										**3**	**8 A**	**-**
		***Period 2*: Visit 5 and Visit 7**										**6**		
Clinic Visit (C) or Telephone Contact (TC)	**C**	**C**										**TC [24]**	**C**	**C**
Day	**-28 to -1**	***Period 1*: Day 1 and Day 4**										**2**	**15**	**ET[2]**
		***Period 2*: Day 8 and Day 11**										**9**		
Time (*Postdose time points are from the time of the first intranasal spray at t = 0)*	**-**	**Predose**	**0**	**5 min**	**10 min**	**40 min**	**1 hr**	**1.5 hr**	**2 hr**	**3 hr**	**6 hr**	**-**	**-**	
Study Procedures														
Cogstate® computerized test battery and HVLT-R[27]	X[26]	X[6]												
Clinical Laboratory Assessments														
Hematology, Chemistry	X	X [7]											X	X
Total cholesterol		X[13]				X[13]								
Urinalysis	X	X [7]											X	X
Thyroid-stimulating hormone	X													
Serology (HIV, Hepatitis B, Hepatitis C)	X													
Serum pregnancy test	X												X	X
Urine pregnancy test		X												
Urine drug screen	X	X												
Alcohol screen (urine)	X													
Alcohol screen (breath)		X												
Efficacy Assessments														
MADRS (7-day recall) [4, 22]		X[7]											X	X
MADRS (24-hour recall) [4 , 22]												X		
MADRS (2-hour recall) [4, 12, 22]									X[7]					
CGI-S [4]		X							X			X	X	X
QIDS-SR$_{16}$ [5]	X	X[7]											X	X
PHQ-9		X[6]												
PGI-S [5]		X							X			X[19]	X	X
PGI-C [5]												X[19]	X	X
GAD-7 (7-day recall) [5]		X[7]											X	X
EQ-5D-5L [5]		X											X	X
Pharmacokinetics														
Blood sample collection		X [6, 13]				X[13, 14]			X[14]	X[14]	X[14]			
Pharmacogenomics (DNA)														
Blood sample collection[15]		X												
Biomarkers														
Blood sample collection for Inflammatory MAP		X[20]											X	X
Blood sample collection for Metabolomics		X[20]							X[20]				X	X
Ongoing Subject Review														
Concomitant therapy [16]	X	X	X	X	X	X	X	X	X	X		X	X	X
Adverse events [17]	X	X	X	X	X	X	X	X	X	X		X	X	X

Footnotes:

1. With the exception of Day 1, visits can occur +/- 1 day.
2. If a randomized subject that has received at least one dose of study medication discontinues the study prior to completion of the double-blind treatment phase, an ET visit will be conducted at the time of discontinuation.

3. Conducted by a remote, independent rater. The SAFER Interview includes the MADRS, a review of the MGH-ATRQ performed at Screening, and the SAFER Criteria Inventory.
4. Clinician-administered assessment. Note: The MGH-ATRQ will be completed in collaboration with the subject.
5. Subject-completed assessment.
6. Day 1 only.
7. Day 1 and Day 8.
8. Self-administered under direct supervision by the Investigator or designee. Time 0 is defined as the time of the first 100-µL spray.
9. Blood pressure, heart rate, respiratory rate, and temperature (tympanic recommended).
10. On each dosing day, continuous arterial oxygen saturation monitoring by pulse oximetry (SpO_2) starting 5 minutes before first spray and then after the first nasal spray (i.e., t = 0 hour), it will be monitored continuously and documented every 15 minutes for approximately 1 hour postdose.
11. Performed every 5 minutes from predose to 1 hour postdose or longer, if necessary, until the subject has a score of 5. If a score of 5 is reached prior to 1 hour postdose, assessments are to still to continue through 1 hour postdose.
12. The sleep and appetite items will not be assessed. These predose MADRS scores for these items performed on the same day will be carried forward.
13. The plasma concentrations of 4β-hydroxycholesterol will be measured from the PK samples obtained at predose on Day 1 only and at t = 40 min on Day 11 only. A separate blood sample for measurement of total cholesterol, which will support the 4β-hydroxycholesterol analyses, will be obtained at the same time points (Day 1 predose, Day 11 at t = 40 minutes .
14. Day 1 and 11 only. An individual PK sample for the measurement of esketamine and noresketamine plasma concentrations will be obtained. The sample collected at t = 40 minutes can be obtained between t = 30 and 50 minutes. The sample collected at t = 2 hours postdose can be obtained between 1.5 and 2 hours postdose. The sample collected at t = 3 hours postdose can be obtained between 2.5 and 3 hours postdose. The sample collected at t = 6 hours (Panel A: required; Panel B: optional) can be obtained between 5.5 and 6.5 hours postdose. Time 0 is defined as the time of the first 100-mcl spray.
15. A 10 mL blood sample will be collected on Day 1 from all enrolled subjects. The pharmacogenomic (DNA) sample should be collected at the specified time point, however if necessary it may be collected at a later time point without constituting a protocol deviation.
16. Concomitant therapies must be recorded throughout the study beginning with signing of the informed consent until the last follow up visit.
17. All adverse events and special reporting situations, whether serious or non-serious, will be reported from the time a signed and dated informed consent form is obtained until completion of the subject's last study-related procedure (which may include contact for follow-up of safety).
18. Day 4 and 11 only.
19. Subject-reported outcome assessments scheduled for Day 2 and Day 9 will be completed in the subject diary. The diary will be collected and reviewed by study staff at the next scheduled clinic visit (i.e., Day 4 and Day 11). Subjects will be instructed to complete the subject-reported outcome assessments prior to any other assessments scheduled to occur during the telephone contact. If a subject discontinues the double-blind treatment phase prior to Day 11, the diary will be collected at the early termination visit. The diary to be used in the posttreatment phase will be dispensed on Day 15.
20. Inflammation MAP: Day 1 (predose) and Day 4 (predose). Metabolomics: Day 1 at predose and t = 2 hours postdose.
21. If response is not “Yes” at 2 hours postdose, repeat assessment every 30 minutes until a “Yes” response.
22. Administered using the Structured Interview Guide for Montgomery Asberg Depression Rating Scale (SIGMA).
23. The PWC-20 will not be performed in Visit 8A if the subject will be participating in the optional open label treatment phase.
24. For inpatient subjects, telephone contact visits can be performed at the inpatient location.
25. If a subject has a score greater than 18 on the BPIC-SS with no evidence of a urinary tract infection on urinalysis and microscopy, the subject will be referred to a specialist for evaluation.
26. Within 1 week prior to the planned first dose of study medication, Panel A subjects will have two mandatory practice sessions during the screening phase on the Cogstate® computerized test battery performed at the clinical site.
27. Panel A only.

TIME AND EVENTS SCHEDULE 2 (OF 2): OPTIONAL OPEN-LABEL TREATMENT AND POSTTREATMENT PHASES FOR PANEL A AND PANEL B

Phase	**Optional Open-Label Treatment[1]**									**Posttreatment (Follow Up) [1]**			
Visit Number	**Panel A: 8B, 9, 10, 11, 12, 13, 14, 15, 16**									**17**	**18**	**19**	**20**
	Panel B: 8B, 9, 10, 11									**12**	**13**	**14**	**15**
Clinic Visit (C) or Telephone Contact (TC)	**C**									**TC[12]**	**C**	**TC[12]**	**TC[12]**
Day (Visit Number)	**Panel A: 15, 18, 22, 25, 32, 39, 46, 60, 74**									**1 week after last dose**	**2 weeks after last dose**	**4 weeks after last dose**	**8 weeks after last dose**
	Panel B: 15, 18, 22, 25												
Time (Postdose time points are from the time of the first intranasal spray at t = 0)	**Predose**	**0**	**5 min**	**10 min**	**40 min**	**1 hr**	**1.5 hr**	**2 hr**	**3 hr**	**-**	**-**	**-**	**-**
Study Procedures													
Administrative													
Review of completed subject diary											X		
Intranasal Study Drug Administration													
Panel A: Esketamine (starting dose of 56 mg) [2]		X	X[2]	X[2]									
Panel B: Esketamine (starting dose of 56 mg)		X	X[18]										
Safety Assessments													
Physical examination	X[4]										X		
Nasal examination	X[4]										X		
Nasal tolerability questionnaire	X							X					
Body weight	X[4]										X		
Vital signs [5]	X[3]										X		
Vital signs: BP, HR, and respiratory rate only				X	X	X		X					
12-lead ECG	X[3]					X					X		
Pulse oximetry	*Continuous [6]*												
C-SSRS (Since Last Visit version) [7]	X[3]									X	X		
BPRS+ [7]	X				X			X					
CADSS [7]	X				X			X					
MOAA/S [7]	*Every 5 minutes [8]*												
PWC-20 [7]									X[21]	X	X		
Clinical Global Assessment of Alertness							X	X[19]					
BPIC-SS [9, 24]	X[23]										X		
Cogstate® computerized test battery and HVLT-R	X[26]										X[26]		
Clinical Laboratory Assessments													
Hematology, Chemistry	X[22]										X		
Total cholesterol					X[10]								
Urinalysis	X[22]										X		
Serum pregnancy test											X		
Urine pregnancy test	X												
Urine drug screen	X												
Alcohol screen (breath)	X												
Efficacy Assessments													
MADRS (7-day recall) using SIGMA [7]	X[20]									X	X	X	X
CGI-S [7]	X[20]										X	X	X

Phase	Optional Open-Label Treatment[1]									Posttreatment (Follow Up) [1]			
Visit Number	Panel A: 8B, 9, 10, 11, 12, 13, 14, 15, 16									17	18	19	20
	Panel B: 8B, 9, 10, 11									12	13	14	15
Clinic Visit (C) or Telephone Contact (TC)	C									TC[12]	C	TC[12]	TC[12]
Day (Visit Number)	Panel A: 15, 18, 22, 25, 32, 39, 46, 60, 74									1 week after last dose	2 weeks after last dose	4 weeks after last dose	8 weeks after last dose
	Panel B: 15, 18, 22, 25												
Time (Postdose time points are from the time of the first intranasal spray at t = 0)	Predose	0	5 min	10 min	40 min	1 hr	1.5 hr	2 hr	3 hr	-	-	-	-
Study Procedures													
QIDS-SR$_{16}$ [9]	X[20]									X[15]	X		
PHQ-9	X[25]												
PGI-S [9]	X[20]									X[15]	X		
GAD-7 [9]	X[16]										X		
EQ-5D-5L [9]	X [4]										X		
Pharmacokinetics													
Blood sample collection					X[10]			X[11]	X[11]				
Biomarkers													
Blood sample collection for Inflammation MAP								X[17]					
Ongoing Subject Review													
Concomitant therapy [13]	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events [14]	X	X	X	X	X	X	X	X	X	X	X	X	X

Footnotes:

1. Visit window: Day 15 (+/- 1 day), Day 18, 22, and 25 can occur +/- 2 days. The follow up visits can occur +/- 3 days. For Panel A only: Day 32, 39, and 46 can occur +/- 2 days, Day 60 and 74 can occur +/- 3 days.
2. Time 0 is defined as the time of the first 100-mcl spray. The need to administer study medication at.t = 5 min and t = 10 min is determined by the total dose being administered (28, 56, or 84 mg). All subjects start with intranasal esketamine 56 mg on Day 15, and the Day 18, 22, 25, 32 (Panel A), 39 (Panel A), and 46 (Panel A) can be down-titrated to the next lower dose or titrated up to the next higher dose at any visit, if desired based on the Investigator's clinical judgment of efficacy and safety. For Panel A only: No further dose adjustment is permitted after Day 46; the same dose administered on Day 46 will be administered on Day 60 and 74.
3. Not required at predose at Visit 8B (Day 15) if subject completed the double-blind phase (Visit 8A; Day 15) on the same day.
4. Day 18 and 25 only.
5. Blood pressure, heart rate, respiratory rate, and temperature (tympanic recommended).
6. On each dosing day, continuous arterial oxygen saturation monitoring by pulse oximetry (SpO2) placed 5 minutes before first spray and then after the first nasal spray (i.e., t = 0 hour), it will be monitored continuously for approximately 1 hour postdose.
7. Clinician-administered assessment
8. Performed every 5 minutes from predose to 1 hour postdose or longer, if necessary, until the subject has a score of 5. If a score of 5 is reached prior to 1 hour postdose, assessments are to still to continue through 1 hour postdose.
9. Subject-completed assessment
10. Day 25 only. The plasma concentrations of esketamine, noresketamine, and 4β-hydroxycholesterol will be measured from the PK sample obtained at t = 40 minutes (+/- 10 min). A separate blood sample will be collected for measurement of total cholesterol at the same time point [t = 40 minutes (+/- 10 minutes)].
11. Day 25 only. The PK sample collected at t = 2 hours postdose can be obtained between 1.5 and 2 hours postdose. The PK sample collected at t = 3 hours postdose can be obtained between 2.5 and 3 hours postdose. Time 0 is defined as the time of the first 100-mcl spray.
12. For inpatient subjects, telephone contact visits can be performed at the inpatient location.
13. Concomitant therapies must be recorded throughout the study beginning with signing of the informed consent until the last follow up visit.

14. All adverse events and special reporting situations, whether serious or non-serious, will be reported from the time a signed and dated informed consent form is obtained until completion of the subject's last study-related procedure (which may include contact for follow-up of safety.
15. Completed in subject diary. Subjects will be instructed to complete the patient-reported outcome assessments prior to any other assessments scheduled to occur during the telephone contact.
16. Day 22 only. For Panel A, also performed on Day 32, 46, and 74.
17. Day 25 only.
18. Time 0 is defined as the time of the first 100-mcl spray. The need to administer study medication at t = 5 min is determined by the total dose being administered (14 mg, 28 mg, or 56 mg). All subjects start with intranasal esketamine 56 mg on Day 15 and subsequent doses can be down-titrated to the next lower dose or titrated up to the next higher dose at any visit, if desired, based on the Investigator's clinical judgment of efficacy and safety.
19. If response is not "Yes" at 2 hours postdose, repeat assessment every 30 minutes until a "Yes" response.
20. Not performed at Visit 8B.
21. Performed once at the subject's last visit of the optional open label treatment phase.
22. Panel A: Performed on Day 25, 46, and Day 74. Panel B: Performed on Day 18 and 25.
23. Performed on Day 25 (Panel A and Panel B), Day 46 (Panel A only) and Day 74 (Panel A only).
24. If a subject has a score greater than 18 on the BPIC-SS with no evidence of a urinary tract infection on urinalysis and microscopy, the subject will be referred to a specialist for evaluation.
25. Panel A: predose on Day 15, 25, 39, 60, and 74. Panel B: predose on Day 15 and 25.
26. Panel A: predose on Day 25, 46, 74 and 2 weeks after last dose.

Abbreviations: **BPIC-SS** = Bladder Pain/Interstitial Cystitis – Symptom Score; **BPRS+** = Four-item positive symptom subscale of the Brief Psychiatric Rating Scale; **CADSS**= Clinician Administered Dissociative States Scale; **CGI-S** =Clinical Global Impression – Severity (S); **C-SSRS** = Columbia Suicide Severity Rating Scale; **EQ-5D-5L** = EQ-5D™ is a trade mark of the EuroQol Group; 5 level; **GAD-7** = Generalized Anxiety Disorder 7-item scale**; HVLT-R** = Hopkins Verbal Learning Test- Revised; **IDS-C_{30}** = Inventory of Depressive Symptoms-Clinician rated, 30-item; **MADRS** = Montgomery Asberg Depression Rating Scale;
MGH-ATRQ = Massachusetts General Hospital – Antidepressant Treatment Response Questionnaire; **MINI** = Mini International Neuropsychiatric Interview; **MOAA/S** = Modified Observer's Assessment of Alertness/Sedation; **PGI-C** = Patient Global Impression of Change; **PGI-S** = Patient Global Impression – Severity; **PHQ-9** = Patient Health Questionnaire-9; PWC = Physician Withdrawal Checklist; **SAFER** = State vs. Trait, Assessibility, Face Validity, Ecological Validity, Rule of Three P's**; SIGMA** = Structured Interview Guide for Montgomery Asberg Depression Rating Scale; **QIDS-SR_{16}** = 16-item Quick Inventory of Depressive Symptoms- Self Report

ABBREVIATIONS

ANCOVA	analysis of covariance
ASA	American Society of Anesthesiologists
ATRQ	antidepressant treatment response questionnaire
AUC	area under the plasma concentration-time curve
BPIC-SS	Bladder Pain/ Interstitial Cystitis Symptom Score
BPRS	brief psychiatric rating scale
BPRS+	four-item positive symptom subscale of the brief psychiatric rating scale
CADSS	clinician administered dissociative states scale
CGI-S	clinical global impression – severity
C_{max}	maximum plasma concentration
CRF	case report form (paper or electronic as appropriate for this study)
C-SSRS	columbia suicide severity rating scale
CVMP	committee for medicinal products for veterinary use
CYP	hepatic cytochrome P450
DSM-IV-TR	diagnostic and statistical manual of mental disorders (4th edition), text revised
DSM-5	diagnostic and statistical manual of mental disorders (5th edition)
ECG	electrocardiogram
eDC	electronic data capture
EDTA	ethylenediaminetetraacetic acid
EQ-5D-5L	EuroQol Group; 5 dimension; 5 level
EQ-VAS	EuroQol Group: visual analogue scale
FT4	free thyroxine
GAD-7	generalized anxiety disorder 7-item scale
GCP	good clinical practice
HBsAg	hepatitis B surface antigen
HVLT-R	Hopkins Verbal Learning Test-Revised
HIV	human immunodeficiency virus
ICD-10	10^{th} revision of the "international statistical classification of diseases and related health problems"
ICF	informed consent form
ICH	international conference on harmonisation
IDS-C_{30}	inventory of depressive symptomatology-clinician rated, 30-item
IEC	independent ethics committee
IRB	institutional review board
IV	intravenous
IWRS	interactive web response system
LC-MS/MS	liquid chromatography/mass spectrometry/mass spectrometry
LD_{50}	median lethal dose
MADRS	montgomery asberg depression rating scale
MAO	monoamine oxidase
MDD	major depressive disorder
MedDRA	medical dictionary for regulatory activities
MGH-ATRQ	massachusetts general hospital – antidepressant treatment response questionnaire
MINI	mini international neuropsychiatric interview
MOAA/S	modified observer's assessment of alertness/sedation
NMDA	n-methyl-d-aspartate
NOAEL	no observed adverse effect level
OCD	Obsessive-compulsive disorder
PD	pharmacodynamic
PGI-C	patient global impression of change
PGI-S	patient global impression - severity
PHQ-9	Patient health questionnaire - 9
PK	pharmacokinetic
PQC	product quality complaint
PRO	patient-reported outcome(s)

PTSD	Post-traumatic stress disorder
PWC-20	physician withdrawal checklist; 20-item
QIDS-SR_{16}	16-item quick inventory of depressive symptoms- self report
SAFER	state vs. trait, assessibility, face validity, ecological validity, rule of three P's
SIGMA	structured interview guide for the montgomery asberg depression rating scale
TEAEs	treatment-emergent adverse events
TRD	treatment-resistant depression
USP	United States pharmacopeia

1. INTRODUCTION

Major depression is associated with significant social, educational, and vocational impairment; high utilization of social and health care services; and increased medical morbidity and mortality. While there are a number of treatments available for the treatment of depression, nearly 30 to 50% of patients do not remit from current biogenic amine-based antidepressant drugs (Preston 2013; Trivedi 2006). Recent advances have begun to shed light on this common and debilitating illness. There are consistent reports of decreased size of brain regions implicated in depression, as well as neuronal atrophy, including loss of synapses in MDD and rodent chronic stress models (Manji 2001; Price 2012). Converging lines of evidence suggest that major depression is associated with abnormalities in glutamatergic synaptic transmission resulting in loss of synaptic plasticity in mood and emotion circuits (Kavalali 2012; Sanacora 2008). Ketamine/esketamine are inhibitors of the glutamatergic NMDA receptor. Preclinical studies have suggested that ketamine is associated with fast induction of synaptogenesis in rodents and reversal of the atrophy caused by chronic stress (Li 2010).

Ketamine is a racemate of R-(-)-ketamine and S-(+)-ketamine. Esketamine (JNJ-54135419) is the S-enantiomer of the ketamine racemate. Ketamine and esketamine are approved medications in several countries for the induction of general anesthesia and for use in addition to other anesthetics. The mechanism of action of ketamine and esketamine results from a noncompetitive binding to the N-methyl-D-aspartate (NMDA)-receptor (ligand-gated calcium channel) at the phencyclidine binding site (Anand 2011). Both also have additional binding sites (NMDA and non-NMDA glutamate receptors, nicotinic and muscarinic, cholinergic, and monoaminergic and opioid receptors, voltage-dependent sodium and L-type calcium channels).

Janssen Research and Development is developing esketamine for intranasal administration. Esketamine has been shown to be well and rapidly absorbed in the systemic circulation via the intranasal route (Clinical Study Report ESKETINTRD1001, in preparation). Intranasal administration has several advantages over intravenous administration, including convenience for patients, safety in terms of limited amount of drug given at any one time, and reduced dosing errors. The physiology of the nasal mucosa makes the rapid and non-invasive delivery of systemic drugs possible. Its large surface area, uniform temperature, high permeability, and extensive vascularization facilitate rapid absorption of drugs into the bloodstream (Turker 2004).

The efficacy of the racemic ketamine administered as a 40-minute intravenous infusion has been evaluated in subjects with treatment-resistant depression (TRD) (see section on Clinical Studies below). In addition, the sponsor recently completed a Phase 2 study (Clinical Study Report ESKETIVTRD2001, in preparation) that assessed the efficacy of 0.2 mg/kg and 0.4 mg/kg (both as 40-minute intravenous infusions) of esketamine in this population. A second ongoing Phase 2 study (Clinical Protocol KETIVTRD2002) that is being conducted by the sponsor in the United States will explore different dose frequencies using 0.5 mg of racemic ketamine as a 40-minute intravenous infusion.

For the most comprehensive nonclinical and clinical information regarding esketamine, refer to the latest version of the Investigator's Brochure for esketamine (IB esketamine hydrochloride 2013).

The term "sponsor" used throughout this document refers to the entities listed in the Contact Information page(s), which will be provided as a separate document.

1.1. Background

1.1.1. Nonclinical Studies

Toxicology

Single Dose Toxicity Studies

The median lethal dose (LD_{50}) of racemic ketamine ranges between 140 mg/kg (rat, intraperitoneal injection) and 616 mg/kg (mouse, oral administration) (Committee for Medicinal Products for Veterinary Use [CVMP] 1997). These high LD_{50}-values do not signal a concern regarding the clinical study at hand.

Repeat-Dose Toxicity Studies

In a 1-month repeat-dose toxicity study, rats were treated intranasally with 75, 90, or 105-mg/kg/day of racemic ketamine in the presence of 0.002% benzalkonium chloride. In all treated groups, dose-related clinical signs were noted including ataxia, hypoactivity, hyperactivity and recumbency. Furthermore, oral discharge was observed without any histomorphologic correlate in the nares. Adverse effects were limited to the 90 and 105 mg/kg/day groups and included increased incidences of irregular respiration and rough haircoat. Body weight gains were decreased in all treated groups. This finding correlated with decreased food consumption in males.

Based on the findings above, the no observed adverse effect level (NOAEL) was set at 75 mg/kg/day. A toxicokinetic examination was not included.

In a 1-month repeat-dose toxicity study, dogs were treated intranasally with racemic ketamine at dosages of 4, 20, and 60 mg/kg/day in the presence of 0.002% benzalkonium chloride. Transient clinical observations included salivation at all dosages, wobbly gait and inability to stand at 20 and 60 mg/kg/day, and right/left/ventral recumbency, vocalization, tremors and increased activity at 60 mg/kg/day. Gross necropsy revealed multiple red foci or reddened mucosa in the urinary bladder of males at 20 and 60 mg/kg/day and in all female dose groups. Microscopically, a treatment-related minimal chronic inflammation in the urinary bladder was seen in all dogs at 20 and 60 mg/kg/day, often accompanied by hyperplasia of the transitional epithelium and hemorrhage. One female dog dosed at 4 mg/kg/day showed urinary bladder lesions with discrete foci of lymphocytic proliferation. Resolution of the bladder inflammation was apparent at 2 weeks after cessation of treatment.

On Day 27, the mean C_{max} values of racemic ketamine were 0.161, 0.570, and 2.958 μg/mL in male dogs, and 0.213, 0.729, and 2.827 μg/mL in female dogs, at 4, 20, and 60 mg/kg/day, respectively. The mean AUC_{all} values were 0.040, 0.396, and 2.215 μg.h/mL, respectively, in male dogs, and 0.088, 0.359, and 2.569 μg.h/mL, respectively, in female dogs.

Safety Pharmacology

In chronically instrumented dogs with autonomic nervous system blockade, intravenously administered ketamine induced a decrease in myocardial contractility. Concomitant decreases in global isovolumetric indices of contractility, regional myocardial function, and cardiac output were also observed. The negative inotropic effect of ketamine was a direct action and independent of the autonomic nervous system (Pagel 1992).

Additional information regarding the effects of ketamine on the cardiovascular system, including effects on blood pressure, is summarized in the esketamine IB (IB esketamine hydrochloride 2013).

Neurotoxicity

Parenterally administered racemic ketamine has been reported to induce neuronal vacuolation and apoptotic cell death in the posterior cingulate and retrosplenial cortex of adult rats (40 to 60 mg/kg, single subcutaneous or intraperitoneal dose) (Olney 1989; Jevtovic-Torodovic 2000, 2001, 2005) and adult mice (50 mg/kg/day intraperitoneally for 7 days) (Zuo 2007).

In juvenile rodents, racemic ketamine induces a more widespread apoptotic neurodegeneration than in adult rodents involving several major regions of the developing brain. The time window of vulnerability to this neuroapoptosis-inducing action coincides with the period of synaptogenesis. In rats, this period begins at 1 day after birth and terminates at approximately 14 days after birth. In humans, it spans the last 3 months of pregnancy and extends into the first several years postnatally.

As in rodents, the window of vulnerability to the neurotoxic effect of ketamine in the nonhuman primate is restricted to the period of rapid synaptogenesis, which occurs at least by 75% of gestation lasting to postnatal Day 35. Rhesus monkeys at 3 stages of development (122 days of gestation [early third trimester; full term at gestation Day 165], and postnatal Days 5 and 35) received racemic ketamine at a high anesthetic dose. Apoptotic and necrotic cell death were noted in the frontal cortex of gestation Day 122 and postnatal Day 5 animals, but not in postnatal Day 35 animals. The plasma levels of racemic ketamine averaged approximately 5 to 15 μg/mL. The rhesus monkey brain at gestation Day 120 is considered to be at a developmental stage equivalent to the brain of a mid- to late-third trimester human fetus (or prematurely born infant), and the postnatal Day 5 to 6 rhesus monkey brain is considered equivalent to that of a 4- to 6 month-old human infant (Brambrink 2012; Slikker 2007).

The relevance to humans of “Olney lesions” is not known. The doses being evaluated in the study would produce exposure that is less than 10x the exposure where lesions occurred.

Developmental or Reproductive Toxicity Studies

In rats, an embryo-fetal developmental toxicity study was conducted with intranasally administered racemic ketamine at dosages of 15, 50, or 150 mg/kg/day in the presence of 0.002% benzalkonium chloride. Dose-related clinical observations were seen at 50 and 150 mg/kg/day and included salivation, wobbly gait, ocular discharge, and dark material around the nose. A rough coat was seen in 1 animal in each of the groups, and nystagmus, impaired mobility and decreased activity were seen in the 150-mg/kg/day group. No treatment-related change in corrected maternal body weight gain was noted. There was no effect of treatment on pregnancy parameters. There were no treatment-related changes at external, visceral or skeletal examination in the fetuses either.

On Day 6 of gestation, the mean C_{max} and $AUC_{0-\infty}$ values of racemic ketamine were 1.37, 7.96, and 10.65 μg/mL, respectively, and 0.83, 5.18, and 6.86 μg.h/mL, respectively. On Day 17 of gestation, the mean C_{max} and $AUC_{0-\infty}$ values were 1.95, 14.13, and 21.73 μg/mL, respectively, and 0.92, 7.77, and 12.95 μg.h/mL, respectively.

In rats, a dose of 15 mg/kg/day was considered the no observed effect level for maternal toxicity and a dose of 150 mg/kg/day was considered the no observed effect level for developmental toxicity.

In an embryo-fetal developmental toxicity study, pregnant rabbits were intranasally dosed with racemic ketamine at dosages of 10, 30, or 100 mg/kg/day in the presence of 0.002% benzalkonium chloride. Due to mortality in the high-dose group, the high dose was reduced from 100 to 50 mg/kg/day during the course of treatment.

Clinical observations were mainly noted in the 30 and 100/50 mg/kg/day groups and included wobbly gait, decreased activity, wet around nares, and a low incidence of post-dose salivation, dilated pupils and ocular discharge. Furthermore, a low incidence of nasal discharge, decreased food consumption and few feces (small in size) were observed in the 30- and 100/50-mg/kg/day groups. Lateral recumbency, shallow breathing, and eyes dark in color were seen in the high-dose group. The corrected mean body weight gain appeared decreased at all dosages, with little effect at 10 mg/kg/day, and much greater effects at the mid- and high-dose (no body weight gain at 100/50 mg/kg/day).

Mean fetal body weights were only decreased in the 30 mg/kg/day female fetuses. There were no treatment-related differences in external, visceral and skeletal malformations or variations seen in fetuses in the test article-treated groups as compared with controls.

On Day 6 of gestation, the mean C_{max} values were 0.12, 1.50, and 11.8 μg/mL, respectively, and the mean $AUC_{0-\infty}$ values were 0.03 (AUC_{all}), 1.71, and 5.46 μg.h/mL, respectively. On Day 18 of gestation, the mean C_{max} values were 0.05, 1.90, and 2.31 μg/mL, respectively, and the mean and $AUC_{0-\infty}$ values were 0.01 (AUC_{all}), 0.76, and 1.53 (AUC_{all}) μg.h/mL, respectively.

In rabbits, based on these results, 10 mg/kg/day was considered the no observed adverse effect level for maternal and developmental toxicity, respectively.

Reproduction studies in dogs, injected with 25 mg/kg ketamine intramuscularly 6 times during 1 trimester of pregnancy (twice a week over a 3-week period), did not show apparent adverse effects. Rats were injected during the pre-mating period (10 mg/kg intravenously), the period of organogenesis (20 mg/kg intramuscularly) and the perinatal period (20 mg intramuscularly), and rabbits were injected during the period of organogenesis (20 mg/kg intramuscularly). Ketamine did not affect reproduction (CVMP 1997).

Genetic Toxicity Studies

In an Ames test, racemic ketamine did not show a mutagenic effect. Racemic ketamine has been reported to show genotoxic potential in an in vitro sister chromatic exchange assay in Chinese Hamster ovary cells (Adhvaryu 1986; CVMP 1997) and an in vitro micronucleus test in Chinese Hamster lung fibroblasts (Toyama 2006). The latter 2 tests do not meet current standards of testing. A positive signal was found in an in vitro mouse lymphoma assay with racemic ketamine in the presence of metabolic activation. An in vitro mouse micronucleus test with esketamine also showed positive in the presence of metabolic activation. (CVMP 1997).

Additional information regarding the effects of ketamine/esketamine on preclinical safety is summarized in the esketamine IB (IB esketamine hydrochloride 2013).

1.1.2. Clinical Studies

1.1.2.1. Summary of Ketamine/Esketamine Efficacy

The efficacy of subanesthetic doses (0.2 to 0.5 mg/kg) of intravenous ketamine has been evaluated in approximately 150 TRD subjects, including 2 studies in bipolar depressed subjects (reviewed in: Mathew 2012; Bunney 2011). In earlier pilot studies, a single intravenous infusion dose (0.5 mg/kg over 40 minutes) of the NMDA-receptor antagonist ketamine produced a rapid (i.e., same day) and robust antidepressant effect lasting on average 5 days in patients with TRD (Zarate 2006) and similarly, responders to the first dose showed improvement with repeated doses for 2 weeks for an average of 19 days (aan het Rot 2010). Psychotomimetic side-effects were minimal and of short duration (Berman 2000; Messer 2010; Zarate 2006).

Preliminary data from a recently completed phase 2 study with IV esketamine (ESKETIVTRD2001 2012) suggests a similar, rapid, and robust antidepressant effect as seen with IV ketamine. This double-blind, double-randomization, placebo-controlled, multiple-dose titration study enrolled 30 adult subjects with TRD: 10 in the IV placebo group, 9 in the IV esketamine 0.20 mg/kg group, and 11 in the IV esketamine 0.40 mg/kg group (based on Day 1 randomization). The preliminary intention-to-treat analysis of the primary efficacy variable, change in MADRS total score from baseline Day 1 to Day 2, indicated that the improvement in both esketamine dose groups was statistically significant (1-sided p-value = 0.001 in both dose groups) when compared with the placebo group. The mean (standard deviation) change from baseline Day 1 to Day 2 in MADRS total score was -4.9 (4.72) in the placebo group, -16.8

(10.12) in the esketamine 0.20 mg/kg group, and -17.8 (9.45) in the esketamine 0.40 mg/kg group.

1.1.2.2. Summary of Ketamine/Esketamine Safety Profile

Ketamine is a rapidly-acting general anesthetic that is approved and widely used intravenously or intramuscularly for the induction and maintenance of anesthesia in children and adults. In Europe, ketamine is marketed as a racemic mixture and in some European Union countries also as the S-enantiomer, esketamine. Ketamine was first introduced as an anesthetic in 1963 and is considered to have an excellent medical safety profile (Haas 1992; Ketalar® Summary of Product Characteristics [SPC] 2011; Ketanest®S SPC 2011; Reich 1989; Sinner 2008).

Adverse Events Associated With Acute Use:

Adverse events reported for ketamine with anesthetic dosages include frequent elevation of blood pressure and pulse, which resolves immediately after the infusion is discontinued. The risk is higher in patients with untreated hypertension, patients with severe cardiac disease, patients at risk of a stroke, and patients with raised intracranial pressure. Although respiration is frequently stimulated, it is reported that severe depression of respiration or apnea may occur after rapid intravenous administration of high dosages of ketamine. Diplopia and nystagmus have been noted after ketamine administration, and it also may cause a slight elevation in intraocular pressure measurement. Anorexia, nausea, and vomiting have been observed; however, this is not usually severe. In some patients, enhanced skeletal muscle tone may be manifested by tonic and clonic movements, sometimes resembling seizures (Ketalar® SPC 2011).

According to the SPC for esketamine (Ketanest®S SPC 2011), the following are reported as common adverse effects: transient tachycardia, vivid dreams (including nightmares), nausea and vomiting, increased blood pressure, increased salivation, blurred vision, dizziness, motor unrest, increase in vascular resistance in pulmonary circulation and increase in mucus secretion, increased oxygen consumption, laryngospasms, and temporary respiratory depression. It is reported that the risk of respiratory depression typically depends on the dosage and injection speed (Ketanest®S SPC 2011). Esketamine may also have potential tolerability advantages over its racemic mixture (Sinner 2008). However, more research needs to be conducted in this area to fully evaluate tolerability of esketamine versus ketamine.

As patients emerge from ketamine anesthesia, perceptual alterations such as dissociative experiences (sense of observing one's body from a distance) and illusions (misinterpretation of a real, external sensory stimulus) have been reported (Sinner 2008). Subanesthetic doses of ketamine induce a range of transient dose-related dissociative, psychotomimetic, and cognitive effects in healthy human subjects that resemble some of the symptoms associated with schizophrenia (Krystal 2003). The schizophrenia-like symptoms include perceptual and mood changes and impairments in memory, attention, and abstract reasoning (Honey 2005; Krystal 1994, 1999, 2000, 2003, 2005; Morgan 2004; Rowland 2005). In humans, a single dose of ketamine induces marked, dose-dependent impairments in working and episodic memory at a range of doses which would impact profoundly on users' ability to function (Morgan 2006). A prior comprehensive report looked at the safety of studies with subanesthetic dosages of

ketamine in medically healthy subjects with no personal or familial Axis I psychotic spectrum disorders who were administered subanesthetic dosages of ketamine by intravenous infusion in a series of clinical investigations from 1989 to 2005 (Perry 2007). A reported 469 subjects were included; 833 active ketamine and 621 placebo infusions were administered to these subjects. All ketamine doses were administered intravenously in a bolus-plus-infusion paradigm or a continuous infusion alone. The bolus doses ranged from 0.081 mg/kg over 10 minutes to 0.26 mg/kg over 1 minute and continuous infusion doses ranged from 0.04 mg/kg to 0.75 mg/kg over 60 to 120 minutes all by intravenous route. Ten adverse mental status events were documented in 9 subjects/infusions that were deemed related to ketamine administration (2% of subjects). All but 1 adverse event resolved by the end of the test session, with the adverse events in the remaining individual no longer clinically significant within 4 days of the test session. No residual sequelae were observed. The mental status adverse events included 3 medically stable subjects who became unresponsive to verbal stimuli. All became responsive again within minutes of discontinuation of ketamine infusion and were back to their baselines by the end of the study day. Six subjects reported distress related to the mental status effects of ketamine, resulting in discontinuation of ketamine infusion. The distress, which resolved within minutes after termination of ketamine infusion, was described variously as "no control", "not a good feeling", "feeling panicky", "very unpleasant", "weird", "too high", "walls were closing in", "felt out of my element", "distorted", and "too intense". Two subjects became tearful. The effects reported by these subjects were experienced by other subjects without the same degree of distress or the need for intervention.

Longer-term follow-up data (up to 6 months from the last infusion of ketamine) from this study found no evidence of ketamine abuse by subjects after study participation and no evidence of subsequent psychiatric problems related to ketamine exposure (alone or in combination with other study drugs) (Perry 2007). Specifically, 100 subjects were contacted for follow-up assessments at 1 week after study participation, 39 subjects at 1 month, 50 subjects at 3 months, and 34 subjects at 6 months. Although these subjects comprised a relatively small subsample of ketamine study subjects, the data collected yielded no reports of emotional or psychological problems, cognitive deficits, medical or neurologic problems, cravings for ketamine, use of ketamine outside the research setting, unusual perceptions, sluggishness, flashbacks, or paranoid thoughts.

Similarly, a prior analysis of these data failed to find evidence of sensitization to the effects of ketamine in those subjects who had more than one exposure to this drug (Cho 2005). These findings are consistent with the lack of long-term effects reported with anesthetic doses of ketamine, (Corssen 1971; Moretti 1984), and further document the safety of subanesthetic doses of ketamine as a psychopharmacologic probe in healthy subjects (Perry 2007).

Adverse Events Associated with Chronic Use:

Much of the literature on chronic use of ketamine comes from the data gathered from street users of the drug rather than systematically conducted clinical studies. Data therefore should be interpreted with caution, as in many cases, no baseline predrug data are available and drug exposure is poorly documented. Recently, Morgan and Curran conducted a comprehensive

review to survey and integrate the research literature on physical, psychological, and social harms of both acute and chronic ketamine use (Morgan 2011). Chronic physical effects reported include ketamine-induced ulcerative cystitis, hydronephrosis, and abdominal cramps.

In the 1-year longitudinal study of 150 individuals (Morgan 2010), Morgan and colleagues divided 30 subjects into 5 groups: frequent ketamine users (more than 4 times per week), infrequent ketamine users (at least once a month), abstinent users (abstinent for at least 1 month), polydrug controls, and non-users of illicit drugs. Eighty percent of the participants were retested at the end of 1 year. Cognitive deficits were mainly observed in frequent users and not with the infrequent users. Short-lasting dose-dependent effects of psychosis were associated with ketamine users. There was no increase in symptoms over time and symptoms were completely reversible upon stopping use of ketamine. As noted, these data should be interpreted with caution, as baseline data predating drug use were not available. Furthermore, in their recent review, Morgan and Curran report that there is little evidence of any link between chronic, heavy use of ketamine and diagnosis of a psychotic disorder.

Given that the principal action of ketamine is at the NMDA receptor, the consequences of ketamine use on cognition have been fairly widely investigated. Several studies have examined cognitive function in infrequent and frequent ketamine users (Curran 2000; Morgan 2006; Morgan 2009; Narendran 2005). Overall, infrequent or recreational ketamine use does not appear to be associated with long-term cognitive impairment (Narendran 2005). The most robust findings are that frequent ketamine users (more than 5 times a week) exhibit impairments in both short- and long-term memory (Morgan 2006). Although dosages have varied, dosages reported by ketamine users in this study were much higher than the dosages intended for use in treating TRD. Memory impairments may be reversible when individuals stop using the drug, as they were not found in a group of 30 ex-ketamine users who had been abstinent for at least a year (Morgan 2011).

Ketamine-induced ulcerative cystitis is a recently identified complication. The most common symptoms are frequency and urgency of urination, dysuria, urge incontinence and occasionally painful haematuria (blood in urine). Computerized tomography scans of these individuals revealed a marked thickening of the bladder wall, a small bladder capacity and perivesicular stranding consistent with severe inflammation. At cystoscopy all patients had severe ulcerative cystitis. Biopsies in four of these cases found denuded urothelial mucosa with thin layers of reactive and regenerating epithelial cells and ulcerations with vascular granulation tissue and scattered inflammatory cells. Cessation of ketamine use provided some relief of symptoms. Most of the described cases are in near daily users of ketamine for recreational purposes. The prevalence is difficult to determine as it is seen in recreational users who often don’t seek help.

The majority of cases resolve after stopping ketamine use, one-third remaining static. The aetiology of ketamine-induced ulcerative cystitis is unclear. It appears to be most common in those misusing the drug frequently, mainly daily, over an extended period. (Morgan 2011). In the current study, the patient reported BPIC-SS has been added at specific timepoints in the study to evaluate for potential treatment emergent cystitis.

Dependence and Withdrawal:

There are a number of reports of ketamine dependence in the literature (Hurt 1994; Jansen 1990; Moore 1999; Pal 2002) but no large-scale studies, and so the incidence of ketamine dependence is largely unknown (Morgan 2011). An interview study of 90 ketamine users found that 57% of frequent users, 43% of infrequent users, and 60% of ex-users expressed concerns about ketamine addiction (Muetzelfeldt 2008). The majority of frequent users in that study reported using the drug without stopping until supplies ran out, so compulsive patterns of behavior are also a concern. Oral ketamine has also been evaluated as a positive control in human abuse potential studies, with dosages of 65 mg and 110 mg reported as appropriate for use as positive controls for future abuse potential studies of compounds with a similar mechanism of action or with possible perception-altering or euphoric effects (Shram 2011). There is conflicting evidence of the existence of a "withdrawal syndrome" after cessation of ketamine use (Morgan 2011). Cravings seem to be a key problem in frequent users: 28 of the 30 daily users in 1 study reported having tried to stop taking the drug but failed; all reported ketamine cravings as the reason for failure. The same study found that 12 of the 30 daily users reported withdrawal symptoms characterized by anxiety, shaking, sweating, and palpitations when they stopped using. A few published case studies also show craving and somatic and psychological aspects of anxiety as withdrawal symptoms (Critchlow 2006; Lim 2003). However, a specific ketamine withdrawal syndrome has not yet been described (Morgan 2011).

Safety Profile in Subjects with TRD and Bipolar Disorder

To date, subanesthetic dosages of intravenous ketamine (mainly single-dose) have been evaluated in several studies in subjects with TRD and bipolar disorder (Berman 2000; Diazgranados 2010; Mathew 2010; Zarate 2012; Zarate 2006). The safety profile observed in these studies is consistent with what has been observed previously with ketamine. For example, the first study in subjects with mood disorder described a rapid antidepressant effect after a single intravenous dose of ketamine, and reported significant but transient increases in psychotomimetic symptoms, as reflected in the BPRS, particularly the positive symptoms subscale (Berman 2000).

One study tested the tolerability, safety, and efficacy of repeat-dose open-label intravenous ketamine (6 infusions over 12 days) in 10 medication-free symptomatic subjects with TRD who had previously shown a meaningful antidepressant response to a single dose (aan het Rot 2010). In this study, ketamine elicited minimal positive psychotic symptoms. Three subjects experienced significant but transient dissociative symptoms and side effects during and after each ketamine infusion; these were generally mild. Throughout the study, all hemodynamic parameters were considered manageable by the administering anesthesiologist and did not necessitate termination of the infusions. After the first infusion, 3 subjects verbally reported headache of mild-to-moderate severity. During subsequent infusions, headache was reported 4 times by 4 different subjects. The Systematic Assessment for Treatment Emergent Effects Self-Report Inventory (SAFTEE-SI) data from the naturalistic follow-up visits was available for 7 of the 9 repeat-dose subjects. One subject reported a moderate increase in "sleep disturbance" from the preketamine baseline at the first follow-up visit but no longer did so at the second visit.

Another subject reported a moderate increase in "blurred vision" at the third follow-up visit after missing the first 2; this subject had no further visits. No other symptoms increased more than mildly (1 degree of severity) from baseline. There were no increases in "poor memory", ”trouble concentrating", or "word-finding difficulties", suggesting that ketamine did not have any persistent cognitive impact as per self-report.

Abuse Liability

Ketamine is a Schedule III drug (Food & Drug Administration 2012). Substances in this schedule have a potential for abuse less than substances in schedules I or II and abuse may lead to moderate or low physical dependence or high psychological dependence. In Belgium, ketamine is a controlled substance according to Chapter 2 of the 1998 Royal Decree on the regulation of psychotropic substances.

Safety Results from Study ESKETINTRD1001

The Phase 1 study ESKETINTRD1001 evaluating 4 single dose regimens of intranasal esketamine (28, 56, 84, and 112 mg), was conducted in healthy subjects. The regimens included in the present study were administered in study ESKETINTRD1001. Safety was assessed from the time of consent until the end of the study. The following safety assessments were collected: adverse events, vital signs, pulse oximetry, clinical laboratory results, physical examinations, and targeted nasal examinations coupled with assessment of nasal tolerability, electrocardiograms (ECGs) that were singular (Cohorts 1, 2, and 3) or continuous (Cohort 3, only), effects of intranasal esketamine on dissociative symptoms, psychosis-like side effects, and suicidal ideation and behavior. The subjects either returned to the study center approximately 11 days after the last dose of study medication for End-of-Study assessments or participated in these assessments at the time of early withdrawal.

Preliminary analyses indicated the following safety outcomes:

- No serious treatment-emergent adverse events (TEAEs), no TEAEs leading to discontinuation, and no deaths occurred.
- One subject with a previous history of psychosis participating in the study was discontinued once the history became known.
- The most common TEAEs were vertigo (29 [50.0%] of 58 subjects), dysgeusia (23 [39.7%] of 58 subjects), dizziness (22 [37.9%] of 58 subjects), and somnolence (22 [37.9%] of 58 subjects).
- Transient dissociative symptoms were seen, consistent with the published literature on ketamine. Dissociative symptoms generally resolved within 2 hours from the start of the dosing. The severity of dissociation was measure on the CADSS. All subjects at the 84 mg dose group had a transient increase in the CADSS scores. 2 of 15 subjects had a transient increase in CADSS scores at the 28 mg dose group and 5 of 15 subjects had a transient increase in CADSS scores at 56mg dose group. These symptoms were reported as adverse events by occurred in 23 (39.7%) of 58 subjects.

- Sedation was mild and transient typically up to 1 hour from the start of the dose.
- No treatment-emergent psychosis-like symptoms were seen.
- No clinically significant changes were seen in ECG assessments in any dose groups.
- No clinically significant changes were seen in laboratory assessments.

Safety Results from Study ESKETIVTRD2001

This placebo-controlled study evaluated 2 doses of IV esketamine (0.2 and 0.4 mg/kg) in patients with TRD (n = 30). During the first infusion period (up to Day 4, before the second infusion), which was the period associated with the primary efficacy endpoint, preliminary key safety results were as follows:

- No serious TEAEs, TEAEs leading to discontinuation, or deaths occurred.
- The most common TEAEs were headache, nausea, and dissociation:

Headache was reported by 7 subjects: 2 (20.0%) of 10 in the placebo group, 2 (22.2%) of 9 in the 0.20 mg/kg group, and 3 (27.3%) of 11 in the 0.40 mg/kg group.

Nausea was reported by 6 subjects: 2 (20.0%) of 10 in the placebo group, 3 (33.3%) of 9 in the 0.20 mg/kg group, and 1 (9.1%) of 11 in the 0.40 mg/kg group.

Dissociation was reported by 3 subjects: 0 of 10 in the placebo group, 1 (11.1%) of 9 in the 0.20 mg/kg group, and 2 (18.2%) of 11 in the 0.40 mg/kg group.

1.1.2.3. Human Pharmacokinetics of Intravenous Ketamine/Esketamine

Esketamine exhibits multiexponential compartmental pharmacokinetics after intravenous administration. The mean systemic clearance, steady-state distribution volume, and terminal half-life values were 26.3 mL/min/kg, 2.7 L/kg, and 146 minutes, respectively, after intravenous administration of esketamine to 10 healthy subjects (Ihmsen 2001). Esketamine does not invert to the R-enantiomer (Geisslinger 1993). The intravenous administration of 20 mg/hour and 40 mg/hour (both per 70 kg; 10 healthy subjects per regimen) of esketamine produced dose-proportional mean plasma Cmax values of 150.4 ng/mL and 304.7 ng/mL, respectively (Noppers 2011). Ketamine undergoes extensive metabolism by hepatic cytochrome P450 (CYP). In humans, N-demethylation to norketamine is the major route of metabolism, which can undergo further metabolism to form hydroxynorketamine. Ketamine and norketamine are extensively hydroxylated to a series of 6 hydroxynorketamine metabolites and 2 hydroxyketamine metabolites (Woolf 1987). Like ketamine, norketamine is a noncompetitive antagonist at the NMDA receptor (Ebert 1997; Holtman 2008). Norketamine has a half-life in plasma of approximately 5 hours (Hagelberg 2010). An inverse relationship has been reported between ketamine metabolites and psychotomimetic or dissociative side effects; i.e., higher dehydronorketamine and hydroxynorketamine concentrations were associated with lower scores on rating scales measuring psychosis symptoms (BPRS+) and dissociative symptoms (CADSS scores) (Zarate 2012). The major human hepatic CYPs that catalyze ketamine N-demethylation in vitro are CYP2B6 and CYP3A4, although there is ambiguity as to their comparative contributions to clinical ketamine metabolism (Yanagihara 2001; Hijazi 2002; Portmann 2010).

The CYP enzymes responsible for the formation of norketamine metabolites include CYP2A6 and CYP2B6 (Portmann 2010).

The administration of a 5-day oral regimen of rifampin (a potent inducer of hepatic CYP3A activity) to 10 healthy subjects before their receiving an intravenous infusion of esketamine produced a 13% and 200% increase in the elimination of esketamine and noresketamine, respectively, relative to treatment with placebo (Noppers 2011). A 4-day regimen of clarithromycin (a potent inhibitor of hepatic CYP3A activity) increased mean esketamine C_{max} and AUC values by 3.6-fold and 2.6-fold (relative to placebo), respectively, in 10 healthy subjects who received esketamine by the oral route (Hagelberg 2010). The effects of potent inducers and inhibitors of CYP on the pharmacokinetics of intranasally administered esketamine are expected to be smaller, relative to oral esketamine, since a smaller fraction of the dose is subjected to "first-pass" metabolism after intranasal delivery.

Pharmacokinetic Results from Studies ESKETINTRD1001 and ESKETIVTRD2001

Uncertainty exists with regards to the extent that plasma maximum concentration (C_{max}) and area under the plasma concentration-time curve (AUC) of ketamine (or esketamine) contributes to its effectiveness in patients with depression. Previously completed studies have demonstrated that a 0.5-mg/kg dose of ketamine intravenously infused over 40 minutes or 100 minutes improves depression scores in patients (Katalinic 2013; Rasmussen 2013). These results would suggest that the AUC plays a role, although the onset of efficacy appeared to be later (after up to 4 doses) when the 0.50 mg/kg dose was given over 100 minutes versus the typical response within a day after a 40-minute infusion. Moreover, a lower ketamine dose of 0.20 mg/kg administered as short (1-2 minute) IV infusion has also provided rapid benefit to patients with depression indicating that C_{max} values may contribute to the onset of response (Katalinic 2013).

[redacted]

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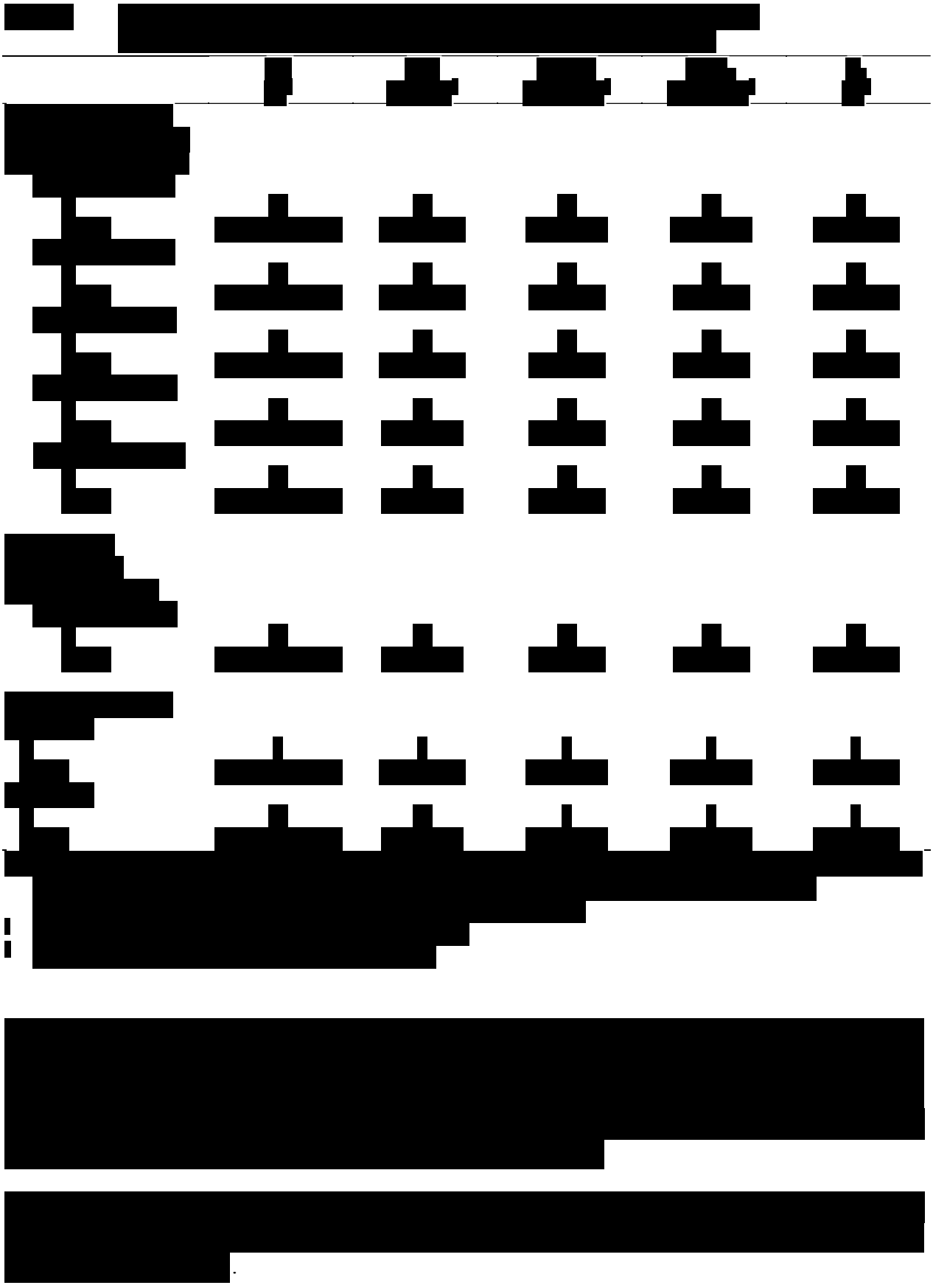


1.2. Overall Rationale for the Study

This is the first study to evaluate the efficacy, dose response, safety, and pharmacokinetics of intranasal esketamine in adult TRD patients. The results will guide the selection of intranasal dose regimens for further clinical development.

2. OBJECTIVES AND HYPOTHESIS

2.1. Objectives

Primary Objective

To assess the efficacy and dose response of intranasal esketamine (Panel A: 28 mg, 56 mg, 84 mg; Panel B: 14 mg and 56 mg) compared with placebo in improving depressive symptoms in subjects with TRD, as assessed by a change from baseline in the Montgomery-Asberg Depression Rating Scale (MADRS) total score for the combined periods in the double-blind treatment phase.

Secondary Objectives

The secondary objectives are:

1. To evaluate sustained response (≥50% reduction from baseline in MADRS total score) with onset by Day 2 through the end of the double-blind phase (Day 15).
2. To investigate the safety and tolerability of intranasal esketamine in TRD subjects, with special attention to:
    a. Local nasal tolerability, using a nasal tolerability questionnaire and nasal examinations
    b. Effects on heart rate, blood pressure, respiratory rate, and blood oxygen saturation (SpO_2)
    c. Effects on suicidal ideation/behavior measured by the Columbia Suicide Severity Rating Scale (C-SSRS);
    d. Effects on alertness and sedation measured by the Modified Observer's Assessment of Alertness/Sedation (MOAA/S) and Clinical Global Assessment of Alertness
    e. Psychosis-like side effects by using a four-item positive symptom subscale of the Brief Psychiatric Rating Scale (BPRS+) consisting of: suspiciousness, hallucinations, unusual thought content, and conceptual disorganization;
    f. Effects on dissociative symptoms using the Clinician Administered Dissociative States Scale (CADSS);
    g. Potential withdrawal symptoms following cessation of intranasal esketamine treatment, as measured by the clinician-administered 20-item Physician Withdrawal Checklist (PWC-20)
    h. Potential symptoms of cystitis using the Bladder Pain/ Interstitial Cystitis Symptom Score (BPIC-SS)
    i. Cognition, using the Cogstate® computerized test battery and the Hopkins Verbal Learning Test-Revised (HVLT-R) (Panel A only)

3. To assess the effect of intranasal esketamine compared to intranasal placebo on:
    a. Depressive symptoms, as assessed by the 16-item Quick Inventory of Depressive Symptomatology- Self Report ($QIDS\text{-}SR_{16}$)
    b. Remission, defined as a MADRS total score ≤10
    c. Response, defined as a ≥50% reduction from baseline in MADRS total score
    d. The severity of illness using the Clinical Global Impression - Severity (CGI-S) and the Patient Global Impression - Severity (PGI-S)
    e. Symptoms of anxiety as assessed by the Generalized Anxiety Disorder 7-item Scale (GAD-7)
4. To evaluate the pharmacokinetics (PK) of intranasal esketamine in subjects with TRD

Exploratory Objectives

The exploratory objectives are:

1. Subject perspective of global change in MDD from baseline, as measured by the Patient Global Impression of Change (PGI-C)
2. To assess the effect of intranasal esketamine compared to intranasal placebo on depressive symptoms as assessed by the 9-item patient health questionnaire (PHQ-9)
3. Impact on health status as assessed using the EuroQol-5D, 5-level version (EQ-5D-5L)
4. To evaluate whether pretreatment concentrations of inflammatory and neurotrophic markers, and plasma glycine correlate with the magnitude of clinical change, as measured by the MADRS, following intranasal administration of esketamine.
5. To assess the impact of intranasal esketamine on plasma inflammatory and neurotrophic markers and glutamatergic pathway metabolic markers

2.2. Hypothesis

The primary hypothesis is that intranasal esketamine (Panel A: 28 mg, 56 mg, 84 mg; Panel B: 14 mg and 56 mg) is superior to intranasal placebo in improving depressive symptoms in adult subjects with TRD, as assessed by the change from baseline in the MADRS total score for the combined periods in the double-blind treatment phase.

3. STUDY DESIGN AND RATIONALE

3.1. Overview of Study Design

This is a 2-panel, doubly-randomized, double-blind, placebo-controlled, multicenter study conducted in approximately 100 male and female adult subjects with TRD.

Panel A will be conducted in approximately 60 subjects in the United States and Belgium. Panel B will be conducted in approximately 40 Japanese subjects in Japan.

In both panels, each subject will participate in up to 4 phases:

- A screening phase of up to 4 weeks,
- A double-blind treatment phase (Day 1 to Day 15) which includes two 1-week treatment periods (Period 1 and Period 2),
- An optional open-label treatment phase (Panel A: Day 15 to 74 ; Panel B: Day 15 to 25), and
- An 8-week posttreatment (follow up) phase

The duration of the subject's participation will be approximately 14 to 23 weeks for Panel A and 14 to 16 weeks for Panel B. The end of study will occur when the last subject in the trial completes his/her last study assessment.

Panel A and B may be conducted in parallel; but Panel B will only be initiated after completion of the planned Phase 1 study to assess the pharmacokinetics in Japanese subjects.

Screening Phase (Day -28 to Day -1)

The screening phase for Panel A and B is the same.

After giving informed consent, subjects that are 20 to 64 years of age (inclusive), will be screened within 4 weeks of Day 1 (i.e., Day -28 to Day -1) to determine eligibility for study participation.

Subjects must meet Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition-Text Revised (DSM-IV-TR) diagnostic criteria for MDD, without psychotic features, based upon clinical assessment (DSM-IV-TR 296.22, 296.23, 296.32, or 296.33) and confirmed by the Mini International Neuropsychiatric Interview (MINI). Subjects must have an Inventory of Depressive Symptomatology 30-item Clinician-rated ($IDS\text{-}C_{30}$) total score ≥34. In addition, the major depressive episode must be deemed valid using the "State vs. Trait, Assessibility, Face Validity, Ecological Validity, Rule of Three P's" (SAFER) criteria interview, administered by remote, independent raters.

Subjects must have had an inadequate response to at least 2 antidepressants, at least one of which is in the current episode of depression, assessed using the Massachusetts General Hospital – Antidepressant Treatment Response Questionnaire (MGH-ATRQ) and prior medication history.

Note: Subjects that are not currently taking an antidepressant at Screening are eligible to participate in this study (i.e., subjects may participate in the study whether or not they are taking an antidepressant).

- Subjects who are taking an antidepressant at the Screening visit must have been receiving a stable dose (i.e., dose has been unchanged) for at least 2 weeks before the Screening visit.
    - With the exception of MAO inhibitors, which are prohibited, the subject may continue the antidepressant at the same dose during the double-blind treatment phase, or
    - The subject can discontinue the antidepressant within 1 week, or within a period at least 5 times the drug's half-life [exception: at least 4 weeks for fluoxetine and at

least 2 weeks for MAO inhibitors], whichever is longer, before the planned first dose of study drug.

- The decision to continue or discontinue the current antidepressant will be made by the subject and investigator (based on their clinical judgment). Antidepressant medication will not be discontinued for the sole purpose of allowing subjects to participate in the study.

Disallowed therapies, including psychotropic medications, are described in the exclusion criteria, Section 8 (Prohibitions and Restrictions), and Attachment 1.

Within 1 week prior to the planned first dose of study medication, Panel A subjects will have two mandatory practice sessions on the Cogstate® computerized test battery performed at the clinical site.

Other screening evaluations (e.g., biomarker, safety) will be performed as per the Time and Events Schedule.

All adverse events and special reporting situations, whether serious or non-serious, will be reported from the time a signed and dated ICF is obtained until completion of the subject's last study-related procedure (which may include contact for follow-up of safety).

Double-Blind Treatment Phase

In both panels, all subjects will participate in two treatment periods (Period 1 and Period 2) during the double-blind treatment phase. Efficacy, safety, pharmacokinetic, biomarker, and/or pharmacogenomic evaluations performed at each visit are described in the Time and Events Schedule. If a subject withdraws before the end of the double-blind treatment phase, for reasons other than withdrawal of consent, the Early Termination visit should be conducted at the time of discontinuation, followed by completion of the posttreatment phase.

Prior to the first dose, subjects will practice spraying (into the air) a demonstration intranasal device that is filled with water.

All subjects will self-administer the study medication in Period 1 and Period 2 under the direct supervision of the investigator or designee (Refer to Section 6, Dosage and Administration). On all dosing days, all subjects must remain at the clinical site for at least 2 hours postdose; a longer duration will be required on Day 1 and 11 (up to 6 hours postdose for study procedures) and on Day 25 (3 hours postdose for study procedures) and should be accompanied by a responsible adult when released from the clinical site. In addition, subjects must not drive a car or work with machines for 24 hours after study medication dosing.

For Panel A, if the placebo response or other assumptions at the end of Period 1 are not as expected, up to a maximum of 78 subjects (i.e., up to 18 additional subjects) may be enrolled to ensure that an adequate number of subjects are re-randomized in Period 2, based on the ongoing assessment of the numbers of subjects that are re-randomized as well as drop-out rates.

For Panel B, if the placebo response or other assumptions at the end of Period 1 are not as expected, up to a maximum of 52 subjects (i.e., up to 12 additional subjects) may be enrolled to ensure that an adequate number of subjects are re-randomized in Period 2, based on the ongoing assessment of the numbers of subjects who are re-randomized as well as drop-out rates.

Panel A

Period 1

On Day 1, subjects (n = 60) will be randomized using a 3:1:1:1 ratio to 1 of the following 4 treatment groups: Intranasal placebo (n = 30), intranasal esketamine 28 mg (n = 10), intranasal esketamine 56 mg (n = 10), or intranasal esketamine 84 mg (n = 10) administered on Day 1 and Day 4.

Period 2

Subjects that received intranasal esketamine 28, 56, or 84 mg in Period 1 will receive the same treatment in Period 2 on Day 8 and Day 11.

Period 2 treatment for subjects that received placebo in Period 1 will be determined by the QIDS-SR_{16} score on Day 8 (predose).

- Subjects that received intranasal placebo in Period 1 and have a QIDS-SR_{16} score < 11 (mild or no symptoms) will receive intranasal placebo again in Period 2 on Day 8 and Day 11.
- Subjects that received intranasal placebo in Period 1 and have a QIDS-SR_{16} score ≥11 (moderate to severe) will be re-randomized to receive intranasal placebo or intranasal esketamine 28 mg, 56 mg, or 84 mg in a 1:1:1:1 ratio on Day 8 and Day 11. The randomization will be stratified by the QIDS-SR_{16} score [moderate (QIDS-SR_{16} score 11 to 16) or severe (QIDS-SR_{16} score > 16)].

Panel B

Period 1

On Day 1, subjects (n = 40) will be randomized using a 2:1:1 ratio to 1 of the following 3 treatment groups: Intranasal placebo (n = 20), intranasal esketamine 14 mg (n = 10), or intranasal esketamine 56 mg (n = 10) administered on Day 1 and Day 4.

Period 2

Subjects that received intranasal esketamine 14 or 56 mg in Period 1 will receive the same treatment in Period 2 on Day 8 and Day 11.

Period 2 treatment for subjects that received placebo in Period 1 will be determined by the QIDS-SR_{16} score on Day 8 (predose):

- Subjects that received intranasal placebo in Period 1 and have a QIDS-SR_{16} score < 11 (mild or no symptoms) will receive intranasal placebo again in Period 2 on Day 8 and Day 11.

- Subjects that received intranasal placebo in Period 1 and have a QIDS-SR_{16} score ≥11 (moderate to severe) will be re-randomized to receive intranasal placebo, intranasal esketamine 14 mg, or intranasal esketamine 56 mg in a 1:1:1 ratio on Day 8 and Day 11. The randomization will be stratified by the QIDS-SR_{16} score [moderate (QIDS-SR_{16} score 11 to 16) or severe (QIDS-SR_{16} score > 16)].

Optional Open-Label Treatment Phase

In both panels, on Day 15, following completion of the double-blind treatment phase, subjects may participate in an optional open-label treatment phase. Subjects that discontinue early from the double-blind treatment phase are not eligible to participate in the optional open label treatment phase. The Investigator must agree and confirm the subject is eligible to participate based on his/her clinical judgment.

The efficacy, safety, and biomarker evaluations that will be performed at each visit during the optional open-label treatment phase are described in the Time and Event Schedule. On all dosing days, all subjects must remain at the clinical site for at least 2 hours postdose; a longer duration will be required on Day 1 and 11 (up to 6 hours postdose for study procedures) and on Day 25 (3 hours postdose for study procedures) and should be accompanied by a responsible adult when released from the clinical site. In addition, subjects must not drive a car or work with machines for 24 hours after study medication dosing.

During the optional open-label treatment phase, Panel A subjects can receive up to 9 single doses of intranasal esketamine on Days 15, 18, 22, 25, 32, 39, 46, 60, and 74. Panel B subjects can receive up to 4 single doses of intranasal esketamine on Days 15, 18, 22, and 25.

If a subject discontinues the optional open-label treatment phase prior to receiving all 9 (Panel A) or 4 (Panel B) doses, for reasons other than withdrawal of consent,the subject would continue into the posttreatment phase (see “Posttreatment Phase” below).

Panel A

The doses for the optional open label treatment phase include 28 mg, 56 mg, and/or 84 mg.

All subjects will start with intranasal esketamine 56 mg on Day 15. Subsequent doses on Days 18, 22, 25, 32, 39, and 46 can be down-titrated to the next lower dose or titrated up to the next higher dose if clinically indicated, based on the Investigator’s clinical judgment of efficacy and safety. The two remaining doses of esketamine administered after Day 46 (i.e., Day 60 and 74) will remain stable. No further dose adjustment is permitted after Day 46, the same dose administered on Day 46 will be administered on Day 60 and 74.

Panel B

The doses for the optional open label treatment phase include 14 mg, 28 mg, and/or 56 mg.

All subjects will start with intranasal esketamine 56 mg on Day 15. Subsequent doses on Days 18, 22, and 25 can be down-titrated to the next lower dose or titrated up to the next higher dose at any visit, if desired, based on the Investigator's clinical judgment of efficacy and safety.

Posttreatment Phase

The posttreatment phase for Panel A and B is the same.

The efficacy and safety evaluations that will be performed at each visit during the posttreatment phase are described in the Time and Event Schedule.

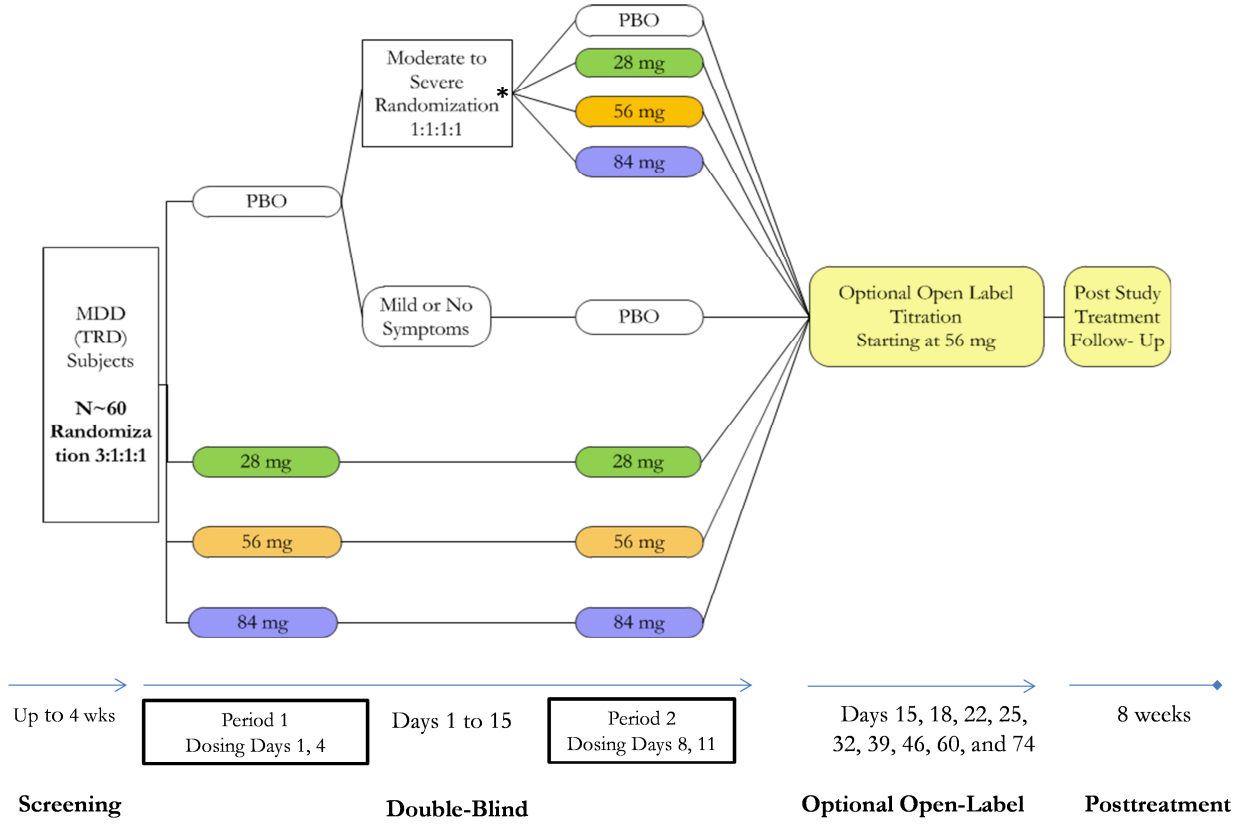
All subjects who receive at least 1 dose of study medication should have 4 follow up visits conducted at 1, 2, 4, and 8 weeks after the last dose of study medication. The follow up visits at 1, 4, and 8 weeks after the last dose of study medication will be telephone contacts. The follow up visit at 2 weeks after the last dose of study medication is a clinic visit.

Any clinically significant abnormalities persisting at the end of the study will be followed by the investigator until resolution or until a clinically stable endpoint is reached.

All adverse events and special reporting situations, whether serious or non-serious, will be reported until completion of the subject's last study-related procedure (which may include contact for follow-up of safety).

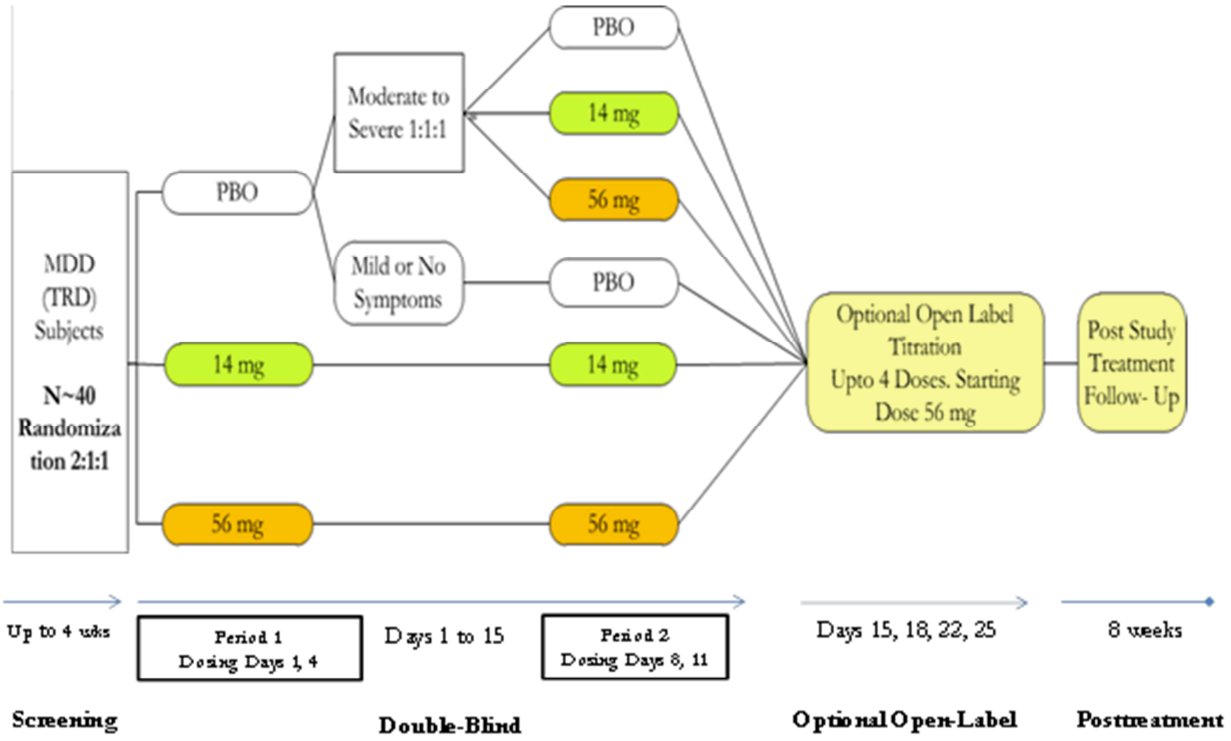
Diagrams of the study design for Panel A and Panel B are provided below (Figure 1 and Figure 2).

Figure 1: Panel A Study Design



* Randomization stratified by QIDS–SR_{16} total score [Moderate = 11 to 16; Severe > 16]

Figure 2: Panel B Study Design



* Randomization stratified by QIDS–SR_{16} total score [Moderate = 11 to 16; Severe > 16]

3.2. Study Design Rationale

Study Population

The study population will consist of men and women that meet DSM-IV-TR diagnostic criteria for MDD, without psychotic features (DSM-IV-TR 296.22, 296.23, 296.32, or 296.33), based upon clinical assessment and confirmed by the MINI. A SAFER Interview, which will confirm the validity of the subject's major depressive episode, will be performed for each subject by a remote, independent rater.

Subjects from the United States, Belgium, and Japan will be included in the study to support a global Phase 3 clinical development program. Subjects must have an IDS-C_{30} total score ≥34 at Screening and predose on Day 1, which corresponds to at least moderate or higher severity depression. The age range of 20 to 64 years old was selected for this study as it represents a general adult age range.

Pilot studies with ketamine and esketamine have shown potentially robust efficacy in patients with TRD. The patient population in this study is consistent with the previously studied population.

Eligible subjects who are currently receiving an antidepressant or other medications do not need to be tapered off them (except for prohibited medications) to come into the study. The investigator and subject will make the decision whether or not to continue the current antidepressant or not based on the subject's clinical status. Antidepressant medication will not be discontinued for the sole purpose of allowing subjects to participate in the study. Subjects will be free of any disallowed pharmacotherapy (prior or concomitant), including any prohibited psychotropic medications, within 1 week (or longer, if specified) before the planned first dose of study drug in order to minimize any interactions (e.g., pharmacodynamic, pharmacokinetic) that would make it difficult to interpret the results of the study.

Blinding and Randomization

For each panel, a placebo control will be used in the double-blind treatment phase to establish the frequency and magnitude of changes in clinical endpoints that may occur in the absence of active treatment.

Randomization will be used to minimize bias in the assignment of subjects to treatment groups, to increase the likelihood that known and unknown subject attributes (e.g., demographic and baseline characteristics) are evenly balanced across treatment groups, and to enhance the validity of statistical comparisons across treatment groups. In Period 1, Panel A subjects are randomized in a 3:1:1:1 ratio (placebo: 28, 56, or 84 mg esketamine) and Panel B subjects are randomized in a 2:1:1 ratio (placebo: 14 or 56 mg esketamine) to reduce expectation bias by increasing the likelihood of receiving placebo and to ensure that there are enough placebo subjects with a moderate or severe QIDS-SR_{16} score available to be re-randomized into Period 2.

Subjects who are re-randomized on Day 8 will be stratified by the Day 8, predose QIDS-SR_{16} score. The self-report QIDS-SR_{16} will be used to determine stratification rather than the clinician-rated MADRS so as to reduce bias on the primary endpoint at this time point.

Study Phases

All subjects will undergo a screening period of up to 4 weeks, which will provide adequate time to assess their eligibility per inclusion/exclusion criteria for the study. The duration of screening phase will also allow an adequate wash out period for prohibited medications, if necessary.

The duration of double-blind treatment phase (Day 1 to 15), which includes two periods (Period 1 and Period 2), was chosen because, based on prior studies with ketamine and esketamine, a rapid antidepressant response is expected within one day and two 1-week periods allow for evaluation of sustained response as well as assessing those with a potential slower onset of response. The double-randomization delayed start design allows for a smaller sample size than a standard parallel-group design while preserving a low chance of type II error and assesses the efficacy, dose response, and safety of intranasal esketamine in subjects with TRD. The key aim of the design is to only include placebo subjects from Period 1 who require treatment in the second period and to re-randomize them to receive one of 3 doses (Panel A) or 2 doses (Panel B) of intranasal esketamine or intranasal placebo. At the end of the trial, efficacy data from both randomizations (Day 1 and Day 8) are combined in an integrated analysis within

each Panel. Additional dose response analyses may be conducted by pooling data from Panel A and Panel B, but further details of this dose response analysis will be presented in the Statistical Analysis Plan. Further details regarding the efficacy analysis can be found within the statistical methods section. The placebo subjects requiring treatment are expected to be more sensitive to treatment with the active drug. Only including placebo subjects from Period 1 who require treatment in the second period addresses the issues with high placebo responses observed in psychiatric clinical trials (Liu 2012).

On Day 15, following completion of the double-blind treatment phase, all subjects may participate in an optional open-label treatment phase. During the optional open-label treatment phase, subjects in Panel A can receive up to 9 doses of intranasal esketamine and subjects in Panel B can receive up to 4 single doses of intranasal esketamine under supervision by the Investigator or designee. For those subjects that may receive placebo in the double-blind treatment phase, this optional open label treatment phase provides an opportunity to receive active treatment. The duration of the optional open label treatment phase has been extended by 7 weeks for Panel A in order to allow the exploration of the efficacy and safety of intranasal esketamine when tapered from twice-weekly dosing to once per week dosing and then dosing once every other week. This additional information will be informative for understanding how the antidepressant effects of esketamine can be sustained for a longer duration with a lower frequency of dosing and to inform the planning and design of the Phase 3 maintenance study.

The 8-week duration of the posttreatment phase allows sufficient time to assess the safety and tolerability of multiple doses, including potential withdrawal symptoms, following the last dose of study medication. In addition, the posttreatment phase allows additional data regarding the duration of efficacy to be evaluated.

Treatment Groups

Panel A

The treatment groups in the double-blind treatment phase are intranasal esketamine 28 mg, 56 mg, or 84 mg, or placebo. The 28 mg, 56 mg, and 84 mg doses of esketamine were selected based on preliminary studies described earlier with the intranasal formulation and IV esketamine (see Section 1) and to allow further exploration of dose response prior to Phase 3 with the intent of carrying forward only the efficacious and well tolerated doses into future studies.

The treatment groups in the optional open label treatment phase are intranasal esketamine 28 mg, 56 mg, and 84 mg. All subjects will start at 56 mg and, if necessary, the dose can be modified based on tolerability and/or efficacy up to Day 46 (inclusive), based on the Investigator's clinical judgment.

Panel B

The treatment groups in the double-blind treatment phase are placebo and intranasal esketamine 14 mg and 56mg. The 14 mg dose was included to assess the minimal efficacious dose. The data obtained for the 14 mg dose in Panel B is considered sufficient to make this determination

without the need to also evaluate this dose in Panel A, thereby exposing less subjects to a potentially ineffective dose. The 56 mg dose was included to have one dose group in common with Panel A to assess for similarity of treatment response across regions. This will allow an exploration of dose response prior to Phase 3 with the intent of carrying forward only the efficacious and well tolerated doses into future studies.

The treatment groups in the optional open label treatment phase are intranasal esketamine 14 mg, 28 mg, and 56 mg. All subjects will start at 56 mg and the dose can be modified, if necessary, based on tolerability and/or efficacy, based on the Investigator's judgment.

Dose and Dose Administration Interval

Based on preliminary results from the 2 clinical studies (ESKETINTRD1001, ESKETIVTRD2001) summarized in Section 1.1.2, the key pharmacokinetic parameters of the 28- and 56-mg regimens of intranasal esketamine substantially overlapped those produced by IV infusion of 0.20 and 0.40 mg/kg esketamine over 40 minutes, respectively. Both of these IV doses were efficacious and generally well tolerated. Therefore, Panel A of this study will evaluate the efficacy of 28, 56 and 84 mg. In Panel B, the 14mg dose is included. The 14mg is included in order to provide an evaluation of what the minimal effective dose (MED) would be. The aim is to take two of the efficacious and well tolerated doses predicated upon the outcome of this study into phase 3 studies. The intranasal esketamine dose regimens in this Phase 2 study are expected to be well tolerated based on the available results from Study ESKETINTRD1001. Preliminary data from the phase 2a study with IV esketamine in TRD (ESKETIVTRD2001) suggests that dosing twice weekly is sufficient to sustain and perhaps improve the antidepressant effect.

Efficacy Measures

MADRS

The 10-item clinician-administered MADRS was designed to be used in subjects with MDD to measure the overall severity of depressive symptoms (Montgomery and Asberg 1979). The scale has been validated, is reliable, and is acceptable to regulatory health authorities as a primary scale to determine efficacy in major depression.

The structured interview guide for the Montgomery Asberg Depression Rating Scale (SIGMA) will be used for each administration (Williams 2008). Using structured interview guides have previously been shown to increase the reliability of given scales.

Modified MADRS assessments (recall period: 2 hours, 24 hours) will be used at some visits to further assess the time to onset and duration of antidepressant effect (i.e., relapse) at assessment time points occurring less than the typical 7-day recall period for the MADRS. The modified MADRS with a 24-hour recall period assessment contains the same 10-items but permits a shorter recall period. The modified MADRS with a 2-hour recall period assessment contains the same 10-items, but the sleep and appetite items will not be assessed. Instead, the predose MADRS scores for the sleep and appetite items recorded earlier on the same day will be carried forward.

In depression, 'response' is commonly defined as a ≥50% reduction in the initial symptom score and remission is typically defined as a total score of ≤10 (Montgomery 1994). The primary efficacy evaluation will be the change from baseline in the MADRS total score in each period in the double-blind treatment phase.

QIDS-SR_{16}

The patient administered QIDS-SR_{16} is designed to be used in patients with MDD to measure the overall severity of depressive symptoms (Rush 2003; Trivedi 2004) over the last 7 days.

PHQ-9

The patient completed PHQ-9 is a 9-item patient-reported measure of depressive symptomatology (Spitzer 1999). Each item is rated on a 4-point scale (0 = Not at all, 1 = Several Days, 2 = More than half the days, and 4 = Nearly every day), with a total score range of 0-27. The recall period is 2 weeks. The PHQ-9 was added to provide data on this measure in the event it may be used as the self-report depression rating scale in the phase 3 program.

CGI-S

The CGI-S will provide an overall clinician-determined summary measure that takes into account all available information, including knowledge of the subject's history, psychosocial circumstances, symptoms, behavior, and the impact of the symptoms on the subject's ability to function (Guy 1976). The CGI evaluates the severity of psychopathology from 1 to 7.

GAD-7

The 7-item patient-reported GAD-7 is a brief and valid measure of overall anxiety (Spitzer 2006). Each item is rated on a 4-point scale (0 to 3), with the total score range from 0-21 (higher scores indicating more anxiety). The standard recall period used is 2 weeks, but in the current study we plan to use a 7-day recall.

PGI-S and PGI-C

Patient Global Impression scales are commonly used measures of symptom severity, treatment response and the efficacy of treatments.

The PGI-S will provide an overall patient-rated summary measure that assesses the severity of the subject's MDD.

The PGI-C will provide an overall patient-rated summary that assesses subject perception of change in their MDD since starting study treatment (Rush 2005).

EQ-5D-5L

The EQ-5D is a standardized 2-part instrument for use as a measure of health outcome, primarily designed for self-completion by respondents. The EQ-5D-5L descriptive system comprises the following 5 dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Each dimension has 5 levels: no problems, slight problems, moderate problems, severe problems, and extreme problems. The descriptive system can be represented as a health state. The EQ visual analog scale (EQ-VAS) self-rating records the respondent's own assessment of their health status (www.euroqol.org/about-eq-5d.html).

Biomarker Evaluations

Metabolomics

Metabolomics is a rapidly emerging technique which captures the metabolic concentration of small molecules (i.e., metabolome) present in biological samples. Studies performed in patients with different CNS disorders have shown that metabolomics might be a promising tool to discover novel biomarkers for drug exposure, safety, and response.

Recently, it has been shown that pretreatment plasma concentrations of glycine are significantly different in depressed patients who respond to citalopram compared to non-responders, and that glycine concentrations might also predict response to drugs which target the glutamatergic system (Ji 2011).

Blood samples will be collected to allow for the exploratory analysis of novel predictive and drug exposure biomarkers. The goals are to investigate whether pretreatment glycine concentrations predict antidepressant response and whether this effect is present only in subjects who respond to ketamine and not to placebo. An additional goal of this is research is to try to identify a glutamatergic metabolic "signature" which might help characterizing the acute and late biological effects of IV ketamine in patients with depression.

Human Inflammation Multi-Analyte Panel (MAP)

Novel multiplex immunoassay platforms allow quantifying simultaneously and reliably several different protein markers in peripheral biological samples. Such techniques have been successfully used to discover novel diagnostic and prognostic biomarkers in patients suffering from different medical conditions, such as coronary artery disease, ovarian cancer, Alzheimer's disease, and schizophrenia (Ray 2007; Schwarz 2011). Inflammatory peripheral markers have been indicated as novel potential diagnostic and prognostic markers in patients with MDD and as possible modulators of NMDA receptor function (Li 2011; Müller 2011).

Blood samples will be collected to allow for an exploratory pharmacodynamics evaluation using the Human Inflammation MAP, a panel of 45 proteins involved in key biologically inflammatory and neurotrophic pathways.

Pharmacokinetic Assessments

PK samples will be obtained during the study for measurement of the plasma concentration of esketamine, noresketamine, and/or additional metabolites, if warranted.

The potential effect of esketamine on the expression of cytochrome P450 enzymes was evaluated in cultured cryopreserved primary human hepatocytes.(Janssen nonclinical study report 2013) Esketamine induced hepatic CYP 3A4 in human hepatocytes. The induction of rat hepatic cytochromes by ketamine was investigated ex vivo in rat livers from male Wistar rats dosed with 10, 20, 40, or 80 mg/kg ketamine IP twice daily for 4 days (Chan 2005). In both, a functional and genomic assay, ketamine showed induction of CYP3A at 80 mg/kg.

The oxysterol 4β-hydroxycholesterol is formed by hepatic CYP 3A4 and 3A5 and has been suggested as a marker for the activity of these drug-metabolizing enzymes (Bodin 2001; Kanebratt 2008). Subjects treated with drugs known to be potent inducers of hepatic cytochrome P450 3A4 and 3A5 have elevated concentrations of 4β-hydroxycholesterol in plasma. Therefore, the concentrations of 4β-hydroxycholesterol on each pharmacokinetic sampling day will be compared to assess the potential for repeated administration of intranasally administered esketamine to influence the activity of the hepatic cytochrome P450 3A4 and 3A5 enzymes. Plasma pharmacokinetic samples collected from subjects in each treatment group will be used for this analysis. In addition, total cholesterol will be measured from a separate blood sample at the same time points to ensure that changes in 4β-hydroxycholesterol are not attributable to changes in total cholesterol.

Safety Evaluations

Physical examination, body weight, vital signs, 12-lead ECG, pulse oximetry, clinical laboratory tests, and evaluation of adverse events and concomitant therapies will be performed throughout the study to monitor subject safety. A nasal examination and a nasal tolerability questionnaire will also be conducted.

The C-SSRS will be performed to assess suicidal ideation and behavior, the CADSS will be administered to assess treatment-emergent dissociative symptoms, the BPRS+ will be administered to assess treatment-emergent psychotic symptoms, the MOAA/S will be used to measure treatment-emergent sedation, the Clinical Global Assessment of Alertness will be used to measure the subject's level of alertness, and the PWC-20 will be administered to assess potential withdrawal symptoms after cessation of esketamine treatment.

Even though it is anticipated that the risk for cystitis is very low based upon the doses to be used in this study, subjects will be assessed for symptoms of cystitis Bladder pain/Interstitial Cystitis using the Bladder Pain/Interstitial Cystitis Symptom Score (BPIC-SS) (Humphrey et al 2012). A score greater than 18 on the BPIC-SS scale is considered as probable cystitis and any subjects meeting this cut-off will have a urine analysis and culture conducted at that visit to assess for potential urinary tract infection, Those without evidence of an ongoing urinary tract infection will be referred to a specialist for diagnostic workup. There are no definitive tests for diagnosing cystitis. If cystitis is considered to be associated with esketamine, subjects will be discontinued

from the study and followed up with appropriate medical care. On dosing days, subjects must remain at the site until at least 2 hours postdose; a longer duration will be required on Day 1 and 11 (up to 6 hours postdose for study procedures) and on Day 25 (3 hours postdose for study procedures) and should be accompanied by a responsible adult when released from the clinical site after study medication dosing. Subjects must not drive a car or work with machines for 24 hours after study medication dosing.

The effect of longer term use of intranasal esketamine on cognition will be assessed in Panel A using the Cogstate® computerized test battery and Hopkins Verbal Learning Test-Revised (HVLT-R).

The Cogstate® test battery will provide assessment of multiple cognitive domains including attention, visual learning and memory, and executive function. The Cogstate® test battery uses playing card stimuli and a maze task, enabling use in multilingual/multicultural settings. Five Cogstate® tests will be administered: Detection, Identification, One Card Learning, One Back, and the Groton Maze Learning Test.

The HVLT-R, a measure of verbal learning and memory, is a 12-item word list recall test. Administration includes 3 learning trials, a 24-word recognition list including 12 target and 12 foil words, and a delayed recall (20 minute) trial (Benedict 1998).

DNA Collection

It is recognized that genetic variation can be an important contributory factor to interindividual differences in drug distribution and response and can also serve as a marker for disease susceptibility and prognosis. Pharmacogenomic research may help to explain interindividual variability in clinical outcomes and may help to identify population subgroups that respond differently to a drug. The goal of the pharmacogenomic component is to collect DNA to allow the identification of genetic factors that may influence the pharmacokinetics, pharmacodynamics, efficacy, safety, or tolerability of esketamine.

The *CYP2B6* gene is known to carry genetic polymorphisms that can influence pharmacokinetics of CYP2B6 substrates (Thorn 2010). Since esketamine is metabolized in part by the CYP2B6 enzyme, DNA samples will be analyzed for the *CYP2B6* gene.

DNA samples may be used to help address emerging issues and to enable the development of safer, more effective, and ultimately individualized therapies.

4. SUBJECT POPULATION

Screening for eligible subjects will be performed within 28 days before administration of the study drug.

The inclusion and exclusion criteria for enrolling subjects in Panel A and Panel B of this study are described in the following 2 subsections. Panel A will be conducted in approximately 60 subjects in the United States and Belgium. Panel B will be conducted in approximately 40 Japanese subjects in Japan.

If there is a question about the inclusion or exclusion criteria below, the investigator should consult with the appropriate sponsor representative before enrolling a subject in the study.

For a discussion of the statistical considerations of subject selection, refer to Section 11.2, Sample Size Determination.

4.1. Inclusion Criteria

Each potential subject must satisfy all of the following criteria to be enrolled in the study.

1. Subject must be a man or woman, 20 to 64 years of age, inclusive.

2. Subject must be medically stable on the basis of physical examination, medical history, vital signs, and 12-lead ECG performed at screening. If there are abnormalities, the subject may be included only if the investigator judges the abnormalities or deviations from normal to be not clinically significant. This determination must be recorded in the subject's source documents and initialed by the investigator.

3. Subject must be medically stable on the basis of clinical laboratory tests performed at screening. If the results of the serum chemistry panel, hematology, or urinalysis are outside the normal reference ranges, the subject may be included only if the investigator judges the abnormalities or deviations from normal to be not clinically significant. This determination must be recorded in the subject's source documents and initialed by the investigator.

    - Retesting of an abnormal laboratory value, at the discretion of the Investigator, that may lead to exclusion will be allowed only once during the screening phase. Retesting will take place during an unscheduled visit in the screening phase.
    - For those without a pre-existing history of hypothyroidism, a normal thyroid-stimulating hormone [TSH] is required at screening.
    - Subjects with hypothyroidism who are on stable treatment for 3 months prior to Screening are required to have TSH and free thyroxine (FT4) obtained. If the TSH value is out of range, but FT4 is normal, such cases should be discussed directly with the medical monitor before the subject is enrolled. If the FT4 value is out of range, the subject is not eligible.

4. Subject must meet Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition-Text Revised (DSM-IV-TR) diagnostic criteria for MDD, without psychotic features, based upon clinical assessment (DSM-IV-TR 296.22, 296.23, 296.32, or 296.33), and confirmed by the Mini International Neuropsychiatric Interview (MINI).

5. The subject’s major depressive episode and treatment response must be deemed "valid" using the SAFER criteria interview (which includes the MADRS, a review of the MGH-ATRQ performed at Screening, and SAFER Criteria Inventory) administered by remote, independent raters

6. Subjects must have had an inadequate response to at least 2 antidepressants, at least one of which is in the current episode of depression. The ATRQ will be used to assess antidepressant treatment response during the current episode. Prior medication history will be used to determine antidepressant treatment response in prior episode(s).

7. Have an IDS-C_{30} total score ≥34 at Screening and predose at Day 1.

8. Comfortable with self-administration of intranasal medication and able to follow instructions provided.

9. Before Period 1 randomization, a woman must be either:

   - Not of childbearing potential: postmenopausal (>45 years of age with amenorrhea for at least 12 months or any age with amenorrhea for at least 6 months and a serum follicle stimulating hormone (FSH) level >40 IU/mL); permanently sterilized (e.g., tubal occlusion, hysterectomy, bilateral salpingectomy); or otherwise be incapable of pregnancy,
   - Of childbearing potential and practicing a highly effective method of birth control consistent with local regulations regarding the use of birth control methods for subjects participating in clinical studies: e.g., established use of oral, injected or implanted hormonal methods of contraception; placement of an intrauterine device (IUD) or intrauterine system (IUS); barrier methods: condom with spermicidal foam/gel/film/cream/suppository or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/suppository; male partner sterilization (the vasectomized partner should be the sole partner for that subject); true abstinence (when this is in line with the preferred and usual lifestyle of the subject)

     Note: If the childbearing potential changes after start of the study (e.g., woman who is not heterosexually active becomes active) a woman must begin a highly effective method of birth control, as described above.

     Women must agree to continue using these methods of contraception throughout the study and for at least 3 months after receiving the last dose of study medication.

10. A woman of childbearing potential must have a negative serum (β-human chorionic gonadotropin [β-hCG]) at Screening and a negative urine pregnancy test prior to Period 1 randomization on Day 1.

11. A woman must agree not to donate eggs (ova, oocytes) for the purposes of assisted reproduction during the study and for at least 3 months after receiving the last dose of study drug.

12. A man who is sexually active with a woman of childbearing potential and has not had a vasectomy must agree to use an effective method of birth control (e.g. condom with spermicide), and all men must also not donate sperm during the study and for 3 months after receiving the last dose of study drug.

13. Subject must be willing and able to adhere to the prohibitions and restrictions specified in this protocol.

14. Each subject must sign an informed consent form (ICF) indicating that he or she understands the purpose of and procedures required for the study and are willing to participate in the study.

4.2. Exclusion Criteria

Any potential subject who meets any of the following criteria will be excluded from participating in the study.

1. Subject has a history of, or current signs and symptoms of, liver or renal insufficiency; significant cardiac, vascular, pulmonary, gastrointestinal, endocrine, neurologic, hematologic, rheumatologic, metabolic disturbances, or fibromyalgia.

    - Subjects with non-insulin dependent diabetes mellitus who are adequately controlled (not on insulin) may participate in the study.

2. Subject has uncontrolled hypertension (SBP > 160 mmHg or DBP > 90 mmHg) despite diet, exercise or a stable dose of a permitted anti-hypertensive treatment at Screening or Day 1 prior to Period 1 randomization; or any past history of hypertensive crisis.

3. Subject has alanine aminotransferase (ALT) or aspartate aminotransferase (AST) values ≥2 x the upper limit of normal at Screening.

4. Subject has a current DSM-IV-TR diagnosis of bipolar or related disorders, intellectual disability, or cluster b personality disorder (e.g., borderline personality disorder, antisocial personality disorder, histrionic personality disorder, and narcissistic personality disorder).

5. Subject has a current or prior DSM-IV-TR diagnosis of a psychotic disorder, MDD with psychosis, post-traumatic stress disorder (PTSD), or obsessive compulsive disorder (OCD).

6. Subject has suicidal ideation with intent to act during Screening phase or on Day 1 (prior to Period 1 randomization) based on the C-SSRS or per Investigator's clinical judgment, or has a history of suicidal behavior within the past year as assessed on the C-SSRS; or subject has homicidal ideation/intent at Screening or on Day 1.

7. Subject has a history of previous non-response of depressive symptoms to esketamine and/or ketamine.

8. Subject has not responded to treatment with electroconvulsive therapy in the current episode of depression.

9. Subject has any significant primary sleep disorder. If the subject has a score of 25 or greater on the Modified Berlin Questionnaire at Screening, obstructive sleep apnea must be ruled out. Subjects adequately treated for obstructive sleep apnea are not excluded.

10. Anatomical or medical conditions that may impede delivery or absorption of study medication (e.g., undergone facial reconstruction, rhinoplasty, significant structural or functional abnormalities of the nose or upper airway; obstructions or mucosal lesions of the nostrils or nasal passages; undergone sinus surgery in the previous 2 years;).

11. Has an abnormal or deviated nasal septum with any 1 or more of the following symptoms: blockage of 1 or both nostrils, nasal congestion (especially 1-sided), frequent nosebleeds, frequent sinus infections, and at times has facial pain, headaches, and postnasal drip.

12. Has a history of substance abuse (drug or alcohol) or dependence (except nicotine or caffeine) within the previous 1 year of screening visit.

13. Subject has a positive test result(s) for drugs of abuse (including barbiturates, methadone, opiates, cocaine, phencyclidine, and amphetamine/methamphetamine) at Screening or predose on Day 1. In addition to the drugs of abuse previously mentioned, cannabinoids will also be tested on Day 1.

    - Subjects that have a positive test result at Screening due to prescribed opiates, barbiturates, or amphetamines may be permitted to continue the Screening phase if the prohibited medication is discontinued at least 1 week or 5 half-lives, whichever is longer, before the first dose of study medication. Provided the Day 1 predose test for drugs of abuse result is negative, the subject may be enrolled. Retesting is not permitted for positive test result(s) from non-prescription use of drugs of abuse.
    - A positive test result for cannabinoids predose on Day 1 is exclusionary.

14. Subject has a positive test result(s) for alcohol at Screening or predose on Day 1.

    - Subjects with a positive alcohol screen at Screening may have the test repeated once during the screening phase, based on the investigator's discretion. This determination, and the reason for permitting a repeat test, must be recorded in the subject's source documents and initialed by the investigator. A positive, repeat alcohol screen is exclusionary.

15. Subject has a history of malignancy within 5 years before screening (exceptions are squamous and basal cell carcinomas of the skin and carcinoma in situ of the cervix, or malignancy that in the opinion of the investigator, with concurrence with the sponsor's medical monitor, is considered cured with minimal risk of recurrence).

16. Subject has known allergies, hypersensitivity, intolerance, or contraindication to

esketamine or its excipients (refer to Investigator's Brochure, product label).

17. Subject has received any disallowed therapies as noted in Section 8, Pre-study and Concomitant Therapy before the specific time relative to the planned first dose of study drug.

18. Subject has received an investigational drug (including investigational vaccines) or used an invasive investigational medical device within 60 days before the planned first dose of study drug or is currently enrolled in an investigational study.

19. Subject is a woman who is pregnant, breast-feeding, or planning to become pregnant while enrolled in this study or within 3 months after the last dose of study drug.

20. Subject is a man who plans to father a child while enrolled in this study or within 3 months after the last dose of study drug.

21. Subject has any condition for which, in the opinion of the investigator, participation would not be in the best interest of the subject (e.g., compromise the well-being) or that could prevent, limit, or confound the protocol-specified assessments.

22. Subject has had major surgery, (e.g., requiring general anesthesia) within 2 weeks before screening, or will not have fully recovered from surgery, or has surgery planned during the time the subject is expected to participate in the study.

    Note: subjects with planned surgical procedures to be conducted under local anesthesia may participate.

23. Subject is an employee of the investigator or study site, with direct involvement in the proposed study or other studies under the direction of that investigator or study site, as well as family members of the employees or the investigator.

24. Subject has a history of human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg) or hepatitis C antibody (anti-HCV) positive, or other clinically active liver disease, or tests positive for HIV, HBsAg, or anti-HCV at Screening.

25. Has clinically significant ECG abnormalities, defined as:

- QTcB interval ≥470 msec at Screening or predose on Day 1
- Evidence of 2nd and 3rd degree AV block, or 1st degree AV block with PR interval >200ms, left bundle branch block (LBBB) or right bundle branch block (RBBB) at Screening or predose on Day 1.
- At Screening, history of additional risk factors for torsades des pointes (e.g. heart failure, hypokalemia, family history of Long QT Syndrome, or the use of concomitant medications that prolong the QT/QTc interval)

NOTE: Investigators should ensure that all study enrollment criteria have been met at screening. If a subject's status changes (including laboratory results or receipt of additional medical records) after screening but before the first dose of study drug is given such that he or she no longer meets all eligibility criteria, then the subject should be excluded from participation in the study. Section 17.4, Source Documentation, describes the required documentation to support meeting the enrollment criteria.

4.3. Prohibitions and Restrictions

Potential subjects must be willing and able to adhere to the following prohibitions and restrictions during the course of the study to be eligible for participation:

1. A woman of childbearing potential who is heterosexually active must remain on a highly effective method of birth control (see inclusion criteria).

2. A man who is sexually active with a woman of childbearing potential must use an effective method of birth control (e.g., condom with spermicide) and all men must also not donate sperm during the study and for 3 months after receiving the last dose of study drug.

3. Subjects will be instructed to refrain from blowing their nose for at least 1 hour after the last intranasal spray.

4. Subjects must abstain from using alcohol within 24 hours before and after study drug administration. Subjects must abstain from prohibited drugs or substances from Screening through to the end of the treatment phase (double-blind and optional open-

label treatment phase, if applicable).

5. On all dosing days, all subjects must remain at the clinical site until at least 2 hours postdose; a longer duration will be required on Day 1 and 11 (up to 6 hours postdose for study procedures) and on Day 25 (3 hours postdose for study procedures) and should be accompanied by a responsible adult when released from the clinical site. Subjects must not drive a car or work with machines for 24 hours after study medication dosing.

6. Subjects should not ingest grapefruit juice, Seville oranges, or quinine for 72 hours before an intranasal dose of esketamine is to be administered.

7. Refer to Section 8, Prestudy and Concomitant Therapies and Attachment 1 (Prohibited Therapies) for disallowed therapies during study participation.

8. Potent CYP3A4 inhibitors are not permitted within 1 week, or within a period less than 5 times the drug's half-life, whichever is longer, before the first dose of study medication and until at least 1 day after the last dose of study medication. Examples of potent CYP3A4 inhibitors are provided in Attachment 1.

9. Potent CYP3A4 inducers are not permitted for 30 days prior to the first dose of study medication and until at least 1 day after the last dose of study medication. Examples of potent CYP3A4 inducers are provided in Attachment 1.

10. Intranasally-administered decongestants are prohibited from 12 hours prior to each study medication administration.

11. Benzodiazepines are prohibited from 12 hours prior to the start of each study drug administration.

12. Non-benzodiazepine sleep aids are prohibited from 12 hours prior to the start of cognition testing (i.e., Cogstate® computerized test battery and HVLT-R).

13. Benztropine is prohibited from 8 hours prior to the start of cognition testing (i.e., Cogstate® computerized test battery and HVLT-R).

5. TREATMENT ALLOCATION AND BLINDING

Treatment Allocation

Procedures for Randomization and Stratification

Panel A

On Day 1 in Period 1, subjects will be randomly assigned to a treatment group based on the first of two computer-generated randomization schedules (Period 1 and Period 2) prepared for the Panel by or under the supervision of the sponsor. The randomization will be balanced by using

randomly permuted blocks and will be stratified by study center with an allocation ratio of 3:1:1:1 to placebo and esketamine 28, 56, and 84 mg.

In order to maintain the blind, subjects who have completed Period 1 and continue into Period 2 will not know if they have been re-randomized in Period 2. The randomization will be maintained within the IWRS system and will not be disclosed until after the study has completed and the database has been finalized.

Those subjects who were randomly assigned to treatment with esketamine in Period 1 will continue to receive the same dose of intranasal esketamine in Period 2. Those subjects who were randomly assigned to placebo in Period 1 with a Day 8 (predose) QIDS-SR_{16} score of < 11 will continue to receive placebo in Period 2. Subjects who were randomly assigned to placebo in Period 1 with a Day 8 (predose) QIDS-SR_{16} score ≥11 will be re-randomized on Day 8 based on the second of the two computer-generated randomization schedules in a 1:1:1:1 ratio to either placebo or 28, 56, or 84 mg of esketamine. The second randomization will be balanced using randomly permuted blocks stratifying by study center and QIDS-SR_{16} [moderate (QIDS-SR_{16} score 11 to 16) or severe (QIDS-SR_{16} score > 16)].

Panel B

On Day 1 in Period 1, subjects will be randomly assigned to a treatment group based on the first of two computer-generated randomization schedules (Period 1 and Period 2) prepared for the Panel by or under the supervision of the sponsor. The randomization will be balanced by using randomly permuted blocks and will be stratified by study center with an allocation ratio of 2:1:1 to placebo and esketamine 14 mg and 56 mg.

In order to maintain the blind, subjects who have completed Period 1 and continue into Period 2 will not know if they have been re-randomized in Period 2. The randomization will be maintained within the IWRS system and will not be disclosed until after the study has completed and the database has been finalized.

Those subjects who were randomly assigned to treatment with esketamine in Period 1 will continue to receive the same dose of intranasal esketamine in Period 2. Those subjects who were randomly assigned to placebo in Period 1 with a Day 8 (predose) QIDS-SR_{16} score of < 11 will continue to receive placebo in Period 2. Subjects who were randomly assigned to placebo in Period 1 with a Day 8 (predose) QIDS-SR_{16} score ≥11 will be re-randomized on Day 8 based on the second of the two computer-generated randomization schedules in a 1:1:1 ratio to either placebo or 14 mg or 56 mg of esketamine. The second randomization will be balanced using randomly permuted blocks stratifying by study center and QIDS-SR_{16} [moderate (QIDS-SR_{16} score 11 to 16) or severe (QIDS-SR_{16} score > 16)].

Based on this randomization code, the study drug for the double-blind treatment phase will be packaged and labeled. Unique medication identification numbers will be preprinted on the study drug labels and assigned as subjects qualify for the study and are assigned to treatment.

Central randomization will be implemented in this study. The interactive web response system (IWRS) will assign a unique treatment code, which will dictate the treatment assignment and matching study drug kit for the subject. The requestor must use his or her own user identification and personal identification number when contacting the IWRS, and will then give the relevant subject details to uniquely identify the subject.

Blinding

The investigator will not be provided with randomization codes. The codes will be maintained within the IWRS, which has the functionality to allow the investigator to break the blind for an individual subject.

Data that may potentially unblind the treatment assignment (e.g., study drug plasma concentrations, study drug accountability data) will be handled with special care to ensure that the integrity of the blind is maintained and the potential for bias is minimized. This can include making special provisions, such as segregating the data in question from view by the investigators, clinical team, or others as appropriate until the time of database lock and unblinding.

Under normal circumstances, the blind should not be broken until all subjects have completed the study and the database is finalized. Otherwise, the blind should be broken only if specific emergency treatment/course of action would be dictated by knowing the treatment status of the subject. In such cases, the investigator may in an emergency determine the identity of the treatment by contacting the IWRS. It is recommended that the investigator contact the sponsor or its designee if possible to discuss the particular situation, before breaking the blind. Telephone contact with the sponsor or its designee will be available 24 hours per day, 7 days per week. In the event the blind is broken, the sponsor must be informed as soon as possible. The date, time, and reason for the unblinding must be documented by the IWRS, and in the source document. The documentation received from the IWRS indicating the code break must be retained with the subject's source documents in a secure manner.

In general, randomization codes will be disclosed fully only if the study is completed and the clinical database is closed. However, if an interim analysis is specified, the randomization codes and, if required, the translation of randomization codes into treatment and control groups will be disclosed to those authorized and only for those subjects included in the interim analysis.

In the optional open label treatment phase, blinding procedures are not applicable.

6. DOSAGE AND ADMINISTRATION

All doses of study medication will be self-administered under the direct supervision of the investigator or designee. Instructions for use of the intranasal device will be provided as a separate document.

The clinical site must have access to a clinician experienced with ventilation management. In addition, equipment for supportive ventilation and resuscitation needs to be present at the site.

Each individual device contains 28 mg (i.e., 2 sprays).

The placebo solution will be provided as a clear, colorless intranasal solution of water for injection with a bittering agent (denatonium benzoate [Bitrex®] at a final concentration of 0.001 mg/mL) added to simulate the taste of the intranasal solution with active drug. Benzalkonium chloride is added as a preservative at a concentration of 0.3 mg/mL.

Prior to the first dose, subjects will practice spraying (into the air) a demonstration intranasal device that is filled with water.

Food will be restricted for at least 2 hours before each administration of study medication. Drinking of any fluids will be restricted for at least 30 minutes before the first nasal spray.

Subjects will be instructed to refrain from blowing their nose for at least 1 hour after the last intranasal spray.

If the subject has nasal congestion on the dosing day, it is recommended the dosing day be delayed (per the permitted visit window). If an intranasal decongestant is used to reduce congestion, it cannot be used within 12 hours prior to study medication dosing. If a delay is not feasible, the subject should be instructed to blow his/her nose prior to the first intranasal spray.

On all dosing days, all subjects must remain at the clinical site until at least 2 hours postdose; a longer duration will be required on Day 1 and 11 (up to 6 hours postdose for study procedures) and on Day 25 (3 hours postdose for study procedures) and should be accompanied by a responsible adult when released from the clinical site. Subjects must not drive a car or work with machines for 24 hours after study medication dosing.

Double-blind treatment phase

Refer to the Section 3.1 (*Overview of Study Design- Double-Blind Treatment Phase*) for a description of the study design for Panel A and Panel B.

Subjects in Panel A and Panel B will self-administer intranasal esketamine or intranasal placebo on Days 1, 4, 8, and 11.

Panel A

On each dosing day, all subjects will self-administer 1 spray into each nostril at t = 0, 5, and 10 minutes. Time 0 is defined as the time of the first 100-mcl spray. Each subject will use 2 sprays per device per time point (t = 0, 5, and 10 minutes) on each dosing day. Sprays to each

nostril should be delivered per the Instructions for Use document at the scheduled time points. Table 2 describes how each treatment will be administered in the double-blind treatment phase.

Table 2: Panel A: Dose Administration in the Double-Blind Treatment Phase

Intranasal Treatment	Time of Administration (Time 0 is defined as the time of the first 100-mcl spray)		
	0	**5 minutes**	**10 minutes**
Placebo	1 spray of placebo to each nostril	1 spray of placebo to each nostril	1 spray of placebo to each nostril
Esketamine 28 mg	1 spray of esketamine to each nostril	1 spray of placebo to each nostril	1 spray of placebo to each nostril
Esketamine 56 mg	1 spray of esketamine to each nostril	1 spray of esketamine to each nostril	1 spray of placebo to each nostril
Esketamine 84 mg	1 spray of esketamine to each nostril	1 spray of esketamine to each nostril	1 spray of esketamine to each nostril

Panel B

On each dosing day, all subjects will self-administer 1 spray into each nostril at t = 0 and 5 minutes. Time 0 is defined as the time of the first 100-mcl spray. Each subject will use 1 spray per device (i.e., 2 devices per time point at t = 0 and 5 minutes) on each dosing day. Sprays to each nostril should be delivered per the Instructions for Use document at the scheduled time points. Table 3 describes how each treatment will be administered in the double-blind treatment phase.

Table 3: Panel B: Dose Administration in the Double-Blind Treatment Phase

Intranasal Treatment	Time of Administration (Time 0 is defined as the time of the first 100-mcl spray)			
	0		**5 minutes**	
	Device 1	Device 2	Device 3	Device 4
Placebo	1 spray of placebo into one nostril	1 spray of placebo into the other nostril	1 spray of placebo into one nostril	1 spray of placebo into the other nostril
Esketamine 14 mg	1 spray of esketamine into one nostril	1 spray of placebo into the other nostril	1 spray of placebo into one nostril	1 spray of placebo into the other nostril
Esketamine 56 mg	1 spray of esketamine into one nostril	1 spray of esketamine into the other nostril	1 spray of esketamine into one nostril	1 spray of esketamine into the other nostril

Optional open-label treatment phase

Refer to the Section 3.1 (*Overview of Study Design- Optional Open Label Treatment Phase*) for a description of the study design for Panel A and Panel B.

Subjects in Panel A will self-administer intranasal esketamine on Days 15, 18, 22, 25, 32, 39, 46, 60, and 74.

Time 0 is defined as the time of the first 100-mcl spray. Sprays to each nostril should be delivered per the Instructions for Use document at the scheduled time points.

Table 4 describes how each treatment will be administered in the optional open-label treatment phase for Panel A.

Table 4: Panel A: Dose Administration in Optional Open-Label Treatment Phase

Intranasal Treatment	Time of Administration (Time 0 is defined as the time of the first 100-mcl spray)		
	0	5 minutes	10 minutes
Esketamine 28 mg	1 spray of esketamine to each nostril	-	-
Esketamine 56 mg	1 spray of esketamine to each nostril	1 spray of esketamine to each nostril	-
Esketamine 84 mg	1 spray of esketamine to each nostril	1 spray of esketamine to each nostril	1 spray of esketamine to each nostril

Subjects in Panel B will self-administer intranasal esketamine on Days 15, 18, 22, and 25.

Time 0 is defined as the time of the first 100-mcl spray. For the 28 mg and 56 mg doses, sprays to each nostril should be delivered per the Instructions for Use document at the scheduled time points.

Table 5 describes how each treatment will be administered in the optional open-label treatment phase for Panel B.

Table 5: Panel B: Dose Administration in Optional Open-Label Treatment Phase

Intranasal Treatment	Time of Administration (Time 0 is defined as the time of the first 100-mcl spray)	
	0	5 minutes
Esketamine 14 mg	1 spray of esketamine to one nostril	-
Esketamine 28 mg	1 spray of esketamine to each nostril	-
Esketamine 56 mg	1 spray of esketamine to each nostril	1 spray of esketamine to each nostril

7. TREATMENT COMPLIANCE

The investigator or designated study-site personnel will maintain a log of all study drug dispensed and returned. Drug supplies for each subject will be inventoried and accounted for throughout the study.

8. PRESTUDY AND CONCOMITANT THERAPY

Prestudy Non-Antidepressant Therapy

Prestudy non-antidepressant therapies administered up to 30 days before the screening visit and any ongoing therapies must be recorded at screening.

Prestudy Antidepressant Therapy

All antidepressant treatment(s), including adjunctive treatment for MDD, used prior to Screening either in the current or prior depressive episodes (known from the subject's psychiatric history or verbal report) that will be used to support subject eligibility (per Inclusion Criterion #6 which requires an inadequate response to at least 2 antidepressant treatments, at least one of which is in the current episode of depression), but that are not continuing at the Screening visit, are to be recorded at Screening on the appropriate antidepressant therapy-specific case report form.

Concomitant therapies (including the current antidepressant treatment(s), if applicable) must be recorded throughout the study beginning with signing of the informed consent (i.e., Screening) until the last follow up visit. Concomitant therapies should also be recorded beyond this time only in conjunction with new or worsening adverse events until resolution of the event.

All therapies (prescription or over-the-counter medications, including vaccines, vitamins, herbal supplements; non-pharmacologic therapies such as electrical stimulation, acupuncture, special diets, exercise regimens) different from the study drug must be recorded in the CRF. Modification of an effective preexisting therapy should not be made for the explicit purpose of entering a subject into the study.

Disallowed therapies:

- Refer to Attachment 1 for a list of prohibited therapies.
- Potent CYP3A4 inhibitors within 1 week, or within a period less than 5 times the drug's half-life, whichever is longer, before the first dose of study medication and until at least 1 day after the last dose of study medication. Examples of potent CYP3A4 inhibitors are provided in Attachment 1.
- Potent CYP3A4 inducers for 30 days prior to the first dose of study medication and until at least 1 day after the last dose of study medication. Examples of potent CYP3A4 inducers are provided in Attachment 1.
- Use of a disallowed pharmacotherapy, including psychotropic medications, within 1 week or within a period less than 5 times the drug's half-life, whichever is longer, before the first dose of study medication throughout the end of the treatment phase(s).
- Any new psychotropic medication(s) started during screening, or any increase in the dose of a currently prescribed (allowed) psychotropic medication(s) during screening until at least 1 day after the last dose of study medication.
- Treatment with any MAO-inhibitor within the past 2 weeks prior to Day 1 dosing until at least 1 day after the last dose of study medication.
- No benzodiazepines should be used within 12 hours prior to the start of the study drug administration.
- Non-benzodiazepine sleep aids are prohibited from 12 hours prior to the start of cognition testing (i.e., Cogstate® computerized test battery and HVLT-R).
- Benztropine is prohibited from 8 hours prior to the start of cognition testing (i.e., Cogstate® computerized test battery and HVLT-R).

- Intranasally-administered decongestants (vasoconstrictors) are prohibited from 12 hours prior to each study medication administration. Intranasal steroids are not prohibited.
- ECT, deep brain stimulation (DBS), transcranial magnetic stimulation (TMS), and vagus nerve stimulation (VNS) are prohibited from Screening to the end of the study.

The sponsor must be notified in advance (or as soon as possible thereafter) of any instances in which prohibited therapies are administered.

Following completion of the double-blind treatment phase and the optional open label treatment phase (if applicable), subjects can be treated according to standard of care.

9. STUDY EVALUATIONS

9.1. Study Procedures

9.1.1. Overview

The Time and Events Schedule summarizes the frequency and timing of efficacy, pharmacokinetic, biomarker, pharmacogenomics, and safety measurements applicable to this study.

The total blood volume to be collected from each subject will be approximately 161 mL (Panel A; Table 6) and 166.5 mL (Panel B; Table 7).

Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.

Additional serum or urine pregnancy tests may be performed, as determined necessary by the investigator or required by local regulation, to establish the absence of pregnancy at any time during the subject's participation in the study.

Table 6: Approximate Volume of Blood to be Collected From Each Panel A Subject

Type of Sample	Volume (mL) per sample	Number of Samples per Subject	Total Volume of Blood (mL)[a]
Screening Phase			
Serum chemistry [b]	2.5	1	2.5
Hematology	2	1	2
Serology - HIV	3.5	1	3.5
Serology – HbsAg, HCV	2.5	1	2.5
Approximate total blood volume for screening phase			10.5
Double-Blind Treatment Phase			
Serum chemistry [d]	2.5	3	7.5
Total cholesterol	2.5	2	5
Hematology	2	3	6
Pharmacokinetic	4	9	36
Pharmacogenomic [c]	10	1	10
Biomarker: Inflammation MAP	10	3	30
Biomarker: Metabolomics	6	3	18
Approximate total blood volume for double-blind treatment phase			112.5
Optional Open Label Treatment Phase			
Serum chemistry	2.5	2	5
Total cholesterol	2.5	1	2.5
Hematology	2	2	4
Pharmacokinetic	4	3	12
Biomarker: Inflammation MAP	10	1	10
Approximate total blood volume for optional open-label treatment phase			33.5
Posttreatment Phase			
Serum chemistry [b]	2.5	1	2.5
Hematology	2	1	2
Approximate total blood volume for posttreatment phase			4.5
Approximate total blood volume for study			**161 mL**

[a] Calculated as number of samples multiplied by amount of blood per sample.
[b] Serum chemistry includes serum β-hCG pregnancy tests (for women of childbearing potential) and TSH.
[c] Required subject participation.
[d] On Day 14 (or Early Termination), serum chemistry includes serum β-hCG pregnancy tests (for women of childbearing potential).

Note: An indwelling intravenous cannula may be used for blood sample collection.

Table 7: Approximate Volume of Blood to be Collected From Each Panel B Subject

Type of Sample	Volume (mL) per sample	Number of Samples per Subject	Total Volume of Blood (mL)[a]
Screening Phase			
Serum chemistry [b]	3	1	3
Hematology	2	1	2
Serology - HIV	3.5	1	3.5
Serology – HbsAg, HCV	3	1	3
Approximate total blood volume for screening phase			11.5
Double-Blind Treatment Phase			
Serum chemistry [d]	3	3	9
Total cholesterol	3	2	6
Hematology	2	3	6
Pharmacokinetic	4	9[e]	36[e]
Pharmacogenomic [c]	10	1	10
Biomarker: Inflammation MAP	10	3	30
Biomarker: Metabolomics	6	3	18
Approximate total blood volume for double-blind treatment phase			115[e]
Optional Open Label Treatment Phase			
Serum chemistry	3	2	6
Total cholesterol	3	1	3
Hematology	2	2	4
Pharmacokinetic	4	3	12
Biomarker: Inflammation MAP	10	1	10
Approximate total blood volume for optional open-label treatment phase			35
Posttreatment Phase			
Serum chemistry [b]	3	1	3
Hematology	2	1	2
Approximate total blood volume for posttreatment phase			5
Approximate total blood volume for study			**166.5 mL**

[a] Calculated as number of samples multiplied by amount of blood per sample.
[b] Serum chemistry includes serum β-hCG pregnancy tests (for women of childbearing potential) and TSH.
[c] Required subject participation.
[d] On Day 14 (or Early Termination), serum chemistry includes serum β-hCG pregnancy tests (for women of childbearing potential).
[e] The 6 hour postdose sample on Day 1 and 11 is optional for Panel B, therefore the blood volumes associated with these 2 samples may not be applicable to all Panel B subjects.

Note: An indwelling intravenous cannula may be used for blood sample collection.

If multiple assessments are scheduled for the same timepoint, it is recommended that procedures be performed in the sequence provided by the Sponsor (Refer to Section 15, Study Specific Materials).

Patient reported outcome (PRO) assessments should be conducted/completed before any tests, procedures, or other consultations scheduled at the same timepoint to prevent influencing subject perceptions. Refer to Attachment 2 for further instructions on completion of patient reported outcomes.

If blood sampling or vital sign measurement is scheduled for the same time point as ECG recording, the procedures should be performed in the following order: ECG(s), vital signs, blood draw.

Actual dates and times of assessments will be recorded in the source documentation and CRF.

9.1.2. Screening Phase

Prior to conducting any study procedure, the investigator (or designated study personnel) will review and explain the written informed consent form (ICF) to each subject. After signing the ICF, the subject will be screened within 4 weeks of Day 1 (i.e., Day -28 to Day -1) to determine eligibility for study participation.

Subjects must be 20 to 64 years of age (inclusive) and meet DSM-IV-TR diagnostic criteria for MDD, without psychotic features, based upon clinical assessment (DSM-IV-TR 296.22, 296.23, 296.32, or 296.33) and confirmed by the MINI. Subjects must have an IDS-C_{30} total score ≥34. In addition, the major depressive episode must be deemed valid using the SAFER criteria interview, administered by remote, independent raters.

Subjects must have had an inadequate response to at least 2 antidepressants, at least one of which is in the current episode of depression, assessed using the MGH-ATRQ and prior medication history.

Note: Subjects that are currently not taking an antidepressant at Screening are eligible to participate in this study (i.e., subjects may participate in the study whether or not they are taking an antidepressant).

- Subjects who are taking an antidepressant at the Screening visit must have been receiving a stable dose (i.e., dose has been unchanged) for at least 2 weeks before the Screening visit. The subject can:
    - With the exception of MAO inhibitors, which are prohibited, the subject can continue the antidepressant at the same dose during the double-blind treatment phase, or
    - The subject can discontinue the antidepressant within 1 week, or within a period at least 5 times the drug's half-life (exception: at least 4 weeks for fluoxetine and at least 2 weeks for MAO inhibitors), whichever is longer, before the planned first dose of study drug.
        - The decision to continue or discontinue the current antidepressant will be made by the subject and investigator, based on their clinical judgment. Antidepressant medication will not be discontinued for the sole purpose of allowing subjects to participate in the study.

Disallowed therapies, including psychotropic medications, are described in the exclusion criteria, Section 8 (Prestudy and Concomitant Therapies), and Attachment 1.

All adverse events and special reporting situations, whether serious or non-serious, will be reported from the time a signed and dated ICF is obtained until completion of the subject's last study-related procedure (which may include contact for follow-up of safety).

Within 1 week prior to the planned first dose of study medication, Panel A subjects will have two mandatory practice sessions on the Cogstate® computerized test battery performed at the clinical site.

Screening evaluations (e.g., biomarker, safety) will be performed as per the Time and Events Schedule.

It is recommended that the patient-reported outcomes and clinician-administered assessments at Screening are performed in the following sequence:

- Patient reported outcomes: QIDS-SR_{16}, Modified Berlin Questionnaire
- Clinician-administered: MINI, IDS-C_{30}, C-SSRS, ATRQ (in collaboration with subject)

9.1.3. Double-Blind Treatment Phase

All subjects will participate in two treatment periods (Period 1 and Period 2) during the double-blind treatment phase. Efficacy, safety, pharmacokinetic, biomarker, and/or pharmacogenomic evaluations performed at each visit are described in the Time and Events Schedule.

Prior to the first dose, subjects will practice spraying (into the air) a demonstration intranasal device that is filled with water.

All subjects will self-administer the study medication in Period 1 and Period 2 under the direct supervision of the investigator or designee (Refer to Section 6, Dosage and Administration).

Refer to the Section 3.1 (*Overview of Study Design*) above for a description of the study design for Panel A and Panel B.

When multiple PRO and clinician-administered assessments are scheduled for the same time point it is recommended they be performed in the following sequence:

PRO: QIDS-SR_{16}, PHQ-9, PGI-S, PGI-C, GAD-7, Nasal Tolerability Questionnaire, BPIC-SS, EQ-5D-5L

Cogstate® computerized test battery and HVLT-R: HVLT-R , detection, identification, one card learning, one back, Groton maze learning test, HVLT-R-delayed

Clinician-administered: IDS-C_{30}, MADRS, CGI-S, C-SSRS, BPRS+, CADSS, MOAA/S, Clinical Global Assessment of Alertness, PWC-20

Subjects will be provided with a diary that includes the subject-completed assessments that are to be completed at home on Day 2 and 9 (i.e., telephone contact visit days) of the double-blind

phase. At the time the diary is dispensed, site staff should review with the subject when the diary is to be completed. During each telephone contact, the site staff will remind the subject to complete their diary that day, prior to any other assessments scheduled to occur during the telephone contact, and bring it to their next clinic visit. The site staff will review the completed diary at the time points noted in the Time and Events Schedule.

For information obtained via telephone contact, written documentation of the communication must be available for review in the source documents. During the telephone contact visits, adverse event and concomitant therapy information will be obtained. In addition, specified clinician-administered assessments will be performed by appropriately qualified staff.

For inpatient subjects, telephone contact visits can be performed at the inpatient location.

Early Termination

If a subject withdraws before the end of the double-blind treatment phase, for reasons other than withdrawal of consent, the Early Termination visit should be conducted at the time of discontinuation, followed by completion of the posttreatment phase.

9.1.4. Optional Open-Label Treatment Phase

On Day 15, following completion of the double-blind treatment phase, all subjects may participate in an optional open-label treatment phase. Subjects that discontinue early from the double-blind treatment phase are not eligible to participate in the optional open label treatment phase. The Investigator must agree and confirm the subject is eligible to participate based on his/her clinical judgment.

The efficacy, safety, and biomarker evaluations that will be performed at each visit during the optional open-label treatment phase are described in the Time and Event Schedule. Where specified in the Time and Events Schedule, study procedures performed on the last day of the double-blind treatment phase (Day 15) that are also required predose on Day 15 of the optional open-label treatment phase will only be performed once.

During the optional open-label treatment phase, Panel A subjects can receive up to 9 doses of intranasal esketamine on Days 15, 18, 22, 25, 32, 39, 46, 60, and 74. Panel B subjects can receive up to 4 doses of intranasal esketamine on Days 15, 18, 22, and 25.

Refer to the Section 3.1 (*Overview of Study Design*) above for a description of the study design for Panel A and Panel B.

If a subject discontinues the optional open-label treatment phase prior to receiving all 9 (Panel A) or 4 (Panel B) doses, for reasons other than withdrawal of consent, the subject would continue into the posttreatment phase.

When multiple PRO and clinician-administered assessments are scheduled for the same time point, it is recommended they be performed in the following sequence:

PRO: QIDS-SR_{16}, PHQ-9, PGI-S, PGI-C, GAD-7, Nasal Tolerability Questionnaire, BPIC-SS, EQ-5D-5L

Cogstate® computerized test battery and HVLT-R: HVLT-R , detection, identification, one card learning, one back, Groton maze learning test, HVLT-R-delayed

Clinician-administered: MADRS, CGI-S, C-SSRS, BPRS+, CADSS, MOAA/S, Clinical Global Assessment of Alertness

9.1.5. Posttreatment Phase (Follow-Up)

The efficacy and safety evaluations that will be performed at each visit during the posttreatment phase are described in the Time and Event Schedule.

Subjects will be provided with a diary that includes the subject-completed assessments that are to be completed at home as part of the first follow up visit (i.e., telephone contact visit 1 week after the last dose of study medication). At the time the diary is dispensed, site staff should review with the subject when the diary is to be completed. During the telephone contact, the site staff will remind the subject to complete their diary that day, prior to any other assessments scheduled to occur during the telephone contact, and bring it to their next clinic visit. The site staff will review the diary at the time points noted in the Time and Events Schedule.

All subjects who receive at least 1 dose of study medication should have 4 follow up visits conducted at 1, 2, 4, and 8 weeks after the last dose of study medication.

The follow up visits at 1, 4, and 8 weeks after the last dose of study medication will be telephone contacts. The follow up visit at 2 weeks after the last dose of study medication is a clinic visit.

For information obtained via telephone contact, written documentation of the communication must be available for review in the source documents. During the telephone contact visit, adverse event and concomitant therapy information will be obtained. In addition, specified clinician-administered assessments will be performed by appropriately qualified staff. For inpatient subjects, telephone contact visits will be performed at the inpatient location.

Any clinically significant abnormalities persisting at the end of the study will be followed by the investigator until resolution or until a clinically stable endpoint is reached.

All adverse events and special reporting situations, whether serious or non-serious, will be reported until completion of the subject's last study-related procedure (which may include contact for follow-up of safety).

9.2. Efficacy

9.2.1. Evaluations

Every effort should be made to ensure that all clinician-administered efficacy assessments are completed by the same individual who made the initial baseline determinations.

9.2.1.1. Primary

MADRS

The primary efficacy evaluation will be the MADRS total score.

The MADRS is a clinician-rated scale designed to measure depression severity and detects changes due to antidepressant treatment (Montgomery 1979). The test consists of 10 items, each of which is scored from 0 (item not present or normal) to 6 (severe or continuous presence of the symptoms), for a total possible score of 60. Higher scores represent a more severe condition. The MADRS evaluates apparent sadness, reported sadness, inner tension, sleep, appetite, concentration, lassitude, interest level, pessimistic thoughts, and suicidal thoughts. The test exhibits high inter-rater reliability.

The structured interview guide for the Montgomery Asberg Depression Rating Scale (SIGMA) will be used for each administration (Williams 2008). Using structured interview guides have previously been shown to increase the reliability of given scales.

The typical recall period for the MADRS is 7 days. In this study, the MADRS will also be administered using modified recall periods of 2 hours and 24 hours. For the recall period of 2 hours, the sleep and appetite items will not be assessed (predose scores for these items obtained on the same day will be carried forward).

9.2.1.2. Secondary

QIDS-SR$_{16}$

The QIDS-SR$_{16}$ is a patient reported measure designed to assess the severity of depressive symptoms (Rush 2003; Trivedi 2004). The QIDS-SR$_{16}$ assesses all the criterion symptom domains designated by the DSM-5 to diagnose a major depressive episode. This assessment can be used to screen for depression, although it has been used predominantly as a measure of symptom severity.

Paper and pen format will be used for this study. Subjects provide responses to each item of this instrument with a 4-point Likert scale, with scores ranging from 0 to 3 for each item. The 7-day period prior to assessment is the usual recall period for assessing symptom severity.

The scoring system of the QIDS converts responses to the 16 separate items into the nine DSM-5 symptom criterion domains. The nine domains comprise 1) sad mood; 2) concentration; 3) self-criticism; 4) suicidal ideation; 5) interest; 6) energy/fatigue; 7) sleep disturbance (initial, middle, and late insomnia or hypersomnia); 8) decrease or increase in appetite or weight; and 9) psychomotor agitation or retardation. The total score is obtained by adding the scores for each of the nine symptom domains of the DSM-5 MDD criteria (Rush 2003): 4 items are used to rate sleep disturbance (early, middle, and late insomnia plus hypersomnia); 2 items are used to rate psychomotor agitation and retardation; 4 items are used to rate appetite (increase or decrease and weight increase or decrease). One item is used to rate the remaining 6 domains (sad mood, interest, energy/fatigue, self-criticism, concentration, and suicidal ideation). For symptom

domains that require more than one item, the highest score of the item relevant for each domain is taken. For example, if early insomnia is 0, middle insomnia is 1, late insomnia is 3, and hypersomnia is 0, the sleep disturbance domain is rated 3. The total score ranges from 0 to 27. Using a scale of severity of depression of none, mild, moderate, severe, and very severe, corresponding QIDS-SR_{16} total scores are none 1-5, mild 6-10, moderate 11-16, severe 17-20 and very severe 21-27.

The QIDS-SR_{16} is sensitive to change, with medications, psychotherapy, or somatic treatments. The psychometric properties of both the QIDS-SR_{16}, has been established in various study samples, and is outlined on the developer's website (www.ids-qids.org).

CGI-S

The CGI-S is a clinician-rated scale that is designed to rate the severity of the subject's illness at the time of assessment, relative to the clinician's past experience with subjects who have the same diagnosis and improvement with treatment (Guy 1976). Considering total clinical experience, a subject is assessed on severity of mental illness at the time of rating according to: 0= not assessed; 1=normal (not at all ill); 2=borderline mentally ill; 3=mildly ill; 4=moderately ill; 5=markedly ill; 6=severely ill; 7=among the most extremely ill patients.

PGI-S

The PGI-S is a 4-point scale that requires the subject to rate the severity of their illness at the time of assessment, relative to the subject's past experience. Considering their total experience, the subject assesses the severity of their depression illness at the time of rating as none, mild, moderate, or severe. Paper and pen format will be used for this study.

GAD-7

The GAD-7 is a validated, brief 7-item self-report assessment of anxiety. Each item is scored on a 4-point scale (0-3), with a total score range of 0-21 (Spitzer 2006). The standard recall period used is 2 weeks, but in the current study we plan to use a 7-day recall.

9.2.1.3. Exploratory

PGI-C

The PGI-C is a 7-point scale that requires the subject to assess how much their illness has improved or worsened relative to a baseline state at the beginning of the intervention. The response options are: very much improved; much improved; improved (just enough to make a difference); no change; worse (just enough to make a difference); much worse; or very much worse. Paper and pen format will be used for this study.

PHQ-9

The PHQ-9 is a 9-item, self-report scale assessing depressive symptoms (Spitzer 1999). Each item is rated on a 4-point scale (0 = Not at all, 1 = Several Days, 2 = More than half the days, and 4 = Nearly every day), with a total score range of 0-27. The recall period is 2 weeks. The

scale scores each of the nine symptom domains of the DSM-IV-TR MDD criteria and it has been used both as a screening tool and a measure of response to treatment for depression.

EQ-5D-5L

The EQ-5D-5L is a standardized 2-part instrument for use as a measure of health outcome, primarily designed for self-completion by respondents. It essentially consists of the EQ-5D descriptive system and the EQ visual analogue scale (EQVAS). The EQ-5D-5L descriptive system comprises the following 5 dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Each dimension has 5 levels: no problems, slight problems, moderate problems, severe problems, and extreme problems. The descriptive system can be represented as a health state. The EQ VAS self-rating records the respondent's own assessment of their health status. Subjects select an answer for each of the 5 dimensions considering the response that best matches their health "today". Paper and pen format will be used for this study.

9.2.2. Endpoints

9.2.2.1. Primary Endpoint

The primary efficacy evaluation for each panel will be the MADRS total score as measured by the change from baseline (i.e., Day 1 predose; Day 8 predose) to the 1-week endpoint (i.e., Day 8, predose; Day 15) for the combined periods in the double-blind treatment phase.

9.2.2.2. Secondary Endpoints

- Proportion of subjects with sustained response (≥50% reduction from baseline in MADRS total score) with onset by Day 2 through the end of the double-blind phase (Day 15)
- Proportion of responders (≥50% reduction from baseline in MADRS total score) at each visit
- Proportion of subjects in remission (MADRS ≤10) at each visit
- Change from baseline (i.e., Day 1 predose; Day 8 predose) to the 1-week endpoint (i.e., Day 8, predose; Day 15) for the combined periods in the double-blind treatment phase in subject-reported depressive symptoms using the QIDS-SR_{16}.
- Change from baseline (i.e., Day 1 predose; Day 8 predose) to the 1-week endpoint (i.e., Day 8, predose; Day 15) for the combined periods in the double-blind treatment phase in severity of illness using the CGI-S.
- Change from baseline (i.e., Day 1 predose; Day 8 predose) to the 1-week endpoint (i.e., Day 8, predose; Day 15) for the combined periods in the double-blind treatment phase in severity of illness using the PGI-S.
- Change from baseline (i.e., Day 1 predose; Day 8 predose) to the 1-week endpoint (i.e., Day 8, predose; Day 15) for the combined periods in the double-blind treatment phase in anxiety symptoms, as measured by the GAD-7

9.3. Pharmacokinetics

9.3.1. Evaluations

Venous blood samples of approximately 4 mL will be collected for measurement of plasma concentrations of esketamine and noresketamine and other metabolites (if warranted) at the time points specified in the Time and Events Schedule. The exact dates and times and pharmacokinetic blood sampling must be recorded.

The plasma concentrations of 4β-hydroxycholesterol will be measured in select PK samples as a probe to assess the potential for repeated administration of intranasal esketamine to induce hepatic cytochrome P450 3A4 enzyme activity, compared with placebo. Total cholesterol will be measured in a separate blood sample at these same time points to ensure that changes in 4β-hydroxycholesterol are not attributable to changes in total cholesterol.

Based on the Time and Events Schedule, plasma samples will be divided into 2 aliquots (pharmacokinetics, back-up) or 3 aliquots (pharmacokinetics, a back-up, and 4β-hydroxycholesterol).

9.3.2. Analytical Procedures

Plasma samples will be analyzed to determine concentrations of esketamine and noresketamine using a validated, achiral LC-MS/MS method by or under the supervision of the sponsor.

The concentrations of 4β-hydroxycholesterol will be measured in select plasma samples using a qualified method.

If required, some plasma samples may be analyzed to document the presence of circulating metabolites using a qualified research method. In addition, plasma PK samples may be stored for future analysis of the metabolite profile.

The bioanalytical report, including a description of the assay and a summary of the assay performance data, will be included in the final study report as an addendum.

9.3.3. Pharmacokinetic Parameters

The plasma concentration-time data of esketamine and noresketamine will be analyzed using population pharmacokinetic modeling. Typical population values of basic pharmacokinetic parameters will be estimated together with the inter-individual variability. Effects of subject demographics, laboratory parameter values, and other covariates on the pharmacokinetics of esketamine will be explored. The results of the population pharmacokinetic analyses may be reported separately.

9.4. Pharmacokinetic/Pharmacodynamic Evaluations

The relationship between MADRS total score (and possibly selected adverse events as additional pharmacodynamic parameters) and pharmacokinetic metrics of esketamine may be evaluated. If

there is any visual trend in graphical analysis, suitable models will be applied to describe the exposure-effect relationships.

A pharmacokinetic/pharmacodynamics analysis may be performed after at least 80% of Panel A subjects have completed the double-blind treatment phase. Subject pharmacokinetic data, MADRS total score, and CADSS scores would be matched to the treatment assignment by an authorized external group who are not part of the study team. The data will be anonymized (i.e., identification numbers would be removed or changed). The results of the analysis would be used to assist in the evaluation of potential intranasal esketamine doses for future clinical studies.

The results of the pharmacokinetic/pharmacodynamics evaluation will be presented in a separate report.

9.5. Biomarkers

Human Inflammation Multi-Analyte Panel (MAP)

Novel multiplex immunoassay platforms allow simultaneous and reliable quantification of several different protein markers in peripheral biological samples. Such techniques have been successfully used to discover novel diagnostic and prognostic biomarkers in patients suffering from different medical conditions, such as coronary artery disease, ovarian cancer, Alzheimer's disease, and schizophrenia (Muller 2011; Schwarz 2011). Inflammatory peripheral markers have been indicated as novel potential diagnostic and prognostic markers in patients with MDD and as possible modulators of NMDA receptor function (Li 2011; Muller 2011).

Blood (serum) samples will be collected to allow for the exploratory pharmacodynamic evaluation using the Human Inflammation MAP, a panel of 45 proteins involved in key biologically inflammatory and neurotrophic pathways.

Metabolomics

Metabolomics is a rapidly emerging technique which captures the metabolic concentration of small molecules (i.e., metabolome) present in biological samples. Studies performed in patients with different CNS disorders have shown that metabolomics might be a promising tool to discover novel biomarkers for drug exposure, safety, and response.

Recently, it has been shown that pretreatment plasma levels of glycine are significantly different in depressed patients who respond to citalopram compared to non-responders, and that glycine levels might also predict response to drugs which target the glutamatergic system (Ji 2011).

Blood (plasma) samples will be collected to allow for the exploratory analysis of novel predictive and drug exposure biomarkers. The goals are to investigate whether pretreatment glycine levels predict antidepressant response and whether this effect is present only in subjects who respond to ketamine and not to placebo. An additional goal of this is research is to try to identify a glutamatergic metabolic "signature" which might help characterizing the acute and late biological effects of esketamine in patients with depression.

Stopping Analysis

Biomarker analyses are dependent upon the availability of appropriate biomarker assays and clinical response rates. Biomarker analysis may be deferred or not performed, if during or at the end of the study, it becomes clear that the analysis will not have sufficient scientific value for biomarker evaluation, or if there are not enough samples or responders to allow for adequate biomarker evaluation. In the event the study is terminated early or shows poor clinical efficacy, completion of biomarker assessments is based on justification and intended utility of the data.

9.6. Pharmacogenomic (DNA) Evaluations

DNA samples will be analyzed for the *CYP2B6* gene. Additional analyses may be conducted if it is hypothesized that this may help resolve issues with the clinical data.

DNA samples will be used for research related to esketamine. They may also be used to develop tests/assays related to esketamine. Pharmacogenomic research may consist of the analysis of one or more candidate genes or of the analysis of genetic markers throughout the genome (as appropriate) in relation to esketamine.

9.7. Safety Evaluations

Any clinically relevant changes occurring during the study must be recorded on the Adverse Event section of the CRF.

Any clinically significant abnormalities persisting at the end of the study/early withdrawal will be followed by the investigator until resolution or until a clinically stable endpoint is reached.

The study will include the following evaluations of safety and tolerability according to the time points provided in the Time and Events Schedule:

Adverse Events

Adverse events will be reported by the subject (or, when appropriate, by a caregiver, surrogate, or the subject's legally acceptable representative) for the duration of the study. Adverse events will be followed by the investigator as specified in Section 12, Adverse Event Reporting.

Clinical Laboratory Tests

Blood samples for serum chemistry and hematology and a random urine sample for urinalysis will be collected. The investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the adverse event section of the CRF.

The use of local laboratories is allowed in cases where initiation of treatment or safety follow-up is time-critical and the central laboratory results are not expected to be available before the need to begin dosing or if actions need to be taken for safety reasons.

The following tests will be performed by the central laboratory, unless noted otherwise:

- Hematology Panel

  -hemoglobin
  -hematocrit
  -red blood cell (RBC) count
  -white blood cell (WBC) count with differential
  -platelet count

- Serum Chemistry Panel

  -sodium
  -potassium
  -chloride
  -bicarbonate
  -blood urea nitrogen (BUN)
  -creatinine
  -glucose
  -aspartate aminotransferase (AST)
  -alanine aminotransferase (ALT)
  -gamma-glutamyltransferase (GGT)
  -total bilirubin
  -alkaline phosphatase
  -creatine phosphokinase (CPK)
  -calcium
  - phosphate
  -albumin
  -total protein
  -total cholesterol**

  ***At scheduled time points*

- Urinalysis

  Dipstick
  -specific gravity
  -pH
  -glucose
  -protein
  -blood
  -ketones
  -bilirubin
  -urobilinogen
  -nitrite
  -leukocyte esterase

  Sediment (if dipstick result is abnormal)]
  -red blood cells
  -white blood cells
  -epithelial cells
  -crystals
  -casts
  -bacteria

  If dipstick result is abnormal, flow cytometry or microscopy will be used to measure sediment. In case of discordance between the dipstick results and the flow cytometric results, the sediment will be examined microscopically.

Specific gravity, pH, glucose, protein, blood, ketones, bilirubin, and urobilinogen will be determined using a dipstick. Red blood cells, white blood cells, epithelial cells, crystals, casts, and bacteria will be measured using flow cytometry or microscopy. If there is discordance between the dipstick results and the flow cytometric results, the sediment will be examined microscopically.

- Serum and Urine Pregnancy Testing (for women of childbearing potential only)
- Serology (HIV antibody, hepatitis B surface antigen [HBsAg], and hepatitis C virus antibody)

- Urine Drug Screen: barbiturates, methadone, opiates, cocaine, cannabinoids (not included at Screening, but included at all other time points), phencyclidine, and amphetamine/methamphetamine
    - The central laboratory will analyze the urine drug screen performed at Screening. The local laboratory will be used for all other time points.
- Alcohol test (urine or breath, as specified)
    - The central laboratory will analyze the urine drug screen performed at Screening. The local laboratory will be used for all other time points.
- Thyroid-stimulating hormone (TSH)
- Free thyroxine (FT4), if required for eligibility decision (See Section 4.1, Inclusion Criteria)

Electrocardiogram (ECG)

Single 12-lead ECG

During the collection of ECGs, subjects should be in a quiet setting without distractions (e.g., television, cell phones). Subjects should rest in a supine position for at least 5 minutes before ECG collection and should refrain from talking or moving arms or legs.

If blood sampling or vital sign measurement is scheduled for the same time point as ECG recording, the procedures should be performed in the following order: ECG(s), vital signs, blood draw.

All ECG tracings will be sent to a central ECG laboratory. The ECGs will be read at the scheduled time points and summarized by a central ECG laboratory. The central ECG laboratory will send the sponsor an electronic copy of the data for inclusion in the clinical database. In addition, the investigator or sub-investigator is required to review all ECGs at the study visit to assess for any potential safety concerns or evidence exclusionary conditions prior to dosing.

Vital Signs (temperature, pulse/heart rate, respiratory rate, blood pressure)

Blood pressure and pulse/heart rate measurements will be assessed supine with a completely automated device. Manual techniques will be used only if an automated device is not available.

Blood pressure and pulse/heart rate measurements should be preceded by at least 5 minutes of rest in a quiet setting without distractions (eg, television, cell phones).

Tympanic temperature is recommended.

Pulse Oximetry

Pulse oximetry will be used to measure arterial oxygen saturation (SpO_2). On each dosing day, the device will be attached to the finger, toe, or ear at approximately 5 minutes before the first nasal spray and then, after the first spray it will be monitored and documented every 15 minutes for approximately 1 hour postdose. Values will be recorded before the first spray and at defined time points thereafter. Any arterial oxygen saturation (SpO_2) lower than 91% and lasting for

more than 2 minutes, and confirmed by an additional manual measurement on another part of the body, will be reported as an adverse event.

Physical Examination, Height, and Body Weight

Physical examinations, body weight, and height will be performed/measured as per the Time and Events Schedule.

Targeted Nasal Examinations and Nasal Tolerability Questionnaire

Targeted nasal examinations (including the upper respiratory tract/throat) will be conducted by a qualified healthcare practitioner. The objective of the examination at Screening is to rule out any subjects with anatomical or medical conditions that may impede drug delivery or absorption.

Subsequent examinations will consist of a visual inspection of the nostrils, nasal mucosa, and throat for nasal erythema, rhinorrhea, rhinitis, capillary/blood vessel disruption and epistaxis and graded as follows: none, mild, moderate, or severe. Any treatment emergent change or worsening from baseline examination will be recorded as an adverse event.

In addition, subjects will be asked to complete a nasal tolerability questionnaire.

C-SSRS

The C-SSRS will be performed to assess suicidal ideation and behavior.

The C-SSRS is a low-burden measure of the spectrum of suicidal ideation and behavior that was developed in the National Institute of Mental Health Treatment of Adolescent Suicide Attempters Study to assess severity and track suicidal events through any treatment (Posner 2007). It is a clinical interview providing a summary of both ideation and behavior that can be administered during any evaluation or risk assessment to identify the level and type of suicidality present. The C-SSRS can also be used during treatment to monitor for clinical worsening.

CADSS

The CADSS will be administered to assess treatment-emergent dissociative symptoms.

The CADSS is an instrument for the measurement of present-state dissociative symptoms (Bremner 1998).

The CADSS comprises 23 subjective items, divided into 3 components: depersonalization (items 3 to 7, 20, 23), derealization (items 1, 2, 8 to 13, 16 to 19, 21) and amnesia (items 14 and 15, 22). Participant's responses are coded on a 5-point scale (0 = "Not at all" through to 4 = "Extremely"). CADSS has excellent inter-rater reliability and internal consistency of the CADSS.

BPRS+

Four items of the BPRS will be administered to assess treatment-emergent psychotic symptoms.

The BPRS (Overall 1962) is an 18-item rating scale which is used to assess a range of psychotic and affective symptoms rated from both observation of the subject and the subject's own report. It reportedly provides a rapid and efficient evaluation of treatment response in clinic drug studies and in clinical settings (Rugani 2012).

Only the 4-item positive symptom subscale (consisting of: suspiciousness, hallucinations, unusual thought content, and conceptual disorganization) will be used in this study. It is highly sensitive to change, and excellent inter-rater reliability can be achieved with training and a standard interview procedure.

MOAA/S

The MOAA/S will be used to measure treatment-emergent sedation with correlation to levels of sedation defined by the American Society of Anesthesiologists (ASA) continuum as described previously (Pambianco 2011).

The MOAA/S scores range from 0 [No response to painful stimulus; corresponds to ASA continuum for general anesthesia] to 5 [Readily responds to name spoken in normal tone (awake); corresponds to ASA continuum for minimal sedation].

On each dosing day, if a score of 5 is reached prior to the 1 hour postdose time point, assessments are to still to continue through 1 hour postdose.

Clinical Global Assessment of Alertness

The Clinical Global Assessment of Alertness will be used to measure the subject's current level of alertness.

The clinician will answer "Yes" or "No" to the question "*"Is the subject's level of alertness considered to be within the normal range*?"

PWC-20

The PWC-20 will be administered to assess potential withdrawal symptoms following cessation of intranasal esketamine treatment.

The PWC-20 is a 20-item simple and accurate method to assess potential development of discontinuation symptoms after stopping of study medication. The PWC-20 is a reliable and sensitive instrument for the assessment of discontinuation symptoms (Rickels 2008). Discontinuation symptoms occur early and disappear rather swiftly, depending upon speed of taper, daily medication dose, and drug elimination half-life.

BPIC-SS

The Bladder Pain/Interstitial Cystitis Symptom Score (BPIC-SS) is a patient-reported measure that was developed by ©Pfizer Ltd to identify an appropriate Bladder Pain Syndrome/Interstitial Cystitis (BPS/IC) population for clinical studies to evaluate new treatments for Bladder Pain Syndrome (BPS) (Humphreys 2012).

The BPIC-SS contains 8 questions with a recall period of the past 7 days. Each of the response option check boxes has a number beside it (or below it in the case of question 8). A total score is calculated by adding up the numbers beside the response options chosen by the patient. Questions 1 - 7 are scored from 0 to 4 and Question 8 is scored from 0-10. The range of scores for the scale is 0 to 38. The BPIC-SS does not contain domains.

A total score of 19 or more has demonstrated good sensitivity/specificity and discriminative power versus healthy controls and Overactive Bladder (OAB) patients.

If any items are missing, a total score cannot be calculated.

In the current study, if a subject has a score greater than 18 on the BPIC-SS scale and there is no evidence of urinary tract infection based on urinalysis and microscopy, he/she will be referred to a specialist for further evaluation.

Cogstate® computerized test battery and Hopkins Verbal Learning Test-Revised (HVLT-R)

The Cogstate® computerized test battery provides assessment of multiple cognitive domains including attention, visual learning and memory, and executive function. The tests use playing card stimuli and a maze task, enabling use in multilingual/multicultural settings. Five tests will be administered:

- Detection – simple reaction time task measuring attention and processing speed; mean of the log10 transformed reaction times for correct responses (lower score = better performance)
- Identification – choice reaction time paradigm measuring attention; mean of the log10 transformed reaction times for correct responses (lower score = better performance)
- One Card Learning – visual episodic memory measure; arcsine transformation of the proportion of correct responses (higher score = better performance)
- One Back – "n-back" working memory measure; arcsine transformation of the proportion of correct responses (higher score = better performance)
- Groton Maze Learning Test – executive function measure; total number of errors made in attempting to learn the same hidden pathway on five consecutive trials at a single session (lower score = better performance)

The Cogstate® measures, including GMLT, have been validated against traditional neuropsychological tests (Maruff 2009; Pietrzak 2008) and are sensitive to effects of various drugs on cognitive performance including ETOH and benzodiazepines (Maruff 2006; Snyder 2005).

The HVLT-R, a measure of verbal learning and memory, is a 12-item word list recall test. Administration includes 3 learning trials, a 24-word recognition list including 12 target and 12 foil words, and a delayed recall (20 minute) trial (Benedict 1998). Administration is computer-assisted, instructions and word lists appear on-screen. The test administrator records each word correctly recalled, and scores for learning, short-term, and delayed recall are generated via the test software. The HVLT-R is a well-validated and widely-used measure of verbal episodic memory.

Completing the battery requires approximately 25 minutes.

The tests will be administered in the following order: HVLT-R, detection, identification, one card learning, one back, Groton maze learning test, HVLT-R-delayed.

9.8. Other Evaluations

MINI

The Mini-International Neuropsychiatric Interview (M.I.N.I.) is a short structured diagnostic interview for DSM-IV-TR and ICD-10 psychiatric disorders. It has an administration time of approximately 15 minutes to provide accurate structured psychiatric interview for multicenter clinical trials.

MGH-ATRQ

The MGH-ATRQ is used to determine treatment resistance in major depressive disorder (Desseilles 2011). Information regarding all antidepressant therapies used in the current depressive episode will be recorded on the ATRQ.

The MGH-ATRQ examines the adequacy of duration and dose of current antidepressant treatments in a step-by-step procedure. In addition, the MGH-ATRQ assesses the degree of improvement (in the most efficacious trial or in all trials during the current episode, depending on the version of the instrument) on a scale from 0% (not improved at all) to 100% (completely improved). The ATRQ is completed by the clinician in collaboration with the subject.

Modified Berlin Questionnaire

The Modified Berlin Questionnaire (Netzer 1999) is a 7-item patient-reported measure that is intended to identify the occurrence of risk factors for obstructive sleep apnea. It has items to address snoring frequency and loudness, pauses in breathing, sleepiness and hypertension. Body mass index is also a part of the measure which is calculated, using a provided algorithm, from height and weight. The total score is obtained by adding the score associated with each response option selected by the subject. Score range is from 0 to 46 with scores greater than 25 indicating a high probability of obstructive sleep apnea.

SAFER Criteria Interview

Remote, independent psychiatrists/psychologists will perform the SAFER Interview (Targum 2008) for all subjects to assess the validity of a diagnosis of depression and eligibility for the study.

SAFER refers to:

S =	State versus trait	The identified symptoms must reflect the current state of illness and not longstanding traits. Traits do not generally change in 4–12 weeks.
A =	Assessibility	The patient's symptoms are measurable with standard, reliable

		rating instruments. The symptoms of valid patients can be reliably assessed with standardized measurement tools
F =	Face validity	The patient's presentation is consistent with our knowledge of the illness (symptoms map to the nosological entity; clear change from previous level of function; similar to previous episodes if recurrent)
E =	Ecological validity	The patient's symptoms reflect the characteristics of the illness in a real-world setting (frequency, intensity, duration, course, impact over at least 4 weeks)
R =	Rule of the Three P's	Identified symptoms must be pervasive, persistent, and pathological and interfere with function and quality of life

The interviewer will review subject screening information and conduct a live, remote interview with the subject. The MADRS and SAFER Criteria Inventory will be administered during the interview. The MGH-ATRQ performed at Screening will be reviewed. After the interview, the site will receive information regarding subject eligibility directly from the interviewer.

IDS-C_{30}

The 30-item IDS-C_{30} (Rush 1996) is designed to assess the severity of depressive symptoms. The IDS assesses all the criterion symptom domains designated by the DSM-IV-TR to diagnose a major depressive episode. These assessments can be used to screen for depression, although they have been used predominantly as measures of symptom severity. The 7-day period prior to assessment is the usual time frame for assessing symptom severity. The psychometric properties of the IDS-C_{30} have been established in various study samples (Trivedi 2004).

9.9. Sample Collection and Handling

The actual dates and times of sample collection must be recorded in the CRF or laboratory requisition form. If blood samples are collected via an indwelling cannula, an appropriate amount (1 mL) of serosanguineous fluid slightly greater than the dead space volume of the lock will be removed from the cannula and discarded before each blood sample is taken. After blood sample collection, the cannula will be flushed with 0.9% sodium chloride, United States Pharmacopeia (USP) (or equivalent) and charged with a volume equal to the dead space volume of the lock.

Refer to the Time and Events Schedule for the timing and frequency of all sample collections.

Instructions for the collection, handling, storage, and shipment of samples are found in the laboratory manual that will be provided. Collection, handling, storage, and shipment of samples must be under the specified, and where applicable, controlled temperature conditions as indicated in the laboratory manual.

10. SUBJECT COMPLETION/WITHDRAWAL

10.1. Completion

A subject will be considered to have completed the double-blind phase if he or she has completed assessments at Day 15 of the double-blind treatment phase. Subjects who prematurely discontinue study treatment for any reason before completion of the double-blind phase will not be considered to have completed the study.

A subject will be considered to have completed the optional open label treatment phase if he or she has received all 9 doses (Panel A) or 4 doses (Panel B) of optional open-label treatment.

10.2. Withdrawal From the Study

A subject will be withdrawn from the study for any of the following reasons:

- Lost to follow-up
- Withdrawal of consent
- Lack of efficacy
- Violation of protocol procedures (determined on a case by case basis)
- The investigator or sponsor believes (e.g., that for safety or tolerability reasons such as an adverse event) it is in the best interest of the subject to discontinue the study.
- The subject becomes pregnant
- If a subject misses more than two doses of study medication during the double-blind treatment phase

If a subject discontinues study treatment before the end of the double-blind treatment phase, for reasons other than withdrawal of consent, early termination and posttreatment assessments should be obtained.

If a subject discontinues the optional open-label treatment phase prior to receiving all 9 (Panel A) or 4 (Panel B) doses, for reasons other than withdrawal of consent, the subject would continue into the posttreatment phase.

If a subject is lost to follow-up, every reasonable effort must be made by the study site personnel to contact the subject and determine the reason for discontinuation/withdrawal. The measures taken to follow up must be documented.

When a subject withdraws before completing the study, the reason for withdrawal is to be documented in the CRF and in the source document. Study drug assigned to the withdrawn subject may not be assigned to another subject

Withdrawal From the Use of Samples in Future Research

The subject may withdraw consent for use of samples for research (refer to Section 16.2.5, Long-Term Retention of Samples for Additional Future Research). In such a case, samples will

be destroyed after they are no longer needed for the clinical study. Details of the sample retention for research are presented in the main ICF.

11. STATISTICAL METHODS

Statistical analysis will be done by the sponsor or under the authority of the sponsor. A general description of the statistical methods to be used to analyze the efficacy and safety data is outlined below. Specific details will be provided in the Statistical Analysis Plan. Each panel will be analyzed upon completion.

11.1. Subject Information

Intent-to-Treat Analysis Set: For each period in the double-blind treatment phase, an intent-to-treat (ITT) analysis set will be defined to include all randomized subjects who receive at least one dose of study drug and have both the baseline and at least one post baseline MADRS total score within that period. The efficacy analyses of data in Period 1 and Period 2 will be based on each respective ITT analysis set.

Safety Analysis Set: The primary population for safety consists of all randomized subjects who receive at least one dose of double-blind study drug. For the optional open-label treatment phase, the safety analysis set will be defined to include all subjects who receive at least 1 dose of study drug during that phase. The same analyses of safety and tolerability will be conducted for the double-blind treatment phase and the optional open-label treatment phase. Select safety summaries may be provided by Period 1 and Period 2 separately.

11.2. Sample Size Determination

Panel A

The sample size for Panel A is determined based on the following treatment differences between intranasal esketamine and placebo for the mean change from baseline in MADRS total score: a 9 point treatment difference was assumed for Period 1 (Day 8), a 7 point treatment difference for Period 2 (Day 15) was assumed for subjects with a moderate QIDS-SR_{16} score (QIDS-SR_{16} score = 11 to 16) and a 9 point treatment difference for Period 2 (Day 15) was assumed for subjects with a severe QIDS-SR_{16} score (QIDS-SR_{16} score >16). Based on results of a previous esketamine IV study (ESKETIVRD2001), it is estimated that 40% of placebo subjects at the end of Period 1 (Day 8 predose) will have a moderate QIDS-SR_{16} score and 55% will have a severe QIDS-SR_{16} score. Additional assumptions for the sample size calculation included a standard deviation of 10, 92.5% power for the combined data from both Day 8 and Day 15, an overall 1-sided significance level of 0.05, and a 5% drop-out rate for Period 1. It is calculated that this panel of the doubly-randomized, outcome based design will require 60 subjects to be randomly assigned to treatment on Day 1 in a 3:1:1:1 ratio (30 subjects on placebo and 10 subjects per intranasal esketamine dose group).

For Panel A, if the placebo response or other assumptions at the end of Period 1 are not as expected, up to a maximum of 78 subjects (i.e., up to 18 additional subjects) may be enrolled to ensure that an adequate number of subjects are re-randomized in Period 2. The assessment will

be based on a blinded IWRS review of the number of subjects who are re-randomized as well as dropout rates.

Panel B

The sample size for Panel B is determined using the same assumptions for MADRS total score, QIDS-SR_{16}, and drop-out rate as was used for Panel A. Additional assumptions for this panel for the sample size calculation included 90% power for the combined data from both Day 8 and Day 15, and an overall 1-sided significance level of 0.1. It is calculated that this panel of the doubly-randomized, outcome based design will require 40 subjects to be randomly assigned to treatment on Day 1 in a 2:1:1 ratio (20 subjects on placebo and 10 subjects per intranasal esketamine dose group).

For Panel B, if the placebo response or other assumptions at the end of Period 1 are not as expected, up to a maximum of 52 subjects (i.e., up to 12 additional subjects) may be enrolled to ensure that an adequate number of subjects are re-randomized in Period 2. The assessment will be based on a blinded IWRS review of the number of subjects who are re-randomized as well as dropout rates.

11.3. Efficacy Analyses

Panel A and Panel B will be analyzed separately for efficacy. The same statistical methodology applies to both panels. The efficacy analyses will be based on the combination of efficacy data from the two periods within each panel of the double-blind treatment phase, unless specified otherwise.

Primary Endpoint

For the primary efficacy analysis, change from baseline (Day 1 predose) in MADRS total score to Day 8 predose assessment of the double-blind treatment phase will be analyzed using an analysis of covariance (ANCOVA) model, with factors for treatment, center, and Period 1 baseline MADRS total score as the continuous covariate. Data from all randomized, treated subjects with change values during Period 1 will be included in the analysis of Period 1. Change from baseline in MADRS total score in Period 2 (Day 8, predose to Day 15) will be analyzed using an ANCOVA model with factors for treatment, center, Period 2 baseline QIDS-SR_{16} score (moderate or severe), and Period 2 baseline MADRS total score as the continuous covariate. Only data from Period 1 placebo subjects who are re-randomized (moderate and severe QIDS-SR_{16} scores) who continue into Period 2 and have a change value during Period 2 will be included in the analysis of Period 2. The comparison of intranasal esketamine dose groups with placebo will be carried out by a combined test on the weighted test statistics for each period in the double-blind treatment phase. The ‘adaptive’ weight will be based on the actual sample sizes for the final analysis (Liu 2012).

Descriptive statistics for values and changes from baseline will be provided at each time point within each period of the double-blind treatment phase.

Details of the dose response analyses will be provided in the Statistical Analysis Plan. In addition to conducting the dose response analyses separately for Panel A and Panel B, the analysis may be conducted by pooling data from Panel A and Panel B.

Secondary Endpoints

For all continuous endpoints descriptive statistics of actual values and changes from baseline by treatment group within each period will be provided. The change from baseline for QIDS-SR_{16} total score, CGI-S, PGI-S, and GAD-7 will be analyzed in the same way as for the MADRS total score. There will be no adjustments for multiplicity in the evaluation of these other efficacy endpoints.

A frequency table for the number and percentage of subjects meeting criteria for sustained response (≥ 50% reduction from baseline in MADRS total score with onset by Day 2 through the end of the double-blind phase (Day 15) will be provided for subjects who remain on the same treatment for the duration of the double-blind phase. Frequency tables for the number and percentage of subjects meeting criteria for response (≥50% reduction from baseline in MADRS total score) and remission (MADRS total score of ≤10) will be provided at each time point.

Descriptive statistics for values and changes from baseline for the MADRS total score will be provided for the group of subjects who are randomized at Period 1 to either intranasal esketamine or placebo and remain on the same treatment for the duration of the double-blind treatment phase (Day 15 or early withdrawal). Placebo subjects who are re-randomized in Period 2 and receive esketamine will not be included in these summaries.

Efficacy data from the open-label phase will be summarized descriptively.

Details of the exploratory analyses will be provided in the Statistical Analysis Plan.

11.4. Pharmacokinetic Analyses

Plasma esketamine, noresketamine, 4β-hydroxycholesterol, and cholesterol concentrations will be listed for all subjects by esketamine treatment and study day. All concentrations below the lowest quantifiable concentration in a sample or missing data will be labeled as such in the concentration data presentations. Concentrations below the lower quantifiable concentration will be treated as zero in the summary statistics. Subjects will be excluded from the pharmacokinetic analysis if their data do not allow for accurate assessment (e.g., incomplete administration of the study drug; missing information of dosing and sampling times). All subjects and samples excluded from the analysis will be clearly documented in the study report.

Descriptive statistics will be used to summarize esketamine, noresketamine, concentrations at each sampling time point. For each esketamine treatment and day, descriptive statistics, including arithmetic mean, SD, coefficient of variation, median, minimum, and maximum will be calculated for each analyte at each sampling time.

Descriptive statistics will be used to summarize 4β-hydroxycholesterol, and cholesterol concentrations at each sampling time point. For each esketamine treatment and day, descriptive

statistics, including arithmetic mean, SD, coefficient of variation, median, minimum, and maximum will be calculated for each analyte at each sampling time. The concentrations of 4β-hydroxycholesterol will be compared visually among treatment groups (placebo and active dose groups) to assess the potential for repeated administration of intranasally administered esketamine to influence the activity of the hepatic cytochrome P450 3A4 and 3A5 enzymes. Additional analysis will be done if deemed appropriate.

Population PK analysis of plasma concentration-time data of esketamine will be performed using nonlinear mixed-effects modeling. Data may be combined with those of other selected studies to support a relevant structural model. Available baseline subject characteristics (demographics, laboratory variables, genotypes, race, etc.) will be tested as potential covariates affecting PK parameters. Details will be given in a population PK analysis plan and the results of the population PK analysis will be presented in a separate report.

11.5. Pharmacokinetic/Pharmacodynamic Analyses

The relationship between MADRS score (and possibly selected adverse events as additional pharmacodynamic parameters) and PK metrics of esketamine may be evaluated. If there is any visual trend in graphical analysis, suitable models will be applied to describe the exposure-effect relationships.

A pharmacokinetic/pharmacodynamics analysis may be performed after at least 80% of Panel A subjects have completed the double-blind treatment phase. Subject pharmacokinetic data, MADRS total score, and CADSS scores would be matched to the treatment assignment by an authorized external group who are not part of the study team. The data will be anonymized (i.e., identification numbers would be removed or changed). The results of the analysis would be used to assist in the evaluation of potential intranasal esketamine doses for future clinical studies.

The results of the pharmacokinetic/pharmacodynamics evaluation will be presented in a separate report.

The results of this analysis would be presented in a separate report.

11.6. Biomarker Analyses

Metabolomics (if analyzed)

Spearman rank correlation coefficients between pretreatment glycine levels and the MADRS total score percentage change from Day 1 (baseline) at all scheduled time points will be calculated to investigate whether pretreatment concentrations of glycine correlate with the magnitude of clinical change following the administration of intranasal esketamine or placebo. Changes in glutamate metabolic pathway markers induced by esketamine or placebo will be investigated using a pattern classifier algorithm. Samples from different studies on ketamine and esketamine in treatment resistant depression will be pooled for analysis.

Human Inflammation MAP (if analyzed)

Statistical analyses of the markers from the Human Inflammation MAP will use both a univariate and a multivariate approach to identify the least number of markers which yield the highest accuracy in separating responders from non-responders to intranasal esketamine. A similar approach will be used to identify the pharmacodynamic effects of esketamine and placebo on inflammatory and neurotrophic markers.

Results of Human Inflammation MAP and metabolomics will be presented in a separate report.

11.7. Pharmacogenomic Analyses

A composite genotype and predicted phenotype will be derived from the raw genotyping data for *CYP2B6*. Allele and genotype frequencies will be tabulated. No formal statistical tests will be performed. Genetic results from other analyzed genes will be pooled together with data from other suitable studies for a meta-analysis.

Results of the pharmacogenomic analysis will be listed and summarized with other clinical studies in a separate pharmacogenomics report.

Results will be presented in a separate report.

11.8. Safety Analyses

Safety data from Panels A and B will be pooled for analysis as well as analyzed separately for the safety summaries

Adverse Events

The verbatim terms used in the CRF by investigators to identify adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). All reported adverse events with onset during the treatment phase (i.e., treatment-emergent adverse events, and adverse events that have worsened since baseline) will be included in the analysis. For each adverse event, the percentage of subjects who experience at least 1 occurrence of the given event will be summarized by treatment group.

Summaries, listings, datasets, or subject narratives may be provided, as appropriate, for those subjects who die, who discontinue treatment due to an adverse event, or who experience a severe or a serious adverse event.

Clinical Laboratory Tests

Laboratory data will be summarized by type of laboratory test. Reference ranges and markedly abnormal results (specified in the Statistical Analysis Plan) will be used in the summary of laboratory data. Descriptive statistics will be calculated for each laboratory analyte at baseline and at each scheduled time point.

Electrocardiogram (ECG)

The effects on cardiovascular variables will be evaluated using descriptive statistics and frequency tabulations. These tables will include observed values and changes from baseline values.

The ECG variables that will be analyzed are heart rate, PR interval, QRS interval, QT interval, and corrected QT (QTc) interval using the following correction methods: QT corrected according to Bazett's formula (QTcB), and QT corrected according to Fridericia's formula (QTcF).

Descriptive statistics of QTc intervals and changes from baseline will be summarized at each scheduled time point. The percentage of subjects with QTc interval >450 ms, >480 ms, or >500 ms will be summarized, as will the percentage of subjects with QTc interval increases from baseline >30 ms or >60 ms.

All important abnormalities in ECG waveform that are changes from the baseline readings will be reported (e.g., changes in T-wave morphology or the occurrence of U-waves.

Vital Signs

Descriptive statistics of temperature, pulse/heart rate, respiratory rate, pulse oximetry, and blood pressure (systolic and diastolic) (supine) values and changes from baseline will be summarized at each scheduled time point. The percentage of subjects with values beyond clinically important limits will be summarized.

Nasal Exam and Nasal Tolerability Questionnaire

Changes in findings from the baseline nasal examination (including the upper respiratory tract/throat) will be listed by treatment group. Examinations will provide ratings (none, mild, moderate, or severe) that are based on a visual inspection of the nostrils, nasal mucosa, and throat for nasal erythema, rhinorrhea, rhinitis, capillary/blood vessel disruption and epistaxis. A shift table for changes in rating for each examination will be presented by treatment group.

In addition, scoring from the nasal tolerability questionnaire will be summarized descriptively by treatment group and period.

C-SSRS

Suicide-related thoughts and behaviors based on the C-SSRS will be summarized by treatment group in incidence and shift tables. Separate endpoints for suicidal ideation and suicidal behavior will be defined and summarized descriptively by treatment group. Missing scores will not be imputed.

CADSS, BPRS+, BPIC-SS, and MOAA/S

Descriptive statistics of each of the scores and changes from predose will be summarized at each scheduled time point.

Clinical Global Assessment of Alertness

The Clinical Global Assessment of Alertness will be analyzed descriptively.

PWC-20

The PWC-20 rating scale will be analyzed descriptively.

Cogstate® cognitive test battery and HVLT-R

Descriptive statistics of each of the cognitive domain scores and changes from baseline will be summarized at each scheduled time point.

11.9. Interim Analysis

An interim analysis may be performed for each panel as needed. If required, details will be provided in a separate charter and interim analysis plan.

12. ADVERSE EVENT REPORTING

Timely, accurate, and complete reporting and analysis of safety information from clinical studies are crucial for the protection of subjects, investigators, and the sponsor, and are mandated by regulatory agencies worldwide. The sponsor has established Standard Operating Procedures in conformity with regulatory requirements worldwide to ensure appropriate reporting of safety information; all clinical studies conducted by the sponsor or its affiliates will be conducted in accordance with those procedures.

12.1. Definitions

12.1.1. Adverse Event Definitions and Classifications

Adverse Event

An adverse event is any untoward medical occurrence in a clinical study subject administered a medicinal (investigational or non-investigational) product. An adverse event does not necessarily have a causal relationship with the treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal finding), symptom, or disease temporally associated with the use of a medicinal (investigational or non-investigational) product, whether or not related to that medicinal (investigational or non-investigational) product. (Definition per International Conference on Harmonisation [ICH]).

This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition, or abnormal results of diagnostic procedures, including laboratory test abnormalities.

Note: The sponsor collects adverse events starting with the signing of the ICF (refer to Section 12.3.1, All Adverse Events, for time of last adverse event recording).

Serious Adverse Event

A serious adverse event based on ICH and EU Guidelines on Pharmacovigilance for Medicinal Products for Human Use is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening
  (The subject was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe.)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is a suspected transmission of any infectious agent via a medicinal product
- Is Medically Important*

*Medical and scientific judgment should be exercised in deciding whether expedited reporting is also appropriate in other situations, such as important medical events that may not be immediately life threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These should usually be considered serious.

If a serious and unexpected adverse event occurs for which there is evidence suggesting a causal relationship between the study drug and the event (e.g., death from anaphylaxis), the event must be reported as a serious and unexpected suspected adverse reaction even if it is a component of the study endpoint (e.g., all-cause mortality).

Unlisted (Unexpected) Adverse Event/Reference Safety Information

An adverse event is considered unlisted if the nature or severity is not consistent with the applicable product reference safety information. For esketamine, the expectedness of an adverse event will be determined by whether or not it is listed in the Investigator's Brochure.

Adverse Event Associated With the Use of the Drug

An adverse event is considered associated with the use of the drug if the attribution is possible, probable, or very likely by the definitions listed in Section 12.1.2.

12.1.2. Attribution Definitions

Not Related

An adverse event that is not related to the use of the drug.

Doubtful

An adverse event for which an alternative explanation is more likely, e.g., concomitant drug(s), concomitant disease(s), or the relationship in time suggests that a causal relationship is unlikely.

Possible

An adverse event that might be due to the use of the drug. An alternative explanation, e.g., concomitant drug(s), concomitant disease(s), is inconclusive. The relationship in time is reasonable; therefore, the causal relationship cannot be excluded.

Probable

An adverse event that might be due to the use of the drug. The relationship in time is suggestive (e.g., confirmed by dechallenge). An alternative explanation is less likely, e.g., concomitant drug(s), concomitant disease(s).

Very Likely

An adverse event that is listed as a possible adverse reaction and cannot be reasonably explained by an alternative explanation, e.g., concomitant drug(s), concomitant disease(s). The relationship in time is very suggestive (e.g., it is confirmed by dechallenge and rechallenge).

12.1.3. Severity Criteria

An assessment of severity grade will be made using the following general categorical descriptors:

Mild: Awareness of symptoms that are easily tolerated, causing minimal discomfort and not interfering with everyday activities.

Moderate: Sufficient discomfort is present to cause interference with normal activity.

Severe: Extreme distress, causing significant impairment of functioning or incapacitation. Prevents normal everyday activities.

The investigator should use clinical judgment in assessing the severity of events not directly experienced by the subject (e.g., laboratory abnormalities).

12.2. Special Reporting Situations

Safety events of interest on a sponsor study drug that may require expedited reporting and/or safety evaluation include, but are not limited to:

- Overdose of a sponsor study drug
- Suspected abuse/misuse of a sponsor study drug
- Inadvertent or accidental exposure to a sponsor study drug
- Medication error involving a sponsor product (with or without subject/patient exposure to the sponsor study drug, e.g., name confusion)

Special reporting situations should be recorded in the CRF. Any special reporting situation that meets the criteria of a serious adverse event should be recorded on the serious adverse event page of the CRF.

12.3. Procedures

12.3.1. All Adverse Events

All adverse events and special reporting situations, whether serious or non-serious, will be reported from the time a signed and dated ICF is obtained until completion of the subject's last study-related procedure (which may include contact for follow-up of safety). Serious adverse events, including those spontaneously reported to the investigator from the time a signed and dated ICF is obtained until completion of the subject's last study-related procedure or within 30 days after the last dose of study drug (if last study-related procedure is less than 30 days after the last dose of study drug), must be reported using the Serious Adverse Event Form. The sponsor will evaluate any safety information that is spontaneously reported by an investigator beyond the time frame specified in the protocol.

All events that meet the definition of a serious adverse event will be reported as serious adverse events, regardless of whether they are protocol-specific assessments.

All adverse events, regardless of seriousness, severity, or presumed relationship to study drug, must be recorded using medical terminology in the source document and the CRF. Whenever possible, diagnoses should be given when signs and symptoms are due to a common etiology (e.g., cough, runny nose, sneezing, sore throat, and head congestion should be reported as "upper respiratory infection"). Investigators must record in the CRF their opinion concerning the relationship of the adverse event to study therapy. All measures required for adverse event management must be recorded in the source document and reported according to sponsor instructions.

The sponsor assumes responsibility for appropriate reporting of adverse events to the regulatory authorities. The sponsor will also report to the investigator (and the head of the investigational institute where required) all serious adverse events that are unlisted (unexpected) and associated with the use of the study drug. The investigator (or sponsor where required) must report these events to the appropriate Independent Ethics Committee/Institutional Review Board (IEC/IRB) that approved the protocol unless otherwise required and documented by the IEC/IRB.

For all studies in which a subject will be outpatient (i.e., not confined to the clinical site for the duration of the study), including open-label studies, the subject must be provided with a "wallet (study) card" and instructed to carry this card with them for the duration of the study indicating the following:

- Study number
- Statement, in the local language(s), that the subject is participating in a clinical study
- Investigator's name and 24-hour contact telephone number
- Local sponsor's name and 24-hour contact telephone number (for medical staff only)
- Site number
- Subject number

- Any other information that is required to do an emergency breaking of the blind

12.3.2. Serious Adverse Events

All serious adverse events occurring during the study must be reported to the appropriate sponsor contact person by study-site personnel within 24 hours of their knowledge of the event.

Information regarding serious adverse events will be transmitted to the sponsor using the Serious Adverse Event Form, which must be completed and signed by a physician from the study site, and transmitted to the sponsor within 24 hours. The initial and follow-up reports of a serious adverse event should be made by facsimile (fax).

All serious adverse events that have not resolved by the end of the study, or that have not resolved upon discontinuation of the subject's participation in the study, must be followed until any of the following occurs:

- The event resolves
- The event stabilizes
- The event returns to baseline, if a baseline value/status is available
- The event can be attributed to agents other than the study drug or to factors unrelated to study conduct
- It becomes unlikely that any additional information can be obtained (subject or health care practitioner refusal to provide additional information, lost to follow-up after demonstration of due diligence with follow-up efforts)

Suspected transmission of an infectious agent by a medicinal product will be reported as a serious adverse event. Any event requiring hospitalization (or prolongation of hospitalization) that occurs during the course of a subject's participation in a study must be reported as a serious adverse event, except hospitalizations for the following:

- Hospitalizations not intended to treat an acute illness or adverse event (e.g., social reasons such as pending placement in long-term care facility)
- Surgery or procedure planned before entry into the study (must be documented in the CRF). Note: Hospitalizations that were planned before the signing of the ICF, and where the underlying condition for which the hospitalization was planned has not worsened, will not be considered serious adverse events. Any adverse event that results in a prolongation of the originally planned hospitalization is to be reported as a new serious adverse event.
- For convenience, the investigator may choose to hospitalize the subject for treatment, with Sponsor’s approval in advance.

The cause of death of a subject in a study, whether or not the event is expected or associated with the study drug, is considered a serious adverse event.

12.3.3. Pregnancy

All initial reports of pregnancy must be reported to the sponsor by the study-site personnel within 24 hours of their knowledge of the event using the appropriate pregnancy notification form. Abnormal pregnancy outcomes (e.g., spontaneous abortion, stillbirth, and congenital anomaly) are considered serious adverse events and must be reported using the Serious Adverse Event Form. Any subject who becomes pregnant during the study must be promptly withdrawn from the study and discontinue further study treatment.

Because the effect of the study drug on sperm is unknown, pregnancies in partners of male subjects included in the study will be reported by the study-site personnel within 24 hours of their knowledge of the event using the appropriate pregnancy notification form.

Follow-up information regarding the outcome of the pregnancy and any postnatal sequelae in the infant will be required.

12.4. Contacting Sponsor Regarding Safety

The names (and corresponding telephone numbers) of the individuals who should be contacted regarding safety issues or questions regarding the study are listed on the Contact Information page(s), which will be provided as a separate document.

13. PRODUCT QUALITY COMPLAINT HANDLING

A product quality complaint (PQC) is defined as any suspicion of a product defect related to manufacturing, labeling, or packaging, i.e., any dissatisfaction relative to the identity, quality, durability, or reliability of a product, including its labeling or package integrity. A PQC may have an impact on the safety and efficacy of the product. Timely, accurate, and complete reporting and analysis of PQC information from studies are crucial for the protection of subjects, investigators, and the sponsor, and are mandated by regulatory agencies worldwide. The sponsor has established procedures in conformity with regulatory requirements worldwide to ensure appropriate reporting of PQC information; all studies conducted by the sponsor or its affiliates will be conducted in accordance with those procedures.

13.1. Procedures

All initial PQCs must be reported to the sponsor by the study-site personnel within 24 hours after being made aware of the event.

If the defect is combined with a serious adverse event, the study-site personnel must report the PQC to the sponsor according to the serious adverse event reporting timelines (refer to Section 12.3.2, Serious Adverse Events). A sample of the suspected product should be maintained for further investigation if requested by the sponsor.

13.2. Contacting Sponsor Regarding Product Quality

The names (and corresponding telephone numbers) of the individuals who should be contacted regarding product quality issues are listed on the Contact Information page(s), which will be provided as a separate document.

14. STUDY DRUG INFORMATION

14.1. Physical Description of Study Drug(s)

The esketamine supplied for this study is a clear, colorless intranasal solution of esketamine hydrochloride (16.14% weight/volume [w/v]; equivalent to 14% w/v of esketamine base). The solution will consist of 161.4 mg/mL esketamine hydrochloride (equivalent to 140 mg of esketamine base) formulated in 0.12 mg/mL EDTA and 1.5 mg/mL citric acid at a pH of 4.5 in water for injection. It is provided in a nasal spray pump, which delivers 16.14 mg esketamine hydrochloride (14 mg esketamine base) per 100 mcl spray. Each individual nasal spray pump (device) contains a total of 28 mg (i.e., 2 sprays). It will be manufactured and provided under the responsibility of the sponsor. Refer to the Investigator's Brochure for a list of excipients.

The placebo supplied for this study is a clear, colorless intranasal solution of water for injection with a bittering agent (denatonium benzoate [Bitrex®] at a final concentration of 0.001 mg/mL) added to simulate the taste of the intranasal solution with active drug. Benzalkonium chloride is added as a preservative at a concentration of 0.3 mg/mL.

14.2. Packaging

Study drug (i.e., intranasal esketamine and placebo solution) will be supplied by the sponsor in a bidose nasal spray device. The devices will contain 200 mcl. Each device delivers 16.14 mg esketamine hydrochloride (14 mg esketamine base) or 0.1 μg of denatonium benzoate per 100 mcl spray. Esketamine will be provided as bulk supplies (i.e., not packaged for individual subject numbers).

14.3. Labeling

Study drug labels will contain information to meet the applicable regulatory requirements.

14.4. Preparation, Handling, and Storage

Study medication will be stored at the study site in a secure area with restricted access until dispensed to the subjects. Before dispensing, the study medication to be administered to the subject will be labeled with the subject’s randomization numbers by the appropriate study staff.

All study drug must be stored at controlled temperatures as indicated on the product specific labeling.

14.5. Drug Accountability

The investigator is responsible for ensuring that all study drug received at the site is inventoried and accounted for throughout the study. The dispensing of study drug to the subject, and the return of study drug from the subject (if applicable), must be documented on the drug accountability form. Subjects must be instructed to return all original containers, whether empty or containing study drug. All study drug will be stored and disposed of according to the sponsor's instructions. Study-site personnel must not combine contents of the study drug containers.

Study drug must be handled in strict accordance with the protocol and the container label, and must be stored at the study site in a limited-access area or in a locked cabinet under appropriate environmental conditions. Unused study drug, and study drug returned by the subject, must be available for verification by the sponsor's study site monitor during on-site monitoring visits. The return to the sponsor of unused study drug, or used returned study drug for destruction, will be documented on the drug return form. When the study site is an authorized destruction unit and study drug supplies are destroyed on-site, this must also be documented on the drug return form.

Potentially hazardous materials such as used ampules, needles, syringes and vials containing hazardous liquids, should be disposed of immediately in a safe manner and therefore will not be retained for drug accountability purposes.

Study drug should be dispensed under the supervision of the investigator or a qualified member of the study-site personnel, or by a hospital/clinic pharmacist. Study drug will be supplied only to subjects participating in the study. Returned study drug must not be dispensed again, even to the same subject. Study drug may not be relabeled or reassigned for use by other subjects. The investigator agrees neither to dispense the study drug from, nor store it at, any site other than the study sites agreed upon with the sponsor.

15. STUDY-SPECIFIC MATERIALS

The investigator will be provided with the following supplies:

- Study medication
- Practice intranasal devices
- Investigator Brochure for esketamine
- Pharmacy manual
- Laboratory manual and materials
- Clinician-administered and subject-completed/patient-reported outcome assessments
- IWRS Manual
- ECG Manual
- Intranasal dose administration instructions
- Recommended order of procedures
- Subject diary
- Rater qualifications/requirements for clinician-administered assessments
- Guidance document for ATRQ
- Guidance for minimum requirements for site staff and equipment on dosing days
- Cogstate® computerized test battery and Hopkins Verbal Learning Test-Revised, and all associated equipment and materials

16. ETHICAL ASPECTS

16.1. Study-Specific Design Considerations

Clinical Study in Treatment-Resistant Major Depression

Major depressive disorder is a common, severe, chronic and often life-threatening illness. It is now the leading cause of disability worldwide. There is a clear need to develop novel and improved therapeutics for major depression.

Ketamine and esketamine have shown antidepressant-like effects in a number of small studies and has been well tolerated and safe in these clinical studies.

Selection of Subjects

The primary aim of the study is to evaluate the efficacy of intranasal esketamine for the treatment of TRD. Thus, the study cannot be completed in healthy subjects. Subjects selected in the study will have adequate capacity to give consent for participation in the study.

Justification for Using Placebo

Assessment of the potential efficacy of a new compound for the treatment of major depression requires adequate and well-controlled clinical studies. For a new compound, this can be achieved either through a placebo-controlled study or through a study comparing it to an active comparator through a non-inferiority design. For non-inferiority studies, previous placebo-controlled studies have to show consistently the superiority of the active standard drug to placebo. Nearly half of the studies with antidepressants fail even with previously proven antidepressants, making assay sensitivity difficult to establish and thus, a non-inferiority design invalid (Laughren 2001). Of note however, with the current design, most subjects will receive the active treatment at some point during the double blind phase and subjects will be offered open label treatment with IN esketamine for an additional 2 weeks after completing the double blind phase of the study. Recent analyses have shown response to placebo varies considerably from 10% to 55%. Therefore, there is a concern that randomized, controlled studies that rely on comparison with standard antidepressants alone will generate unreliable results with limited assay sensitivity.

However, some have considered it unethical to do placebo-controlled studies in major depression due to the potential risk of irreversible harm (Rothman 1994). In a meta-analysis (Khan 2000) of drug studies conducted in major depression, it was reported that adult subjects did not have higher rates of suicide behaviors or attempts in the placebo group compared with those receiving an active antidepressant. These studies show annual suicide rates of 0.8% on the investigational drug, 0.7% on the active comparator, and 0.4% on placebo. The risk of irreversible harm is not higher in the placebo arm compared to the active control arms. Some subjects may decide not to participate due to the potential for increased distress and dysfunction due to prolonged depression.

Therefore, the use of a placebo-controlled study remains the gold standard for assessment of efficacy of new compound to allow for scientifically meaningful results. Placebo-controlled

studies in major depression have been argued to be ethically and scientifically justifiable (Adam 2005; Temple 2000; Laughren 2001).

Moreover, the duration of the double-blind treatment phase is relatively short, approximately 2 weeks (Day 1 to Day 15). The subjects will continue existing and allowed anti-depressant medications. They will visit the study site at least twice weekly during the double-blind study period and their symptoms will be carefully monitored during each study visit. Safety evaluations will include evaluation of suicidal ideation/behavior at each clinic visit. At any point in the study the subject may withdraw consent or be removed from the study by the investigator if there are any clinical concerns. Subjects who complete the double-blind treatment phase may receive optional open-label intranasal esketamine treatment for up to an additional 2 weeks (up to 4 additional doses) for subjects in Panel B, and up to an additional 9 weeks (up to 9 additional doses) for subjects in Panel A. The study medication, intranasal esketamine, will not be available after the study, however, following completion of the double-blind treatment phase and the optional open label treatment phase (if applicable), subjects can be treated according to standard of care.

Precautions to Ensure Subject Safety in the Study

Subjects may participate in the study only if they have adequate capacity to give consent and after fully understanding the potential risks and giving an informed consent. Determination of capacity will be made by the study investigator. Subjects may discontinue the study at any time. The probability of receiving placebo and the concept of random assignment will be explained to the subject. The duration of the study is short, minimizing the time on placebo. Potential disadvantages and adverse events of participating in the study and alternative treatment options will be discussed. For subjects who do not respond during the study and are not willing or able to receive additional open-label esketamine treatment for 9 (Panel A) or 2 (Panel B) weeks, clinical care will be arranged between the study investigator and or their physician.

Compensation for any procedure will be fair per local standards and approved by the participating sites IRB in order to not offer any undue incentive to participate in the study.

Subjects who are unable to tolerate study drug during the double-blind treatment phase will be discontinued from the study. Subjects who are unable to tolerate study drug during the optional open label treatment phase can have their dose decreased to a permitted dosage (not applicable to Panel A doses on Day 60 and 74) or can discontinue from the optional open label treatment phase. If the investigator judges it to be necessary to immediately stop study drug, he or she has the option to do so.

Only subjects who have not adequately responded to their antidepressant where a clinician would consider changing it for lack of response or poor tolerability in addition to meeting the severity criteria for the study will be enrolled.

Only highly qualified and experienced investigators will participate in the study.

Potential subjects will be fully informed of the risks and requirements of the study and, during the study, subjects will be given any new information that may affect their decision to continue participation. They will be told that their consent to participate in the study is voluntary and may be withdrawn at any time with no reason given and without penalty or loss of benefits to which they would otherwise be entitled. Only subjects who are fully able to understand the risks, benefits, and potential adverse events of the study, and provide their consent voluntarily will be enrolled.

The total blood volume to be collected is considered to be within the normal range allowed for this subject population over this time frame. The maximum blood volume to be collected is approximately 153 mL which will be less than a Red Cross blood donation.

16.2. Regulatory Ethics Compliance

16.2.1. Investigator Responsibilities

The investigator is responsible for ensuring that the study is performed in accordance with the protocol, current ICH guidelines on Good Clinical Practice (GCP), and applicable regulatory and country-specific requirements.

Good Clinical Practice is an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety, and well-being of study subjects are protected, consistent with the principles that originated in the Declaration of Helsinki, and that the study data are credible.

16.2.2. Independent Ethics Committee or Institutional Review Board

Before the start of the study, the investigator (or sponsor where required) will provide the IEC/IRB with current and complete copies of the following documents (as required by local regulations):

- Final protocol and, if applicable, amendments
- Sponsor-approved ICF (and any other written materials to be provided to the subjects)
- Investigator's Brochure (or equivalent information) and amendments/addenda
- Sponsor-approved subject recruiting materials
- Information on compensation for study-related injuries or payment to subjects for participation in the study, if applicable
- Investigator's curriculum vitae or equivalent information (unless not required, as documented by the IEC/IRB)
- Information regarding funding, name of the sponsor, institutional affiliations, other potential conflicts of interest, and incentives for subjects
- Any other documents that the IEC/IRB requests to fulfill its obligation

This study will be undertaken only after the IEC/IRB has given full approval of the final protocol, amendments (if any, excluding the ones that are purely administrative, with no

consequences for subjects, data or study conduct), the ICF, applicable recruiting materials, and subject compensation programs, and the sponsor has received a copy of this approval. This approval letter must be dated and must clearly identify the IEC/IRB and the documents being approved.

During the study the investigator (or sponsor where required) will send the following documents and updates to the IEC/IRB for their review and approval, where appropriate:

- Protocol amendments (excluding the ones that are purely administrative, with no consequences for subjects, data or study conduct)
- Revision(s) to ICF and any other written materials to be provided to subjects
- If applicable, new or revised subject recruiting materials approved by the sponsor
- Revisions to compensation for study-related injuries or payment to subjects for participation in the study, if applicable
- New edition(s) of the Investigator's Brochure and amendments/addenda
- Summaries of the status of the study at intervals stipulated in guidelines of the IEC/IRB (at least annually)
- Reports of adverse events that are serious, unlisted/unexpected, and associated with the study drug
- New information that may adversely affect the safety of the subjects or the conduct of the study
- Deviations from or changes to the protocol to eliminate immediate hazards to the subjects
- Report of deaths of subjects under the investigator's care
- Notification if a new investigator is responsible for the study at the site
- Development Safety Update Report and Line Listings, where applicable
- Any other requirements of the IEC/IRB

For all protocol amendments (excluding the ones that are purely administrative, with no consequences for subjects, data or study conduct), the amendment and applicable ICF revisions must be submitted promptly to the IEC/IRB for review and approval before implementation of the change(s).

At least once a year, the IEC/IRB will be asked to review and reapprove this study. The reapproval should be documented in writing (excluding the ones that are purely administrative, with no consequences for subjects, data, or study conduct).

At the end of the study, the investigator (or sponsor where required) will notify the IEC/IRB about the study completion.

16.2.3. Informed Consent

Each subject must give written consent according to local requirements after the nature of the study has been fully explained. The ICF(s) must be signed before performance of any study-related activity. The ICF(s) that is/are used must be approved by both the sponsor and by the reviewing IEC/IRB and be in a language that the subject can read and understand. The informed consent should be in accordance with principles that originated in the Declaration of Helsinki, current ICH and GCP guidelines, applicable regulatory requirements, and sponsor policy.

Before enrollment in the study, the investigator or an authorized member of the study-site personnel must explain to potential subjects the aims, methods, reasonably anticipated benefits, and potential hazards of the study, and any discomfort participation in the study may entail. Subjects will be informed that their participation is voluntary and that they may withdraw consent to participate at any time. They will be informed that choosing not to participate will not affect the care the subject will receive for the treatment of his or her disease. Subjects will be told that alternative treatments are available if they refuse to take part and that such refusal will not prejudice future treatment. Finally, they will be told that the investigator will maintain a subject identification register for the purposes of long-term follow up if needed and that their records may be accessed by health authorities and authorized sponsor personnel without violating the confidentiality of the subject, to the extent permitted by the applicable law(s) or regulations. By signing the ICF the subject is authorizing such access, and agrees to allow his or her study physician to recontact the subject for the purpose of obtaining consent for additional safety evaluations, if needed.

The subject will be given sufficient time to read the ICF and the opportunity to ask questions. After this explanation and before entry into the study, consent should be appropriately recorded by means of the subject's personally dated signature. After having obtained the consent, a copy of the ICF must be given to the subject.

Where local regulations require, a separate ICF may be used for the required DNA component of the study.

16.2.4. Privacy of Personal Data

The collection and processing of personal data from subjects enrolled in this study will be limited to those data that are necessary to fulfill the objectives of the study.

These data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations. Appropriate technical and organizational measures to protect the personal data against unauthorized disclosures or access, accidental or unlawful destruction, or accidental loss or alteration must be put in place. Sponsor personnel whose responsibilities require access to personal data agree to keep the identity of subjects confidential.

The informed consent obtained from the subject includes explicit consent for the processing of personal data and for the investigator/institution to allow direct access to his or her original medical records (source data/documents) for study-related monitoring, audit, IEC/IRB review, and regulatory inspection. This consent also addresses the transfer of the data to other entities and to other countries.

The subject has the right to request through the investigator access to his or her personal data and the right to request rectification of any data that are not correct or complete. Reasonable steps will be taken to respond to such a request, taking into consideration the nature of the request, the conditions of the study, and the applicable laws and regulations.

Exploratory DNA, biomarker, and PK research is not conducted under standards appropriate for the return of data to subjects. In addition, the sponsor cannot make decisions as to the significance of any findings resulting from exploratory research. Therefore, exploratory research data will not be returned to subjects or investigators, unless required by law or local regulations. Privacy and confidentiality of data generated in the future on stored samples will be protected by the same standards applicable to all other clinical data.

16.2.5. Long-Term Retention of Samples for Additional Future Research

Samples collected in this study may be stored for up to 15 years (or according to local regulations) for additional research. Samples will only be used to understand esketamine, to understand depression, to understand differential drug responders, and to develop tests/assays related to esketamine and depression. The research may begin at any time during the study or the post-study storage period.

Stored samples will be coded throughout the sample storage and analysis process and will not be labeled with personal identifiers. Subjects may withdraw their consent for their samples to be stored for research (refer to Section 10.2, Withdrawal From the Study (Withdrawal From the Use of Samples in Future Research).

16.2.6. Country Selection

This study will only be conducted in those countries where the intent is to launch or otherwise help ensure access to the developed product, unless explicitly addressed as a specific ethical consideration in Section 16.1, Study-Specific Design Considerations.

17. ADMINISTRATIVE REQUIREMENTS

17.1. Protocol Amendments

Neither the investigator nor the sponsor will modify this protocol without a formal amendment by the sponsor. All protocol amendments must be issued by the sponsor, and signed and dated by the investigator. Protocol amendments must not be implemented without prior IEC/IRB approval, or when the relevant competent authority has raised any grounds for non-acceptance, except when necessary to eliminate immediate hazards to the subjects, in which case the amendment must be promptly submitted to the IEC/IRB and relevant competent authority.

Documentation of amendment approval by the investigator and IEC/IRB must be provided to the sponsor. When the change(s) involves only logistic or administrative aspects of the study, the IRB (and IEC where required) only needs to be notified.

During the course of the study, in situations where a departure from the protocol is unavoidable, the investigator or other physician in attendance will contact the appropriate sponsor representative (see Contact Information page(s) provided separately). Except in emergency situations, this contact should be made before implementing any departure from the protocol. In all cases, contact with the sponsor must be made as soon as possible to discuss the situation and agree on an appropriate course of action. The data recorded in the CRF and source documents will reflect any departure from the protocol, and the source documents will describe this departure and the circumstances requiring it.

17.2. Regulatory Documentation

17.2.1. Regulatory Approval/Notification

This protocol and any amendment(s) must be submitted to the appropriate regulatory authorities in each respective country, if applicable. A study may not be initiated until all local regulatory requirements are met.

17.2.2. Required Prestudy Documentation

The following documents must be provided to the sponsor before shipment of study drug to the study site:

- Protocol and amendment(s), if any, signed and dated by the principal investigator
- A copy of the dated and signed (or sealed, where appropriate per local regulations), written IEC/IRB approval of the protocol, amendments, ICF, any recruiting materials, and if applicable, subject compensation programs. This approval must clearly identify the specific protocol by title and number and must be signed (or sealed, where appropriate per local regulations) by the chairman or authorized designee.
- Name and address of the IEC/IRB, including a current list of the IEC/IRB members and their function, with a statement that it is organized and operates according to GCP and the applicable laws and regulations. If accompanied by a letter of explanation, or equivalent, from the IEC/IRB, a general statement may be substituted for this list. If an investigator or a member of the study-site personnel is a member of the IEC/IRB, documentation must be obtained to state that this person did not participate in the deliberations or in the vote/opinion of the study.
- Regulatory authority approval or notification, if applicable
- Signed and dated statement of investigator (e.g., Form FDA 1572), if applicable
- Documentation of investigator qualifications (e.g., curriculum vitae)
- Completed investigator financial disclosure form from the principal investigator, where required
- Signed and dated clinical trial agreement, which includes the financial agreement

- Any other documentation required by local regulations

The following documents must be provided to the sponsor before enrollment of the first subject:

- Completed investigator financial disclosure forms from all subinvestigators
- Documentation of subinvestigator qualifications (e.g., curriculum vitae)
- Name and address of any local laboratory conducting tests for the study, and a dated copy of current laboratory normal ranges for these tests, if applicable
- Local laboratory documentation demonstrating competence and test reliability (e.g., accreditation/license), if applicable

17.3. Subject Identification, Enrollment, and Screening Logs

The investigator agrees to complete a subject identification and enrollment log to permit easy identification of each subject during and after the study. This document will be reviewed by the sponsor study-site contact for completeness.

The subject identification and enrollment log will be treated as confidential and will be filed by the investigator in the study file. To ensure subject confidentiality, no copy will be made. All reports and communications relating to the study will identify subjects by subject identification and date of birth. In cases where the subject is not randomized into the study, the date seen and date of birth will be used.

The investigator must also complete a subject screening log, which reports on all subjects who were seen to determine eligibility for inclusion in the study.

17.4. Source Documentation

At a minimum, source documentation must be available for the following to confirm data collected in the CRF: subject identification, eligibility, and study identification; study discussion and date of signed informed consent; dates of visits; results of safety and efficacy parameters as required by the protocol; record of all adverse events and follow-up of adverse events; concomitant medication; drug receipt/dispensing/return records; study drug administration information; and date of study completion and reason for early discontinuation of study drug or withdrawal from the study, if applicable.

In addition, the author of an entry in the source documents should be identifiable.

At a minimum, the type and level of detail of source data available for a subject should be consistent with that commonly recorded at the study site as a basis for standard medical care. Specific details required as source data for the study will be reviewed with the investigator before the study and will be described in the monitoring guidelines (or other equivalent document).

The following data will be recorded directly into the CRF and will be considered source data:

- Race

The minimum source documentation requirements for Section 4.1, Inclusion Criteria and Section 4.2, Exclusion Criteria that specify a need for documented medical history are as follows:

- Referral letter from treating physician or
- Complete history of medical notes at the site
- Discharge summaries
- Antidepressant treatment in the current episode of depression

Inclusion and exclusion criteria not requiring documented medical history must be verified at a minimum by subject interview or other protocol required assessment (e.g., physical examination, laboratory assessment) and documented in the source documents.

17.5. Case Report Form Completion

Case report forms are provided for each subject in electronic format.

Electronic Data Capture (eDC) will be used for this study. The study data will be transcribed by study-site personnel from the source documents onto an electronic CRF, and transmitted in a secure manner to the sponsor within the timeframe agreed upon between the sponsor and the study site. The electronic file will be considered to be the CRF.

Worksheets may be used for the capture of some data to facilitate completion of the CRF. Any such worksheets will become part of the subject's source documentation. All data relating to the study must be recorded in CRFs prepared by the sponsor. Data must be entered into CRFs in English. Study site personnel must complete the CRF as soon as possible after a subject visit, and the forms should be available for review at the next scheduled monitoring visit.

The investigator must verify that all data entries in the CRFs are accurate and correct.

All CRF entries, corrections, and alterations must be made by the investigator or other authorized study-site personnel. If necessary, queries will be generated in the eDC tool. The investigator or study-site personnel must adjust the CRF (if applicable) and complete the query.

If corrections to a CRF are needed after the initial entry into the CRF, this can be done in 3 different ways:

- Study site personnel can make corrections in the eDC tool at their own initiative or as a response to an auto query (generated by the eDC tool).
- Study site manager can generate a query for resolution by the study-site personnel.
- Clinical data manager can generate a query for resolution by the study-site personnel.

17.6. Data Quality Assurance/Quality Control

Steps to be taken to ensure the accuracy and reliability of data include the selection of qualified investigators and appropriate study sites, review of protocol procedures with the investigator and study-site personnel before the study, periodic monitoring visits by the sponsor, and direct transmission of clinical laboratory data from a central laboratory into the sponsor's data base. Written instructions will be provided for collection, handling, storage, and shipment of samples.

Guidelines for CRF completion will be provided and reviewed with study-site personnel before the start of the study.

The sponsor will review CRFs for accuracy and completeness during on-site monitoring visits and after transmission to the sponsor; any discrepancies will be resolved with the investigator or designee, as appropriate. After upload of the data into the study database they will be verified for accuracy and consistency with the data sources.

17.7. Record Retention

In compliance with the ICH/GCP guidelines, the investigator/institution will maintain all CRFs and all source documents that support the data collected from each subject, as well as all study documents as specified in ICH/GCP Section 8, Essential Documents for the Conduct of a Clinical Trial, and all study documents as specified by the applicable regulatory requirement(s). The investigator/institution will take measures to prevent accidental or premature destruction of these documents.

Essential documents must be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents will be retained for a longer period if required by the applicable regulatory requirements or by an agreement with the sponsor. It is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

If the responsible investigator retires, relocates, or for other reasons withdraws from the responsibility of keeping the study records, custody must be transferred to a person who will accept the responsibility. The sponsor must be notified in writing of the name and address of the new custodian. Under no circumstance shall the investigator relocate or dispose of any study documents before having obtained written approval from the sponsor.

If it becomes necessary for the sponsor or the appropriate regulatory authority to review any documentation relating to this study, the investigator/institution must permit access to such reports.

17.8. Monitoring

The sponsor will perform on-site monitoring visits as frequently as necessary. The monitor will record dates of the visits in a study site visit log that will be kept at the study site. The first

post-initiation visit will be made as soon as possible after enrollment has begun. At these visits, the monitor will compare the data entered into the CRFs with the hospital or clinic records (source documents). The nature and location of all source documents will be identified to ensure that all sources of original data required to complete the CRF are known to the sponsor and study-site personnel and are accessible for verification by the sponsor study-site contact. If electronic records are maintained at the study site, the method of verification must be discussed with the study-site personnel.

Direct access to source documentation (medical records) must be allowed for the purpose of verifying that the data recorded in the CRF are consistent with the original source data. Findings from this review of CRFs and source documents will be discussed with the study-site personnel. The sponsor expects that, during monitoring visits, the relevant study-site personnel will be available, the source documentation will be accessible, and a suitable environment will be provided for review of study-related documents. The monitor will meet with the investigator on a regular basis during the study to provide feedback on the study conduct.

17.9. Study Completion/Termination

17.9.1. Study Completion

The study is considered completed with the last study assessment for the last subject participating in the study. The final data from the study site will be sent to the sponsor (or designee) after completion of the final subject assessment at that study site, in the time frame specified in the Clinical Trial Agreement.

17.9.2. Study Termination

The sponsor reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the sponsor. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the sponsor or investigator may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IEC/IRB or local health authorities, the sponsor's procedures, or GCP guidelines
- Inadequate recruitment of subjects by the investigator
- Discontinuation of further study drug development

17.10. On-Site Audits

Representatives of the sponsor's clinical quality assurance department may visit the study site at any time during or after completion of the study to conduct an audit of the study in compliance

with regulatory guidelines and company policy. These audits will require access to all study records, including source documents, for inspection and comparison with the CRFs. Subject privacy must, however, be respected. The investigator and study-site personnel are responsible for being present and available for consultation during routinely scheduled study-site audit visits conducted by the sponsor or its designees.

Similar auditing procedures may also be conducted by agents of any regulatory body, either as part of a national GCP compliance program or to review the results of this study in support of a regulatory submission. The investigator should immediately notify the sponsor if he or she has been contacted by a regulatory agency concerning an upcoming inspection.

17.11. Use of Information and Publication

All information, including but not limited to information regarding esketamine or the sponsor's operations (e.g., patent application, formulas, manufacturing processes, basic scientific data, prior clinical data, formulation information) supplied by the sponsor to the investigator and not previously published, and any data, including pharmacogenomic or exploratory biomarker research data, generated as a result of this study, are considered confidential and remain the sole property of the sponsor. The investigator agrees to maintain this information in confidence and use this information only to accomplish this study, and will not use it for other purposes without the sponsor's prior written consent.

The investigator understands that the information developed in the study will be used by the sponsor in connection with the continued development of esketamine, and thus may be disclosed as required to other clinical investigators or regulatory agencies. To permit the information derived from the clinical studies to be used, the investigator is obligated to provide the sponsor with all data obtained in the study.

The results of the study will be reported in a Clinical Study Report generated by the sponsor and will contain CRF data from all study sites that participated in the study. Recruitment performance or specific expertise related to the nature and the key assessment parameters of the study will be used to determine a coordinating investigator. Results of pharmacogenomics or exploratory biomarker analyses performed after the Clinical Study Report has been issued will be reported in a separate report and will not require a revision of the Clinical Study Report. Study subject identifiers will not be used in publication of results. Any work created in connection with performance of the study and contained in the data that can benefit from copyright protection (except any publication by the investigator as provided for below) shall be the property of the sponsor as author and owner of copyright in such work.

Consistent with Good Publication Practices and International Committee of Medical Journal Editors guidelines, the sponsor shall have the right to publish such primary (multicenter) data and information without approval from the investigator. The investigator has the right to publish study site-specific data after the primary data are published. If an investigator wishes to publish information from the study, a copy of the manuscript must be provided to the sponsor for review at least 60 days before submission for publication or presentation. Expedited reviews will be arranged for abstracts, poster presentations, or other materials. If requested by the sponsor in

writing, the investigator will withhold such publication for up to an additional 60 days to allow for filing of a patent application. In the event that issues arise regarding scientific integrity or regulatory compliance, the sponsor will review these issues with the investigator. The sponsor will not mandate modifications to scientific content and does not have the right to suppress information. For multicenter study designs and substudy approaches, secondary results generally should not be published before the primary endpoints of a study have been published. Similarly, investigators will recognize the integrity of a multicenter study by not submitting for publication data derived from the individual study site until the combined results from the completed study have been submitted for publication, within 12 months of the availability of the final data (tables, listings, graphs), or the sponsor confirms there will be no multicenter study publication. Authorship of publications resulting from this study will be based on the guidelines on authorship, such as those described in the Uniform Requirements for Manuscripts Submitted to Biomedical Journals, which state that the named authors must have made a significant contribution to the design of the study or analysis and interpretation of the data, provided critical review of the paper, and given final approval of the final version.

Registration of Clinical Studies and Disclosure of Results

The sponsor will register and/or disclose the existence of and the results of clinical studies as required by law.

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Attachment 1: Prohibited Therapies

Allowed (Y) and Not Allowed (N)

The table below is intended for general guidance, please discuss with the study team regarding any specific concomitant therapies for a subject.

The pharmacotherapies listed below are excluded due to potential impact on efficacy evaluation and/or patient safety or because they are indicated for exclusionary conditions.

Except where specifically noted in the protocol, the prohibited therapies listed in this table are prohibited from Screening until at least 1 day after the last dose of study medication.

Drug Class	Episodic Use (PRN)	Continuous Use	Comments
Allopurinol	N	Y	
Amantadine	N	N	
Analgesics (e.g., NSAIDS, acetaminophen), except opioids	Y	Y	See "Opioids" row below.
Anorexiants (e.g., phenteramine)	N	N	
Antacids	Y	Y	
Anti-anginal agents	N	N	Subjects with angina are excluded
Anti-arrhythmics	N	N	Subjects with any history of cardiovascular arrhythmias excluded
Anticholinesterase inhibitors	N	N	
Anticoagulants	N	N	
Anticonvulsants	N	N	Subjects with seizures are excluded. Anticonvulsants used for other indications may be allowed (e.g., valproate for migraine, lamotrigine for mood disorder)
Antidepressants (*except* monoamine oxidase inhibitors)	N	Y	
Antidepressants: Monoamine oxidase inhibitors	N	N	
Antidiarrheal preparations	Y	N	
Anti-emetics	Y	N	
Anti-inflammatory drugs, except steroids	Y	Y	See "Steroid" rows below.
Antipsychotics	N	Y	Use of antipsychotics for treatment of depression is not exclusionary. It would be excluded if being used for psychotic symptoms.
Aspirin	Y	Y	
Benzodiazepines	Y	Y	PRN use: Not permitted within 12 hours of study medication dosing
Benztropine	Y	Y	Not permitted within 8 hours of cognition testing (Panel A only)
Calcium Channel Blockers	Y	Y	
Chloral hydrate	N	N	
Chloramphenicol	N	N	
Clonidine	N	N	
Cough/Cold preparations (except those containing diphenhydramine or dextromethorphan)	Y	N	Intranasally-administered decongestants (vasoconstrictors) are prohibited from 12 hours prior to each study medication administration. Intranasal steroids are not prohibited.

			Cough/cold preparations containing diphenhydramine or dextromethorphan are prohibited within 12 hours of study medication dosing.
CYP3A4 inhibitors - Potent	N	N	Examples (not all-inclusive): indinavir, nelfinavir, ritonavir, clarithromycin, itraconazole, ketoconazole, nefazodone, saquinavir, telithromycin
CYP3A4 inducers - Potent	N	N	Examples (not all-inclusive): efavirenz, nevirapine, barbiturates, carbamazepine, glucocorticoids, modafinil, oxcarbazepine, phenobarbital, phenytoin, rifabutin, rifampin, St. John's wort
DHEA	Y	Y	
Fish oils	Y	Y	
Ginko	Y	Y	
Ginseng	Y	Y	
Guanabenz	N	N	
Guanadrel	N	N	
Guanethidine	N	N	
Guanfacine	N	N	
HIV antiviral drugs	N	N	Subjects testing positive for HIV excluded
Hormones (e.g., contraceptives, thyroid hormones etc.)	N	Y	
Ketanserin	N	N	
Lithium	N	N	
Methyldopa	N	N	
Metyrosine	N	N	
Opioids	N	N	
Omega-3-fatty acids	Y	Y	
Psychostimulants (e.g, amphetamines)	N	N	
Reserpine	N	N	
Scopolamine	N	N	
Sleep-aids (non-benzodiazepine)	Y	Y	Not permitted within 12 hours of cognition testing (Panel A only)
St. John’s Wort	N	N	
Steroids (inhaled, topical, ophthalmic only)	Y	Y	
Steroids (oral)	N	N	
Thyroid hormone supplement	N	Y	Subjects needing supplements must be on a stable thyroid supplement dose for at least 3 months prior to Day 1 of the double-blind treatment phase.

Attachment 2: Instructions for the Completion of the Patient-Reported Outcome (PRO) Case Report Forms

The following instructions are intended to assist investigators, study coordinators, and those with monitoring responsibilities with the completion of all patient self-administered (PRO) assessments.

I. General Instructions

1. Patients should complete the patient-reported outcome (PRO) assessments in a quiet, semi-private location with access to study staff for questions.
2. Patients should be allowed approximately 30 minutes to orient him/herself and to self-administer all PRO assessments.
3. Patients should be literate in the language of the PRO assessment(s). Patients must not have any developmental, learning, or behavioral disabilities.
4. Patients should complete all PRO assessments using a black ballpoint pen. Have the patient press firmly and print legibly when writing to ensure that all copies are clear and legible. Have the patient place a piece of cardboard between the pages to ensure no 'run through' pages.
5. Explain to patients the reasons why they are being asked to complete the PRO assessment(s), i.e., they are part of the overall medical assessment and are designed to find out more information about how having their disease has affected their life.
6. Indicate to patients that all of the information on the PRO assessment(s) is confidential, and that someone from the study staff will only check for completeness and not share the results with other clinical staff.
7. Indicate to patients that there are no right or wrong answers.
8. Provide patients with the set of instructions that are provided with the PRO assessment(s) material
9. Have patients read the instructions prior to completing the assessment(s). For almost all items, it is necessary for patients to check the box next to the answer that applies. Occasionally, patients will be asked to write in some additional information. Not all of the questions apply to every patient. Where a particular question or set of questions does not apply, there will be instructions on which question to answer next.

II. Assessment Times

1. Each PRO assessment asks the patient for an evaluation of a specified period of time. Therefore, it is important to minimize the influence of feedback from the clinic visit itself. Insofar as it is possible, it is also important to have the PRO assessment(s) be conducted within the flow of the protocol-specified study visit.
2. The PRO instruments have been placed in the correct order of completion in the study CRF. Please ask the subject to complete the PROs in the sequence that they appear in the booklet.

Screening:

PRO assessment(s) during this time period should be completed immediately after the patient provides his/her informed consent, but before any clinical tests are taken or assessments associated with the study visit are conducted.

Post-Screening visits:

Patient reported outcome (PRO) assessments should be conducted/completed before any tests, procedures, or other consultations scheduled at the same timepoint to prevent influencing subject perceptions.

III. Quality Control

When the subject returns the completed PRO assessments, check for any questions that might have been left blank. If an item has been omitted, point this out and ask that these items be completed. Occasionally, subjects mark more than one answer per item. In such instances, ask the subject if he/she will reconsider the question and try to choose one answer only.

INVESTIGATOR AGREEMENT

I have read this protocol and agree that it contains all necessary details for carrying out this study. I will conduct the study as outlined herein and will complete the study within the time designated.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure that they are fully informed regarding the study drug, the conduct of the study, and the obligations of confidentiality.

Coordinating Investigator (where required):

Name (typed or printed): _____

Institution and Address: _____

_____

_____

_____

Signature: _____ Date: _____

(Day Month Year)

Principal (Site) Investigator:

Name (typed or printed): _____

Institution and Address: _____

_____

_____

_____

Telephone Number: _____

Signature: _____ Date: _____

(Day Month Year)

Sponsor's Responsible Medical Officer:

Name (typed or printed): Ella Daly, M.D.

Institution: Janssen Research & Development

Signature: ELLA DALY Date: _____

(Day Month Year)

Note: If the address or telephone number of the investigator changes during the course of the study, written notification will be provided by the investigator to the sponsor, and a protocol amendment will not be required.

LAST PAGE

Janssen Research & Development *

Clinical Protocol

A Double-Blind, Doubly-Randomized, Placebo-Controlled Study of Intranasal Esketamine in an Adaptive Treatment Protocol to Assess Safety and Efficacy in Treatment-Resistant Depression (SYNAPSE)

Protocol ESKETINTRD2003; Phase 2a

JNJ-54135419 (esketamine)

*Janssen Research & Development is a global organization that operates through different legal entities in various countries. Therefore, the legal entity acting as the sponsor for Janssen Research & Development studies may vary, such as, but not limited to Janssen Biotech, Inc.; Janssen Products, LP; Janssen Biologics, BV; Janssen-Cilag International NV; Janssen, Inc; Janssen Infectious Diseases BVBA; Janssen R&D Ireland; or Janssen Research & Development, LLC. The term "sponsor" is used throughout the protocol to represent these various legal entities; the sponsor is identified on the Contact Information page that accompanies the protocol.

This study will be conducted under US Food & Drug Administration IND regulations (21 CFR Part 312).

EudraCT NUMBER: 2013-004005-11

Status: Approved
Date: 8 October 2013
Prepared by: Janssen Research & Development, LLC
EDMS No & Version: EDMS-ERI-71950055

GCP Compliance: This study will be conducted in compliance with Good Clinical Practice, and applicable regulatory requirements.

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SYNOPSIS

A Double-Blind, Doubly-Randomized, Placebo-Controlled Study of Intranasal Esketamine in an Adaptive Treatment Protocol to Assess Safety and Efficacy in Treatment-Resistant Depression (SYNAPSE)

Esketamine (JNJ54135419) is the S-enantiomer of the ketamine racemate (1:1 racemic mixture of R-ketamine and esketamine). Ketamine and esketamine are approved medications in several countries for the induction of general anesthesia and for use as adjunct to other anesthetics.

The mechanism of action results from a noncompetitive binding to the N-methyl-D-aspartate (NMDA)-receptor (ligand-gated calcium channel) at the phencyclidine binding site. It also has additional binding sites (NMDA and non-NMDA glutamate receptors, nicotinic and muscarinic, cholinergic, and monoaminergic and opioid receptors, and voltage-dependent sodium and L-type calcium channels).

Esketamine has been shown to have a 3- to 4-times stronger binding affinity for the phencyclidine site of the NMDA receptor than the R-enantiomer, making it more feasible for intranasal delivery.

Intranasal esketamine is being developed for the treatment of treatment-resistant depression (TRD).

OBJECTIVES AND HYPOTHESIS

Primary Objective

To assess the efficacy and dose response of intranasal esketamine (28 mg, 56 mg, and 84 mg) compared with placebo in improving depressive symptoms in subjects with treatment-resistant depression (TRD), as assessed by a change from baseline in the Montgomery-Asberg Depression Rating Scale (MADRS) total score for the combined periods in the double-blind treatment phase.

Secondary Objectives

1. To evaluate sustained response (≥50% reduction from baseline in MADRS total score) with onset by Day 2 through the end of the double-blind phase (Day 15).
2. To investigate the safety and tolerability of intranasal esketamine in TRD subjects, with special attention to:
    a. Local nasal tolerability, using a nasal tolerability questionnaire and nasal examinations
    b. Effects on heart rate, blood pressure, and blood oxygen saturation (SpO2)
    c. Effects on suicidal ideation/behavior measured by the Columbia Suicide Severity Rating Scale (C-SSRS);
    d. Effects on alertness and sedation measured by the Modified Observer's Assessment of Alertness/Sedation (MOAA/S)
    e. Psychosis-like side effects by using a four-item positive symptom subscale of the Brief Psychiatric Rating Scale (BPRS+) consisting of: suspiciousness, hallucinations, unusual thought content, and conceptual disorganization;
    f. Effects on dissociative symptoms using the Clinician Administered Dissociative States Scale (CADSS);
    g. Potential withdrawal symptoms following cessation of intranasal esketamine treatment, as measured by the clinician-administered 20-item Physician Withdrawal Checklist (PWC-20)

3. To assess the effect of intranasal esketamine compared to intranasal placebo on:
    a. Depressive symptoms, as assessed by the 16-item Quick Inventory of Depressive Symptomatology- Self Report (QIDS-SR_{16})
    b. Remission, defined as a MADRS total score ≤10
    c. Response, defined as a ≥50% reduction from baseline in MADRS total score
    d. The severity of illness using the Clinical Global Impression - Severity (CGI-S) and the Patient Global Impression - Severity (PGI-S)
    e. Symptoms of anxiety as assessed by the Generalized Anxiety Disorder 7-item Scale (GAD-7)
4. To evaluate the pharmacokinetics (PK) of intranasal esketamine in subjects with TRD

Exploratory Objectives

1. Subject perspective of global change in major depressive disorder (MDD) from baseline, as measured by the Patient Global Impression of Change (PGI-C)
2. Impact on function as assessed by the health status using the EuroQol-5D, 5-level version (EQ-5D-5L)
3. To evaluate whether pretreatment concentrations of inflammatory and neurotrophic markers and plasma glycine correlate with the magnitude of clinical change, as measured by the MADRS, following intranasal administration of esketamine
4. To assess the impact of intranasal esketamine on plasma inflammatory and neurotrophic markers and glutamatergic pathway metabolic markers

Hypothesis

The primary hypothesis is that intranasal esketamine (28 mg, 56 mg, 84 mg) is superior to intranasal placebo in improving depressive symptoms in adult subjects with TRD, as assessed by the change from baseline in the MADRS total score for the combined periods in the double-blind treatment phase.

OVERVIEW OF STUDY DESIGN

This is a doubly-randomized, double-blind, placebo-controlled, multicenter study in 60 male and female adult subjects with TRD.

Each subject will participate in up to 4 phases:

- A screening phase of up to 4 weeks,
- A double-blind treatment phase (Day 1 to Day 15) which includes two 1-week treatment periods (Period 1 and Period 2),
- An optional open-label treatment phase (Day 15 to 25), and
- An 8-week posttreatment (follow up) phase

The duration of the subject's participation will be approximately 8 to 16 weeks. The end of study will occur when the last subject in the trial completes his/her last study assessment.

Screening Phase (Day -28 to Day -1)

After giving informed consent, subjects who are 18 to 64 years of age (inclusive), will be screened within 4 weeks of Day 1 (i.e., Day -28 to Day -1) to determine eligibility for study participation.

Subjects must meet Diagnostic and Statistical Manual of Mental Disorders – Fifth Edition (DSM-5) diagnostic criteria for MDD, without psychotic features, based upon clinical assessment (DSM-5 296.22, 296.23, 296.32, or 296.33) and confirmed by the Mini International Psychiatric Interview (MINI). Subjects must have an Inventory of Depressive Symptomatology 30-item Clinician-rated (IDS-C_{30}) total score ≥34. In addition, the major depressive episode must be deemed valid using the "State vs. Trait, Assessibility, Face Validity, Ecological Validity, Rule of Three P's" (SAFER) criteria interview, administered by remote, independent raters.

Subjects must have had an inadequate response to at least 2 antidepressants, at least one of which is in the current episode of depression, assessed using the Massachusetts General Hospital – Antidepressant Treatment Response Questionnaire (MGH-ATRQ) and prior medication history.

- Subjects who are not currently taking an antidepressant at Screening are eligible to participate.
- Subjects taking an antidepressant at the Screening visit must have been receiving a stable dose (i.e., dose has been unchanged) for at least 2 weeks before the Screening visit.
    - With the exception of monoamine oxidase (MAO) inhibitors, which are prohibited, the subject may continue the antidepressant at the same dose during the double-blind treatment phase, or
    - The subject can discontinue the antidepressant within 1 week, or within a period less than 5 times the drug's half-life [exception: At least 4 weeks for fluoxetine and 2 weeks for MAO inhibitors], whichever is longer, before the planned first dose of study drug.

The decision to continue or discontinue the current antidepressant will be made by the subject and investigator, based on their clinical judgment. Antidepressant medication will not be discontinued for the sole purpose of allowing subjects to participate in the study.

Disallowed therapies, including psychotropic medications, are described in the exclusion criteria, Section 8 (Prohibitions and Restrictions), and Attachment 1.

Other screening evaluations (e.g., biomarker, safety) will be performed as per the Time and Events Schedule.

All adverse events and special reporting situations, whether serious or non-serious, will be reported from the time a signed and dated informed consent form (ICF) is obtained until completion of the subject's last study-related procedure (which may include contact for follow-up of safety).

Double-Blind Treatment Phase

All subjects will participate in two treatment periods (Period 1 and Period 2) during the double-blind treatment phase. Efficacy, safety, PK, biomarker, and/or pharmacogenomic evaluations performed at each visit are described in the Time and Events Schedule. If a subject withdraws before the end of the double-blind treatment phase, the Early Termination visit should be conducted at the time of discontinuation, followed by completion of the posttreatment phase.

All subjects will self-administer the study medication in Period 1 and Period 2 under the direct supervision of the investigator or designee (Refer to Section 6, Dosage and Administration). On all dosing days, all subjects must remain at the clinical site until at least 2 hours postdose (or longer if required for study procedures) and should be accompanied by a responsible adult when discharged from the clinical site. In addition, subjects must not drive a car or work with machines for 24 hours after study medication dosing.

If the placebo response or other assumptions at the end of Period 1 are not as expected, up to a maximum of 78 subjects (i.e., up to 18 additional subjects) may be enrolled to ensure that an adequate number of

subjects are re-randomized in Period 2, based on the ongoing assessment of the numbers of subjects who are re-randomized as well as drop-out rates.

Period 1

On Day 1, subjects (n = 60) will be randomized using a 3:1:1:1 ratio to 1 of the following 4 treatment groups: Intranasal placebo (n = 30), intranasal esketamine 28 mg (n = 10), intranasal esketamine 56 mg (n = 10), or intranasal esketamine 84 mg (n = 10) administered on Day 1 and Day 4.

Period 2

Subjects that received intranasal esketamine 28, 56, or 84 mg in Period 1 will receive the same treatment in Period 2 on Day 8 and Day 11.

Period 2 treatment for subjects that received placebo in Period 1 will be determined by the QIDS-SR_{16} score on Day 8 (predose):

- Subjects that received intranasal placebo in Period 1 and have a QIDS-SR_{16} score < 11 (mild or no symptoms) will receive intranasal placebo again in Period 2 on Day 8 and Day 11.
- Subjects that received intranasal placebo in Period 1 and have a QIDS-SR_{16} score ≥11 (moderate to severe) will be re-randomized to receive intranasal placebo or intranasal esketamine 28 mg, 56 mg, or 84 mg in a 1:1:1:1 ratio on Day 8 and Day 11. The randomization will be stratified by the QIDS-SR_{16} score [moderate (QIDS-SR_{16} score 11 to 16) or severe (QIDS-SR_{16} score > 16)].

***Optional* Open-Label Treatment Phase**

On Day 15, following completion of the double-blind treatment phase assessments, subjects may participate in an optional open-label treatment phase. Subjects that discontinue early from the double-blind treatment phase are not eligible to participate in the optional open-label treatment phase. The Investigator must agree and confirm the subject is eligible to participate based on his/her clinical judgment.

The efficacy, safety, and biomarker evaluations that will be performed at each visit during the optional open-label treatment phase are described in the Time and Event Schedule. Where specified in the Time and Events Schedule, study procedures performed on the last day of the double-blind treatment phase (Day 15) that are also required predose on Day 15 of the optional open-label treatment phase will only be performed once.

During the optional open-label treatment phase, subjects can receive up to 4 single doses of intranasal esketamine on Days 15, 18, 22, and 25. On all dosing days, all subjects must remain at the clinical site until at least 2 hours postdose (or longer if required for study procedures) and should be accompanied by a responsible adult when discharged from the clinical site. In addition, subjects must not drive a car or work with machines for 24 hours after study medication dosing.

If a subject withdraws before the end of the optional open-label treatment phase, an Early Termination visit is not required. The subject will continue into the posttreatment phase (see "Posttreatment Phase" below).

All subjects will start with intranasal esketamine 56 mg on Day 15, and subsequent doses on Days 18, 22, and 25 can be titrated up to 84 mg at any time point, if desired. If an intranasal esketamine 56 mg or 84 mg dose is not tolerated, the next dose can be lowered to 28 mg or 56 mg, respectively.

Posttreatment Phase

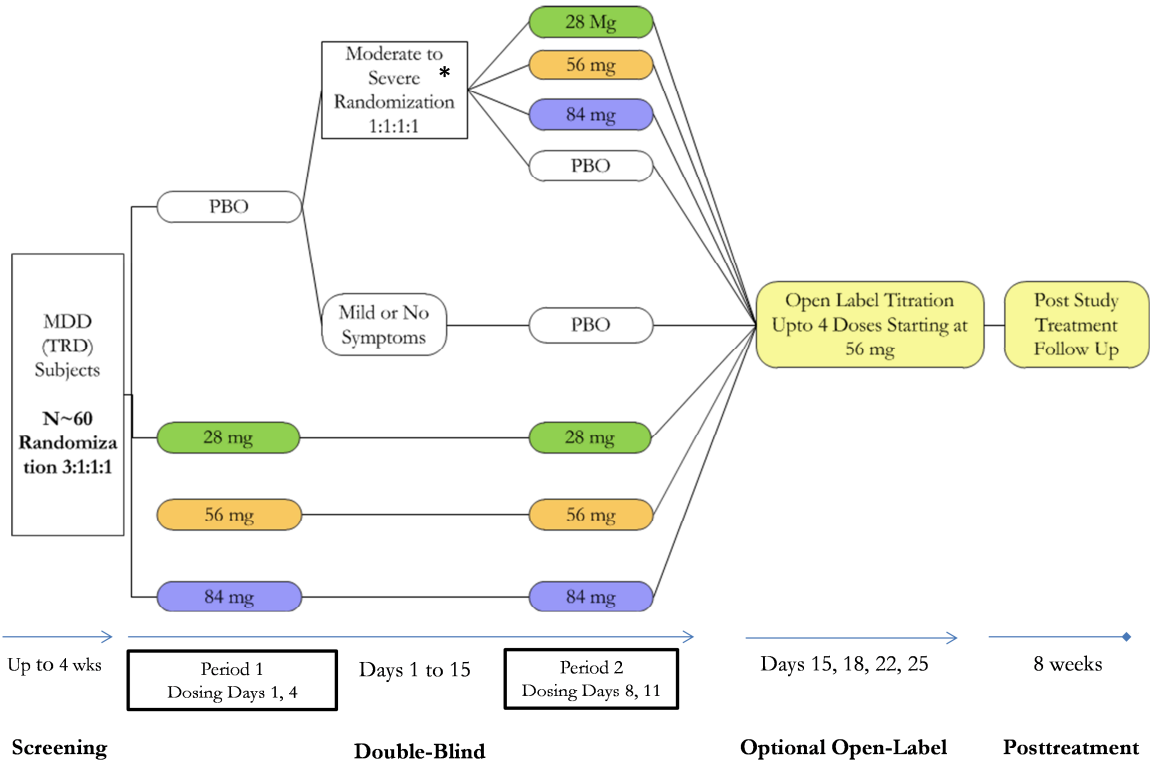
The efficacy and safety evaluations that will be performed at each visit during the posttreatment phase are described in the Time and Event Schedule.

All subjects who receive at least 1 dose of study medication should have 4 follow up visits conducted at 1, 2, 4, and 8 weeks after the last dose of study medication. The follow up visits at 1, 4, and 8 weeks after the last dose of study medication will be telephone contacts. The follow up visit at 2 weeks after the last dose of study medication is a clinic visit.

Any clinically significant abnormalities persisting at the end of the study will be followed by the investigator until resolution or until a clinically stable endpoint is reached.

All adverse events and special reporting situations, whether serious or non-serious, will be reported until completion of the subject's last study-related procedure (which may include contact for follow-up of safety).

A diagram of the study design is provided below.



* Randomization stratified by QIDS–SR_{16} total score [Moderate = 11 to 16; Severe > 16]

SUBJECT POPULATION

Key Inclusion Criteria

- Man or woman, 18 to 64 years of age, inclusive.
- Subject must meet Diagnostic and Statistical Manual of Mental Disorders – Fifth Edition (DSM-5) diagnostic criteria for MDD, without psychotic features, based upon clinical assessment (DSM-5 296.22, 296.23, 296.32, or 296.33), and confirmed by the Mini International Psychiatric Interview (MINI).

- The subject's major depressive episode and treatment response must be deemed "valid" using the SAFER criteria interview (which includes the MADRS, a review of the MGH-ATRQ performed at Screening, and SAFER Criteria Inventory) administered by remote, independent raters.
- Subject must have had an inadequate response to at least 2 antidepressants, at least one of which is in the current episode of depression. The ATRQ will be used to assess antidepressant treatment response during the current episode. Prior medication history will be used to determine antidepressant treatment response in prior episode(s).
- Have an IDS-C_{30} total score ≥34 at Screening and predose at Day 1.

Key Exclusion Criteria

- Subject has a current diagnosis of bipolar and related disorders, intellectual disability, or cluster b personality disorder (e.g., borderline personality disorder, antisocial personality disorder, histrionic personality disorder, and narcissistic personality disorder).
- Subject has a current or prior diagnosis of a psychotic disorder or MDD with psychosis.
- Anatomical or medical conditions that may impede delivery or absorption of study medication (e.g., undergone facial reconstruction, significant structural or functional abnormalities of the nose or upper airway; obstructions or mucosal lesions of the nostrils or nasal passages; undergone sinus surgery in the previous 2 years; signs and symptoms of rhinitis).
- Has an abnormal or deviated nasal septum with any 1 or more of the following symptoms: blockage of 1 or both nostrils, nasal congestion (especially 1-sided), frequent nosebleeds, frequent sinus infections, and at times has facial pain, headaches, and postnasal drip.
- Subject meets criteria for substance or alcohol use disorder, except tobacco or caffeine, according to DSM-5 criteria at Screening.
- Subject has known allergies, hypersensitivity, intolerance, or contraindication to esketamine/ketamine or its excipients.

DOSAGE AND ADMINISTRATION

All doses of study medication will be self-administered under the direct supervision of the investigator or designee. Instructions for use of the intranasal device will be provided as a separate document.

[redacted]. It is provided in a nasal spray pump, which delivers 16.14 mg esketamine (14 mg esketamine base) per 100 μL spray. Each individual device delivers 28 mg (i.e., 2 sprays).

The placebo solution will be provided as a clear, colorless intranasal solution of water for injection with a bittering agent (denatonium benzoate [Bitrex®] at a final concentration of 0.001 mg/mL) added to simulate the taste of the intranasal solution with active drug. Benzalkonium chloride is added as a preservative at a concentration of 0.3 mg/mL.

Food will be restricted for at least 2 hours before each administration of study medication. Drinking of water or any other permitted beverage will be restricted for at least 30 minutes before the first nasal spray.

Subjects will be instructed to refrain from blowing their nose for at least 1 hour after the last intranasal spray.

On all dosing days, all subjects must remain at the clinical site until at least 2 hours postdose (or longer if required for study procedures) and should be accompanied by a responsible adult when discharged from the clinical site. Subjects must not drive a car or work with machines for 24 hours after study medication dosing.

Double-blind treatment phase

Refer to the "*Overview of Study Design- Double-Blind Treatment Phase*" section above for a description of the study design.

Subjects will self-administer intranasal esketamine on Days 1, 4, 8, and 11.

On each dosing day, all subjects will self-administer 1 spray into each nostril at t = 0, 5, and 10 minutes. Time 0 is defined as the time of the first 100-µL spray. Sprays to each nostril should be delivered in rapid succession at the scheduled time points (i.e., there should be no waiting between sprays to the right and left nostrils at each time point). The table below describes how each treatment will be administered in the double-blind treatment phase.

Dose Administration in the Double-Blind Treatment Phase

Intranasal Treatment	Time of Administration (Time 0 is defined as the time of the first 100-µL spray)		
	0	5 minutes	10 minutes
Placebo	1 spray of placebo to each nostril	1 spray of placebo to each nostril	1 spray of placebo to each nostril
Esketamine 28 mg	1 spray of esketamine to each nostril	1 spray of placebo to each nostril	1 spray of placebo to each nostril
Esketamine 56 mg	1 spray of esketamine to each nostril	1 spray of esketamine to each nostril	1 spray of placebo to each nostril
Esketamine 84 mg	1 spray of esketamine to each nostril	1 spray of esketamine to each nostril	1 spray of esketamine to each nostril

Optional open-label treatment phase

Refer to the "*Overview of Study Design- Optional Open Label Treatment Phase*" section above for a description of the study design.

Subjects will self-administer intranasal esketamine on Days 15, 18, 22, and 25.

Time 0 is defined as the time of the first 100-µL spray. Sprays to each nostril should be delivered in rapid succession at the scheduled time points (i.e., there should be no waiting between sprays in each nostril at each time point). The table below describes how each treatment will be administered in the optional open-label treatment phase.

Dose Administration in Optional Open-Label Treatment Phase

Intranasal Treatment	Time of Administration (Time 0 is defined as the time of the first 100-µL spray)		
	0	5 minutes	10 minutes
Esketamine 28 mg	1 spray of esketamine to each nostril	-	-
Esketamine 56 mg	1 spray of esketamine to each nostril	1 spray of esketamine to each nostril	-
Esketamine 84 mg	1 spray of esketamine to each nostril	1 spray of esketamine to each nostril	1 spray of esketamine to each nostril

EFFICACY EVALUATIONS

The primary efficacy evaluation will be the MADRS total score.

Other secondary and exploratory efficacy evaluations include the QIDS-SR_{16}, CGI-S, PGI-S, PGI-C, GAD-7, and the EQ-5D-5L.

PHARMACOKINETIC EVALUATIONS

Venous blood samples will be collected for determination of the plasma concentrations of esketamine, noresketamine, and other metabolites (if warranted) at the time points specified in the Time and Events Schedule. The exact dates and times of study medication dosing and PK blood sampling must be recorded.

The plasma concentrations of 4β-hydroxycholesterol will be measured in select PK samples as a probe to assess the potential for repeated administration of intranasal esketamine to induce hepatic cytochrome P450 3A4 enzyme activity. Total cholesterol will be measured in a separate blood sample at these same time points to ensure that changes in 4β-hydroxycholesterol are not attributable to changes in total cholesterol.

BIOMARKER EVALUATIONS

Human Inflammation Multi-Analyte Panel (MAP)

Blood (serum) samples will be collected to allow for an exploratory pharmacodynamic evaluation using the Human Inflammation MAP, a panel of 45 proteins involved in key biologically inflammatory and neurotrophic pathways.

Metabolomics

Blood (plasma) samples will be collected to allow for the exploratory analysis of novel predictive and drug exposure biomarkers.

PHARMACOGENOMIC (DNA) EVALUATIONS

A pharmacogenomic blood sample will be collected from all enrolled subjects on Day 1. DNA samples will be analyzed for the *CYP2B6* gene. Additional analyses may also be conducted if it is hypothesized that this may help resolve issues with the clinical data.

DNA samples will be used for research related to esketamine or depression. They may also be used to develop tests/assays related to esketamine and depression. Pharmacogenomic research may consist of the analysis of one or more candidate genes or of the analysis of genetic markers throughout the genome (as appropriate) in relation to esketamine or depression clinical endpoints.

SAFETY EVALUATIONS

Safety evaluations will be performed at the time points specified in the Time and Events Schedule.

Safety evaluations include adverse events, clinical laboratory tests, electrocardiograms (ECG), vital signs (blood pressure, heart rate, respiratory rate, tympanic temperature), pulse oximetry, physical examination, nasal examination, and a nasal tolerability questionnaire.

In addition, the C-SSRS will be performed to assess suicidal ideation and behavior, the CADSS will be administered to assess treatment-emergent dissociative symptoms, the BPRS+ will be administered to assess treatment-emergent psychotic symptoms, the MOAA/S will be used to measure treatment-emergent sedation, and the PWC-20 will be administered to assess potential withdrawal symptoms following cessation of intranasal esketamine treatment.

STATISTICAL METHODS

Sample Size Determination

The sample size is determined based on the following treatment differences between intranasal esketamine and placebo for the mean change from baseline in MADRS total score: a 9 point treatment difference was assumed for Period 1 (Day 8), a 7 point treatment difference for Period 2 (Day 15) was assumed for subjects with a moderate QIDS-SR_{16} score (QIDS-SR_{16} score = 11 to 16) and a 9 point treatment difference for Period 2 (Day 15) was assumed for subjects with a severe QIDS-SR_{16} score (QIDS-SR_{16} score > 16). Based on results of a previous esketamine IV study (ESKETIVTRD2001), it is estimated that 40% of placebo subjects at the end of Period 1 (Day 8 predose) will have a moderate QIDS-SR_{16} score and 55% will have a severe QIDS-SR_{16} score. Additional assumptions for the sample size calculation included a standard deviation of 10, 92.5% power for the combined data from both Day 8 and Day 15, an overall 1-sided significance level of 0.05, and a 5% drop-out rate for Period 1. It is calculated that this doubly-randomized design will require 60 subjects to be randomly assigned to treatment on Day 1 in a 3:1:1:1 ratio (30 subjects on placebo and 10 subjects per intranasal esketamine dose group).

Efficacy Analysis

Efficacy analyses will be based on the combination of efficacy data from the two periods of the double-blind treatment phase, unless specified otherwise.

For each period in the double-blind treatment phase, an intent-to-treat (ITT) analysis set will be defined to include all randomized subjects who receive at least 1 dose of study drug and have both the baseline and at least one post-baseline MADRS total score within that period. The efficacy analyses of data in Period 1 and Period 2 will be based on each respective ITT analysis set.

For the primary efficacy analysis, change from baseline (Day 1 predose) in MADRS total score to Day 8 predose assessment of the double-blind treatment phase will be analyzed using an analysis of covariance (ANCOVA) model, with factors for treatment, center, and Period 1 baseline MADRS total score as the continuous covariate. Data from all randomized, treated subjects with change values during Period 1 will be included in the analysis of Period 1. Change from baseline in MADRS total score in Period 2 (Day 8, predose to Day 15) will be analyzed using an ANCOVA model with factors for treatment, center, Period 2 baseline QIDS-SR_{16} score (moderate or severe), and Period 2 baseline MADRS total score as the continuous covariate. Only data from Period 1 placebo subjects who are re-randomized (those with moderate and severe QIDS-SR_{16} scores on predose Day 8) who continue into Period 2 and have a change value during Period 2 will be included in the analysis of Period 2. The comparison of intranasal esketamine dose groups with placebo will be carried out by a combined test on the weighted test statistics for each period in the double-blind treatment phase. The 'adaptive' weight will be based on the actual sample sizes for the final analysis (Liu 2012). Further details of the dose response analysis will be presented in the Statistical Analysis Plan.

Descriptive statistics for values and changes from baseline will be provided at each time point within each period of the double-blind treatment phase.

For all continuous secondary endpoints, descriptive statistics of actual values and changes from baseline by treatment group within each period will be provided. The change from baseline for QIDS-SR_{16} total score, CGI-S, PGI-S, and GAD-7 will be analyzed in the same way as for the MADRS total score. There will be no adjustments for multiplicity in the evaluation of these other efficacy endpoints.

A frequency table for the number and percentage of subjects meeting criteria for sustained response ≥50% reduction from baseline in MADRS total score with onset by Day 2 through the end of the double-blind phase (Day 15)] will be provided for subjects who remain on the same treatment for the duration of the double-blind phase. Frequency tables for the number and percentage of subjects meeting criteria for

response (≥50% reduction from baseline in MADRS total score) and remission (MADRS total score of ≤10) will be provided at each time point.

Descriptive statistics for values and changes from baseline for the MADRS total score will be provided for the group of subjects who are randomized at Period 1 to either intranasal esketamine or placebo and remain on the same treatment for the duration of the double-blind treatment phase (Day 15 or early withdrawal). Placebo subjects who are re-randomized in Period 2 and receive esketamine will not be included in these summaries.

Efficacy data from the optional open-label treatment phase will be summarized descriptively.

Details of the exploratory efficacy analyses will be provided in the Statistical Analysis Plan.

Pharmacokinetic Analysis

Plasma esketamine, noresketamine, 4β-hydroxycholesterol, and total cholesterol concentrations will be listed for all subjects by esketamine treatment and study day. All concentrations below the lowest quantifiable concentration in a sample or missing data will be labeled as such in the concentration data presentations. Concentrations below the lower quantifiable concentration will be treated as zero in the summary statistics.

Subjects will be excluded from the pharmacokinetic analysis if their data do not allow for accurate assessment (e.g., incomplete administration of the study drug; missing information of dosing and sampling times). All subjects and samples excluded from the analysis will be clearly documented in the study report.

Descriptive statistics will be used to summarize esketamine, noresketamine, 4β-hydroycholesterol, and total cholesterol concentrations at each sampling time point. For each esketamine treatment and day, descriptive statistics, including arithmetic mean, standard deviation (SD), coefficient of variation, median, minimum, and maximum will be calculated for each analyte at each sampling time.

Population PK analysis of plasma concentration-time data of esketamine will be performed using nonlinear mixed-effects modeling. Data may be combined with those of other selected studies to support a relevant structural model. Available baseline subject characteristics (demographics, laboratory variables, genotypes, race, etc.) will be tested as potential covariates affecting PK parameters. Details will be given in a population PK analysis plan and results of the population analyses will be presented in a separate report.

Pharmacokinetic and Pharmacodynamic Analysis

The relationship between MADRS total score (and possibly selected adverse events as additional pharmacodynamic parameters) and PK metrics of esketamine may be evaluated. If there is any visual trend in graphical analysis, suitable models will be applied to describe the exposure-effect relationships. The results of this analysis would be presented in a separate report.

Biomarker Analysis

Metabolomics (if analyzed)

Spearman rank correlation coefficients between pretreatment glycine concentrations and the MADRS total score percentage change from Day 1 (baseline) at all scheduled time points will be calculated to investigate whether pretreatment concentrations of glycine correlate with the magnitude of clinical change following the administration of intranasal esketamine or placebo. Changes in glutamate metabolic pathway markers induced by esketamine or placebo will be investigated using a pattern classifier algorithm. Samples from different studies on ketamine and esketamine in treatment resistant depression will be pooled for analysis.

Human Inflammation MAP (if analyzed)

Statistical analyses of the markers from the Human Inflammation MAP will use both a univariate and a multivariate approach to identify the least number of markers which yield the highest accuracy in separating responders from non-responders to intranasal esketamine. A similar approach will be used to identify the pharmacodynamic effects of esketamine and placebo on inflammatory and neurotrophic markers.

Results of Human Inflammation MAP and metabolomics will be presented in a separate report.

Pharmacogenomic Analyses

A composite genotype and predicted phenotype will be derived from the raw genotyping data for *CYP2B6*. Allele and genotype frequencies will be tabulated. No formal statistical tests will be performed. Genetic results from other analyzed genes will be pooled together with data from other suitable studies for a meta-analysis.

Results of the pharmacogenomic analysis will be listed and summarized with other clinical studies in a separate pharmacogenomics report.

Safety Analyses

For each period in the double-blind treatment phase, the safety analysis set will be defined to include all randomized subjects who receive at least 1 dose of study drug within that period. For the optional open-label treatment phase, the safety analysis set will be defined to include all subjects who receive at least 1 dose of study drug within that period. The same analyses of safety and tolerability will be conducted for the double-blind Period 1 and Period 2 separately, as well as the optional open-label treatment phase.

Adverse Events

The verbatim terms used in the CRF by investigators to identify adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). All reported adverse events with onset during the treatment phase (i.e., treatment-emergent adverse events, and adverse events that have worsened since baseline) will be included in the analysis. For each adverse event, the percentage of subjects who experience at least 1 occurrence of the given event will be summarized by treatment group.

Clinical Laboratory Tests

Laboratory data will be summarized by type of laboratory test. Reference ranges and markedly abnormal results (specified in the SAP) will be used in the summary of laboratory data. Descriptive statistics will be calculated for each laboratory analyte at baseline and at each scheduled time point.

ECG

The ECGs will be summarized with descriptive statistics on heart rate, RR, PR interval, QRS interval, QT interval, and corrected QT (QTc) interval using the following correction methods: QT corrected according to Bazett's formula (QTcB) and QT corrected according to Fridericia's formula (QTcF).

The frequency and percentage of subjects with QTc interval >450 milliseconds (ms), >480 ms, or >500 ms will be summarized, as will the percentage of subjects with QTc interval increases from baseline >30 ms or >60 ms.

All important abnormalities in ECG waveform that are changes from the baseline readings will be reported (e.g., occurrence of U waves).

Vital Signs

Descriptive statistics of temperature, pulse/heart rate, respiratory rate, pulse oximetry, and blood pressure (systolic and diastolic) values and changes from baseline will be provided at each scheduled time point. The percentage of subjects with values beyond clinically important limits will be summarized.

Nasal Exam and Nasal Tolerability Questionnaire

Changes in findings from the baseline nasal examination (including the upper respiratory tract/throat) will be listed by treatment group and period. Examinations will provide ratings (none, mild, moderate, or severe) that are based on a visual inspection of the nostrils, nasal mucosa, and throat for nasal erythema, rhinorrhea, rhinitis, capillary/blood vessel disruption and epistaxis. A shift table for changes in rating for each examination will be presented by treatment group and period.

In addition, scoring from the nasal tolerability questionnaire will be summarized descriptively by treatment group and period.

Physical Examination

Abnormal findings from the physical examinations will be listed.

C-SSRS

Suicide-related thoughts and behaviors based on the C-SSRS will be summarized by treatment group in incidence and shift tables. Separate endpoints for suicidal ideation and suicidal behavior will be defined and summarized descriptively by treatment group. Missing scores will not be imputed.

CADSS, BPRS+, and MOAA/S

Descriptive statistics of each of the scores and changes from predose will be summarized at each scheduled time point.

PWC-20

The PWC-20 rating scale will be analyzed descriptively.

Interim Analysis

An interim analysis may be performed as needed. If one is required, further details will be provided in a separate charter and interim analysis plan.

TIME AND EVENTS SCHEDULE 1 (OF 2): SCREENING AND DOUBLE-BLIND TREATMENT PHASES

Phase	**Screening**	**Double-Blind Treatment [1]**										
Visit Number	**1**	***Period 1*: Visit 2 and Visit 4**								**3**	**8 A**	**-**
		***Period 2*: Visit 5 and Visit 7**								**6**		
Clinic Visit (C) or Telephone Contact (TC)	**C**	**C**								**TC**	**C**	**C**
Day	**-28 to -1**	***Period 1*: Day 1 and Day 4**								**2**	**15**	**ET[2]**
		***Period 2*: Day 8 and Day 11**								**9**		
Time	**-**	**Predose**	**0**	**5 min**	**10 min**	**40 min (+/- 10 min)**	**1 hr**	**2 hr**	**3 hr**	**-**	**-**	
Study Procedures												
Screening/Administrative												
Informed consent	X											
Inclusion/exclusion criteria	X											
Medical history and demographics	X											
Prestudy therapy	X											
SAFER Interview [3]	X											
MINI [4]	X											
IDS-C_{30} [4]	X	X[6]										
MGH-ATRQ [4]	X											
Modified Berlin Questionnaire [5]	X											
Dispense subject diary [19]								X[6]				
Review of subject diary		X[18]									X	X
Intranasal Study Drug Administration												
Randomization		X[7]										
Placebo or esketamine 28 mg, 56 mg, or 84 mg [8]			X	X	X							
Safety Assessments												
Physical examination	X	X[7]									X	X
Nasal examination	X	X[7]						X[7]			X	X
Nasal tolerability questionnaire [5]		X						X				
Height	X											
Body weight	X	X[7]									X	X
Vital signs [9]	X										X	X
Vital signs: BP and HR only		X				X	X	X				
12-lead ECG	X	X									X	X
Pulse oximetry		*Continuous[10]*										
C-SSRS (Screening/Baseline version) [4]	X											
C-SSRS (Since Last Visit version) [4]		X									X	X
MOAA/S [4]		*Every 5 minutes [11]*										
BPRS+ [4]		X				X		X				
CADSS[4]		X				X		X				
PWC-20 [4]											X	X
Clinical Laboratory Assessments												
Hematology, Chemistry	X	X[7]									X	X
Total cholesterol		X[13]										
Urinalysis	X	X[7]									X	X
Thyroid-stimulating hormone	X											

Phase	**Screening**	**Double-Blind Treatment [1]**										
Visit Number	**1**	***Period 1*: Visit 2 and Visit 4**								**3**	**8 A**	**-**
		***Period 2*: Visit 5 and Visit 7**								**6**		
Clinic Visit (C) or Telephone Contact (TC)	**C**	**C**								**TC**	**C**	**C**
Day	**-28 to -1**	***Period 1*: Day 1 and Day 4**								**2**	**15**	**ET[2]**
		***Period 2*: Day 8 and Day 11**								**9**		
Time	**-**	**Predose**	**0**	**5 min**	**10 min**	**40 min (+/- 10 min)**	**1 hr**	**2 hr**	**3 hr**	**-**	**-**	
Study Procedures												
Serology (HIV, Hepatitis B, Hepatitis C)	X											
Serum pregnancy test	X										X	X
Urine pregnancy test		X										
Urine drug screen	X	X										
Alcohol screen (urine)	X											
Alcohol screen (breath)		X										
Efficacy Assessments												
MADRS (7-day recall) [4]		X[7]									X	X
MADRS (24-hour recall) [4]										X		
MADRS (2-hour recall) [4, 12]								X				
CGI-S [4]		X						X		X	X	X
QIDS-SR$_{16}$ [5]	X	X[7]									X	X
PGI-S [5]		X						X		X[19]	X	X
PGI-C [5]										X[19]	X	X
GAD-7 (7-day recall) [5]		X[7]									X	X
EQ-5D-5L [5]		X									X	X
Pharmacokinetics												
Blood sample collection		X[6, 13]				X[13, 14]		X[14]	X[14]			
Pharmacogenomics (DNA)												
Blood sample collection[15]		X										
Biomarkers												
Blood sample collection for Inflammatory MAP		X[20]									X	X
Blood sample collection for Metabolomics		X[20]									X	X
Ongoing Subject Review												
Concomitant therapy [16]	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events [17]	X	X	X	X	X	X	X	X	X	X	X	X

Footnotes:

1. With the exception of Day 1, visits can occur +/- 1 day.
2. If a randomized subject that has received at least one dose of study medication discontinues the study prior to completion of the double-blind treatment phase, an ET visit will be conducted at the time of discontinuation.
3. Conducted by a remote, independent rater. The SAFER Interview includes the MADRS, a review of the MGH-ATRQ performed at Screening, and the SAFER Criteria Inventory.
4. Clinician-administered assessment. Note: The MGH-ATRQ will be completed in collaboration with the subject.
5. Subject-completed assessment.
6. Day 1 only.
7. Day 1 and Day 8.
8. Self-administered under direct supervision by the Investigator or designee. Time 0 is defined as the time of the first 100-µL spray. Subjects will administer 1 spray into each nostril at each time point on each dosing day.
9. Blood pressure, heart rate, respiratory rate, and tympanic temperature.

10. Continuous arterial oxygen saturation monitoring by pulse oximetry (SpO_2) starting 5 minutes before first spray and monitored continuously for approximately 1 hour postdose.
11. Performed every 5 minutes from predose to 1 hour postdose or longer, if necessary, until the subject has a score of 5.
12. The sleep and appetite items will not be assessed. These predose MADRS scores performed on the same day will be carried forward.
13. The plasma concentrations of 4β-hydroxycholesterol will be measured from the PK samples obtained at predose on Day 1 only and at t = 40 min (+/- 10 min) on Day 11 only. A separate blood sample for measurement of total cholesterol will be obtained at the same time points (Day 1 predose, Day 11 at t = 40 minutes (+/- 10 min).
14. Day 1 and 11 only. An individual PK sample for the measurement of esketamine and noresketamine plasma concentrations will be obtained. The sample collected at t = 2 hours postdose can be obtained between 1.5 and 2 hours postdose. The sample collected at t = 3 hours postdose can be obtained between 2.5 and 3 hours postdose. Time 0 is defined as the time of the first 100-µL spray.
15. A 10 mL blood sample will be collected on Day 1 from all enrolled subjects. The pharmacogenomic (DNA) sample should be collected at the specified time point, however if necessary it may be collected at a later time point without constituting a protocol deviation.
16. Concomitant therapies must be recorded throughout the study beginning with signing of the informed consent until the last follow up visit.
17. All adverse events and special reporting situations, whether serious or non-serious, will be reported from the time a signed and dated informed consent form is obtained until completion of the subject's last study-related procedure (which may include contact for follow-up of safety).
18. Day 4 and 11 only.
19. Patient-reported outcome assessments scheduled for Day 2 and Day 9 will be completed in the subject diary.
20. Inflammation MAP: Day 1 (predose) and Day 4 (predose). Metabolomics: Day 1 at predose and t = 2 hours postdose.

TIME AND EVENTS SCHEDULE 2 (OF 2): OPTIONAL OPEN-LABEL TREATMENT AND POSTTREATMENT PHASES

Phase	**Optional Open-Label Treatment[1]**								**Posttreatment (Follow Up) [1]**			
Visit Number	**8B, 9, 10, and 11**								**12**	**13**	**14**	**15**
Clinic Visit (C) or Telephone Contact (TC)	**C**								**TC**	**C**	**TC**	**TC**
Day (Visit Number)	**15, 18, 22, 25**								**1 week after last dose**	**2 weeks after last dose**	**4 weeks after last dose**	**8 weeks after last dose**
Time	**Predose**	**0**	**5 min**	**10 min**	**40 min (+/- 10 min)**	**1 hour**	**2 hour**	**3 hour**	-	-	-	-
Study Procedures												
Administrative												
Review of subject diary										X		
Intranasal Study Drug Administration												
Esketamine 28 mg, 56, or 84 mg [2]		X										
Safety Assessments												
Physical examination	X[4]									X		
Nasal examination	X[4]									X		
Nasal tolerability questionnaire	X						X					
Body weight	X[4]									X		
Vital signs [5]	X[3]									X		
Vital signs: BP and HR only					X	X	X					
12-lead ECG	X[3]				X	X				X		
Pulse oximetry	*Continuous [6]*											
C-SSRS (Since Last Visit version) [7]	X[3]								X	X		
BPRS+ [7]	X				X		X					
CADSS [7]	X				X		X					
MOAA/S [7]	*Every 5 minutes [8]*											
PWC-20 [7]									X			
Clinical Laboratory Assessments												
Hematology, Chemistry	X[4]									X		
Total cholesterol					X[10]							
Urinalysis	X[4]									X		
Serum pregnancy test										X		
Urine pregnancy test	X											
Urine drug screen	X											
Alcohol screen (breath)	X											
Efficacy Assessments												
MADRS (7-day recall) [7]	X[3]								X	X	X	X
CGI-S [7]	X[3]									X	X	X
QIDS-SR$_{16}$ [9]	X[3]								X[15]	X		
PGI-S [9]	X[3]									X		
PGI-C [9]	X[3]								X[15]	X		
GAD-7 [9]	X[3, 16]									X		
EQ-5D-5L [9]	X [4]									X		
Pharmacokinetics												
Blood sample collection					X[10]		X[11]	X[11]				
Biomarkers												

Phase	**Optional Open-Label Treatment[1]**								**Posttreatment (Follow Up) [1]**			
Visit Number	**8B, 9, 10, and 11**								**12**	**13**	**14**	**15**
Clinic Visit (C) or Telephone Contact (TC)	**C**								**TC**	**C**	**TC**	**TC**
Day (Visit Number)	**15, 18, 22, 25**								**1 week after last dose**	**2 weeks after last dose**	**4 weeks after last dose**	**8 weeks after last dose**
Time	**Predose**	**0**	**5 min**	**10 min**	**40 min (+/- 10 min)**	**1 hour**	**2 hour**	**3 hour**	-	-	-	-
Study Procedures												
Blood sample collection for Inflammation MAP							X[17]					
Ongoing Subject Review												
Concomitant therapy [13]	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events [14]	X	X	X	X	X	X	X	X	X	X	X	X

Footnotes:

1. Visit window: Day 15 (+/- 1 day), all other visits can occur +/- 2 days.
2. Time 0 is defined as the time of the first 100-µL spray. All subjects start with intranasal esketamine 56 mg on Day 15, and subsequent doses can be titrated up to 84 mg at any time point, if desired. If an intranasal esketamine 56 mg or 84 mg dose is not tolerated, the next dose can be lowered to 28 mg or 56 mg, respectively.
3. Not required on Day 15 if subject completed the double-blind phase (Day 15) on the same day.
4. Day 18 and 25 only.
5. Blood pressure, heart rate, respiratory rate, and tympanic temperature.
6. Continuous arterial oxygen saturation monitoring by pulse oximetry (SpO2) placed 5 minutes before first spray and monitored continuously for approximately 1 hour postdose.
7. Clinician-administered assessment
8. Performed every 5 minutes from predose to 1 hour postdose or longer, if necessary, until the subject has a score of 5.
9. Subject-completed assessment
10. Day 25 only. The plasma concentrations of esketamine, noresketamine, and 4β-hydroxycholesterol will be measured from the PK sample obtained at t = 40 minutes (+/- 10 min). A separate blood sample will be collected for measurement of total cholesterol at the same time point [t = 40 minutes (+/- 10 minutes)].
11. Day 25 only. The PK sample collected at t = 2 hours postdose can be obtained between 1.5 and 2 hours postdose. The PK sample collected at t = 3 hours postdose can be obtained between 2.5 and 3 hours postdose. Time 0 is defined as the time of the first 100-µL spray.
12. Day 15 only.
13. Concomitant therapies must be recorded throughout the study beginning with signing of the informed consent until the last follow up visit.
14. All adverse events and special reporting situations, whether serious or non-serious, will be reported from the time a signed and dated informed consent form is obtained until completion of the subject's last study-related procedure (which may include contact for follow-up of safety.
15. Completed in subject diary.
16. Day 22 only.
17. Day 25 only.

Abbreviations: **BPRS+** = Four-item positive symptom subscale of the Brief Psychiatric Rating Scale; **CADSS**= Clinician Administered Dissociative States Scale; **CGI-S** =Clinical Global Impression – Severity (S); **C-SSRS** = Columbia Suicide Severity Rating Scale; **EQ-5D-5L** = EQ-5D™ is a trade mark of the EuroQol Group; 5 level; **GAD-7** = Generalized Anxiety Disorder 7-item scale**; IDS-C_{30}** = Inventory of Depressive Symptoms-Clinician rated, 30-item; **MADRS** = Montgomery Asberg Depression Rating Scale; **MGH-ATRQ** = Massachusetts General Hospital – Antidepressant Treatment Response Questionnaire; **MINI** = Mini International Psychiatric Interview; **MOAA/S** = Modified Observer's Assessment of Alertness/Sedation; **PGI-C** = Patient Global Impression of Change; **PGI-S** = Patient Global Impression – Severity; **PWC** = Physician Withdrawal Checklist; **SAFER** = State vs. Trait, Assessibility, Face Validity, Ecological Validity, Rule of Three P's**; QIDS-SR_{16}** = 16-item Quick Inventory of Depressive Symptoms- Self Report

ABBREVIATIONS

ANCOVA	analysis of covariance
ATRQ	antidepressant treatment response questionnaire
AUC	area under the plasma concentration-time curve
BPRS	brief psychiatric rating scale
BPRS+	four-item positive symptom subscale of the brief psychiatric rating scale
CADSS	clinician administered dissociative states scale
CGI-S	clinical global impression – severity
C_{max}	maximum plasma concentration
CRF	case report form (paper or electronic as appropriate for this study)
C-SSRS	columbia suicide severity rating scale
CVMP	committee for medicinal products for veterinary use
CYP	hepatic cytochrome P450
DSM-IV	diagnostic and statistical manual of mental disorders (4th edition)
DSM-5	diagnostic and statistical manual of mental disorders (5th edition)
ECG	electrocardiogram
eDC	electronic data capture
EDTA	ethylenediaminetetraacetic acid
EQ-5D-5L	EuroQol Group; 5 dimension; 5 level
EQ-VAS	EuroQol Group: visual analogue scale
FT4	free thyroxine
GAD-7	generalized anxiety disorder 7-item scale
GCP	good clinical practice
HBsAg	hepatitis B surface antigen
HIV	human immunodeficiency virus
ICD-10	10^{th} revision of the "international statistical classification of diseases and related health problems"
ICF	informed consent form
ICH	international conference on harmonisation
IDS-C_{30}	inventory of depressive symptoms-clinician rated, 30-item
IEC	independent ethics committee
IRB	institutional review board
IV	intravenous
IVRS	interactive voice response system
IWRS	interactive web response system
LC-MS/MS	liquid chromatography/mass spectrometry/mass spectrometry
LD_{50}	median lethal dose
MADRS	montgomery asberg depression rating scale
MAO	monoamine oxidase
MDD	major depressive disorder
MedDRA	medical dictionary for regulatory activities
MGH-ATRQ	massachusetts general hospital – antidepressant treatment response questionnaire
MINI	mini international psychiatric interview
MOAA/S	modified observer's assessment of alertness/sedation
NMDA	n-methyl-d-aspartate
NOAEL	no observed adverse effect level
PD	pharmacodynamic
PGI-C	patient global impression of change
PGI-S	patient global impression - severity
PK	pharmacokinetic
PQC	product quality complaint
PRO	patient-reported outcome(s)
PWC-20	physician withdrawal checklist; 20-item
QIDS-SR_{16}	16-item quick inventory of depressive symptoms- self report
SAFER	state vs. trait, assessibility, face validity, ecological validity, rule of three P's
SIGMA	structured interview guide for the montgomery asberg depression rating scale

TEAEs	treatment-emergent adverse events
TRD	treatment-resistant depression
USP	United States pharmacopeia

1. INTRODUCTION

Major depression is associated with significant social, educational, and vocational impairment; high utilization of social and health care services; and increased medical morbidity and mortality. While there are a number of treatments available for the treatment of depression, nearly 30 to 50% of patients do not remit from current biogenic amine-based antidepressant drugs (Preston 2013; Trivedi 2006). Recent advances have begun to shed light on this common and debilitating illness. There are consistent reports of decreased size of brain regions implicated in depression, as well as neuronal atrophy, including loss of synapses in MDD and rodent chronic stress models (Manji 2001; Price 2012). Converging lines of evidence suggest that major depression is associated with abnormalities in glutamatergic synaptic transmission resulting in loss of synaptic plasticity in mood and emotion circuits (Kavalali 2012; Sanacora 2008). Ketamine/esketamine are an inhibitor of the glutamatergic NMDA receptor. Preclinical studies have suggested that ketamine is associated with fast induction of synaptogenesis in rodents and reversal of the atrophy caused by chronic stress (Li 2010).

Ketamine is a racemate of R-(-)-ketamine and S-(+)-ketamine. Esketamine (JNJ-54135419) is the S-enantiomer of the ketamine racemate. Ketamine and esketamine are approved medications in several countries for the induction of general anesthesia and for use in addition to other anesthetics. The mechanism of action of ketamine and esketamine results from a noncompetitive binding to the N-methyl-D-aspartate (NMDA)-receptor (ligand-gated calcium channel) at the phencyclidine binding site (Anand 2011). Both also have additional binding sites (NMDA and non-NMDA glutamate receptors, nicotinic and muscarinic, cholinergic, and monoaminergic and opioid receptors, voltage-dependent sodium and L-type calcium channels).

Janssen Research and Development is developing esketamine for intranasal administration. Esketamine has been shown to be well and rapidly absorbed in the systemic circulation via the intranasal route (Clinical Study Report ESKETINTRD1001, in preparation). Intranasal administration has several advantages over intravenous administration, including convenience for patients, safety in terms of limited amount of drug given at any one time, and reduced dosing errors. The physiology of the nasal mucosa makes the rapid and non-invasive delivery of systemic drugs possible. Its large surface area, uniform temperature, high permeability, and extensive vascularization facilitate rapid absorption of drugs into the bloodstream (Turker 2004).

The efficacy of the racemic ketamine administered as a 40-minute intravenous infusion has been evaluated in subjects with treatment-resistant depression (TRD) (see section on Clinical Studies below). In addition, the sponsor recently completed a Phase 2 study (Clinical Study Report ESKETIVTRD2001, in preparation) that assessed the efficacy of 0.2 mg/kg and 0.4 mg/kg (both as 40-minute intravenous infusions) of esketamine in this population. A second ongoing Phase 2 study (Clinical Protocol KETIVTRD2002) that is being conducted by the sponsor in the United States will explore different dose frequencies using 0.5 mg of racemic ketamine as a 40-minute intravenous infusion.

For the most comprehensive nonclinical and clinical information regarding esketamine, refer to the latest version of the Investigator's Brochure for esketamine (IB esketamine hydrochloride 2013).

The term "sponsor" used throughout this document refers to the entities listed in the Contact Information page(s), which will be provided as a separate document.

1.1. Background

1.1.1. Nonclinical Studies

Toxicology

Single Dose Toxicity Studies

The median lethal dose (LD_{50}) of racemic ketamine ranges between 140 mg/kg (rat, intraperitoneal injection) and 616 mg/kg (mouse, oral administration) (Committee for Medicinal Products for Veterinary Use [CVMP] 1997). These high LD_{50}-values do not signal a concern regarding the clinical study at hand.

Repeat-Dose Toxicity Studies

In a1-month repeat-dose toxicity study, rats were treated intranasally with 75, 90, or 105-mg/kg/day of racemic ketamine in the presence of 0.002% benzalkonium chloride. In all treated groups, dose-related clinical signs were noted including ataxia, hypoactivity, hyperactivity and recumbency. Furthermore, oral discharge was observed without any histomorphologic correlate in the nares. Adverse effects were limited to the 90 and 105 mg/kg/day groups and included increased incidences of irregular respiration and rough haircoat. Body weight gains were decreased in all treated groups. This finding correlated with decreased food consumption in males.

Based on the findings above, the no observed adverse effect level (NOAEL) was set at 75 mg/kg/day. A toxicokinetic examination was not included.

In a 1-month repeat-dose toxicity study, dogs were treated intranasally with racemic ketamine at dosages of 4, 20, and 60 mg/kg/day in the presence of 0.002% benzalkonium chloride. Transient clinical observations included salivation at all dosages, wobbly gait and inability to stand at 20 and 60 mg/kg/day, and right/left/ventral recumbency, vocalization, tremors and increased activity at 60 mg/kg/day. Gross necropsy revealed multiple red foci or reddened mucosa in the urinary bladder of males at 20 and 60 mg/kg/day and in all female dose groups. Microscopically, a treatment-related minimal chronic inflammation in the urinary bladder was seen in all dogs at 20 and 60 mg/kg/day, often accompanied by hyperplasia of the transitional epithelium and hemorrhage. One female dog dosed at 4 mg/kg/day showed urinary bladder lesions with discrete foci of lymphocytic proliferation. Resolution of the bladder inflammation was apparent at 2 weeks after cessation of treatment.

On Day 27, the mean C_{max} values of racemic ketamine were 0.161, 0.570, and 2.958 μg/mL in male dogs, and 0.213, 0.729, and 2.827 μg/mL in female dogs, at 4, 20, and 60 mg/kg/day, respectively. The mean AUC_{all} values were 0.040, 0.396, and 2.215 μg.h/mL, respectively, in male dogs, and 0.088, 0.359, and 2.569 μg.h/mL, respectively, in female dogs.

Safety Pharmacology

In chronically instrumented dogs with autonomic nervous system blockade, intravenously administered ketamine induced a decrease in myocardial contractility. Concomitant decreases in global isovolumetric indices of contractility, regional myocardial function, and cardiac output were also observed. The negative inotropic effect of ketamine was a direct action and independent of the autonomic nervous system (Pagel 1992).

Additional information regarding the effects of ketamine on the cardiovascular system, including effects on blood pressure, is summarized in the esketamine IB (IB esketamine hydrochloride 2013).

Neurotoxicity

Parenterally administered racemic ketamine has been reported to induce neuronal vacuolation and apoptotic cell death in the posterior cingulate and retrosplenial cortex of adult rats (40 to 60 mg/kg, single subcutaneous or intraperitoneal dose) (Olney 1989; Jevtovic-Torodovic 2000, 2001, 2005) and adult mice (50 mg/kg/day intraperitoneally for 7 days) (Zuo 2007).

In juvenile rodents, racemic ketamine induces a more widespread apoptotic neurodegeneration than in adult rodents involving several major regions of the developing brain. The time window of vulnerability to this neuroapoptosis-inducing action coincides with the period of synaptogenesis. In rats, this period begins at 1 day after birth and terminates at approximately 14 days after birth. In humans, it spans the last 3 months of pregnancy and extends into the first several years postnatally.

As in rodents, the window of vulnerability to the neurotoxic effect of ketamine in the nonhuman primate is restricted to the period of rapid synaptogenesis, which occurs at least by 75% of gestation lasting to postnatal Day 35. Rhesus monkeys at 3 stages of development (122 days of gestation [early third trimester; full term at gestation Day 165], and postnatal Days 5 and 35) received racemic ketamine at a high anesthetic dose. Apoptotic and necrotic cell death were noted in the frontal cortex of gestation Day 122 and postnatal Day 5 animals, but not in postnatal Day 35 animals. The plasma levels of racemic ketamine averaged approximately 5 to 15 μg/mL. The rhesus monkey brain at gestation Day 120 is considered to be at a developmental stage equivalent to the brain of a mid- to late-third trimester human fetus (or prematurely born infant), and the postnatal Day 5 to 6 rhesus monkey brain is considered equivalent to that of a 4- to 6 month-old human infant (Brambrink 2012; Slikker 2007).

The relevance to humans of “Olney lesions” is not known. The doses being evaluated in the study would produce exposure that is less than 10x the exposure where lesions occurred.

Developmental or Reproductive Toxicity Studies

In rats, an embryo-fetal developmental toxicity study was conducted with intranasally administered racemic ketamine at dosages of 15, 50, or 150 mg/kg/day in the presence of 0.002% benzalkonium chloride. Dose-related clinical observations were seen at 50 and 150 mg/kg/day and included salivation, wobbly gait, ocular discharge, and dark material around the nose. A rough coat was seen in 1 animal in each of the groups, and nystagmus, impaired mobility and decreased activity were seen in the 150-mg/kg/day group. No treatment-related change in corrected maternal body weight gain was noted. There was no effect of treatment on pregnancy parameters. There were no treatment-related changes at external, visceral or skeletal examination in the fetuses either.

On Day 6 of gestation, the mean C_{max} and $AUC_{0-\infty}$ values of racemic ketamine were 1.37, 7.96, and 10.65 μg/mL, respectively, and 0.83, 5.18, and 6.86 μg.h/mL, respectively. On Day 17 of gestation, the mean C_{max} and $AUC_{0-\infty}$ values were 1.95, 14.13, and 21.73 μg/mL, respectively, and 0.92, 7.77, and 12.95 μg.h/mL, respectively.

In rats, a dose of 15 mg/kg/day was considered the no observed effect level for maternal toxicity and a dose of 150 mg/kg/day was considered the no observed effect level for developmental toxicity.

In an embryo-fetal developmental toxicity study, pregnant rabbits were intranasally dosed with racemic ketamine at dosages of 10, 30, or 100 mg/kg/day in the presence of 0.002% benzalkonium chloride. Due to mortality in the high-dose group, the high dose was reduced from 100 to 50 mg/kg/day during the course of treatment.

Clinical observations were mainly noted in the 30 and 100/50 mg/kg/day groups and included wobbly gait, decreased activity, wet around nares, and a low incidence of post-dose salivation, dilated pupils and ocular discharge. Furthermore, a low incidence of nasal discharge, decreased food consumption and few feces (small in size) were observed in the 30- and 100/50-mg/kg/day groups. Lateral recumbency, shallow breathing, and eyes dark in color were seen in the high-dose group. The corrected mean body weight gain appeared decreased at all dosages, with little effect at 10 mg/kg/day, and much greater effects at the mid- and high-dose (no body weight gain at 100/50 mg/kg/day).

Mean fetal body weights were only decreased in the 30 mg/kg/day female fetuses. There were no treatment-related differences in external, visceral and skeletal malformations or variations seen in fetuses in the test article-treated groups as compared with controls.

On Day 6 of gestation, the mean C_{max} values were 0.12, 1.50, and 11.8 μg/mL, respectively, and the mean $AUC_{0-\infty}$ values were 0.03 (AUC_{all}), 1.71, and 5.46 μg.h/mL, respectively. On Day 18 of gestation, the mean C_{max} values were 0.05, 1.90, and 2.31 μg/mL, respectively, and the mean and $AUC_{0-\infty}$ values were 0.01 (AUC_{all}), 0.76, and 1.53 (AUC_{all}) μg.h/mL, respectively.

In rabbits, based on these results, 10 and 100/50 mg/kg/day were considered the no observed adverse effect level for maternal and developmental toxicity, respectively.

Reproduction studies in dogs, injected with 25 mg/kg ketamine intramuscularly 6 times during 1 trimester of pregnancy (twice a week over a 3-week period), did not show apparent adverse effects. Rats were injected during the pre-mating period (10 mg/kg intravenously), the period of organogenesis (20 mg/kg intramuscularly) and the perinatal period (20 mg intramuscularly), and rabbits were injected during the period of organogenesis (20 mg/kg intramuscularly). Ketamine did not affect reproduction (CVMP 1997).

Genetic Toxicity Studies

In an Ames test, racemic ketamine did not show a mutagenic effect. Racemic ketamine has been reported to show genotoxic potential in an in vitro sister chromatic exchange assay in Chinese Hamster ovary cells (Adhvaryu 1986; CVMP 1997) and an in vitro micronucleus test in Chinese Hamster lung fibroblasts (Toyama 2006). The latter 2 tests do not meet current standards of testing. A positive signal was found in an in vitro mouse lymphoma assay with racemic ketamine in the presence of metabolic activation. An in vitro mouse micronucleus test with esketamine also showed positive in the presence of metabolic activation. (CVMP 1997).

Additional information regarding the effects of ketamine/esketamine on preclinical safety is summarized in the esketamine IB (IB esketamine hydrochloride 2013).

1.1.2. Clinical Studies

1.1.2.1. Summary of Ketamine/Esketamine Efficacy

The efficacy of subanesthetic doses (0.2 to 0.5 mg/kg) of intravenous ketamine has been evaluated in approximately 150 TRD subjects, including 2 studies in bipolar depressed subjects (reviewed in: Mathew 2012; Bunney 2011). In earlier pilot studies, a single intravenous infusion dose (0.5 mg/kg over 40 minutes) of the NMDA-receptor antagonist ketamine produced a rapid (i.e., same day) and robust antidepressant effect lasting on average 5 days in patients with TRD (Zarate 2006) and similarly, responders to the first dose showed improvement with repeated doses for 2 weeks for an average of 19 days (aan het Rot 2010). Psychotomimetic side-effects were minimal and of short duration (Berman 2000; Messer 2010; Zarate 2006).

Preliminary data from a recently completed phase 2 study with IV esketamine (ESKETIVTRD2001 2012) suggests a similar, rapid, and robust antidepressant effect as seen with IV ketamine. This double-blind, double-randomization, placebo-controlled, multiple-dose titration study enrolled 30 adult subjects with TRD: 10 in the IV placebo group, 9 in the IV esketamine 0.20 mg/kg group, and 11 in the IV esketamine 0.40 mg/kg group (based on Day 1 randomization). The preliminary intention-to-treat analysis of the primary efficacy variable, change in MADRS total score from baseline Day 1 to Day 2, indicated that the improvement in both esketamine dose groups was statistically significant (1-sided p-value = 0.001 in both dose groups) when compared with the placebo group. The mean (standard deviation) change from baseline Day 1 to Day 2 in MADRS total score was -4.9 (4.72) in the placebo group, -16.8

(10.12) in the esketamine 0.20 mg/kg group, and -17.8 (9.45) in the esketamine 0.40 mg/kg group.

1.1.2.2. Summary of Ketamine/Esketamine Safety Profile

Ketamine is a rapidly-acting general anesthetic that is approved and widely used intravenously or intramuscularly for the induction and maintenance of anesthesia in children and adults. In Europe, ketamine is marketed as a racemic mixture and in some European Union countries also as the S-enantiomer, esketamine. Ketamine was first introduced as an anesthetic in 1963 and is considered to have an excellent medical safety profile (Haas 1992; Ketalar® Summary of Product Characteristics [SPC] 2011; Ketanest®S SPC 2011; Reich 1989; Sinner 2008).

Adverse Events Associated With Acute Use:

Adverse events reported for ketamine with anesthetic dosages include frequent elevation of blood pressure and pulse, which resolves immediately after the infusion is discontinued. The risk is higher in patients with untreated hypertension, patients with severe cardiac disease, patients at risk of a stroke, and patients with raised intracranial pressure. Although respiration is frequently stimulated, it is reported that severe depression of respiration or apnea may occur after rapid intravenous administration of high dosages of ketamine. Diplopia and nystagmus have been noted after ketamine administration, and it also may cause a slight elevation in intraocular pressure measurement. Anorexia, nausea, and vomiting have been observed; however, this is not usually severe. In some patients, enhanced skeletal muscle tone may be manifested by tonic and clonic movements, sometimes resembling seizures (Ketalar® SPC 2011).

According to the SPC for esketamine (Ketanest®S SPC 2011), the following are reported as common adverse effects: transient tachycardia, vivid dreams (including nightmares), nausea and vomiting, increased blood pressure, increased salivation, blurred vision, dizziness, motor unrest, increase in vascular resistance in pulmonary circulation and increase in mucus secretion, increased oxygen consumption, laryngospasms, and temporary respiratory depression. It is reported that the risk of respiratory depression typically depends on the dosage and injection speed (Ketanest®S SPC 2011). Esketamine may also have potential tolerability advantages over its racemic mixture (Sinner 2008). However, more research needs to be conducted in this area to fully evaluate tolerability of esketamine versus ketamine.

As patients emerge from ketamine anesthesia, perceptual alterations such as dissociative experiences (sense of observing one's body from a distance) and illusions (misinterpretation of a real, external sensory stimulus) have been reported (Sinner 2008). Subanesthetic doses of ketamine induce a range of transient dose-related dissociative, psychotomimetic, and cognitive effects in healthy human subjects that resemble some of the symptoms associated with schizophrenia (Krystal 2003). The schizophrenia-like symptoms include perceptual and mood changes and impairments in memory, attention, and abstract reasoning (Honey 2005; Krystal 1994, 1999, 2000, 2003, 2005; Morgan 2004; Rowland 2005). In humans, a single dose of ketamine induces marked, dose-dependent impairments in working and episodic memory at a range of doses which would impact profoundly on users' ability to function (Morgan 2006). A prior comprehensive report looked at the safety of studies with subanesthetic dosages of

ketamine in medically healthy subjects with no personal or familial Axis I psychotic spectrum disorders who were administered subanesthetic dosages of ketamine by intravenous infusion in a series of clinical investigations from 1989 to 2005 (Perry 2007). A reported 469 subjects were included; 833 active ketamine and 621 placebo infusions were administered to these subjects. All ketamine doses were administered intravenously in a bolus-plus-infusion paradigm or a continuous infusion alone. The bolus doses ranged from 0.081 mg/kg over 10 minutes to 0.26 mg/kg over 1 minute and continuous infusion doses ranged from 0.04 mg/kg to 0.75 mg/kg over 60 to 120 minutes all by intravenous route. Ten adverse mental status events were documented in 9 subjects/infusions that were deemed related to ketamine administration (2% of subjects). All but 1 adverse event resolved by the end of the test session, with the adverse events in the remaining individual no longer clinically significant within 4 days of the test session. No residual sequelae were observed. The mental status adverse events included 3 medically stable subjects who became unresponsive to verbal stimuli. All became responsive again within minutes of discontinuation of ketamine infusion and were back to their baselines by the end of the study day. Six subjects reported distress related to the mental status effects of ketamine, resulting in discontinuation of ketamine infusion. The distress, which resolved within minutes after termination of ketamine infusion, was described variously as "no control", "not a good feeling", "feeling panicky", "very unpleasant", "weird", "too high", "walls were closing in", "felt out of my element", "distorted", and "too intense". Two subjects became tearful. The effects reported by these subjects were experienced by other subjects without the same degree of distress or the need for intervention.

Longer-term follow-up data (up to 6 months from the last infusion of ketamine) from this study found no evidence of ketamine abuse by subjects after study participation and no evidence of subsequent psychiatric problems related to ketamine exposure (alone or in combination with other study drugs) (Perry 2007). Specifically, 100 subjects were contacted for follow-up assessments at 1 week after study participation, 39 subjects at 1 month, 50 subjects at 3 months, and 34 subjects at 6 months. Although these subjects comprised a relatively small subsample of ketamine study subjects, the data collected yielded no reports of emotional or psychological problems, cognitive deficits, medical or neurologic problems, cravings for ketamine, use of ketamine outside the research setting, unusual perceptions, sluggishness, flashbacks, or paranoid thoughts.

Similarly, a prior analysis of these data failed to find evidence of sensitization to the effects of ketamine in those subjects who had more than one exposure to this drug (Cho 2005). These findings are consistent with the lack of long-term effects reported with anesthetic doses of ketamine, (Corssen 1971; Moretti 1984), and further document the safety of subanesthetic doses of ketamine as a psychopharmacologic probe in healthy subjects (Perry 2007).

Adverse Events Associated with Chronic Use:

Much of the literature on chronic use of ketamine comes from the data gathered from street users of the drug rather than systematically conducted clinical studies. Data therefore should be interpreted with caution, as in many cases, no baseline predrug data are available and drug exposure is poorly documented. Recently, Morgan and Curran conducted a comprehensive

review to survey and integrate the research literature on physical, psychological, and social harms of both acute and chronic ketamine use (Morgan 2011). Chronic physical effects reported include ketamine-induced ulcerative cystitis, hydronephrosis, and abdominal cramps.

In the 1-year longitudinal study of 150 individuals (Morgan 2010), Morgan and colleagues divided 30 subjects into 5 groups: frequent ketamine users (more than 4 times per week), infrequent ketamine users (at least once a month), abstinent users (abstinent for at least 1 month), polydrug controls, and non-users of illicit drugs. Eighty percent of the participants were retested at the end of 1 year. Cognitive deficits were mainly observed in frequent users and not with the infrequent users. Short-lasting dose-dependent effects of psychosis were associated with ketamine users. There was no increase in symptoms over time and symptoms were completely reversible upon stopping use of ketamine. As noted, these data should be interpreted with caution, as baseline data predating drug use were not available. Furthermore, in their recent review, Morgan and Curran report that there is little evidence of any link between chronic, heavy use of ketamine and diagnosis of a psychotic disorder.

Given that the principal action of ketamine is at the NMDA receptor, the consequences of ketamine use on cognition have been fairly widely investigated. Several studies have examined cognitive function in infrequent and frequent ketamine users (Curran 2000; Morgan 2006; Morgan 2009; Narendran 2005). Overall, infrequent or recreational ketamine use does not appear to be associated with long-term cognitive impairment (Narendran 2005). The most robust findings are that frequent ketamine users (more than 5 times a week) exhibit impairments in both short- and long-term memory (Morgan 2006). Although dosages have varied, dosages reported by ketamine users in this study were much higher than the dosages intended for use in treating TRD. Memory impairments may be reversible when individuals stop using the drug, as they were not found in a group of 30 ex-ketamine users who had been abstinent for at least a year (Morgan 2011).

Ketamine-induced ulcerative cystitis is a recently identified complication. The most common symptoms are frequency and urgency of urination, dysuria, urge incontinence and occasionally painful haematuria (blood in urine). Computerized tomography scans of these individuals revealed a marked thickening of the bladder wall, a small bladder capacity and perivesicular stranding consistent with severe inflammation. At cystoscopy all patients had severe ulcerative cystitis. Biopsies in four of these cases found denuded urothelial mucosa with thin layers of reactive and regenerating epithelial cells and ulcerations with vascular granulation tissue and scattered inflammatory cells. Cessation of ketamine use provided some relief of symptoms. Most of the described cases are in near daily users of ketamine for recreational purposes. The prevalence is difficult to determine as it is seen in recreational users who often don’t seek help.

The majority of cases resolve after stopping ketamine use, one-third remaining static. The aetiology of ketamine-induced ulcerative cystitis is unclear. It appears to be most common in those misusing the drug frequently, mainly daily, over an extended period. (Morgan 2011)

Dependence and Withdrawal:

There are a number of reports of ketamine dependence in the literature (Hurt 1994; Jansen 1990; Moore 1999; Pal 2002) but no large-scale studies, and so the incidence of ketamine dependence is largely unknown (Morgan 2011). An interview study of 90 ketamine users found that 57% of frequent users, 43% of infrequent users, and 60% of ex-users expressed concerns about ketamine addiction (Muetzelfeldt 2008). The majority of frequent users in that study reported using the drug without stopping until supplies ran out, so compulsive patterns of behavior are also a concern. Oral ketamine has also been evaluated as a positive control in human abuse potential studies, with dosages of 65 mg and 110 mg reported as appropriate for use as positive controls for future abuse potential studies of compounds with a similar mechanism of action or with possible perception-altering or euphoric effects (Shram 2011). There is conflicting evidence of the existence of a "withdrawal syndrome" after cessation of ketamine use (Morgan 2011). Cravings seem to be a key problem in frequent users: 28 of the 30 daily users in 1 study reported having tried to stop taking the drug but failed; all reported ketamine cravings as the reason for failure. The same study found that 12 of the 30 daily users reported withdrawal symptoms characterized by anxiety, shaking, sweating, and palpitations when they stopped using. A few published case studies also show craving and somatic and psychological aspects of anxiety as withdrawal symptoms (Critchlow 2006; Lim 2003). However, a specific ketamine withdrawal syndrome has not yet been described (Morgan 2011).

Safety Profile in Subjects with TRD and Bipolar Disorder

To date, subanesthetic dosages of intravenous ketamine (mainly single-dose) have been evaluated in several studies in subjects with TRD and bipolar disorder (Berman 2000; Diazgranados 2010; Mathew 2010; Zarate 2012; Zarate 2006). The safety profile observed in these studies is consistent with what has been observed previously with ketamine. For example, the first study in subjects with mood disorder described a rapid antidepressant effect after a single intravenous dose of ketamine, and reported significant but transient increases in psychotomimetic symptoms, as reflected in the BPRS, particularly the positive symptoms subscale (Berman 2000).

One study tested the tolerability, safety, and efficacy of repeat-dose open-label intravenous ketamine (6 infusions over 12 days) in 10 medication-free symptomatic subjects with TRD who had previously shown a meaningful antidepressant response to a single dose (aan het Rot 2010). In this study, ketamine elicited minimal positive psychotic symptoms. Three subjects experienced significant but transient dissociative symptoms and side effects during and after each ketamine infusion; these were generally mild. Throughout the study, all hemodynamic parameters were considered manageable by the administering anesthesiologist and did not necessitate termination of the infusions. After the first infusion, 3 subjects verbally reported headache of mild-to-moderate severity. During subsequent infusions, headache was reported 4 times by 4 different subjects. The Systematic Assessment for Treatment Emergent Effects Self-Report Inventory (SAFTEE-SI) data from the naturalistic follow-up visits was available for 7 of the 9 repeat-dose subjects. One subject reported a moderate increase in "sleep disturbance" from the preketamine baseline at the first follow-up visit but no longer did so at the second visit.

Another subject reported a moderate increase in "blurred vision" at the third follow-up visit after missing the first 2; this subject had no further visits. No other symptoms increased more than mildly (1 degree of severity) from baseline. There were no increases in "poor memory", ”trouble concentrating", or "word-finding difficulties", suggesting that ketamine did not have any persistent cognitive impact as per self-report.

Abuse Liability

Ketamine is a Schedule III drug (Food & Drug Administration 2012). Substances in this schedule have a potential for abuse less than substances in schedules I or II and abuse may lead to moderate or low physical dependence or high psychological dependence. In Belgium, ketamine is a controlled substance according to Chapter 2 of the 1998 Royal Decree on the regulation of psychotropic substances.

Safety Results from Study ESKETINTRD1001

The Phase 1 study ESKETINTRD1001 evaluating 4 single dose regimens of intranasal esketamine (28, 56, 84, and 112 mg) was conducted in healthy subjects. The regimens included in the present study were administered in study ESKETINTRD1001. Safety was assessed from the time of consent until the end of the study. The following safety assessments were collected: adverse events, vital signs, pulse oximetry, clinical laboratory results, physical examinations, and targeted nasal examinations coupled with assessment of nasal tolerability, electrocardiograms (ECGs) that were singular (Cohorts 1, 2, and 3) or continuous (Cohort 3, only), effects of intranasal esketamine on dissociative symptoms, psychosis-like side effects, and suicidal ideation and behavior. The subjects either returned to the study center approximately 11 days after the last dose of study medication for End-of-Study assessments or participated in these assessments at the time of early withdrawal.

Preliminary analyses indicated the following safety outcomes:

- No serious treatment-emergent adverse events (TEAEs), no TEAEs leading to discontinuation, and no deaths occurred.
- One subject with a previous history of psychosis participating in the study was discontinued once the history became known.
- The most common TEAEs were vertigo (29 [50.0%] of 58 subjects), dysgeusia (23 [39.7%] of 58 subjects), dizziness (22 [37.9%] of 58 subjects), and somnolence (22 [37.9%] of 58 subjects).
- Transient dissociative symptoms were seen, consistent with the published literature on ketamine. Dissociative symptoms generally resolved within 2 hours from the start of the dosing. The severity of dissociation was measure on the CADSS. All subjects at the 84 mg dose group had a transient increase in the CADSS scores. 2 of 15 subjects had a transient increase in CADSS scores at the 28 mg dose group and 5 of 15 subjects had a transient increase in CADSS scores at 56mg dose group. These symptoms were reported as adverse events by occurred in 23 (39.7%) of 58 subjects.

- Sedation was mild and transient typically up to 1 hour from the start of the dose.
- No treatment-emergent psychosis-like symptoms were seen.
- No clinically significant changes were seen in ECG assessments in any dose groups.
- No clinically significant changes were seen in laboratory assessments.

Safety Results from Study ESKETIVTRD2001

This placebo-controlled study evaluated 2 doses of IV esketamine (0.2 and 0.4 mg/kg) in patients with TRD (n = 30). During the first infusion period (up to Day 4, before the second infusion), which was the period associated with the primary efficacy endpoint, preliminary key safety results were as follows:

- No serious TEAEs, TEAEs leading to discontinuation, or deaths occurred.
- The most common TEAEs were headache, nausea, and dissociation:
  - Headache was reported by 7 subjects: 2 (20.0%) of 10 in the placebo group, 2 (22.2%) of 9 in the 0.20 mg/kg group, and 3 (27.3%) of 11 in the 0.40 mg/kg group.
  - Nausea was reported by 6 subjects: 2 (20.0%) of 10 in the placebo group, 3 (33.3%) of 9 in the 0.20 mg/kg group, and 1 (9.1%) of 11 in the 0.40 mg/kg group.
  - Dissociation was reported by 3 subjects: 0 of 10 in the placebo group, 1 (11.1%) of 9 in the 0.20 mg/kg group, and 2 (18.2%) of 11 in the 0.40 mg/kg group.

1.1.2.3. Human Pharmacokinetics of Intravenous Ketamine/Esketamine

Esketamine exhibits multiexponential compartmental pharmacokinetics after intravenous administration. The mean systemic clearance, steady-state distribution volume, and terminal half-life values were 26.3 mL/min/kg, 2.7 L/kg, and 146 minutes, respectively, after intravenous administration of esketamine to 10 healthy subjects (Ihmsen 2001). Esketamine does not invert to the R-enantiomer (Geisslinger 1993). The intravenous administration of 20 mg/hour and 40 mg/hour (both per 70 kg; 10 healthy subjects per regimen) of esketamine produced dose-proportional mean plasma Cmax values of 150.4 ng/mL and 304.7 ng/mL, respectively (Noppers 2011). Ketamine undergoes extensive metabolism by hepatic cytochrome P450 (CYP). In humans, N-demethylation to norketamine is the major route of metabolism, which can undergo further metabolism to form hydroxynorketamine. Ketamine and norketamine are extensively hydroxylated to a series of 6 hydroxynorketamine metabolites and 2 hydroxyketamine metabolites (Woolf 1987). Like ketamine, norketamine is a noncompetitive antagonist at the NMDA receptor (Ebert 1997; Holtman 2008). Norketamine has a half-life in plasma of approximately 5 hours (Hagelberg 2010). An inverse relationship has been reported between ketamine metabolites and psychotomimetic or dissociative side effects; i.e., higher dehydronorketamine and hydroxynorketamine concentrations were associated with lower scores on rating scales measuring psychosis symptoms (BPRS+) and dissociative symptoms (CADSS scores) (Zarate 2012). The major human hepatic CYPs that catalyze ketamine N-demethylation in vitro are CYP2B6 and CYP3A4, although there is ambiguity as to their comparative contributions to clinical ketamine metabolism (Yanagihara 2001; Hijazi 2002; Portmann 2010).

The CYP enzymes responsible for the formation of norketamine metabolites include CYP2A6 and CYP2B6 (Portmann 2010).

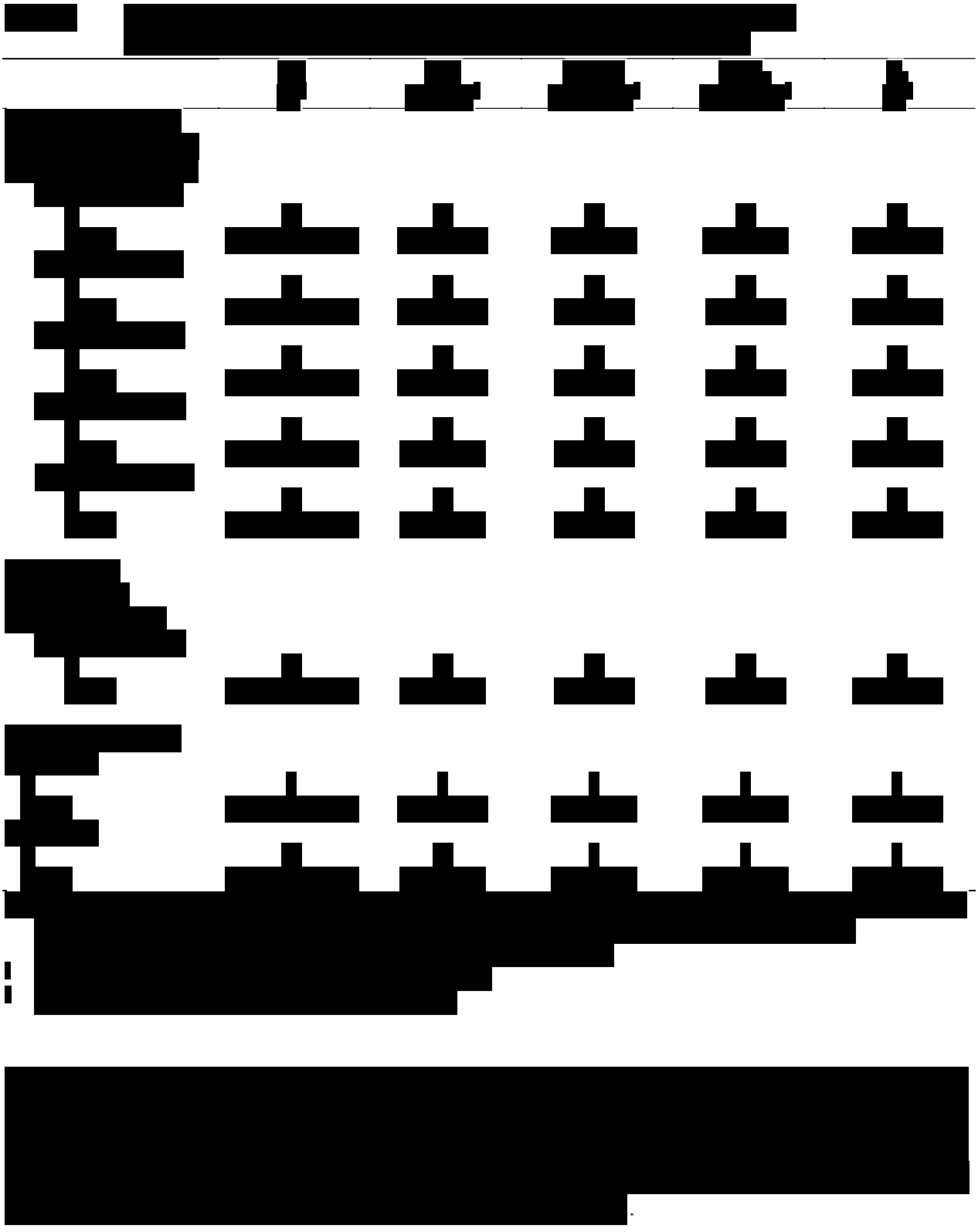
The administration of a 5-day oral regimen of rifampin (a potent inducer of hepatic CYP3A activity) to 10 healthy subjects before their receiving an intravenous infusion of esketamine produced a 13% and 200% increase in the elimination of esketamine and noresketamine, respectively, relative to treatment with placebo (Noppers 2011). A 4-day regimen of clarithromycin (a potent inhibitor of hepatic CYP3A activity) increased mean esketamine C_{max} and AUC values by 3.6-fold and 2.6-fold (relative to placebo), respectively, in 10 healthy subjects who received esketamine by the oral route (Hagelberg 2010). The effects of potent inducers and inhibitors of CYP on the pharmacokinetics of intranasally administered esketamine are expected to be smaller, relative to oral esketamine, since a smaller fraction of the dose is subjected to "first-pass" metabolism after intranasal delivery.

Pharmacokinetic Results from Studies ESKETINTRD1001 and ESKETIVTRD2001

Uncertainty exists with regards to the extent that plasma maximum concentration (C_{max}) and area under the plasma concentration-time curve (AUC) of ketamine (or esketamine) contributes to its effectiveness in patients with depression. Previously completed studies have demonstrated that a 0.5-mg/kg dose of ketamine intravenously infused over 40 minutes or 100 minutes improves depression scores in patients (Katalinic 2013; Rasmussen 2013). These results would suggest that the AUC plays a role, although the onset of efficacy appeared to be later (after up to 4 doses) when the 0.50 mg/kg dose was given over 100 minutes versus the typical response within a day after a 40-minute infusion. Moreover, a lower ketamine dose of 0.20 mg/kg administered as short (1-2 minute) IV infusion has also provided rapid benefit to patients with depression indicating that C_{max} values may contribute to the onset of response (Katalinic 2013).

[redacted]

[redacted]



1.2. Overall Rationale for the Study

This is the first study to evaluate the efficacy, dose response, safety, and pharmacokinetics of intranasal esketamine in adult TRD patients. The results will guide the selection of intranasal dose regimens for further clinical development.

2. OBJECTIVES AND HYPOTHESIS

2.1. Objectives

Primary Objective

To assess the efficacy and dose response of intranasal esketamine (28 mg, 56 mg, 84 mg) compared with placebo in improving depressive symptoms in subjects with TRD, as assessed by a change from baseline in the Montgomery-Asberg Depression Rating Scale (MADRS) total score for the combined periods in the double-blind treatment phase.

Secondary Objectives

The secondary objectives are:

1. To evaluate sustained response (≥50% reduction from baseline in MADRS total score) with onset by Day 2 through the end of the double-blind phase (Day 15).
2. To investigate the safety and tolerability of intranasal esketamine in TRD subjects, with special attention to:
    a. Local nasal tolerability, using a nasal tolerability questionnaire and nasal examinations
    b. Effects on heart rate, blood pressure, and blood oxygen saturation (SpO_2)
    c. Effects on suicidal ideation/behavior measured by the Columbia Suicide Severity Rating Scale (C-SSRS);
    d. Effects on alertness and sedation measured by the Modified Observer's Assessment of Alertness/Sedation (MOAA/S)
    e. Psychosis-like side effects by using a four-item positive symptom subscale of the Brief Psychiatric Rating Scale (BPRS+) consisting of: suspiciousness, hallucinations, unusual thought content, and conceptual disorganization;
    f. Effects on dissociative symptoms using the Clinician Administered Dissociative States Scale (CADSS);
    g. Potential withdrawal symptoms following cessation of intranasal esketamine treatment, as measured by the clinician-administered 20-item Physician Withdrawal Checklist (PWC-20)
3. To assess the effect of intranasal esketamine compared to intranasal placebo on:
    a. Depressive symptoms, as assessed by the 16-item Quick Inventory of Depressive Symptomatology- Self Report ($QIDS\text{-}SR_{16}$)
    b. Remission, defined as a MADRS score ≤10
    c. Response, defined as a ≥50% reduction from baseline in MADRS total score

d. The severity of illness using the Clinical Global Impression - Severity (CGI-S) and the Patient Global Impression - Severity (PGI-S)

e. Symptoms of anxiety as assessed by the Generalized Anxiety Disorder 7-item Scale (GAD-7)

4. To evaluate the pharmacokinetics (PK) of intranasal esketamine in subjects with TRD

Exploratory Objectives

The exploratory objectives are:

1. Subject perspective of global change in MDD from baseline, as measured by the Patient Global Impression of Change (PGI-C)
2. Impact on function as assessed by the health status using the EuroQol-5D, 5-level version (EQ-5D-5L)
3. To evaluate whether pretreatment concentrations of inflammatory and neurotrophic markers, and plasma glycine correlate with the magnitude of clinical change, as measured by the MADRS, following intranasal administration of esketamine.
4. To assess the impact of intranasal esketamine on plasma inflammatory and neurotrophic markers and glutamatergic pathway metabolic markers

2.2. Hypothesis

The primary hypothesis is that intranasal esketamine (28 mg, 56 mg, 84 mg) is superior to intranasal placebo in improving depressive symptoms in adult subjects with TRD, as assessed by the change from baseline in the MADRS total score for the combined periods in the double-blind treatment phase.

3. STUDY DESIGN AND RATIONALE

3.1. Overview of Study Design

This is a doubly-randomized, double-blind, placebo-controlled, multicenter study conducted in 60 male and female adult subjects with TRD.

Each subject will participate in up to 4 phases:

- A screening phase of up to 4 weeks,
- A double-blind treatment phase (Day 1 to Day 15) which includes two 1-week treatment periods (Period 1 and Period 2),
- An optional open-label treatment phase (Day 15 to 25), and
- An 8-week posttreatment (follow up) phase

The duration of the subject's participation will be approximately 8 to 16 weeks. The end of study will occur when the last subject in the trial completes his/her last study assessment.

Screening Phase (Day -28 to Day -1)

After giving informed consent, subjects that are 18 to 64 years of age (inclusive), will be screened within 4 weeks of Day 1 (i.e., Day -28 to Day -1) to determine eligibility for study participation.

Subjects must meet Diagnostic and Statistical Manual of Mental Disorders – Fifth Edition (DSM-5) diagnostic criteria for MDD, without psychotic features, based upon clinical assessment (DSM-5 296.22, 296.23, 296.32, or 296.33) and confirmed by the Mini International Psychiatric Interview (MINI). Subjects must have an Inventory of Depressive Symptomatology 30-item Clinician-rated (IDS-C_{30}) total score ≥34. In addition, the major depressive episode must be deemed valid using the "State vs. Trait, Assessibility, Face Validity, Ecological Validity, Rule of Three P's" (SAFER) criteria interview, administered by remote, independent raters.

Subjects must have had an inadequate response to at least 2 antidepressants, at least one of which is in the current episode of depression, assessed using the Massachusetts General Hospital – Antidepressant Treatment Response Questionnaire (MGH-ATRQ) and prior medication history.

- Subjects that are not currently taking an antidepressant at Screening are eligible to participate.
- Subjects taking an antidepressant at the Screening visit must have been receiving a stable dose (i.e., dose has been unchanged) for at least 2 weeks before the Screening visit.
    - With the exception of MAO inhibitors, which are prohibited, the subject may continue the antidepressant at the same dose during the double-blind treatment phase, or
    - The subject can discontinue the antidepressant within 1 week, or within a period less than 5 times the drug's half-life [exception: at least 4 weeks for fluoxetine and at least 2 weeks for MAO inhibitors], whichever is longer, before the planned first dose of study drug.

    The decision to continue or discontinue the current antidepressant will be made by the subject and investigator (based on their clinical judgment). Antidepressant medication will not be discontinued for the sole purpose of allowing subjects to participate in the study.

Disallowed therapies, including psychotropic medications, are described in the exclusion criteria, Section 8 (Prohibitions and Restrictions), and Attachment 1.

Other screening evaluations (e.g., biomarker, safety) will be performed as per the Time and Events Schedule.

All adverse events and special reporting situations, whether serious or non-serious, will be reported from the time a signed and dated ICF is obtained until completion of the subject's last study-related procedure (which may include contact for follow-up of safety).

Double-Blind Treatment Phase

All subjects will participate in two treatment periods (Period 1 and Period 2) during the double-blind treatment phase. Efficacy, safety, pharmacokinetic, biomarker, and/or pharmacogenomic evaluations performed at each visit are described in the Time and Events Schedule. If a subject withdraws before the end of the double-blind treatment phase, the Early Termination visit should be conducted at the time of discontinuation, followed by completion of the posttreatment phase.

All subjects will self-administer the study medication in Period 1 and Period 2 under the direct supervision of the investigator or designee (Refer to Section 6, Dosage and Administration). On all dosing days, all subjects must remain at the clinical site for at least 2 hours postdose (or longer if required for study procedures) and should be accompanied by a responsible adult when discharged from the clinical site. In addition, subjects must not drive a car or work with machines for 24 hours after study medication dosing.

If the placebo response or other assumptions at the end of Period 1 are not as expected, up to a maximum of 78 subjects (i.e., up to 18 additional subjects) may be enrolled to ensure that an adequate number of subjects are re-randomized in Period 2, based on the ongoing assessment of the numbers of subjects that are re-randomized as well as drop-out rates.

Period 1

On Day 1, subjects (n = 60) will be randomized using a 3:1:1:1 ratio to 1 of the following 4 treatment groups: Intranasal placebo (n = 30), intranasal esketamine 28 mg (n = 10), intranasal esketamine 56 mg (n = 10), or intranasal esketamine 84 mg (n = 10) administered on Day 1 and Day 4.

Period 2

Subjects that received intranasal esketamine 28, 56, or 84 mg in Period 1 will receive the same treatment in Period 2 on Day 8 and Day 11.

Period 2 treatment for subjects that received placebo in Period 1 will be determined by the QIDS-SR_{16} score on Day 8 (predose).

- Subjects that received intranasal placebo in Period 1 and have a QIDS-SR_{16} score < 11 (mild or no symptoms) will receive intranasal placebo again in Period 2 on Day 8 and Day 11.
- Subjects that received intranasal placebo in Period 1 and have a QIDS-SR_{16} score ≥11 (moderate to severe) will be re-randomized to receive intranasal placebo or intranasal esketamine 28 mg, 56 mg, or 84 mg in a 1:1:1:1 ratio on Day 8 and Day 11. The randomization will be stratified by the QIDS-SR_{16} score [moderate (QIDS-SR_{16} score 11 to 16) or severe (QIDS-SR_{16} score > 16)].

Optional Open-Label Treatment Phase

On Day 15, following completion of the double-blind treatment phase, subjects may participate in an optional open-label treatment phase. Subjects that discontinue early from the double-blind treatment phase are not eligible to participate in the optional open label treatment phase. The Investigator must agree and confirm the subject is eligible to participate based on his/her clinical judgment.

The efficacy, safety, and biomarker evaluations that will be performed at each visit during the optional open-label treatment phase are described in the Time and Event Schedule. On all dosing days, all subjects must remain at the clinical site for at least 2 hours postdose (or longer if required for study procedures) and should be accompanied by a responsible adult when discharged from the clinical site. In addition, subjects must not drive a car or work with machines for 24 hours after study medication dosing.

During the optional open-label treatment phase, subjects can receive up to 4 single doses of intranasal esketamine on Days 15, 18, 22, and 25.

If a subject withdraws before the end of the optional open-label treatment phase, an Early Termination visit is not required. The subject would continue into the posttreatment phase (see "Posttreatment Phase" below).

All subjects will start with intranasal esketamine 56 mg on Day 15, and subsequent doses on Days 18, 22, and 25 can be titrated up to 84 mg at any time point, if desired. If an intranasal esketamine 56 mg or 84 mg dose is not tolerated, the next dose can be lowered to 28 mg or 56 mg, respectively.

Posttreatment Phase

The efficacy and safety evaluations that will be performed at each visit during the posttreatment phase are described in the Time and Event Schedule.

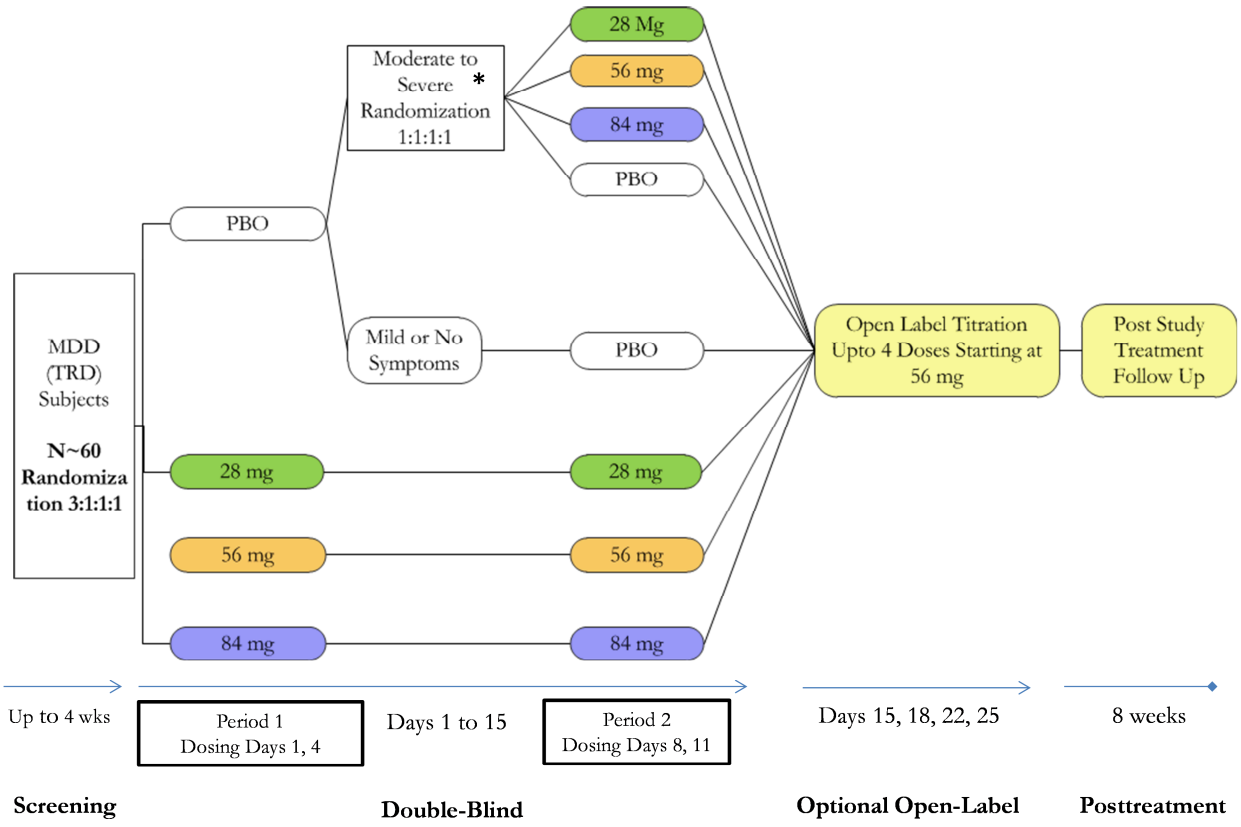
All subjects who receive at least 1 dose of study medication should have 4 follow up visits conducted at 1, 2, 4, and 8 weeks after the last dose of study medication. The follow up visits at 1, 4, and 8 weeks after the last dose of study medication will be telephone contacts. The follow up visit at 2 weeks after the last dose of study medication is a clinic visit.

Any clinically significant abnormalities persisting at the end of the study will be followed by the investigator until resolution or until a clinically stable endpoint is reached.

All adverse events and special reporting situations, whether serious or non-serious, will be reported until completion of the subject's last study-related procedure (which may include contact for follow-up of safety).

A diagram of the study design is provided below (Figure 1).

Figure 1: Study Design



* Randomization stratified by QIDS–SR_{16} total score [Moderate = 11 to 16; Severe > 16]

3.2. Study Design Rationale

Study Population

The study population will consist of men and women that meet DSM-5 diagnostic criteria for MDD, without psychotic features (DSM-5 296.22, 296.23, 296.32, or 296.33), based upon clinical assessment and confirmed by the MINI. A SAFER Interview, which will confirm the validity of the subject's major depressive episode, will be performed for each subject by a remote, independent rater.

Subjects must have an IDS-C_{30} total score ≥34 at Screening and predose on Day 1, which corresponds to at least moderate or higher severity depression. The age range of 18 to 64 years old was selected for this study as it represents a general adult age range.

Pilot studies with ketamine and esketamine have shown potentially robust efficacy in patients with TRD. The patient population in this study is consistent with the previously studied population.

Eligible subjects who are currently receiving an antidepressant or other medications do not need to be tapered off them (except for prohibited medications) to come into the study. The investigator and subject will make the decision whether or not to continue the current

antidepressant or not based on the subject's clinical status. Antidepressant medication will not be discontinued for the sole purpose of allowing subjects to participate in the study. Subjects will be free of any disallowed therapy (prior or concomitant), including psychotropic medications, within 1 week (or longer, if specified) before the planned first dose of study drug in order to minimize any interactions (e.g., pharmacodynamic, pharmacokinetic) that would make it difficult to interpret the results of the study.

Blinding and Randomization

A placebo control will be used in the double-blind treatment phase to establish the frequency and magnitude of changes in clinical endpoints that may occur in the absence of active treatment.

Randomization will be used to minimize bias in the assignment of subjects to treatment groups, to increase the likelihood that known and unknown subject attributes (e.g., demographic and baseline characteristics) are evenly balanced across treatment groups, and to enhance the validity of statistical comparisons across treatment groups. In Period 1, subjects are randomized in a 3:1:1:1 ratio (placebo: 28, 56, or 84 esketamine) to reduce expectation bias by increasing the likelihood of receiving placebo and to ensure that there are enough placebo subjects with a moderate or severe QIDS-SR_{16} score available to be re-randomized into Period 2.

Subjects who are re-randomized on Day 8 will be stratified by the Day 8, predose QIDS-SR_{16} score. The self-report QIDS-SR_{16} will be used to determine stratification rather than the clinician-rated MADRS so as to reduce bias on the primary endpoint at this time point.

Study Phases

All subjects will undergo a screening period of up to 4 weeks, which will provide adequate time to assess their eligibility per inclusion/exclusion criteria for the study. The duration of screening phase will also allow an adequate wash out period for prohibited medications, if necessary.

The duration of double-blind treatment phase (Day 1 to 15), which includes two periods (Period 1 and Period 2), was chosen because, based on prior studies with ketamine and esketamine, a rapid antidepressant response is expected within one day and two 1-week periods allow for evaluation of sustained response as well as assessing those with a potential slower onset of response. The double-randomization delayed start design allows for a smaller sample size than a standard parallel-group design while preserving a low chance of type II error and assesses the efficacy, dose response, and safety of intranasal esketamine in subjects with TRD. The key aim of the design is to only include placebo subjects from Period 1 who require treatment in the second period and re-randomize them to receive one of 3 doses of intranasal esketamine or intranasal placebo. At the end of the trial, efficacy data from both randomizations (Day 1 and Day 8) are combined in an integrated analysis. Further details regarding the analysis can be found within the statistical methods section. The placebo subjects requiring treatment are expected to be more sensitive to treatment with the active drug. Only including placebo subjects from Period 1 who require treatment in the second period addresses the issues with high placebo responses observed in psychiatric clinical trials (Liu 2012).

On Day 15, following completion of the double-blind treatment phase, all subjects may participate in an optional open-label treatment phase. During the optional open-label treatment phase, subjects can receive up to 4 single doses of intranasal esketamine on Days 15, 18, 22, and 25 under supervision by the Investigator or designee. For those subjects that may receive placebo in the double-blind treatment phase, this optional open label treatment phase provides an opportunity to receive active treatment.

The 8-week duration of the posttreatment phase allows sufficient time to assess the safety and tolerability of multiple doses, including potential withdrawal symptoms, following the last dose of study medication. In addition, this allows additional data regarding the duration of efficacy to be evaluated.

Treatment Groups

The treatment groups in the double-blind treatment phase are intranasal esketamine 28 mg, 56 mg, or 84 mg, or placebo. The 28 mg, 56 mg, and 84 mg doses of esketamine were selected based on preliminary studies described earlier with the intranasal formulation and IV esketamine (see Section 1) and to allow further exploration of dose response prior to Phase 3 with the intent of carrying forward only the efficacious and well tolerated doses into future study.

The treatment groups in the optional open label treatment phase are intranasal esketamine 28 mg, 56 mg, and 84 mg. In this phase, all subjects can modify the dose based on tolerability, if necessary.

Dose and Dose Administration Interval

Based on preliminary results from the 2 clinical studies (ESKETINTRD1001, ESKETIVTRD2001) summarized in Section 1.1.2.1, the key pharmacokinetic parameters of the 28- and 56-mg regimens of intranasal esketamine substantially overlapped those produced by IV infusion of 0.20 and 0.40 mg/kg esketamine over 40 minutes, respectively. Both of these IV doses were efficacious and generally well tolerated. Therefore, this study will evaluate the efficacy of 28, 56 and 84 mg. In addition to confirming efficacy of the intranasal formulation in the TRD population, establishing the efficacious and well tolerated active doses of intranasal esketamine prior to Phase 3 would be another important objective. The aim is to take two of the efficacious and well tolerated doses predicated upon the outcome of this study into phase 3 studies. The intranasal esketamine dose regimens in this Phase 2 study are expected to be well tolerated based on the available results from Study ESKETINTRD1001. Preliminary data from the phase 2a study with IV esketamine in TRD (ESKETIVTRD2001) suggests that dosing twice weekly is sufficient to sustain and perhaps improve the antidepressant effect.

Efficacy Measures

MADRS

The 10-item clinician-administered MADRS was designed to be used in subjects with MDD to measure the overall severity of depressive symptoms (Montgomery and Asberg 1979). The scale

has been validated, is reliable, and is acceptable to regulatory health authorities as a primary scale to determine efficacy in major depression.

The structured interview guide for the Montgomery Asberg Depression Rating Scale (SIGMA) will be used for each administration (Williams 2008). Using structured interview guides have previously been shown to increase the reliability of given scales.

Modified MADRS assessments (recall period: 2 hours, 24 hours) will be used at some visits to further assess the time to onset and duration of antidepressant effect (i.e., relapse) at assessment time points occurring less than the typical 7-day recall period for the MADRS. The modified MADRS assessment contains the same 10-items but permits a shorter recall period.

In depression, 'response' is commonly defined as a ≥50% reduction in the initial symptom score and remission is typically defined as a total score of ≤10 (Montgomery 1994). The primary efficacy evaluation will be the change from baseline in the MADRS total score in each period in the double-blind treatment phase.

QIDS-SR_{16}

The patient administered QIDS-SR_{16} is designed to be used in patients with MDD to measure the overall severity of depressive symptoms (Rush 2003; Trivedi 2004).

CGI-S

The CGI-S will provide an overall clinician-determined summary measure that takes into account all available information, including knowledge of the subject's history, psychosocial circumstances, symptoms, behavior, and the impact of the symptoms on the subject's ability to function (Guy 1976). The CGI evaluates the severity of psychopathology from 1 to 7.

GAD-7

The 7-item patient-reported GAD-7 is a brief and valid measure of overall anxiety (Spitzer 2006). Each item is rated on a 4-point scale (0 to 3), with the total score range from 0-21 (higher scores indicating more anxiety).

PGI-S and PGI-C

Patient Global Impression scales are commonly used measures of symptom severity, treatment response and the efficacy of treatments.

The PGI-S will provide an overall patient-rated summary measure that assesses the severity of the subject's MDD.

The PGI-C will provide an overall patient-rated summary that assesses subject perception of change in their MDD since starting study treatment (Rush 2005).

EQ-5D-5L

The EQ-5D is a standardized 2-part instrument for use as a measure of health outcome, primarily designed for self-completion by respondents. The EQ-5D-5L descriptive system comprises the following 5 dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Each dimension has 5 levels: no problems, slight problems, moderate problems, severe problems, and extreme problems. The descriptive system can be represented as a health state. The EQ visual analog scale (EQ-VAS) self-rating records the respondent's own assessment of their health status (Euroqol website).

Biomarker Evaluations

Metabolomics

Metabolomics is a rapidly emerging technique which captures the metabolic concentration of small molecules (i.e., metabolome) present in biological samples. Studies performed in patients with different CNS disorders have shown that metabolomics might be a promising tool to discover novel biomarkers for drug exposure, safety, and response.

Recently, it has been shown that pretreatment plasma concentrations of glycine are significantly different in depressed patients who respond to citalopram compared to non-responders, and that glycine concentrations might also predict response to drugs which target the glutamatergic system (Ji 2011).

Blood samples will be collected to allow for the exploratory analysis of novel predictive and drug exposure biomarkers. The goals are to investigate whether pretreatment glycine concentrations predict antidepressant response and whether this effect is present only in subjects who respond to ketamine and not to placebo. An additional goal of this is research is to try to identify a glutamatergic metabolic "signature" which might help characterizing the acute and late biological effects of IV ketamine in patients with depression.

Human Inflammation Multi-Analyte Panel (MAP)

Novel multiplex immunoassay platforms allow quantifying simultaneously and reliably several different protein markers in peripheral biological samples. Such techniques have been successfully used to discover novel diagnostic and prognostic biomarkers in patients suffering from different medical conditions, such as coronary artery disease, ovarian cancer, Alzheimer's disease, and schizophrenia (Ray 2007; Schwarz 2011). Inflammatory peripheral markers have been indicated as novel potential diagnostic and prognostic markers in patients with MDD and as possible modulators of NMDA receptor function (Li 2011; Müller 2011).

Blood samples will be collected to allow for an exploratory pharmacodynamics evaluation using the Human Inflammation MAP, a panel of 45 proteins involved in key biologically inflammatory and neurotrophic pathways.

Pharmacokinetic Assessments

PK samples will be obtained during the study for measurement of the plasma concentration of esketamine, noresketamine, and/or additional metabolites, if warranted.

The potential effect of esketamine on the expression of cytochrome P450 enzymes was evaluated in cultured cryopreserved primary human hepatocytes.(Janssen nonclinical study report 2013) Esketamine induced hepatic CYP 3A4 in human hepatocytes. The induction of rat hepatic cytochromes by ketamine was investigated ex vivo in rat livers from male Wistar rats dosed with 10, 20, 40, or 80 mg/kg ketamine IP twice daily for 4 days (Chan 2005). In both, a functional and genomic assay, ketamine showed induction of CYP3A at 80 mg/kg.

The oxysterol 4β-hydroxycholesterol is formed by hepatic CYP 3A4 and 3A5 and has been suggested as a marker for the activity of these drug-metabolizing enzymes (Bodin 2001; Kanebratt 2008). Subjects treated with drugs known to be potent inducers of hepatic cytochrome P450 3A4 and 3A5 have elevated concentrations of 4β-hydroxycholesterol in plasma. Therefore, the concentrations of 4β-hydroxycholesterol on each pharmacokinetic sampling day will be compared to assess the potential for repeated administration of intranasally administered esketamine to influence the activity of the hepatic cytochrome P450 3A4 and 3A5 enzymes. Plasma pharmacokinetic samples collected from subjects in each treatment group will be used for this analysis. In addition, total cholesterol will be measured from a separate blood sample at the same time points to ensure that changes in 4β-hydroxycholesterol are not attributable to changes in total cholesterol.

Safety Evaluations

Physical examination, body weight, vital signs, 12-lead ECG, pulse oximetry, clinical laboratory tests, and evaluation of adverse events and concomitant therapies will be performed throughout the study to monitor subject safety. A nasal examination and a nasal tolerability questionnaire will also be conducted.

The C-SSRS will be performed to assess suicidal ideation and behavior, the CADSS will be administered to assess treatment-emergent dissociative symptoms, the BPRS+ will be administered to assess treatment-emergent psychotic symptoms, the MOAA/S will be used to measure treatment-emergent sedation, and the PWC-20 will be administered to assess potential

On dosing days, subjects must remain at the site until at least 2 hours postdose (or longer if required for study procedures) and should be accompanied by a responsible adult when discharged from the clinical site after study medication dosing. Subjects must not drive a car or work with machines for 24 hours after study medication dosing.

DNA Collection

It is recognized that genetic variation can be an important contributory factor to interindividual differences in drug distribution and response and can also serve as a marker for disease susceptibility and prognosis. Pharmacogenomic research may help to explain interindividual variability in clinical outcomes and may help to identify population subgroups that respond

differently to a drug. The goal of the pharmacogenomic component is to collect DNA to allow the identification of genetic factors that may influence the pharmacokinetics, pharmacodynamics, efficacy, safety, or tolerability of esketamine and to identify genetic factors associated with depression.

The *CYP2B6* gene is known to carry genetic polymorphisms that can influence pharmacokinetics of CYP2B6 substrates (Thorn 2010). Since esketamine is metabolized in part by the CYP2B6 enzyme, DNA samples will be analyzed for the *CYP2B6* gene.

DNA samples may be used to help address emerging issues and to enable the development of safer, more effective, and ultimately individualized therapies.

4. SUBJECT POPULATION

Screening for eligible subjects will be performed within 28 days before administration of the study drug.

The inclusion and exclusion criteria for enrolling subjects in this study are described in the following 2 subsections. If there is a question about the inclusion or exclusion criteria below, the investigator should consult with the appropriate sponsor representative before enrolling a subject in the study.

For a discussion of the statistical considerations of subject selection, refer to Section 11.2, Sample Size Determination.

4.1. Inclusion Criteria

Each potential subject must satisfy all of the following criteria to be enrolled in the study.

1. Subject must be a man or woman, 18 to 64 years of age, inclusive.

2. Subject must be medically stable on the basis of physical examination, medical history, vital signs, and 12-lead ECG performed at screening. If there are abnormalities, the subject may be included only if the investigator judges the abnormalities or deviations from normal to be not clinically significant. This determination must be recorded in the subject's source documents and initialed by the investigator.

3. Subject must be medically stable on the basis of clinical laboratory tests performed at screening. If the results of the serum chemistry panel, hematology, or urinalysis are outside the normal reference ranges, the subject may be included only if the investigator judges the abnormalities or deviations from normal to be not clinically significant. This determination must be recorded in the subject's source documents and initialed by the investigator.

    - Retesting of an abnormal laboratory value, at the discretion of the Investigator, that may lead to exclusion will be allowed only once during the screening phase. Retesting will take place during an unscheduled visit in the screening phase.

- For those without a pre-existing history of hypothyroidism, a normal thyroid-stimulating hormone [TSH] is required at screening.
- Subjects with hypothyroidism who are on stable treatment for 3 months prior to Screening are required to have TSH and free thyroxine (FT4) obtained. If the TSH value is out of range, but FT4 is normal, such cases should be discussed directly with the medical monitor before the subject is enrolled. If the FT4 value is out of range, the subject is not eligible.

4. Subject must meet Diagnostic and Statistical Manual of Mental Disorders – Fifth Edition (DSM-5) diagnostic criteria for MDD, without psychotic features, based upon clinical assessment (DSM-5 296.22, 296.23, 296.32, or 296.33), and confirmed by the Mini International Psychiatric Interview (MINI).

5. The subject's major depressive episode and treatment response must be deemed "valid" using the SAFER criteria interview (which includes the MADRS, a review of the MGH-ATRQ performed at Screening, and SAFER Criteria Inventory) administered by remote, independent raters

6. Subjects must have had an inadequate response to at least 2 antidepressants, at least one of which is in the current episode of depression. The ATRQ will be used to assess antidepressant treatment response during the current episode. Prior medication history will be used to determine antidepressant treatment response in prior episode(s).

7. Have an IDS-C_{30} total score ≥34 at Screening and predose at Day 1.

8. Comfortable with self-administration of intranasal medication and able to follow instructions provided.

9. Before Period 1 randomization, a woman must be either:

   - Not of childbearing potential: postmenopausal (>45 years of age with amenorrhea for at least 12 months or any age with amenorrhea for at least 6 months and a serum follicle stimulating hormone (FSH) level >40 IU/mL); permanently sterilized (e.g., tubal occlusion, hysterectomy, bilateral salpingectomy); or otherwise be incapable of pregnancy,
   - Of childbearing potential and practicing a highly effective method of birth control consistent with local regulations regarding the use of birth control methods for subjects participating in clinical studies: e.g., established use of oral, injected or implanted hormonal methods of contraception; placement of an intrauterine device (IUD) or intrauterine system (IUS); barrier methods: condom with spermicidal foam/gel/film/cream/suppository or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/suppository; male partner sterilization (the vasectomized partner should be the sole partner for that subject); true abstinence (when this is in line with the preferred and usual lifestyle of the subject)

     Note: If the childbearing potential changes after start of the study (e.g., woman who is not heterosexually active becomes active) a woman must begin a highly

effective method of birth control, as described above.

Women must agree to continue using these methods of contraception throughout the study and for at least 3 months after receiving the last dose of study medication.

10. A woman of childbearing potential must have a negative serum (β-human chorionic gonadotropin [β-hCG]) at Screening and a negative urine pregnancy test prior to Period 1 randomization on Day 1.

11. A woman must agree not to donate eggs (ova, oocytes) for the purposes of assisted reproduction during the study and for at least 3 months after receiving the last dose of study drug.

12. A man who is sexually active with a woman of childbearing potential and has not had a vasectomy must agree to use a barrier method of birth control e.g., either condom with spermicidal foam/gel/film/cream/suppository or partner with occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/suppository, and all men must also not donate sperm during the study and for 3 months after receiving the last dose of study drug.

13. Subject must be willing and able to adhere to the prohibitions and restrictions specified in this protocol.

14. Each subject must sign an informed consent form (ICF) indicating that he or she understands the purpose of and procedures required for the study and are willing to participate in the study.

4.2. Exclusion Criteria

Any potential subject who meets any of the following criteria will be excluded from participating in the study.

1. Subject has a history of, or current signs and symptoms of, liver or renal insufficiency; significant cardiac, vascular, pulmonary, gastrointestinal, endocrine, neurologic, hematologic, rheumatologic, or metabolic disturbances.

    - Subjects with non-insulin dependent diabetes mellitus who are adequately controlled (not on insulin) may participate in the study.

2. Subject has uncontrolled hypertension (SBP > 160 mmHg or DBP > 90 mmHg) despite diet, exercise or a stable dose of a permitted anti-hypertensive treatment at Screening or Day 1 prior to Period 1 randomization; or any past history of hypertensive crisis.

3. Subject has alanine aminotransferase (ALT) and aspartate aminotransferase (AST) values ≥2 x the upper limit of normal.

4. Subject has a current diagnosis of bipolar or related disorders, intellectual disability, or cluster b personality disorder (e.g., borderline personality disorder, antisocial personality disorder, histrionic personality disorder, and narcissistic personality disorder).

5. Subject has a current or prior diagnosis of a psychotic disorder or MDD with psychosis.

6. Subject has suicidal ideation with intent to act during Screening phase or on Day 1 (prior to Period 1 randomization) based on the C-SSRS or per Investigator's clinical judgment, or has a history of suicidal behavior within the past year as assessed on the C-SSRS; or subject has homicidal ideation/intent at Screening or on Day 1.

7. Subject has a history of previous non-response of depressive symptoms to esketamine and/or ketamine.

8. Subject has not responded to treatment with electroconvulsive therapy in the current episode of depression.

9. Subject has any significant primary sleep disorder. If the subject has a score of 25 or greater on the Modified Berlin Questionnaire at Screening, obstructive sleep apnea must be ruled out. Subjects adequately treated for obstructive sleep apnea are not excluded.

10. Anatomical or medical conditions that may impede delivery or absorption of study medication (e.g., undergone facial reconstruction, significant structural or functional abnormalities of the nose or upper airway; obstructions or mucosal lesions of the nostrils or nasal passages; undergone sinus surgery in the previous 2 years; or signs and symptoms of rhinitis).

11. Has an abnormal or deviated nasal septum with any 1 or more of the following symptoms: blockage of 1 or both nostrils, nasal congestion (especially 1-sided), frequent nosebleeds, frequent sinus infections, and at times has facial pain, headaches, and postnasal drip.

12. Subject meets criteria for substance or alcohol use disorder, except tobacco or caffeine, according to DSM-5 criteria at Screening.

13. Subject has a positive test result(s) for drugs of abuse (including barbiturates, methadone, opiates, cocaine, cannabinoids, and amphetamine/methamphetamine) at Screening.

    - Subjects that have a positive test result at Screening due to prescribed opiates, barbiturates, or amphetamines may be permitted to continue the Screening phase if the prohibited medication is discontinued at least 1 week or 5 half-lives, whichever is longer, before the first dose of study medication. Provided the Day 1

predose test for drugs of abuse result is negative, the subject may be enrolled. Retesting is not permitted for positive test result(s) from non-prescription use of drugs of abuse.

14. Subject has a positive test result(s) for alcohol at Screening.

    - Subjects with a positive alcohol screen at Screening may have the test repeated once during the screening phase, based on the investigator's discretion. This determination, and the reason for permitting a repeat test, must be recorded in the subject's source documents and initialed by the investigator. A positive, repeat alcohol screen is exclusionary.

15. Subject has a history of malignancy within 5 years before screening (exceptions are squamous and basal cell carcinomas of the skin and carcinoma in situ of the cervix, or malignancy that in the opinion of the investigator, with concurrence with the sponsor's medical monitor, is considered cured with minimal risk of recurrence).

16. Subject has known allergies, hypersensitivity, intolerance, or contraindication to esketamine or its excipients (refer to Investigator's Brochure, product label).

17. Subject has taken any disallowed therapies as noted in Section 8, Pre-study and Concomitant Therapy before the specific time relative to the planned first dose of study drug.

18. Subject has received an investigational drug (including investigational vaccines) or used an invasive investigational medical device within 60 days before the planned first dose of study drug or is currently enrolled in an investigational study.

19. Subject is a woman who is pregnant, breast-feeding, or planning to become pregnant while enrolled in this study or within 3 months after the last dose of study drug.

20. Subject is a man who plans to father a child while enrolled in this study or within 3 months after the last dose of study drug.

21. Subject has any condition for which, in the opinion of the investigator, participation would not be in the best interest of the subject (e.g., compromise the well-being) or that could prevent, limit, or confound the protocol-specified assessments.

22. Subject has had major surgery, (e.g., requiring general anesthesia) within 2 weeks before screening, or will not have fully recovered from surgery, or has surgery planned during the time the subject is expected to participate in the study.

    Note: subjects with planned surgical procedures to be conducted under local anesthesia may participate.

23. Subject is an employee of the investigator or study site, with direct involvement in the proposed study or other studies under the direction of that investigator or study site, as well as family members of the employees or the investigator.

24. Subject has a history of human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg) or hepatitis C antibody (anti-HCV) positive, or other clinically active liver disease, or tests positive for HIV, HBsAg, or anti-HCV at Screening.

NOTE: Investigators should ensure that all study enrollment criteria have been met at screening. If a subject's status changes (including laboratory results or receipt of additional medical records) after screening but before the first dose of study drug is given such that he or she no longer meets all eligibility criteria, then the subject should be excluded from participation in the study. Section 17.4, Source Documentation, describes the required documentation to support meeting the enrollment criteria.

4.3. Prohibitions and Restrictions

Potential subjects must be willing and able to adhere to the following prohibitions and restrictions during the course of the study to be eligible for participation:

1. A woman of childbearing potential who is heterosexually active must remain on a highly effective method of birth control (see inclusion criteria).

2. A man who is sexually active with a woman of childbearing potential must use a double-barrier method of birth control (i.e., male condom, female diaphragm or cervical cap, or condom) and all men must also not donate sperm during the study and for 3 months after receiving the last dose of study drug.

3. Subjects will be instructed to refrain from blowing their nose for at least 1 hour after the last intranasal spray.

4. Subjects must abstain from using alcohol or prohibited drugs from Screening through to the end of the treatment phase (double-blind and optional open-label treatment phase, if applicable).

5. On all dosing days, all subjects must remain at the clinical site until at least 2 hours postdose (or longer if required for study procedures) and should be accompanied by a responsible adult when discharged from the clinical site. Subjects must not drive a car or work with machines for 24 hours after study medication dosing.

6. Subjects should not ingest grapefruit juice, Seville oranges, or quinine for 24 hours before an intranasal dose of esketamine is to be administered.

7. Refer to Section 8, Prestudy and Concomitant Therapies and Attachment 1 (Prohibited Therapies) for disallowed therapies during study participation.

8. Potent CYP3A4 inhibitors are not permitted within 1 week, or within a period less than 5 times the drug's half-life, whichever is longer, before the first dose of study medication and throughout the study

9. Potent CYP3A4 inducers are not permitted for 30 days prior to the first dose of study medication and throughout the study

10. Intranasally-administered decongestants are prohibited from 12 hours prior to each study medication administration.

5. TREATMENT ALLOCATION AND BLINDING

Treatment Allocation

Procedures for Randomization and Stratification

On Day 1 in Period 1, subjects will be randomly assigned to a treatment group based on the first of two computer-generated randomization schedule (Period 1 and Period 2) prepared before the study by or under the supervision of the sponsor. The randomization will be balanced by using randomly permuted blocks and will be stratified by study center with an allocation ratio of 3:1:1:1 to placebo and esketamine 28, 56, and 84 mg.

All subjects who complete Period 1 will receive a randomization number for Period 2 in order to maintain the blind. Those subjects who were randomly assigned to treatment with esketamine in Period 1 will continue to receive the same dose of intranasal esketamine in Period 2. Those subjects who were randomly assigned to placebo in Period 1 with a Day 8 (predose) QIDS-SR_{16} score of < 11 will continue to receive placebo in Period 2. Subjects who were randomly assigned to placebo in Period 1 with a Day 8 (predose) QIDS-SR_{16} score ≥11 will be re-randomized on Day 8 based on the second of the two computer-generated randomization schedules in a 1:1:1:1 ratio to either placebo or 28, 56, or 84 mg of esketamine. The second randomization will be balanced using randomly permuted blocks stratifying by study center and QIDS-SR_{16} [moderate (QIDS-SR_{16} score 11 to 16) or severe (QIDS-SR_{16} score > 16)].

Based on this randomization code, the study drug for the double-blind treatment phase will be packaged and labeled. Unique medication identification numbers will be preprinted on the study drug labels and assigned as subjects qualify for the study and are assigned to treatment.

Central randomization will be implemented in this study. The interactive voice response system (IVRS) will assign a unique treatment code, which will dictate the treatment assignment and matching study drug kit for the subject. The requestor must use his or her own user identification and personal identification number when contacting the IVRS, and will then give the relevant subject details to uniquely identify the subject.

Blinding

The investigator will not be provided with randomization codes. The codes will be maintained within the (IVRS), which has the functionality to allow the investigator to break the blind for an individual subject.

Data that may potentially unblind the treatment assignment (e.g., study drug plasma concentrations, study drug accountability data) will be handled with special care to ensure that the integrity of the blind is maintained and the potential for bias is minimized. This can include making special provisions, such as segregating the data in question from view by the investigators, clinical team, or others as appropriate until the time of database lock and unblinding.

Under normal circumstances, the blind should not be broken until all subjects have completed the study and the database is finalized. Otherwise, the blind should be broken only if specific emergency treatment/course of action would be dictated by knowing the treatment status of the subject. In such cases, the investigator may in an emergency determine the identity of the treatment by contacting the IVRS. It is recommended that the investigator contact the sponsor or its designee if possible to discuss the particular situation, before breaking the blind. Telephone contact with the sponsor or its designee will be available 24 hours per day, 7 days per week. In the event the blind is broken, the sponsor must be informed as soon as possible. The date, time, and reason for the unblinding must be documented by the IVRS, and in the source document. The documentation received from the IVRS indicating the code break must be retained with the subject's source documents in a secure manner.

In general, randomization codes will be disclosed fully only if the study is completed and the clinical database is closed. However, if an interim analysis is specified, the randomization codes and, if required, the translation of randomization codes into treatment and control groups will be disclosed to those authorized and only for those subjects included in the interim analysis.

In the optional open label treatment phase, blinding procedures are not applicable.

6. DOSAGE AND ADMINISTRATION

All doses of study medication will be self-administered under the direct supervision of the investigator or designee. Instructions for use of the intranasal device will be provided as a separate document.


Each individual device delivers 28 mg (i.e., 2 sprays).

The placebo solution will be provided as a clear, colorless intranasal solution of water for injection with a bittering agent (denatonium benzoate [Bitrex®] at a final concentration of 0.001 mg/mL) added to simulate the taste of the intranasal solution with active drug. Benzalkonium chloride is added as a preservative at a concentration of 0.3 mg/mL.

Food will be restricted for at least 2 hours before each administration of study medication. Drinking of water or any other permitted beverage will be restricted for at least 30 minutes before the first nasal spray.

Subjects will be instructed to refrain from blowing their nose for at least 1 hour after the last intranasal spray.

On all dosing days, all subjects must remain at the clinical site until at least 2 hours postdose (or longer if required for study procedures) and should be accompanied by a responsible adult when discharged from the clinical site. Subjects must not drive a car or work with machines for 24 hours after study medication dosing.

Double-blind treatment phase

Refer to the Section 3.1 (*Overview of Study Design- Double-Blind Treatment Phase*) for a description of the study design.

On each dosing day, all subjects will self-administer 1 spray into each nostril at t = 0, 5, and 10 minutes. Time 0 is defined as the time of the first 100-µL spray. Sprays to each nostril should be delivered in rapid succession at the scheduled time points (i.e., there should be no waiting between sprays to the right and left nostrils at each time point). Table 2 describes how each treatment will be administered in the double-blind treatment phase.

Table 2: Dose Administration in the Double-Blind Treatment Phase

Intranasal Treatment	Time of Administration (Time 0 is defined as the time of the first 100-µL spray)		
	0	**5 minutes**	**10 minutes**
Placebo	1 spray of placebo to each nostril	1 spray of placebo to each nostril	1 spray of placebo to each nostril
Esketamine 28 mg	1 spray of esketamine to each nostril	1 spray of placebo to each nostril	1 spray of placebo to each nostril
Esketamine 56 mg	1 spray of esketamine to each nostril	1 spray of esketamine to each nostril	1 spray of placebo to each nostril
Esketamine 84 mg	1 spray of esketamine to each nostril	1 spray of esketamine to each nostril	1 spray of esketamine to each nostril

Optional open-label treatment phase

Refer to the Section 3.1 (*Overview of Study Design- Optional Open Label Treatment Phase*) for a description of the study design.

Subjects will self-administer intranasal esketamine on Days 15, 18, 22, and 25.

Time 0 is defined as the time of the first 100-µL spray. Sprays to each nostril should be delivered in rapid succession at the scheduled time points (i.e., there should be no waiting between sprays in each nostril at each time point). Table 3 describes how each treatment will be administered in the optional open-label treatment phase.

Table 3: Dose Administration in Optional Open-Label Treatment Phase

Intranasal Treatment	Time of Administration (Time 0 is defined as the time of the first 100-µL spray)		
	0	5 minutes	10 minutes
Esketamine 28 mg	1 spray of esketamine to each nostril	-	-
Esketamine 56 mg	1 spray of esketamine to each nostril	1 spray of esketamine to each nostril	-
Esketamine 84 mg	1 spray of esketamine to each nostril	1 spray of esketamine to each nostril	1 spray of esketamine to each nostril

7. TREATMENT COMPLIANCE

The investigator or designated study-site personnel will maintain a log of all study drug dispensed and returned. Drug supplies for each subject will be inventoried and accounted for throughout the study.

8. PRESTUDY AND CONCOMITANT THERAPY

Prestudy therapies administered up to 30 days before screening must be recorded at screening.

Antidepressant therapies used in the current episode will be recorded on the ATRQ and as concomitant therapy(ies). In addition, any prior antidepressant treatment(s) that is known from the subject's psychiatric history or verbal report should also be recorded as prestudy therapy.

Concomitant therapies must be recorded throughout the study beginning with signing of the informed consent until the last follow up visit. Concomitant therapies should also be recorded beyond this time only in conjunction with new or worsening adverse events until resolution of the event.

All therapies (prescription or over-the-counter medications, including vaccines, vitamins, herbal supplements; non-pharmacologic therapies such as electrical stimulation, acupuncture, special diets, exercise regimens) different from the study drug must be recorded in the CRF. Modification of an effective preexisting therapy should not be made for the explicit purpose of entering a subject into the study.

Disallowed therapies:

- Refer to Attachment 1 for a list of prohibited therapies.
- Potent CYP3A4 inhibitors within 1 week, or within a period less than 5 times the drug's half-life, whichever is longer, before the first dose of study medication and throughout the study.

- Potent CYP3A4 inducers for 30 days prior to the first dose of study medication and throughout the study.
- Use of a disallowed therapy, including psychotropic medications, within 1 week or within a period less than 5 times the drug's half-life, whichever is longer, before the first dose of study medication throughout the end of the treatment phase(s).
- Any new psychotropic medication(s) started during screening, or any increase in the dose of a currently prescribed (allowed) psychotropic medication(s) during screening and throughout the study.
- Treatment with any MAO-inhibitor currently or within the past 2 weeks prior to Day 1 dosing and throughout the study.
- No benzodiazepines should be used within 8 hours prior to the start of the study drug administration.
- Intranasally-administered decongestants are prohibited from 12 hours prior to each study medication administration.

The sponsor must be notified in advance (or as soon as possible thereafter) of any instances in which prohibited therapies are administered.

Following completion of the double-blind treatment phase and the optional open label treatment phase (if applicable), subjects can be treated according to standard of care.

9. STUDY EVALUATIONS

9.1. Study Procedures

9.1.1. Overview

The Time and Events Schedule summarizes the frequency and timing of efficacy, pharmacokinetic, biomarker, pharmacogenomics, and safety measurements applicable to this study.

The total blood volume to be collected from each subject will be approximately 153 mL (Table 4).

Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.

Additional serum or urine pregnancy tests may be performed, as determined necessary by the investigator or required by local regulation, to establish the absence of pregnancy at any time during the subject's participation in the study.

Table 4: Approximate Volume of Blood to be Collected From Each Subject

Type of Sample	Volume (mL) per sample	Number of Samples per Subject	Total Volume of Blood (mL)[a]
Screening Phase			
Serum chemistry [b]	2.5	1	2.5
Hematology	2	1	2
Serology - HIV	3.5	1	3.5
Serology – HbsAg, HCV	2.5	1	2.5
Approximate total blood volume for screening phase			10.5
Double-Blind Treatment Phase			
Serum chemistry [d]	2.5	3	7.5
Total cholesterol	2.5	2	5
Hematology	2	3	6
Pharmacokinetic	4	7	28
Pharmacogenomic [c]	10	1	10
Biomarker: Inflammation MAP	10	3	30
Biomarker: Metabolomics	5	3	18
Approximate total blood volume for double-blind treatment phase			104.5
Optional Open Label Treatment Phase			
Serum chemistry	2.5	2	5
Total cholesterol	2.5	1	2.5
Hematology	2	2	4
Pharmacokinetic	4	3	12
Biomarker: Inflammation MAP	10	1	10
Approximate total blood volume for optional open-label treatment phase			33.5
Posttreatment Phase			
Serum chemistry [b]	2.5	1	2.5
Hematology	2	1	2
Approximate total blood volume for posttreatment phase			4.5
Approximate total blood volume for study			**153 mL**

[a] Calculated as number of samples multiplied by amount of blood per sample.
[b] Serum chemistry includes serum β-hCG pregnancy tests (for women of childbearing potential) and TSH.
[c] Required subject participation.
[d] On Day 14 (or Early Termination), serum chemistry includes serum β-hCG pregnancy tests (for women of childbearing potential).

Note: An indwelling intravenous cannula may be used for blood sample collection.

If multiple assessments are scheduled for the same timepoint, it is recommended that procedures be performed in the sequence provided by the Sponsor (Refer to Section 15, Study Specific Materials).

Patient reported outcome (PRO) assessments should be conducted/completed before any tests, procedures, or other consultations scheduled at the same timepoint to prevent influencing subject perceptions. Refer to Attachment 2 for further instructions on completion of patient reported outcomes.

If blood sampling or vital sign measurement is scheduled for the same time point as ECG recording, the procedures should be performed in the following order: ECG(s), vital signs, blood draw.

Blood collections for pharmacokinetic assessments should be kept as close to the specified time as possible. Other measurements may be done earlier than specified time points, if needed.

Actual dates and times of assessments will be recorded in the source documentation and CRF.

9.1.2. Screening Phase

Prior to conducting any study procedure, the investigator (or designated study personnel) will review and explain the written informed consent form (ICF) to each subject. After signing the ICF, the subject will be screened within 4 weeks of Day 1 (i.e., Day -28 to Day -1) to determine eligibility for study participation.

Subjects must be 18 to 64 years of age (inclusive) and meet DSM-5 diagnostic criteria for MDD, without psychotic features, based upon clinical assessment (DSM-5 296.22, 296.23, 296.32, or 296.33) and confirmed by the MINI. Subjects must have an IDS-C_{30} total score ≥34. In addition, the major depressive episode must be deemed valid using the SAFER criteria interview, administered by remote, independent raters.

Subjects must have had an inadequate response to at least 2 antidepressants, at least one of which is in the current episode of depression, assessed using the MGH-ATRQ and prior medication history.

- Subjects that are currently not taking an antidepressant at Screening are eligible to participate.
- Subjects taking an antidepressant at the Screening visit must have been receiving a stable dose (i.e., dose has been unchanged) for at least 2 weeks before the Screening visit. The subject can:
    - With the exception of MAO inhibitors, which are prohibited, the subject can continue the antidepressant at the same dose during the double-blind treatment phase, or
    - The subject can discontinue the antidepressant within 1 week, or within a period less than 5 times the drug's half-life (exception: at least 4 weeks for fluoxetine and at least 2 weeks for MAO inhibitors), whichever is longer, before the planned first dose of study drug.

The decision to continue or discontinue the current antidepressant will be made by the subject and investigator, based on their clinical judgment. Antidepressant medication will not be discontinued for the sole purpose of allowing subjects to participate in the study.

Disallowed therapies, including psychotropic medications, are described in the exclusion criteria, Section 8 (Prestudy and Concomitant Therapies), and Attachment 1.

All adverse events and special reporting situations, whether serious or non-serious, will be reported from the time a signed and dated ICF is obtained until completion of the subject's last study-related procedure (which may include contact for follow-up of safety).

Screening evaluations (e.g., biomarker, safety) will be performed as per the Time and Events Schedule.

It is recommended that the patient-reported outcomes and clinician-administered assessments at Screening are performed in the following sequence:

- Patient reported outcomes: Modified Berlin Questionnaire
- Clinician-administered: MINI, IDS-C_{30}, C-SSRS, ATRQ (in collaboration with subject)

9.1.3. Double-Blind Treatment Phase

All subjects will participate in two treatment periods (Period 1 and Period 2) during the double-blind treatment phase. Efficacy, safety, pharmacokinetic, biomarker, and/or pharmacogenomic evaluations performed at each visit are described in the Time and Events Schedule.

All subjects will self-administer the study medication in Period 1 and Period 2 under the direct supervision of the investigator or designee (Refer to Section 6, Dosage and Administration).

Refer to the Section 3.1 (*Overview of Study Design*) above for a description of the study design.

When multiple PRO and clinician-administered assessments are scheduled for the same time point it is recommended they be performed in the following sequence:

PRO: QIDS-SR_{16}, PGI-S, PGI-C, GAD-7, EQ-5D-5L

Clinician-administered: IDS-C_{30}, MADRS, CGI-S, C-SSRS, BPRS+, CADSS, MOAA/S, PWC-20

Subjects will be provided with a diary that includes the subject-completed assessments that are to be completed at home on Day 2 and 9 (i.e., telephone contact visit days). During each telephone contact, the site staff will remind the subject to complete their diary that day and bring it to their next clinic visit. The site staff will review the diary at each visit.

For information obtained via telephone contact, written documentation of the communication must be available for review in the source documents. During the telephone contact visits, adverse event and concomitant therapy information will be obtained. In addition, specified clinician-administered assessments will be performed by appropriately qualified staff.

Early Termination

If a subject withdraws before the end of the double-blind treatment phase, the Early Termination visit should be conducted at the time of discontinuation, followed by completion of the posttreatment phase.

9.1.4. Optional Open-Label Treatment Phase

On Day 15, following completion of the double-blind treatment phase, all subjects may participate in an optional open-label treatment phase. Subjects that discontinue early from the double-blind treatment phase are not eligible to participate in the optional open label treatment phase. The Investigator must agree and confirm the subject is eligible to participate based on his/her clinical judgment.

The efficacy, safety, and biomarker evaluations that will be performed at each visit during the optional open-label treatment phase are described in the Time and Event Schedule. Where specified in the Time and Events Schedule, study procedures performed on the last day of the double-blind treatment phase (Day 15) that are also required predose on Day 15 of the optional open-label treatment phase will only be performed once.

During the optional open-label treatment phase, subjects can receive up to 4 single doses of intranasal esketamine on Days 15, 18, 22, and 25.

Refer to the Section 3.1 (*Overview of Study Design*) above for a description of the study design.

If a subject withdraws before the end of the optional open-label treatment phase, an Early Termination visit is not required. The subject would continue into the posttreatment phase.

When multiple PRO and clinician-administered assessments are scheduled for the same time point, it is recommended they be performed in the following sequence:

PRO: QIDS-$SR_{16,}$ PGI-S, PGI-C, GAD-7, EQ-5D-5L

Clinician-administered: MADRS, CGI-S, C-SSRS, BPRS+, CADSS, MOAA/S

9.1.5. Posttreatment Phase (Follow-Up)

The efficacy and safety evaluations that will be performed at each visit during the posttreatment phase are described in the Time and Event Schedule.

Subjects will be provided with a diary that includes the subject-completed assessments that are to be completed at home as part of the first follow up visit (i.e., telephone contact visit 1 week after the last dose of study medication). During the telephone contact, the site staff will remind the subject to complete their diary that day and bring it to their next clinic visit. The site staff will review the diary at each subsequent visit.

All subjects who receive at least 1 dose of study medication should have 4 follow up visits conducted at 1, 2, 4, and 8 weeks after the last dose of study medication.

The follow up visits at 1, 4, and 8 weeks after the last dose of study medication will be telephone contacts. The follow up visit at 2 weeks after the last dose of study medication is a clinic visit.

For information obtained via telephone contact, written documentation of the communication must be available for review in the source documents. During the telephone contact visit, adverse

event and concomitant therapy information will be obtained. In addition, specified clinician-administered assessments will be performed by appropriately qualified staff.

Any clinically significant abnormalities persisting at the end of the study will be followed by the investigator until resolution or until a clinically stable endpoint is reached.

All adverse events and special reporting situations, whether serious or non-serious, will be reported until completion of the subject's last study-related procedure (which may include contact for follow-up of safety).

9.2. Efficacy

9.2.1. Evaluations

Every effort should be made to ensure that all clinician-administered efficacy assessments are completed by the same individual who made the initial baseline determinations.

9.2.1.1. Primary

The primary efficacy evaluation will be the MADRS total score.

The MADRS is a clinician-rated scale designed to measure depression severity and detects changes due to antidepressant treatment (Montgomery 1979). The test consists of 10 items, each of which is scored from 0 (item not present or normal) to 6 (severe or continuous presence of the symptoms), for a total possible score of 60. Higher scores represent a more severe condition. The MADRS evaluates apparent sadness, reported sadness, inner tension, sleep, appetite, concentration, lassitude, interest level, pessimistic thoughts, and suicidal thoughts. The test exhibits high inter-rater reliability.

The structured interview guide for the Montgomery Asberg Depression Rating Scale (SIGMA) will be used for each administration (Williams 2008). Using structured interview guides have previously been shown to increase the reliability of given scales.

The typical recall period for the MADRS is 7 days. In this study, the MADRS will also be administered using modified recall periods of 2 hours and 24 hours. For the recall period of 2 hours, the sleep and appetite items will not be assessed (predose scores for these items obtained on the same day will be carried forward).

9.2.1.2. Secondary

QIDS-SR$_{16}$

The QIDS-SR$_{16}$ is a patient reported measure designed to assess the severity of depressive symptoms (Rush 2003; Trivedi 2004). The QIDS-SR$_{16}$ assesses all the criterion symptom domains designated by the DSM-IV to diagnose a major depressive episode. This assessment can be used to screen for depression, although it has been used predominantly as a measure of symptom severity.

Paper and pen format will be used for this study. Subjects provide responses to each item of this instrument with a 4-point Likert scale, with scores ranging from 0 to 3 for each item. The 7-day period prior to assessment is the usual recall period for assessing symptom severity.

The scoring system of the QIDS converts responses to the 16 separate items into the nine DSM-IV symptom criterion domains. The nine domains comprise 1) sad mood; 2) concentration; 3) self-criticism; 4) suicidal ideation; 5) interest; 6) energy/fatigue; 7) sleep disturbance (initial, middle, and late insomnia or hypersomnia); 8) decrease or increase in appetite or weight; and 9) psychomotor agitation or retardation. The total score is obtained by adding the scores for each of the nine symptom domains of the DSM-IV MDD criteria (Rush 2003): 4 items are used to rate sleep disturbance (early, middle, and late insomnia plus hypersomnia); 2 items are used to rate psychomotor agitation and retardation; 4 items are used to rate appetite (increase or decrease and weight increase or decrease). One item is used to rate the remaining 6 domains (sad mood, interest, energy/fatigue, self-criticism, concentration, and suicidal ideation). For symptom domains that require more than one item, the highest score of the item relevant for each domain is taken. For example, if early insomnia is 0, middle insomnia is 1, late insomnia is 3, and hypersomnia is 0, the sleep disturbance domain is rated 3. The total score ranges from 0 to 27. Using a scale of severity of depression of none, mild, moderate, severe, and very severe, corresponding QIDS-SR_{16} total scores are none 1-5, mild 6-10, moderate 11-16, severe 17-20 and very severe 21-27.

The QIDS-SR_{16} is sensitive to change, with medications, psychotherapy, or somatic treatments. The psychometric properties of both the QIDS-SR_{16}, has been established in various study samples, and is outlined on the developer's website (www.ids-qids.org).

CGI-S

The CGI-S is a clinician-rated scale that is designed to rate the severity of the subject's illness at the time of assessment, relative to the clinician's past experience with subjects who have the same diagnosis and improvement with treatment (Guy 1976). Considering total clinical experience, a subject is assessed on severity of mental illness at the time of rating according to: 0= not assessed; 1=normal (not at all ill); 2=borderline mentally ill; 3=mildly ill; 4=moderately ill; 5=markedly ill; 6=severely ill; 7=among the most extremely ill patients.

PGI-S

The PGI-S is a 4-point scale that requires the subject to rate the severity of their illness at the time of assessment, relative to the subject's past experience. Considering their total experience, the subject assesses the severity of their depression illness at the time of rating as none, mild, moderate, or severe. Paper and pen format will be used for this study.

GAD-7

The GAD-7 is a validated, brief 7-item self-report assessment of anxiety. Each item is scored on a 4-point scale (0-3), with a total score range of 0-30 (Spitzer 2006). The standard recall period used is 2 weeks, but in the current study we plan to use a 7-day recall.

9.2.1.3. Exploratory

PGI-C

The PGI-C is a 7-point scale that requires the subject to assess how much their illness has improved or worsened relative to a baseline state at the beginning of the intervention. The response options are: very much improved; much improved; improved (just enough to make a difference); no change; worse (just enough to make a difference); much worse; or very much worse. Paper and pen format will be used for this study

EQ-5D-5L

The EQ-5D-5L is a standardized 2-part instrument for use as a measure of health outcome, primarily designed for self-completion by respondents. It essentially consists of the EQ-5D descriptive system and the EQ visual analogue scale (EQVAS). The EQ-5D-5L descriptive system comprises the following 5 dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Each dimension has 5 levels: no problems, slight problems, moderate problems, severe problems, and extreme problems. The descriptive system can be represented as a health state. The EQ VAS self-rating records the respondent's own assessment of their health status. Subjects select an answer for each of the 5 dimensions considering the response that best matches their health "today". Paper and pen format will be used for this study.

9.2.2. Endpoints

9.2.2.1. Primary Endpoint

The primary efficacy evaluation will be the MADRS total score as measured by the change from baseline (i.e., Day 1 predose; Day 8 predose) to the 1-week endpoint (i.e., Day 8, predose; Day 15) for the combined periods in the double-blind treatment phase.

9.2.2.2. Secondary Endpoints

- Proportion of subjects with sustained response (≥50% reduction from baseline in MADRS total score) with onset by Day 2 through the end of the double-blind phase (Day 15)
- Proportion of responders (≥50% reduction from baseline in MADRS total score) at each visit
- Proportion of subjects in remission (MADRS ≤10) at each visit
- Change from baseline (i.e., Day 1 predose; Day 8 predose) to the 1-week endpoint (i.e., Day 8, predose; Day 15) for the combined periods in the double-blind treatment phase in subject-reported depressive symptoms using the QIDS-SR_{16}.
- Change from baseline (i.e., Day 1 predose; Day 8 predose) to the 1-week endpoint (i.e., Day 8, predose; Day 15) for the combined periods in the double-blind treatment phase in severity of illness using the CGI-S.
- Change from baseline (i.e., Day 1 predose; Day 8 predose) to the 1-week endpoint (i.e., Day 8, predose; Day 15) for the combined periods in the double-blind treatment phase in severity of illness using the PGI-S.

- Change from baseline (i.e., Day 1 predose; Day 8 predose) to the 1-week endpoint (i.e., Day 8, predose; Day 15) for the combined periods in the double-blind treatment phase in anxiety symptoms, as measured by the GAD-7

9.3. Pharmacokinetics

9.3.1. Evaluations

Venous blood samples of approximately 4 mL will be collected for measurement of plasma concentrations of esketamine and noresketamine and other metabolites (if warranted) at the time points specified in the Time and Events Schedule. The exact dates and times and pharmacokinetic blood sampling must be recorded.

The plasma concentrations of 4β-hydroxycholesterol will be measured in select PK samples as a probe to assess the potential for repeated administration of intranasal esketamine to induce hepatic cytochrome P450 3A4 enzyme activity. Total cholesterol will be measured in a separate blood sample at these same time points to ensure that changes in 4β-hydroxycholesterol are not attributable to changes in total cholesterol.

Based on the Time and Events Schedule, plasma samples will be divided into 2 aliquots (pharmacokinetics, back-up) or 3 aliquots (pharmacokinetics, a back-up, and 4β-hydroxycholesterol).

9.3.2. Analytical Procedures

Plasma samples will be analyzed to determine concentrations of esketamine and noresketamine using a validated, achiral LC-MS/MS method by or under the supervision of the sponsor.

The concentrations of 4β-hydroxycholesterol will be measured in select plasma samples using a qualified method.

If required, some plasma samples may be analyzed to document the presence of circulating metabolites using a qualified research method. In addition, plasma PK samples may be stored for future analysis of the metabolite profile.

The bioanalytical report, including a description of the assay and a summary of the assay performance data, will be included in the final study report as an addendum.

9.3.3. Pharmacokinetic Parameters

The plasma concentration-time data of esketamine and noresketamine will be analyzed using population pharmacokinetic modeling. Typical population values of basic pharmacokinetic parameters will be estimated together with the inter-individual variability. Effects of subject demographics, laboratory parameter values, and other covariates on the pharmacokinetics of esketamine will be explored. The results of the population pharmacokinetic analyses may be reported separately.

9.4. Pharmacokinetic/Pharmacodynamic Evaluations

The relationship between MADRS total score (and possibly selected adverse events as additional pharmacodynamic parameters) and pharmacokinetic metrics of esketamine may be evaluated. If there is any visual trend in graphical analysis, suitable models will be applied to describe the exposure-effect relationships. The results of this analysis will be presented in a separate report.

9.5. Biomarkers

Human Inflammation Multi-Analyte Panel (MAP)

Novel multiplex immunoassay platforms allow simultaneous and reliable quantification of several different protein markers in peripheral biological samples. Such techniques have been successfully used to discover novel diagnostic and prognostic biomarkers in patients suffering from different medical conditions, such as coronary artery disease, ovarian cancer, Alzheimer's disease, and schizophrenia (Muller 2011; Schwarz 2011). Inflammatory peripheral markers have been indicated as novel potential diagnostic and prognostic markers in patients with MDD and as possible modulators of NMDA receptor function (Li 2011; Muller 2011).

Blood (serum) samples will be collected to allow for the exploratory pharmacodynamic evaluation using the Human Inflammation MAP, a panel of 45 proteins involved in key biologically inflammatory and neurotrophic pathways.

Metabolomics

Metabolomics is a rapidly emerging technique which captures the metabolic concentration of small molecules (i.e., metabolome) present in biological samples. Studies performed in patients with different CNS disorders have shown that metabolomics might be a promising tool to discover novel biomarkers for drug exposure, safety, and response.

Recently, it has been shown that pretreatment plasma levels of glycine are significantly different in depressed patients who respond to citalopram compared to non-responders, and that glycine levels might also predict response to drugs which target the glutamatergic system (Ji 2011).

Blood (plasma) samples will be collected to allow for the exploratory analysis of novel predictive and drug exposure biomarkers. The goals are to investigate whether pretreatment glycine levels predict antidepressant response and whether this effect is present only in subjects who respond to ketamine and not to placebo. An additional goal of this is research is to try to identify a glutamatergic metabolic "signature" which might help characterizing the acute and late biological effects of esketamine in patients with depression.

Stopping Analysis

Biomarker analyses are dependent upon the availability of appropriate biomarker assays and clinical response rates. Biomarker analysis may be deferred or not performed, if during or at the end of the study, it becomes clear that the analysis will not have sufficient scientific value for biomarker evaluation, or if there are not enough samples or responders to allow for adequate

biomarker evaluation. In the event the study is terminated early or shows poor clinical efficacy, completion of biomarker assessments is based on justification and intended utility of the data.

9.6. Pharmacogenomic (DNA) Evaluations

DNA samples will be analyzed for the *CPY2B6* gene. Additional analyses may be conducted if it is hypothesized that this may help resolve issues with the clinical data.

DNA samples will be used for research related to esketamine or depression. They may also be used to develop tests/assays related to esketamine and depression. Pharmacogenomic research may consist of the analysis of one or more candidate genes or of the analysis of genetic markers throughout the genome (as appropriate) in relation to esketamine or depression clinical endpoints.

9.7. Safety Evaluations

Any clinically relevant changes occurring during the study must be recorded on the Adverse Event section of the CRF.

Any clinically significant abnormalities persisting at the end of the study/early withdrawal will be followed by the investigator until resolution or until a clinically stable endpoint is reached.

The study will include the following evaluations of safety and tolerability according to the time points provided in the Time and Events Schedule:

Adverse Events

Adverse events will be reported by the subject (or, when appropriate, by a caregiver, surrogate, or the subject's legally acceptable representative) for the duration of the study. Adverse events will be followed by the investigator as specified in Section 12, Adverse Event Reporting.

Clinical Laboratory Tests

Blood samples for serum chemistry and hematology and a random urine sample for urinalysis will be collected. The investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the adverse event section of the CRF.

The use of local laboratories is allowed in cases where initiation of treatment or safety follow-up is time-critical and the central laboratory results are not expected to be available before the need to begin dosing or if actions need to be taken for safety reasons.

The following tests will be performed by the central laboratory, unless noted otherwise:

- Hematology Panel

  -hemoglobin
  -hematocrit
  -red blood cell (RBC) count
  -white blood cell (WBC) count with differential
  -platelet count

- Serum Chemistry Panel

-sodium	-alkaline phosphatase
-potassium	-creatine phosphokinase (CPK)
-chloride	
-bicarbonate	
-blood urea nitrogen (BUN)	-calcium
-creatinine	- phosphate
-glucose	-albumin
-aspartate aminotransferase (AST)	-total protein
-alanine aminotransferase (ALT)	-total cholesterol**
-gamma-glutamyltransferase (GGT)	
-total bilirubin	***At scheduled time points*

- Urinalysis

Dipstick	Sediment (if dipstick result is abnormal)]
-specific gravity	-red blood cells
-pH	-white blood cells
-glucose	-epithelial cells
-protein	-crystals
-blood	-casts
-ketones	-bacteria
-bilirubin	
-urobilinogen	
-nitrite	
-leukocyte esterase	

If dipstick result is abnormal, flow cytometry or microscopy will be used to measure sediment. In case of discordance between the dipstick results and the flow cytometric results, the sediment will be examined microscopically.

Specific gravity, pH, glucose, protein, blood, ketones, bilirubin, and urobilinogen will be determined using a dipstick. Red blood cells, white blood cells, epithelial cells, crystals, casts, and bacteria will be measured using flow cytometry or microscopy. If there is discordance between the dipstick results and the flow cytometric results, the sediment will be examined microscopically.

- Serum and Urine Pregnancy Testing (for women of childbearing potential only)
- Serology (HIV antibody, hepatitis B surface antigen [HBsAg], and hepatitis C virus antibody)
- Urine Drug Screen: barbiturates, methadone, opiates, cocaine, cannabinoids, amphetamine/methamphetamine, and benzodiazepines
- Alcohol test (urine or breath, as specified)
- Thyroid-stimulating hormone (TSH)
- Free thyroxine (FT4), if required for eligibility decision (See Section 4.1, Inclusion Criteria)

Electrocardiogram (ECG)

Single 12-lead ECG

During the collection of ECGs, subjects should be in a quiet setting without distractions (e.g., television, cell phones). Subjects should rest in a supine position for at least 5 minutes before ECG collection and should refrain from talking or moving arms or legs.

If blood sampling or vital sign measurement is scheduled for the same time point as ECG recording, the procedures should be performed in the following order: ECG(s), vital signs, blood draw.

All ECG tracings will be sent to a central ECG laboratory. The ECGs will be read at the scheduled time points and summarized by a central ECG laboratory. The central ECG laboratory will send the sponsor an electronic copy of the data for inclusion in the clinical database. In addition, the investigator or sub-investigator is required to review all ECGs at the study visit to assess for any potential safety concerns or evidence exclusionary conditions prior to dosing.

Vital Signs (tympanic temperature, pulse/heart rate, respiratory rate, blood pressure

Blood pressure and pulse/heart rate measurements will be assessed supine with a completely automated device. Manual techniques will be used only if an automated device is not available.

Blood pressure and pulse/heart rate measurements should be preceded by at least 5 minutes of rest in a quiet setting without distractions (eg, television, cell phones).

Pulse Oximetry

Pulse oximetry will be used to measure arterial oxygen saturation (SpO_2). On each dosing day, the device will be attached to the finger, toe, or ear at approximately 5 minutes before the first nasal spray and will be monitored for approximately 1 hour postdose. Values will be recorded before the first spray and at defined time points thereafter. Any arterial oxygen saturation (SpO_2) lower than 91% and lasting for more than 2 minutes, and confirmed by an additional manual measurement on another part of the body, will be reported as an adverse event.

Physical Examination, Height, and Body Weight

Physical examinations, body weight, and height will be performed/measured as per the Time and Events Schedule.

Targeted Nasal Examinations and Nasal Tolerability Questionnaire

Targeted nasal examinations (including the upper respiratory tract/throat) will be conducted by a qualified healthcare practitioner. The objective of the examination at Screening is to rule out any subjects with anatomical or medical conditions that may impede drug delivery or absorption.

Subsequent examinations will consist of a visual inspection of the nostrils, nasal mucosa, and throat for nasal erythema, rhinorrhea, rhinitis, capillary/blood vessel disruption and epistaxis and

graded as follows: none, mild, moderate, or severe. Any treatment emergent change or worsening from baseline examination will be recorded as an adverse event.

In addition, subjects will be asked to complete a nasal tolerability questionnaire.

C-SSRS

The C-SSRS will be performed to assess suicidal ideation and behavior.

The C-SSRS is a low-burden measure of the spectrum of suicidal ideation and behavior that was developed in the National Institute of Mental Health Treatment of Adolescent Suicide Attempters Study to assess severity and track suicidal events through any treatment (Posner 2007). It is a clinical interview providing a summary of both ideation and behavior that can be administered during any evaluation or risk assessment to identify the level and type of suicidality present. The C-SSRS can also be used during treatment to monitor for clinical worsening.

CADSS

The CADSS will be administered to assess treatment-emergent dissociative symptoms.

The CADSS is an instrument for the measurement of present-state dissociative symptoms (Bremner 1998).

The CADSS comprises 23 subjective items, divided into 3 components: depersonalization (items 3 to 7, 20, 23), derealization (items 1, 2, 8 to 13, 16 to 19, 21) and amnesia (items 14 and 15, 22). Participant's responses are coded on a 5-point scale (0 = "Not at all" through to 4 = "Extremely"). CADSS has excellent inter-rater reliability and internal consistency of the CADSS.

BPRS+

Four items of the BPRS will be administered to assess treatment-emergent psychotic symptoms.

The BPRS (Overall 1962) is an 18-item rating scale which is used to assess a range of psychotic and affective symptoms rated from both observation of the subject and the subject's own report. It reportedly provides a rapid and efficient evaluation of treatment response in clinic drug studies and in clinical settings (Rugani 2012).

Only the 4-item positive symptom subscale (consisting of: suspiciousness, hallucinations, unusual thought content, and conceptual disorganization) will be used in this study. It is highly sensitive to change, and excellent inter-rater reliability can be achieved with training and a standard interview procedure.

MOAA/S

The MOAA/S will be used to measure treatment-emergent sedation with correlation to levels of sedation defined by the American Society of Anesthesiologists continuum as described previously (Pambianco 2011).

PWC-20

The PWC-20 will be administered to assess potential withdrawal symptoms following cessation of intranasal esketamine treatment.

The PWC-20 is a 20-item simple and accurate method to assess potential development of discontinuation symptoms after stopping of study medication. The PWC-20 is a reliable and sensitive instrument for the assessment of discontinuation symptoms (Rickels 2008). Discontinuation symptoms occur early and disappear rather swiftly, depending upon speed of taper, daily medication dose, and drug elimination half-life.

9.8. Other Evaluations

MINI

The Mini-International Neuropsychiatric Interview (M.I.N.I.) is a short structured diagnostic interview for DSM-IV and ICD-10 psychiatric disorders. It has an administration time of approximately 15 minutes to provide accurate structured psychiatric interview for multicenter clinical trials.

MGH-ATRQ

The MGH-ATRQ is used to determine treatment resistance in major depressive disorder (Desseilles 2011). The MGH-ATRQ examines the adequacy of duration and dose of current antidepressant treatments in a step-by-step procedure. In addition, the MGH-ATRQ assesses the degree of improvement (in the most efficacious trial or in all trials during the current episode, depending on the version of the instrument) on a scale from 0% (not improved at all) to 100% (completely improved). The ATRQ is completed by the clinician in collaboration with the subject.

Modified Berlin Questionnaire

The Modified Berlin Questionnaire (Netzer 1999) is a 7-item patient-reported measure that is intended to identify the occurrence of risk factors for obstructive sleep apnea. It has items to address snoring frequency and loudness, pauses in breathing, sleepiness and hypertension. Body mass index is also a part of the measure which is calculated, using a provided algorithm, from height and weight. The total score is obtained by adding the score associated with each response option selected by the subject. Score range is from 0 to 46 with scores greater than 25 indicating a high probability of obstructive sleep apnea.

SAFER Criteria Interview

Remote, independent psychiatrists/psychologists will perform the SAFER Interview (Targum 2008) for all subjects to assess the validity of a diagnosis of depression and eligibility for the study.

SAFER refers to:

S =	State versus trait	The identified symptoms must reflect the current state of illness and not longstanding traits. Traits do not generally change in 4–12 weeks.
A =	Assessibility	The patient's symptoms are measurable with standard, reliable rating instruments. The symptoms of valid patients can be reliably assessed with standardized measurement tools
F =	Face validity	The patient's presentation is consistent with our knowledge of the illness (symptoms map to the nosological entity; clear change from previous level of function; similar to previous episodes if recurrent)
E =	Ecological validity	The patient's symptoms reflect the characteristics of the illness in a real-world setting (frequency, intensity, duration, course, impact over at least 4 weeks)
R =	Rule of the Three P's	Identified symptoms must be pervasive, persistent, and pathological and interfere with function and quality of life

The interviewer will review subject screening information and conduct a live, remote interview with the subject. The MADRS and SAFER Criteria Inventory will be administered during the interview. The MGH-ATRQ performed at Screening will be reviewed. After the interview, the site will receive information regarding subject eligibility directly from the interviewer.

IDS-C_{30}

The 30-item IDS-C_{30} (Rush 1996) is designed to assess the severity of depressive symptoms. The IDS assesses all the criterion symptom domains designated by the DSM-IV to diagnose a major depressive episode. These assessments can be used to screen for depression, although they have been used predominantly as measures of symptom severity. The 7-day period prior to assessment is the usual time frame for assessing symptom severity. The psychometric properties of the IDS-C_{30} have been established in various study samples (Trivedi 2004).

9.9. Sample Collection and Handling

The actual dates and times of sample collection must be recorded in the CRF or laboratory requisition form. If blood samples are collected via an indwelling cannula, an appropriate amount (1 mL) of serosanguineous fluid slightly greater than the dead space volume of the lock will be removed from the cannula and discarded before each blood sample is taken. After blood sample collection, the cannula will be flushed with 0.9% sodium chloride, United States Pharmacopeia (USP) (or equivalent) and charged with a volume equal to the dead space volume of the lock.

Refer to the Time and Events Schedule for the timing and frequency of all sample collections.

Instructions for the collection, handling, storage, and shipment of samples are found in the laboratory manual that will be provided. Collection, handling, storage, and shipment of samples must be under the specified, and where applicable, controlled temperature conditions as indicated in the laboratory manual.

10. SUBJECT COMPLETION/WITHDRAWAL

10.1. Completion

A subject will be considered to have completed the study if he or she has completed assessments at Day 15 of the double-blind treatment phase. Subjects who prematurely discontinue study treatment for any reason before completion of the double-blind phase will not be considered to have completed the study.

10.2. Withdrawal From the Study

A subject will be withdrawn from the study for any of the following reasons:

- Lost to follow-up
- Withdrawal of consent
- Lack of efficacy
- Violation of protocol procedures, as per Investigator’s judgment
- The blind is broken by the Investigator
- Discontinuation of study treatment. A subject's study treatment will be discontinued if:
  - The investigator or sponsor believes (e.g., that for safety or tolerability reasons such as an adverse event) it is in the best interest of the subject to discontinue treatment
  - The subject becomes pregnant
  - If a subject misses more than two doses of study medication during the double-blind treatment phase

If a subject discontinues study treatment before the end of the double-blind treatment phase, early termination and posttreatment assessments should be obtained.

If a subject is lost to follow-up, every reasonable effort must be made by the study site personnel to contact the subject and determine the reason for discontinuation/withdrawal. The measures taken to follow up must be documented.

When a subject withdraws before completing the study, the reason for withdrawal is to be documented in the CRF and in the source document. Study drug assigned to the withdrawn subject may not be assigned to another subject. If a subject withdraws from the study before the end of the double-blind treatment phase, early termination and posttreatment assessments should be obtained.

Withdrawal From the Use of Samples in Future Research

The subject may withdraw consent for use of samples for research (refer to Section 16.2.5, Long-Term Retention of Samples for Additional Future Research). In such a case, samples will be destroyed after they are no longer needed for the clinical study. Details of the sample retention for research are presented in the main ICF.

11. STATISTICAL METHODS

Statistical analysis will be done by the sponsor or under the authority of the sponsor. A general description of the statistical methods to be used to analyze the efficacy and safety data is outlined below. Specific details will be provided in the Statistical Analysis Plan.

11.1. Subject Information

Intent-to-Treat Analysis Set: For each period in the double-blind treatment phase, an intent-to-treat (ITT) analysis set will be defined to include all randomized subjects who receive at least one dose of study drug and have both the baseline and at least one post baseline MADRS total score within that period. The efficacy analyses of data in Period 1 and Period 2 will be based on each respective ITT analysis set.

Safety Analysis Set: The primary population for safety consists of all subjects who receive at least one dose of double-blind medication within that period. For the optional open-label treatment phase, the safety analysis set will be defined to include all subjects who receive at least 1 dose of study drug within that period. The same analyses of safety and tolerability will be conducted for the double-blind Period 1 and Period 2 separately, as well as the optional open-label treatment phase.

11.2. Sample Size Determination

The sample size is determined based on the following treatment differences between intranasal esketamine and placebo for the mean change from baseline in MADRS total score: a 9 point treatment difference was assumed for Period 1 (Day 8), a 7 point treatment difference for Period 2 (Day 15) was assumed for subjects with a moderate QIDS-SR_{16} score (QIDS-SR_{16} score = 11 to 16) and a 9 point treatment difference for Period 2 (Day 15) was assumed for subjects with a severe QIDS-SR_{16} score (QIDS-SR_{16} score >16). Based on results of a previous esketamine IV study (ESKETIVRD2001), it is estimated that 40% of placebo subjects at the end of Period 1 (Day 8 predose) will have a moderate QIDS-SR_{16} score and 55% will have a severe QIDS-SR_{16} score. Additional assumptions for the sample size calculation included a standard deviation of 10, 92.5% power for the combined data from both Day 8 and Day 15, an overall 1-sided significance level of 0.05, and a 5% drop-out rate for Period 1. It is calculated that this doubly-randomized, outcome based design will require 60 subjects to be randomly assigned to treatment on Day 1 in a 3:1:1:1 ratio (30 subjects on placebo and 10 subjects per intranasal esketamine dose group).

11.3. Efficacy Analyses

Efficacy analyses will be based on the combination of efficacy data from the two periods of the double-blind treatment phase, unless specified otherwise.

Primary Endpoint

For the primary efficacy analysis, change from baseline (Day 1 predose) in MADRS total score to Day 8 predose assessment of the double-blind treatment phase will be analyzed using an analysis of covariance (ANCOVA) model, with factors for treatment, center, and Period 1 baseline MADRS total score as the continuous covariate. Data from all randomized, treated subjects with change values during Period 1 will be included in the analysis of Period 1. Change from baseline in MADRS total score in Period 2 (Day 8, predose to Day 15) will be analyzed using an ANCOVA model with factors for treatment, center, Period 2 baseline QIDS-SR_{16} score (moderate or severe), and Period 2 baseline MADRS total score as the continuous covariate. Only data from Period 1 placebo subjects who are re-randomized (moderate and severe QIDS-SR_{16} scores) who continue into Period 2 and have a change value during Period 2 will be included in the analysis of Period 2. The comparison of intranasal esketamine dose groups with placebo will be carried out by a combined test on the weighted test statistics for each period in the double-blind treatment phase. The 'adaptive' weight will be based on the actual sample sizes for the final analysis (Liu 2012). Further details of the dose response analysis will be presented in the Statistical Analysis Plan (SAP).

Descriptive statistics for values and changes from baseline will be provided at each time point within each period of the double-blind treatment phase.

Secondary Endpoints

For all continuous endpoints descriptive statistics of actual values and changes from baseline by treatment group within each period will be provided. The change from baseline for QIDS-SR_{16} total score, CGI-S, PGI-S, and GAD-7 will be analyzed in the same way as for the MADRS total score. There will be no adjustments for multiplicity in the evaluation of these other efficacy endpoints.

A frequency table for the number and percentage of subjects meeting criteria for sustained response (≥50% reduction from baseline in MADRS total score with onset by Day 2 through the end of the double-blind phase (Day 15) will be provided for subjects who remain on the same treatment for the duration of the double-blind phase. Frequency tables for the number and percentage of subjects meeting criteria for response (≥50% reduction from baseline in MADRS total score) and remission (MADRS total score of ≤10) will be provided at each time point.

Descriptive statistics for values and changes from baseline for the MADRS total score will be provided for the group of subjects who are randomized at Period 1 to either intranasal esketamine or placebo and remain on the same treatment for the duration of the double-blind treatment phase (Day 15 or early withdrawal). Placebo subjects who are re-randomized in Period 2 and receive esketamine will not be included in these summaries.

Efficacy data from the open-label phase will be summarized descriptively.

Details of the exploratory analyses will be provided in the Statistical Analysis Plan.

11.4. Pharmacokinetic Analyses

Plasma esketamine, noresketamine, 4β-hydroxycholesterol, and cholesterol concentrations will be listed for all subjects by esketamine treatment and study day. All concentrations below the lowest quantifiable concentration in a sample or missing data will be labeled as such in the concentration data presentations. Concentrations below the lower quantifiable concentration will be treated as zero in the summary statistics. Subjects will be excluded from the pharmacokinetic analysis if their data do not allow for accurate assessment (e.g., incomplete administration of the study drug; missing information of dosing and sampling times). All subjects and samples excluded from the analysis will be clearly documented in the study report.

Descriptive statistics will be used to summarize esketamine, noresketamine, 6β-hydroxycholesterol, and cholesterol concentrations at each sampling time point. For each esketamine treatment and day, descriptive statistics, including arithmetic mean, SD, coefficient of variation, median, minimum, and maximum will be calculated for each analyte at each sampling time.

Population PK analysis of plasma concentration-time data of esketamine will be performed using nonlinear mixed-effects modeling. Data may be combined with those of other selected studies to support a relevant structural model. Available baseline subject characteristics (demographics, laboratory variables, genotypes, race, etc.) will be tested as potential covariates affecting PK parameters. Details will be given in a population PK analysis plan and the results of the population PK analysis will be presented in a separate report.

11.5. Pharmacokinetic/Pharmacodynamic Analyses

The relationship between MADRS score (and possibly selected adverse events as additional pharmacodynamic parameters) and PK metrics of esketamine may be evaluated. If there is any visual trend in graphical analysis, suitable models will be applied to describe the exposure-effect relationships. The results of this analysis would be presented in a separate report.

11.6. Biomarker Analyses

Metabolomics (if analyzed)

Spearman rank correlation coefficients between pretreatment glycine levels and the MADRS percentage change from Day 1 (baseline) at all scheduled time points will be calculated to investigate whether pretreatment levels of glycine correlate with the magnitude of clinical change following the administration of intranasal esketamine or placebo. Changes in glutamate metabolic pathway markers induced by esketamine or placebo will be investigated using a pattern classifier algorithm. Samples from different studies on ketamine and esketamine in treatment resistant depression will be pooled for analysis.

Human Inflammation MAP (if analyzed)

Statistical analyses of the markers from the Human Inflammation MAP will use both a univariate and a multivariate approach to identify the least number of markers which yield the highest accuracy in separating responders from non-responders to intranasal esketamine. A similar approach will be used to identify the pharmacodynamic effects of esketamine and placebo on inflammatory and neurotrophic markers.

Results of Human Inflammation MAP and metabolomics will be presented in a separate report.

11.7. Pharmacogenomic Analyses

A composite genotype and predicted phenotype will be derived from the raw genotyping data for *CYP2B6*. Allele and genotype frequencies will be tabulated. No formal statistical tests will be performed. Genetic results from other analyzed genes will be pooled together with data from other suitable studies for a meta-analysis.

Results of the pharmacogenomic analysis will be listed and summarized with other clinical studies in a separate pharmacogenomics report.

Results will be presented in a separate report.

11.8. Safety Analyses

Adverse Events

The verbatim terms used in the CRF by investigators to identify adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). All reported adverse events with onset during the treatment phase (i.e., treatment-emergent adverse events, and adverse events that have worsened since baseline) will be included in the analysis. For each adverse event, the percentage of subjects who experience at least 1 occurrence of the given event will be summarized by treatment group.

Summaries, listings, datasets, or subject narratives may be provided, as appropriate, for those subjects who die, who discontinue treatment due to an adverse event, or who experience a severe or a serious adverse event.

Clinical Laboratory Tests

Laboratory data will be summarized by type of laboratory test. Reference ranges and markedly abnormal results (specified in the Statistical Analysis Plan) will be used in the summary of laboratory data. Descriptive statistics will be calculated for each laboratory analyte at baseline and at each scheduled time point.

Electrocardiogram (ECG)

The effects on cardiovascular variables will be evaluated using descriptive statistics and frequency tabulations. These tables will include observed values and changes from baseline values.

The ECG variables that will be analyzed are heart rate, PR interval, QRS interval, QT interval, and corrected QT (QTc) interval using the following correction methods: QT corrected according to Bazett's formula (QTcB), and QT corrected according to Fridericia's formula (QTcF).

Descriptive statistics of QTc intervals and changes from baseline will be summarized at each scheduled time point. The percentage of subjects with QTc interval >450 ms, >480 ms, or >500 ms will be summarized, as will the percentage of subjects with QTc interval increases from baseline >30 ms or >60 ms.

All important abnormalities in ECG waveform that are changes from the baseline readings will be reported (e.g., changes in T-wave morphology or the occurrence of U-waves.

Vital Signs

Descriptive statistics of temperature, pulse/heart rate, respiratory rate, pulse oximetry, and blood pressure (systolic and diastolic) (supine) values and changes from baseline will be summarized at each scheduled time point. The percentage of subjects with values beyond clinically important limits will be summarized.

Nasal Exam and Nasal Tolerability Questionnaire

Changes in findings from the baseline nasal examination (including the upper respiratory tract/throat) will be listed by treatment group and period. Examinations will provide ratings (none, mild, moderate, or severe) that are based on a visual inspection of the nostrils, nasal mucosa, and throat for nasal erythema, rhinorrhea, rhinitis, capillary/blood vessel disruption and epistaxis. A shift table for changes in rating for each examination will be presented by treatment group and period.

In addition, scoring from the nasal tolerability questionnaire will be summarized descriptively by treatment group and period.

Physical Examination

Abnormal physical examination findings will be listed.

C-SSRS

Suicide-related thoughts and behaviors based on the C-SSRS will be summarized by treatment group in incidence and shift tables. Separate endpoints for suicidal ideation and suicidal behavior will be defined and summarized descriptively by treatment group. Missing scores will not be imputed.

CADSS, BPRS+, and MOAA/S

Descriptive statistics of each of the scores and changes from predose will be summarized at each scheduled time point.

PWC-20

The PWC-20 rating scale will be analyzed descriptively.

11.9. Interim Analysis

An interim analysis may be performed as needed. If one is required, details will be provided in a separate charter and interim analysis plan.

12. ADVERSE EVENT REPORTING

Timely, accurate, and complete reporting and analysis of safety information from clinical studies are crucial for the protection of subjects, investigators, and the sponsor, and are mandated by regulatory agencies worldwide. The sponsor has established Standard Operating Procedures in conformity with regulatory requirements worldwide to ensure appropriate reporting of safety information; all clinical studies conducted by the sponsor or its affiliates will be conducted in accordance with those procedures.

12.1. Definitions

12.1.1. Adverse Event Definitions and Classifications

Adverse Event

An adverse event is any untoward medical occurrence in a clinical study subject administered a medicinal (investigational or non-investigational) product. An adverse event does not necessarily have a causal relationship with the treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal finding), symptom, or disease temporally associated with the use of a medicinal (investigational or non-investigational) product, whether or not related to that medicinal (investigational or non-investigational) product. (Definition per International Conference on Harmonisation [ICH]).

This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition, or abnormal results of diagnostic procedures, including laboratory test abnormalities.

Note: The sponsor collects adverse events starting with the signing of the ICF (refer to Section 12.3.1, All Adverse Events, for time of last adverse event recording).

Serious Adverse Event

A serious adverse event based on ICH and EU Guidelines on Pharmacovigilance for Medicinal Products for Human Use is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening
  (The subject was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe.)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect

- Is a suspected transmission of any infectious agent via a medicinal product
- Is Medically Important*

*Medical and scientific judgment should be exercised in deciding whether expedited reporting is also appropriate in other situations, such as important medical events that may not be immediately life threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These should usually be considered serious.

If a serious and unexpected adverse event occurs for which there is evidence suggesting a causal relationship between the study drug and the event (e.g., death from anaphylaxis), the event must be reported as a serious and unexpected suspected adverse reaction even if it is a component of the study endpoint (e.g., all-cause mortality).

Unlisted (Unexpected) Adverse Event/Reference Safety Information

An adverse event is considered unlisted if the nature or severity is not consistent with the applicable product reference safety information. For esketamine, the expectedness of an adverse event will be determined by whether or not it is listed in the Investigator's Brochure.

Adverse Event Associated With the Use of the Drug

An adverse event is considered associated with the use of the drug if the attribution is possible, probable, or very likely by the definitions listed in Section 12.1.2.

12.1.2. Attribution Definitions

Not Related

An adverse event that is not related to the use of the drug.

Doubtful

An adverse event for which an alternative explanation is more likely, e.g., concomitant drug(s), concomitant disease(s), or the relationship in time suggests that a causal relationship is unlikely.

Possible

An adverse event that might be due to the use of the drug. An alternative explanation, e.g., concomitant drug(s), concomitant disease(s), is inconclusive. The relationship in time is reasonable; therefore, the causal relationship cannot be excluded.

Probable

An adverse event that might be due to the use of the drug. The relationship in time is suggestive (e.g., confirmed by dechallenge). An alternative explanation is less likely, e.g., concomitant drug(s), concomitant disease(s).

Very Likely

An adverse event that is listed as a possible adverse reaction and cannot be reasonably explained by an alternative explanation, e.g., concomitant drug(s), concomitant disease(s). The relationship in time is very suggestive (e.g., it is confirmed by dechallenge and rechallenge).

12.1.3. Severity Criteria

An assessment of severity grade will be made using the following general categorical descriptors:

Mild: Awareness of symptoms that are easily tolerated, causing minimal discomfort and not interfering with everyday activities.

Moderate: Sufficient discomfort is present to cause interference with normal activity.

Severe: Extreme distress, causing significant impairment of functioning or incapacitation. Prevents normal everyday activities.

The investigator should use clinical judgment in assessing the severity of events not directly experienced by the subject (e.g., laboratory abnormalities).

12.2. Special Reporting Situations

Safety events of interest on a sponsor study drug that may require expedited reporting and/or safety evaluation include, but are not limited to:

- Overdose of a sponsor study drug
- Suspected abuse/misuse of a sponsor study drug
- Inadvertent or accidental exposure to a sponsor study drug
- Medication error involving a sponsor product (with or without subject/patient exposure to the sponsor study drug, e.g., name confusion)

Special reporting situations should be recorded in the CRF. Any special reporting situation that meets the criteria of a serious adverse event should be recorded on the serious adverse event page of the CRF.

12.3. Procedures

12.3.1. All Adverse Events

All adverse events and special reporting situations, whether serious or non-serious, will be reported from the time a signed and dated ICF is obtained until completion of the subject's last study-related procedure (which may include contact for follow-up of safety). Serious adverse events, including those spontaneously reported to the investigator within 30 days after the last dose of study drug, must be reported using the Serious Adverse Event Form. The sponsor will evaluate any safety information that is spontaneously reported by an investigator beyond the time frame specified in the protocol.

All events that meet the definition of a serious adverse event will be reported as serious adverse events, regardless of whether they are protocol-specific assessments.

All adverse events, regardless of seriousness, severity, or presumed relationship to study drug, must be recorded using medical terminology in the source document and the CRF. Whenever possible, diagnoses should be given when signs and symptoms are due to a common etiology (e.g., cough, runny nose, sneezing, sore throat, and head congestion should be reported as "upper respiratory infection"). Investigators must record in the CRF their opinion concerning the relationship of the adverse event to study therapy. All measures required for adverse event management must be recorded in the source document and reported according to sponsor instructions.

The sponsor assumes responsibility for appropriate reporting of adverse events to the regulatory authorities. The sponsor will also report to the investigator (and the head of the investigational institute where required) all serious adverse events that are unlisted (unexpected) and associated with the use of the study drug. The investigator (or sponsor where required) must report these events to the appropriate Independent Ethics Committee/Institutional Review Board (IEC/IRB) that approved the protocol unless otherwise required and documented by the IEC/IRB.

For all studies with an outpatient phase, including open-label studies, the subject must be provided with a "wallet (study) card" and instructed to carry this card with them for the duration of the study indicating the following:

- Study number
- Statement, in the local language(s), that the subject is participating in a clinical study
- Investigator's name and 24-hour contact telephone number
- Local sponsor's name and 24-hour contact telephone number (for medical staff only)
- Site number
- Subject number
- Any other information that is required to do an emergency breaking of the blind

12.3.2. Serious Adverse Events

All serious adverse events occurring during the study must be reported to the appropriate sponsor contact person by study-site personnel within 24 hours of their knowledge of the event.

Information regarding serious adverse events will be transmitted to the sponsor using the Serious Adverse Event Form, which must be completed and signed by a physician from the study site, and transmitted to the sponsor within 24 hours. The initial and follow-up reports of a serious adverse event should be made by facsimile (fax).

All serious adverse events that have not resolved by the end of the study, or that have not resolved upon discontinuation of the subject's participation in the study, must be followed until any of the following occurs:

- The event resolves
- The event stabilizes
- The event returns to baseline, if a baseline value/status is available
- The event can be attributed to agents other than the study drug or to factors unrelated to study conduct
- It becomes unlikely that any additional information can be obtained (subject or health care practitioner refusal to provide additional information, lost to follow-up after demonstration of due diligence with follow-up efforts)

Suspected transmission of an infectious agent by a medicinal product will be reported as a serious adverse event. Any event requiring hospitalization (or prolongation of hospitalization) that occurs during the course of a subject's participation in a study must be reported as a serious adverse event, except hospitalizations for the following:

- Hospitalizations not intended to treat an acute illness or adverse event (e.g., social reasons such as pending placement in long-term care facility)
- Surgery or procedure planned before entry into the study (must be documented in the CRF). Note: Hospitalizations that were planned before the signing of the ICF, and where the underlying condition for which the hospitalization was planned has not worsened, will not be considered serious adverse events. Any adverse event that results in a prolongation of the originally planned hospitalization is to be reported as a new serious adverse event.
- For convenience, the investigator may choose to hospitalize the subject for treatment, with Sponsor’s approval.

The cause of death of a subject in a study, whether or not the event is expected or associated with the study drug, is considered a serious adverse event.

12.3.3. Pregnancy

All initial reports of pregnancy must be reported to the sponsor by the study-site personnel within 24 hours of their knowledge of the event using the appropriate pregnancy notification form. Abnormal pregnancy outcomes (e.g., spontaneous abortion, stillbirth, and congenital anomaly) are considered serious adverse events and must be reported using the Serious Adverse Event Form. Any subject who becomes pregnant during the study must be promptly withdrawn from the study and discontinue further study treatment.

Because the effect of the study drug on sperm is unknown, pregnancies in partners of male subjects included in the study will be reported by the study-site personnel within 24 hours of their knowledge of the event using the appropriate pregnancy notification form.

Follow-up information regarding the outcome of the pregnancy and any postnatal sequelae in the infant will be required.

12.4. Contacting Sponsor Regarding Safety

The names (and corresponding telephone numbers) of the individuals who should be contacted regarding safety issues or questions regarding the study are listed on the Contact Information page(s), which will be provided as a separate document.

13. PRODUCT QUALITY COMPLAINT HANDLING

A product quality complaint (PQC) is defined as any suspicion of a product defect related to manufacturing, labeling, or packaging, i.e., any dissatisfaction relative to the identity, quality, durability, or reliability of a product, including its labeling or package integrity. A PQC may have an impact on the safety and efficacy of the product. Timely, accurate, and complete reporting and analysis of PQC information from studies are crucial for the protection of subjects, investigators, and the sponsor, and are mandated by regulatory agencies worldwide. The sponsor has established procedures in conformity with regulatory requirements worldwide to ensure appropriate reporting of PQC information; all studies conducted by the sponsor or its affiliates will be conducted in accordance with those procedures.

13.1. Procedures

All initial PQCs must be reported to the sponsor by the study-site personnel within 24 hours after being made aware of the event.

If the defect is combined with a serious adverse event, the study-site personnel must report the PQC to the sponsor according to the serious adverse event reporting timelines (refer to Section 12.3.2, Serious Adverse Events). A sample of the suspected product should be maintained for further investigation if requested by the sponsor.

13.2. Contacting Sponsor Regarding Product Quality

The names (and corresponding telephone numbers) of the individuals who should be contacted regarding product quality issues are listed on the Contact Information page(s), which will be provided as a separate document.

14. STUDY DRUG INFORMATION

14.1. Physical Description of Study Drug(s)

The esketamine supplied for this study is a clear, colorless intranasal solution of esketamine hydrochloride (16.14% weight/volume [w/v]; equivalent to 14% w/v of esketamine base). The solution will consist of 161.4 mg/mL esketamine hydrochloride (equivalent to 140 mg of esketamine base) formulated in 0.12 mg/mL EDTA and 1.5 mg/mL citric acid at a pH of 4.5 in water for injection. It is provided in a nasal spray pump, which delivers 16.14 mg esketamine hydrochloride (14 mg esketamine base) per 100 µL spray. Each individual nasal spray pump (device) can deliver a total of 28 mg (i.e., 2 sprays). It will be manufactured and provided under the responsibility of the sponsor. Refer to the Investigator's Brochure for a list of excipients.

The placebo supplied for this study is a clear, colorless intranasal solution of water for injection with a bittering agent (denatonium benzoate [Bitrex®] at a final concentration of 0.001 mg/mL)

added to simulate the taste of the intranasal solution with active drug. Benzalkonium chloride is added as a preservative at a concentration of 0.3 mg/mL.

14.2. Packaging

Study drug (i.e., intranasal esketamine and placebo solution) will be supplied by the sponsor in a bidose nasal spray device. The devices will contain 200 μL. Each device delivers 16.14 mg esketamine hydrochloride (14 mg esketamine base) or 0.1 μg of denatonium benzoate per 100 μL spray. Esketamine will be provided as bulk supplies (i.e., not packaged for individual subject numbers).

14.3. Labeling

Study drug labels will contain information to meet the applicable regulatory requirements.

14.4. Preparation, Handling, and Storage

Study medication will be stored at the study site in a secure area with restricted access until dispensed to the subjects. Before dispensing, the study medication to be administered to the subject will be labeled with the subject's randomization numbers by the appropriate study staff.

All study drug must be stored at controlled temperatures as indicated on the product specific labeling.

14.5. Drug Accountability

The investigator is responsible for ensuring that all study drug received at the site is inventoried and accounted for throughout the study. The dispensing of study drug to the subject, and the return of study drug from the subject (if applicable), must be documented on the drug accountability form. Subjects must be instructed to return all original containers, whether empty or containing study drug. All study drug will be stored and disposed of according to the sponsor's instructions. Study-site personnel must not combine contents of the study drug containers.

Study drug must be handled in strict accordance with the protocol and the container label, and must be stored at the study site in a limited-access area or in a locked cabinet under appropriate environmental conditions. Unused study drug, and study drug returned by the subject, must be available for verification by the sponsor's study site monitor during on-site monitoring visits. The return to the sponsor of unused study drug, or used returned study drug for destruction, will be documented on the drug return form. When the study site is an authorized destruction unit and study drug supplies are destroyed on-site, this must also be documented on the drug return form.

Potentially hazardous materials such as used ampules, needles, syringes and vials containing hazardous liquids, should be disposed of immediately in a safe manner and therefore will not be retained for drug accountability purposes.

Study drug should be dispensed under the supervision of the investigator or a qualified member of the study-site personnel, or by a hospital/clinic pharmacist. Study drug will be supplied only to subjects participating in the study. Returned study drug must not be dispensed again, even to

the same subject. Study drug may not be relabeled or reassigned for use by other subjects. The investigator agrees neither to dispense the study drug from, nor store it at, any site other than the study sites agreed upon with the sponsor.

15. STUDY-SPECIFIC MATERIALS

The investigator will be provided with the following supplies:

- Study medication
- Investigator Brochure for esketamine
- Pharmacy manual
- Laboratory manual and materials
- Clinician-administered and subject-completed/patient-reported outcome assessments
- IVRS/IWRS Manual
- ECG Manual
- Intranasal dose administration instructions
- Recommended order of procedures
- Subject diary
- Rater qualifications/requirements for clinician-administered assessments

16. ETHICAL ASPECTS

16.1. Study-Specific Design Considerations

Clinical Study in Treatment-Resistant Major Depression

Major depressive disorder is a common, severe, chronic and often life-threatening illness. It is now the leading cause of disability worldwide. There is a clear need to develop novel and improved therapeutics for major depression.

Ketamine and esketamine have shown antidepressant-like effects in a number of small studies and has been well tolerated and safe in these clinical studies.

Selection of Subjects

The primary aim of the study is to evaluate the efficacy of intranasal esketamine for the treatment of TRD. Thus, the study cannot be completed in healthy subjects. Subjects selected in the study will have adequate capacity to give consent for participation in the study.

Justification for Using Placebo

Assessment of the potential efficacy of a new compound for the treatment of major depression requires adequate and well-controlled clinical studies. For a new compound, this can be achieved either through a placebo-controlled study or through a study comparing it to an active comparator through a non-inferiority design. For non-inferiority studies, previous

placebo-controlled studies have to show consistently the superiority of the active standard drug to placebo. Nearly half of the studies with antidepressants fail even with previously proven antidepressants, making assay sensitivity difficult to establish and thus, a non-inferiority design invalid (Laughren 2001). Of note however, with the current design, most subjects will receive the active treatment at some point during the double blind phase and subjects will be offered open label treatment with IN esketamine for an additional 2 weeks after completing the double blind phase of the study. Recent analyses have shown response to placebo varies considerably from 10% to 55%. Therefore, there is a concern that randomized, controlled studies that rely on comparison with standard antidepressants alone will generate unreliable results with limited assay sensitivity.

However, some have considered it unethical to do placebo-controlled studies in major depression due to the potential risk of irreversible harm (Rothman 1994). In a meta-analysis (Khan 2000) of drug studies conducted in major depression, it was reported that adult subjects did not have higher rates of suicide behaviors or attempts in the placebo group compared with those receiving an active antidepressant. These studies show annual suicide rates of 0.8% on the investigational drug, 0.7% on the active comparator, and 0.4% on placebo. The risk of irreversible harm is not higher in the placebo arm compared to the active control arms. Some subjects may decide not to participate due to the potential for increased distress and dysfunction due to prolonged depression.

Therefore, the use of a placebo-controlled study remains the gold standard for assessment of efficacy of new compound to allow for scientifically meaningful results. Placebo-controlled studies in major depression have been argued to be ethically and scientifically justifiable (Adam 2005; Temple 2000; Laughren 2001).

Moreover, the duration of the double-blind treatment phase is relatively short, approximately 2 weeks (Day 1 to Day 15). The subjects will continue existing and allowed anti-depressant medications. They will visit the study site at least twice weekly during the double-blind study period and their symptoms will be carefully monitored during each study visit. Safety evaluations will include evaluation of suicidal ideation/behavior at each clinic visit. At any point in the study the subject may withdraw consent or be removed from the study by the investigator if there are any clinical concerns. Subjects who complete the double-blind treatment phase may receive open-label intranasal esketamine treatment for up to an additional 2 weeks (up to 4 additional, single doses). The study medication, intranasal esketamine, will not be available after the study, however, following completion of the double-blind treatment phase and the optional open label treatment phase (if applicable), subjects can be treated according to standard of care.

Precautions to Ensure Subject Safety in the Study

Subjects may participate in the study only if they have adequate capacity to give consent and after fully understanding the potential risks and giving an informed consent. Determination of capacity will be made by the study investigator. Subjects may discontinue the study at any time. The probability of receiving placebo and the concept of random assignment will be explained to

the subject. The duration of the study is short, minimizing the time on placebo. Potential disadvantages and adverse events of participating in the study and alternative treatment options will be discussed. For subjects who do not respond during the study and are not willing or able to receive additional open-label ketamine treatment for 2 weeks, clinical care will be arranged between the study investigator and or their physician.

Compensation for any procedure will be fair per local standards and approved by the participating sites IRB in order to not offer any undue incentive to participate in the study.

Subjects who are unable to tolerate study drug during the double-blind treatment phase will be discontinued from the study. Subjects who are unable to tolerate study drug during the optional open label treatment phase can have their dose decreased to a permitted dosage or can discontinue from the optional open label treatment phase. If the investigator judges it to be necessary to immediately stop study drug, he or she has the option to do so.

Only subjects who have not adequately responded to their antidepressant where a clinician would consider changing it for lack of response or poor tolerability in addition to meeting the severity criteria for the study will be enrolled.

Only highly qualified and experienced investigators will participate in the study.

Potential subjects will be fully informed of the risks and requirements of the study and, during the study, subjects will be given any new information that may affect their decision to continue participation. They will be told that their consent to participate in the study is voluntary and may be withdrawn at any time with no reason given and without penalty or loss of benefits to which they would otherwise be entitled. Only subjects who are fully able to understand the risks, benefits, and potential adverse events of the study, and provide their consent voluntarily will be enrolled.

The total blood volume to be collected is considered to be within the normal range allowed for this subject population over this time frame. The maximum blood volume to be collected is approximately 165 mL which will be less than a Red Cross blood donation.

16.2. Regulatory Ethics Compliance

16.2.1. Investigator Responsibilities

The investigator is responsible for ensuring that the study is performed in accordance with the protocol, current ICH guidelines on Good Clinical Practice (GCP), and applicable regulatory and country-specific requirements.

Good Clinical Practice is an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety, and well-being of study subjects are protected, consistent with the principles that originated in the Declaration of Helsinki, and that the study data are credible.

16.2.2. Independent Ethics Committee or Institutional Review Board

Before the start of the study, the investigator (or sponsor where required) will provide the IEC/IRB with current and complete copies of the following documents (as required by local regulations):

- Final protocol and, if applicable, amendments
- Sponsor-approved ICF (and any other written materials to be provided to the subjects)
- Investigator's Brochure (or equivalent information) and amendments/addenda
- Sponsor-approved subject recruiting materials
- Information on compensation for study-related injuries or payment to subjects for participation in the study, if applicable
- Investigator's curriculum vitae or equivalent information (unless not required, as documented by the IEC/IRB)
- Information regarding funding, name of the sponsor, institutional affiliations, other potential conflicts of interest, and incentives for subjects
- Any other documents that the IEC/IRB requests to fulfill its obligation

This study will be undertaken only after the IEC/IRB has given full approval of the final protocol, amendments (if any, excluding the ones that are purely administrative, with no consequences for subjects, data or study conduct), the ICF, applicable recruiting materials, and subject compensation programs, and the sponsor has received a copy of this approval. This approval letter must be dated and must clearly identify the IEC/IRB and the documents being approved.

During the study the investigator (or sponsor where required) will send the following documents and updates to the IEC/IRB for their review and approval, where appropriate:

- Protocol amendments (excluding the ones that are purely administrative, with no consequences for subjects, data or study conduct)
- Revision(s) to ICF and any other written materials to be provided to subjects
- If applicable, new or revised subject recruiting materials approved by the sponsor
- Revisions to compensation for study-related injuries or payment to subjects for participation in the study, if applicable
- New edition(s) of the Investigator's Brochure and amendments/addenda
- Summaries of the status of the study at intervals stipulated in guidelines of the IEC/IRB (at least annually)
- Reports of adverse events that are serious, unlisted/unexpected, and associated with the study drug
- New information that may adversely affect the safety of the subjects or the conduct of the study

- Deviations from or changes to the protocol to eliminate immediate hazards to the subjects
- Report of deaths of subjects under the investigator's care
- Notification if a new investigator is responsible for the study at the site
- Development Safety Update Report and Line Listings, where applicable
- Any other requirements of the IEC/IRB

For all protocol amendments (excluding the ones that are purely administrative, with no consequences for subjects, data or study conduct), the amendment and applicable ICF revisions must be submitted promptly to the IEC/IRB for review and approval before implementation of the change(s).

At least once a year, the IEC/IRB will be asked to review and reapprove this study. The reapproval should be documented in writing (excluding the ones that are purely administrative, with no consequences for subjects, data, or study conduct).

At the end of the study, the investigator (or sponsor where required) will notify the IEC/IRB about the study completion.

16.2.3. Informed Consent

Each subject must give written consent according to local requirements after the nature of the study has been fully explained. The ICF(s) must be signed before performance of any study-related activity. The ICF(s) that is/are used must be approved by both the sponsor and by the reviewing IEC/IRB and be in a language that the subject can read and understand. The informed consent should be in accordance with principles that originated in the Declaration of Helsinki, current ICH and GCP guidelines, applicable regulatory requirements, and sponsor policy.

Before enrollment in the study, the investigator or an authorized member of the study-site personnel must explain to potential subjects the aims, methods, reasonably anticipated benefits, and potential hazards of the study, and any discomfort participation in the study may entail. Subjects will be informed that their participation is voluntary and that they may withdraw consent to participate at any time. They will be informed that choosing not to participate will not affect the care the subject will receive for the treatment of his or her disease. Subjects will be told that alternative treatments are available if they refuse to take part and that such refusal will not prejudice future treatment. Finally, they will be told that the investigator will maintain a subject identification register for the purposes of long-term follow up if needed and that their records may be accessed by health authorities and authorized sponsor personnel without violating the confidentiality of the subject, to the extent permitted by the applicable law(s) or regulations. By signing the ICF the subject is authorizing such access, and agrees to allow his or her study physician to recontact the subject for the purpose of obtaining consent for additional safety evaluations, if needed.

The subject will be given sufficient time to read the ICF and the opportunity to ask questions. After this explanation and before entry into the study, consent should be appropriately recorded

by means of the subject's personally dated signature. After having obtained the consent, a copy of the ICF must be given to the subject.

Where local regulations require, a separate ICF may be used for the required DNA component of the study.

16.2.4. Privacy of Personal Data

The collection and processing of personal data from subjects enrolled in this study will be limited to those data that are necessary to fulfill the objectives of the study.

These data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations. Appropriate technical and organizational measures to protect the personal data against unauthorized disclosures or access, accidental or unlawful destruction, or accidental loss or alteration must be put in place. Sponsor personnel whose responsibilities require access to personal data agree to keep the identity of subjects confidential.

The informed consent obtained from the subject includes explicit consent for the processing of personal data and for the investigator/institution to allow direct access to his or her original medical records (source data/documents) for study-related monitoring, audit, IEC/IRB review, and regulatory inspection. This consent also addresses the transfer of the data to other entities and to other countries.

The subject has the right to request through the investigator access to his or her personal data and the right to request rectification of any data that are not correct or complete. Reasonable steps will be taken to respond to such a request, taking into consideration the nature of the request, the conditions of the study, and the applicable laws and regulations.

Exploratory DNA, biomarker, and PK research is not conducted under standards appropriate for the return of data to subjects. In addition, the sponsor cannot make decisions as to the significance of any findings resulting from exploratory research. Therefore, exploratory research data will not be returned to subjects or investigators, unless required by law or local regulations. Privacy and confidentiality of data generated in the future on stored samples will be protected by the same standards applicable to all other clinical data.

16.2.5. Long-Term Retention of Samples for Additional Future Research

Samples collected in this study may be stored for up to 15 years (or according to local regulations) for additional research. Samples will only be used to understand esketamine, to understand depression, to understand differential drug responders, and to develop tests/assays related to esketamine and depression. The research may begin at any time during the study or the post-study storage period.

Stored samples will be coded throughout the sample storage and analysis process and will not be labeled with personal identifiers. Subjects may withdraw their consent for their samples to be

stored for research (refer to Section 10.2, Withdrawal From the Study (Withdrawal From the Use of Samples in Future Research).

16.2.6. Country Selection

This study will only be conducted in those countries where the intent is to launch or otherwise help ensure access to the developed product, unless explicitly addressed as a specific ethical consideration in Section 16.1, Study-Specific Design Considerations.

17. ADMINISTRATIVE REQUIREMENTS

17.1. Protocol Amendments

Neither the investigator nor the sponsor will modify this protocol without a formal amendment by the sponsor. All protocol amendments must be issued by the sponsor, and signed and dated by the investigator. Protocol amendments must not be implemented without prior IEC/IRB approval, or when the relevant competent authority has raised any grounds for non-acceptance, except when necessary to eliminate immediate hazards to the subjects, in which case the amendment must be promptly submitted to the IEC/IRB and relevant competent authority. Documentation of amendment approval by the investigator and IEC/IRB must be provided to the sponsor. When the change(s) involves only logistic or administrative aspects of the study, the IRB (and IEC where required) only needs to be notified.

During the course of the study, in situations where a departure from the protocol is unavoidable, the investigator or other physician in attendance will contact the appropriate sponsor representative (see Contact Information page(s) provided separately). Except in emergency situations, this contact should be made before implementing any departure from the protocol. In all cases, contact with the sponsor must be made as soon as possible to discuss the situation and agree on an appropriate course of action. The data recorded in the CRF and source documents will reflect any departure from the protocol, and the source documents will describe this departure and the circumstances requiring it.

17.2. Regulatory Documentation

17.2.1. Regulatory Approval/Notification

This protocol and any amendment(s) must be submitted to the appropriate regulatory authorities in each respective country, if applicable. A study may not be initiated until all local regulatory requirements are met.

17.2.2. Required Prestudy Documentation

The following documents must be provided to the sponsor before shipment of study drug to the study site:

- Protocol and amendment(s), if any, signed and dated by the principal investigator

- A copy of the dated and signed (or sealed, where appropriate per local regulations), written IEC/IRB approval of the protocol, amendments, ICF, any recruiting materials, and if applicable, subject compensation programs. This approval must clearly identify the specific protocol by title and number and must be signed (or sealed, where appropriate per local regulations) by the chairman or authorized designee.
- Name and address of the IEC/IRB, including a current list of the IEC/IRB members and their function, with a statement that it is organized and operates according to GCP and the applicable laws and regulations. If accompanied by a letter of explanation, or equivalent, from the IEC/IRB, a general statement may be substituted for this list. If an investigator or a member of the study-site personnel is a member of the IEC/IRB, documentation must be obtained to state that this person did not participate in the deliberations or in the vote/opinion of the study.
- Regulatory authority approval or notification, if applicable
- Signed and dated statement of investigator (e.g., Form FDA 1572), if applicable
- Documentation of investigator qualifications (e.g., curriculum vitae)
- Completed investigator financial disclosure form from the principal investigator, where required
- Signed and dated clinical trial agreement, which includes the financial agreement
- Any other documentation required by local regulations

The following documents must be provided to the sponsor before enrollment of the first subject:

- Completed investigator financial disclosure forms from all subinvestigators
- Documentation of subinvestigator qualifications (e.g., curriculum vitae)
- Name and address of any local laboratory conducting tests for the study, and a dated copy of current laboratory normal ranges for these tests, if applicable
- Local laboratory documentation demonstrating competence and test reliability (e.g., accreditation/license), if applicable

17.3. Subject Identification, Enrollment, and Screening Logs

The investigator agrees to complete a subject identification and enrollment log to permit easy identification of each subject during and after the study. This document will be reviewed by the sponsor study-site contact for completeness.

The subject identification and enrollment log will be treated as confidential and will be filed by the investigator in the study file. To ensure subject confidentiality, no copy will be made. All reports and communications relating to the study will identify subjects by subject identification and date of birth. In cases where the subject is not randomized into the study, the date seen and date of birth will be used.

The investigator must also complete a subject screening log, which reports on all subjects who were seen to determine eligibility for inclusion in the study.

17.4. Source Documentation

At a minimum, source documentation must be available for the following to confirm data collected in the CRF: subject identification, eligibility, and study identification; study discussion and date of signed informed consent; dates of visits; results of safety and efficacy parameters as required by the protocol; record of all adverse events and follow-up of adverse events; concomitant medication; drug receipt/dispensing/return records; study drug administration information; and date of study completion and reason for early discontinuation of study drug or withdrawal from the study, if applicable.

In addition, the author of an entry in the source documents should be identifiable.

At a minimum, the type and level of detail of source data available for a subject should be consistent with that commonly recorded at the study site as a basis for standard medical care. Specific details required as source data for the study will be reviewed with the investigator before the study and will be described in the monitoring guidelines (or other equivalent document).

The following data will be recorded directly into the CRF and will be considered source data:

- Race

The minimum source documentation requirements for Section 4.1, Inclusion Criteria and Section 4.2, Exclusion Criteria that specify a need for documented medical history are as follows:

- Referral letter from treating physician or
- Complete history of medical notes at the site
- Discharge summaries
- Antidepressant treatment in the current episode of depression

Inclusion and exclusion criteria not requiring documented medical history must be verified at a minimum by subject interview or other protocol required assessment (e.g., physical examination, laboratory assessment) and documented in the source documents.

17.5. Case Report Form Completion

Case report forms are provided for each subject in electronic format.

Electronic Data Capture (eDC) will be used for this study. The study data will be transcribed by study-site personnel from the source documents onto an electronic CRF, and transmitted in a secure manner to the sponsor within the timeframe agreed upon between the sponsor and the study site. The electronic file will be considered to be the CRF.

Worksheets may be used for the capture of some data to facilitate completion of the CRF. Any such worksheets will become part of the subject's source documentation. All data relating to the study must be recorded in CRFs prepared by the sponsor. Data must be entered into CRFs in

English. Study site personnel must complete the CRF as soon as possible after a subject visit, and the forms should be available for review at the next scheduled monitoring visit.

The investigator must verify that all data entries in the CRFs are accurate and correct.

All CRF entries, corrections, and alterations must be made by the investigator or other authorized study-site personnel. If necessary, queries will be generated in the eDC tool. The investigator or study-site personnel must adjust the CRF (if applicable) and complete the query.

If corrections to a CRF are needed after the initial entry into the CRF, this can be done in 3 different ways:

- Study site personnel can make corrections in the eDC tool at their own initiative or as a response to an auto query (generated by the eDC tool).
- Study site manager can generate a query for resolution by the study-site personnel.
- Clinical data manager can generate a query for resolution by the study-site personnel.

17.6. Data Quality Assurance/Quality Control

Steps to be taken to ensure the accuracy and reliability of data include the selection of qualified investigators and appropriate study sites, review of protocol procedures with the investigator and study-site personnel before the study, periodic monitoring visits by the sponsor, and direct transmission of clinical laboratory data from a central laboratory into the sponsor's data base. Written instructions will be provided for collection, handling, storage, and shipment of samples.

Guidelines for CRF completion will be provided and reviewed with study-site personnel before the start of the study.

The sponsor will review CRFs for accuracy and completeness during on-site monitoring visits and after transmission to the sponsor; any discrepancies will be resolved with the investigator or designee, as appropriate. After upload of the data into the study database they will be verified for accuracy and consistency with the data sources.

17.7. Record Retention

In compliance with the ICH/GCP guidelines, the investigator/institution will maintain all CRFs and all source documents that support the data collected from each subject, as well as all study documents as specified in ICH/GCP Section 8, Essential Documents for the Conduct of a Clinical Trial, and all study documents as specified by the applicable regulatory requirement(s). The investigator/institution will take measures to prevent accidental or premature destruction of these documents.

Essential documents must be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents will be

retained for a longer period if required by the applicable regulatory requirements or by an agreement with the sponsor. It is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

If the responsible investigator retires, relocates, or for other reasons withdraws from the responsibility of keeping the study records, custody must be transferred to a person who will accept the responsibility. The sponsor must be notified in writing of the name and address of the new custodian. Under no circumstance shall the investigator relocate or dispose of any study documents before having obtained written approval from the sponsor.

If it becomes necessary for the sponsor or the appropriate regulatory authority to review any documentation relating to this study, the investigator/institution must permit access to such reports.

17.8. Monitoring

The sponsor will perform on-site monitoring visits as frequently as necessary. The monitor will record dates of the visits in a study site visit log that will be kept at the study site. The first post-initiation visit will be made as soon as possible after enrollment has begun. At these visits, the monitor will compare the data entered into the CRFs with the hospital or clinic records (source documents). The nature and location of all source documents will be identified to ensure that all sources of original data required to complete the CRF are known to the sponsor and study-site personnel and are accessible for verification by the sponsor study-site contact. If electronic records are maintained at the study site, the method of verification must be discussed with the study-site personnel.

Direct access to source documentation (medical records) must be allowed for the purpose of verifying that the data recorded in the CRF are consistent with the original source data. Findings from this review of CRFs and source documents will be discussed with the study-site personnel. The sponsor expects that, during monitoring visits, the relevant study-site personnel will be available, the source documentation will be accessible, and a suitable environment will be provided for review of study-related documents. The monitor will meet with the investigator on a regular basis during the study to provide feedback on the study conduct.

17.9. Study Completion/Termination

17.9.1. Study Completion

The study is considered completed with the last study assessment for the last subject participating in the study. The final data from the study site will be sent to the sponsor (or designee) after completion of the final subject assessment at that study site, in the time frame specified in the Clinical Trial Agreement.

17.9.2. Study Termination

The sponsor reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the sponsor. Study sites will be closed upon study completion. A

study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the sponsor or investigator may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IEC/IRB or local health authorities, the sponsor's procedures, or GCP guidelines
- Inadequate recruitment of subjects by the investigator
- Discontinuation of further study drug development

17.10. On-Site Audits

Representatives of the sponsor's clinical quality assurance department may visit the study site at any time during or after completion of the study to conduct an audit of the study in compliance with regulatory guidelines and company policy. These audits will require access to all study records, including source documents, for inspection and comparison with the CRFs. Subject privacy must, however, be respected. The investigator and study-site personnel are responsible for being present and available for consultation during routinely scheduled study-site audit visits conducted by the sponsor or its designees.

Similar auditing procedures may also be conducted by agents of any regulatory body, either as part of a national GCP compliance program or to review the results of this study in support of a regulatory submission. The investigator should immediately notify the sponsor if he or she has been contacted by a regulatory agency concerning an upcoming inspection.

17.11. Use of Information and Publication

All information, including but not limited to information regarding esketamine or the sponsor's operations (e.g., patent application, formulas, manufacturing processes, basic scientific data, prior clinical data, formulation information) supplied by the sponsor to the investigator and not previously published, and any data, including pharmacogenomic or exploratory biomarker research data, generated as a result of this study, are considered confidential and remain the sole property of the sponsor. The investigator agrees to maintain this information in confidence and use this information only to accomplish this study, and will not use it for other purposes without the sponsor's prior written consent.

The investigator understands that the information developed in the study will be used by the sponsor in connection with the continued development of esketamine, and thus may be disclosed as required to other clinical investigators or regulatory agencies. To permit the information derived from the clinical studies to be used, the investigator is obligated to provide the sponsor with all data obtained in the study.

The results of the study will be reported in a Clinical Study Report generated by the sponsor and will contain CRF data from all study sites that participated in the study. Recruitment performance or specific expertise related to the nature and the key assessment parameters of the study will be used to determine a coordinating investigator. Results of pharmacogenomics or exploratory biomarker analyses performed after the Clinical Study Report has been issued will be reported in a separate report and will not require a revision of the Clinical Study Report. Study subject identifiers will not be used in publication of results. Any work created in connection with performance of the study and contained in the data that can benefit from copyright protection (except any publication by the investigator as provided for below) shall be the property of the sponsor as author and owner of copyright in such work.

Consistent with Good Publication Practices and International Committee of Medical Journal Editors guidelines, the sponsor shall have the right to publish such primary (multicenter) data and information without approval from the investigator. The investigator has the right to publish study site-specific data after the primary data are published. If an investigator wishes to publish information from the study, a copy of the manuscript must be provided to the sponsor for review at least 60 days before submission for publication or presentation. Expedited reviews will be arranged for abstracts, poster presentations, or other materials. If requested by the sponsor in writing, the investigator will withhold such publication for up to an additional 60 days to allow for filing of a patent application. In the event that issues arise regarding scientific integrity or regulatory compliance, the sponsor will review these issues with the investigator. The sponsor will not mandate modifications to scientific content and does not have the right to suppress information. For multicenter study designs and substudy approaches, secondary results generally should not be published before the primary endpoints of a study have been published. Similarly, investigators will recognize the integrity of a multicenter study by not submitting for publication data derived from the individual study site until the combined results from the completed study have been submitted for publication, within 12 months of the availability of the final data (tables, listings, graphs), or the sponsor confirms there will be no multicenter study publication. Authorship of publications resulting from this study will be based on the guidelines on authorship, such as those described in the Uniform Requirements for Manuscripts Submitted to Biomedical Journals, which state that the named authors must have made a significant contribution to the design of the study or analysis and interpretation of the data, provided critical review of the paper, and given final approval of the final version.

Registration of Clinical Studies and Disclosure of Results

The sponsor will register and/or disclose the existence of and the results of clinical studies as required by law.

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Attachment 1: Prohibited Therapies

Allowed (Y) and Not Allowed (N)

The table below is intended for general guidance, please discuss with the study team regarding any specific concomitant therapies for a subject.

Drug Class	Episodic Use (PRN)	Continuous Use	Comments
Allopurinol	N	Y	
Amantadine	N	N	
Analgesics (e.g., NSAIDS, acetaminophen), except opioids	Y	Y	See "Opioids" row below.
Anorexiants (e.g., phenteramine)	N	N	
Antacids	Y	Y	
Anti-anginal agents	N	N	Subjects with angina are excluded
Anti-arrhythmics	N	N	Subjects with any history of cardiovascular arrhythmias excluded
Anticholinesterase inhibitors	N	N	
Anticoagulants	N	N	
Anticonvulsants	N	N	Subjects with seizures are excluded. Anticonvulsants used for other indications may be allowed (e.g., valproate for migraine, lamotrigine for mood disorder)
Antidepressants (*except* monoamine oxidase inhibitors)	N	Y	
Antidepressants: Monoamine oxidase inhibitors	N	N	
Antidiarrheal preparations	Y	N	
Anti-emetics	Y	N	
Anti-inflammatory drugs, except steroids	Y	Y	See "Steroid" rows below.
Antipsychotics	N	Y	Use of APS for treatment of depression is not excluded.
Aspirin	Y	Y	
Benzodiazepines	Y	N	
Calcium Channel Blockers	Y	Y	
Chloral hydrate	N	N	
Chloramphenicol	N	N	
Clonidine	N	N	
Cough/Cold preparations (except those containing diphenhydramine or dextromethorphan)	Y	N	Intranasally-administered decongestants are prohibited from 12 hours prior to each study medication administration.
CYP3A4 inhibitors - Potent	N	N	
CYP3A4 inducers - Potent	N	N	
DHEA	N	N	
Fish oils	N	N	
Ginko	N	N	
Ginseng	N	N	
Guanabenz	N	N	
Guanadrel	N	N	
Guanethidine	N	N	
Guanfacine	N	N	
HIV antiviral drugs	N	N	Subjects testing positive for HIV excluded
Hormones (e.g., contraceptives, thyroid hormones etc.)	N	Y	

Ketanserin	N	N	
Lithium	N	Y	
Methyldopa	N	N	
Metyrosine	N	N	
Opioids	N	N	
Omega-3-fatty acids	N	N	
Psychostimulants (e.g, amphetamines)	N	N	
Reserpine	N	N	
Scopolamine	N	N	
St. John's Wort	N	N	
Steroids (inhaled, topical, ophthalmic only)	Y	Y	
Steroids (oral)	N	N	
Thyroid hormone supplement	N	Y	Subjects needing supplements must be on a stable thyroid supplement dose for at least 6 weeks prior to Day 1 of the double-blind treatment phase.

Attachment 2: Instructions for the Completion of the Patient-Reported Outcome (PRO) Case Report Forms

The following instructions are intended to assist investigators, study coordinators, and those with monitoring responsibilities with the completion of all patient self-administered (PRO) assessments.

I. General Instructions

1. Patients should complete the patient-reported outcome (PRO) assessments in a quiet, semi-private location with access to study staff for questions.
2. Patients should be allowed approximately 30 minutes to orient him/herself and to self-administer all PRO assessments.
3. Patients should be literate in the language of the PRO assessment(s). Patients must not have any developmental, learning, or behavioral disabilities.
4. Patients should complete all PRO assessments using a black ballpoint pen. Have the patient press firmly and print legibly when writing to ensure that all copies are clear and legible. Have the patient place a piece of cardboard between the pages to ensure no 'run through' pages.
5. Explain to patients the reasons why they are being asked to complete the PRO assessment(s), i.e., they are part of the overall medical assessment and are designed to find out more information about how having their disease has affected their life.
6. Indicate to patients that all of the information on the PRO assessment(s) is confidential, and that someone from the study staff will only check for completeness and not share the results with other clinical staff.
7. Indicate to patients that there are no right or wrong answers.
8. Provide patients with the set of instructions that are provided with the PRO assessment(s) material
9. Have patients read the instructions prior to completing the assessment(s). For almost all items, it is necessary for patients to check the box next to the answer that applies. Occasionally, patients will be asked to write in some additional information. Not all of the questions apply to every patient. Where a particular question or set of questions does not apply, there will be instructions on which question to answer next.

II. Assessment Times

1. Each PRO assessment asks the patient for an evaluation of a specified period of time. Therefore, it is important to minimize the influence of feedback from the clinic visit itself. Insofar as it is possible, it is also important to have the PRO assessment(s) be conducted within the flow of the protocol-specified study visit.
2. The PRO instruments have been placed in the correct order of completion in the study CRF. Please ask the subject to complete the PROs in the sequence that they appear in the booklet.

Screening:

PRO assessment(s) during this time period should be completed immediately after the patient provides his/her informed consent, but before any clinical tests are taken or assessments associated with the study visit are conducted.

Post-Screening visits:

Patient reported outcome (PRO) assessments should be conducted/completed before any tests, procedures, or other consultations scheduled at the same timepoint to prevent influencing subject perceptions.

III. Quality Control

When the subject returns the completed PRO assessments, check for any questions that might have been left blank. If an item has been omitted, point this out and ask that these items be completed. Occasionally, subjects mark more than one answer per item. In such instances, ask the subject if he/she will reconsider the question and try to choose one answer only.

INVESTIGATOR AGREEMENT

I have read this protocol and agree that it contains all necessary details for carrying out this study. I will conduct the study as outlined herein and will complete the study within the time designated.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure that they are fully informed regarding the study drug, the conduct of the study, and the obligations of confidentiality.

Coordinating Investigator (where required):

Name (typed or printed): _____

Institution and Address: _____

_____

_____

_____

Signature: _____ Date: _____
(Day Month Year)

Principal (Site) Investigator:

Name (typed or printed): _____

Institution and Address: _____

_____

_____

_____

Telephone Number: _____

Signature: _____ Date: _____
(Day Month Year)

Sponsor's Responsible Medical Officer:

Name (typed or printed): Ella Daly, M.D.

Institution: Janssen Research & Development, L.L.C.

Signature: [redacted] Date: 7th October 2013
(Day Month Year)

Note: If the address or telephone number of the investigator changes during the course of the study, written notification will be provided by the investigator to the sponsor, and a protocol amendment will not be required.

LAST PAGE

Janssen Research & Development

Statistical Analysis Plan

A Double-Blind, Doubly-Randomized, Placebo-Controlled Study of Intranasal Esketamine in an Adaptive Treatment Protocol to Assess Safety and Efficacy in Treatment-Resistant Depression (SYNAPSE)

Protocol ESKETINTRD2003; Phase 2a

JNJ-54135419 (esketamine)

Status: Approved

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Prepared by: Janssen Research & Development, LLC

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Compliance: The study described in this report was performed according to the principles of Good Clinical Practice (GCP).

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ABBREVIATIONS

AE	adverse event
ANOVA	analysis of variance
ANCOVA	analysis of covariance
BMI	body mass index
BP	Blood Pressure
BPIC-SS	Bladder Pain / Interstitial Cystitis Symptom Score
BPRS	Brief Psychiatric Rating Scale
CADSS	Clinician Administered Dissociative States Scale
CGAA	Clinical Global Assessment of Alertness
CGI-S	Clinical Global Impression – Severity
CI	confidence interval
CRF	case report form
CSR	Clinical Study Report
C-SSRS	Columbia-Suicide Severity Rating Scale
DB	double-blind
DRC	Data Review Committee
ECG	Electrocardiogram
eCRF	electronic case report form
E_{max}	maximum possible response (or effect)
ED_{90}	the dose required to produce 90% of the maximal response (or effect)
EQ-5D-5L	EuroQol Group; 5 dimension; 5 level
EQ-VAS	EuroQol Group: visual analogue scale
FDA	Food and Drug Administration
GAD-7	generalized anxiety disorder 7-item scale
HVLT-R	Hopkins Verbal Learning Test-Revised
ICH	International Conference on Harmonization
IDS-C_{30}	Inventory of Depressive Symptoms-Clinician rated, 30 item
ITT	Intent-to-Treat
IWRS	interactive web response system
LOCF	last observation carried forward
MADRS	Montgomery-Asberg Depression Rating Scale
MedDRA	Medical Dictionary for Regulatory Activities
MINI	Mini International Psychiatric Interview
MGH-ATRQ	Massachusetts General Hospital Antidepressant Treatment History
MMRM	mixed-effects model using repeated measures
PD	Pharmacodynamic
PGI-C	Patient Global Impression of Change
PGI-S	Patient Global Impression - Severity
PHQ-9	Patient health questionnaire – 9
PK	pharmacokinetic(s)
PRO	patient-reported outcome
PWC-20	physician withdrawal checklist; 20 item
QIDS-SR	Quick Inventory of Depressive Symptoms- Self Report
SAE	serious adverse event
SAP	Statistical Analysis Plan
SD	standard deviation
SMQ	Standardized MedDRA Query
SOC	System Organ Class
TEAE	treatment-emergent adverse event
TEMA	treatment-emergent markedly abnormal
TRD	Treatment Resistant Depression

1. INTRODUCTION

This statistical analysis plan (SAP) contains definitions of analysis sets, derived variables, and statistical methods for all planned analyses for the study JNJ54135419-ESKETINTRD2003.

1.1. Trial Objectives

Primary Objective

To assess the efficacy and dose response of intranasal esketamine (Panel A: 28 mg, 56 mg, 84 mg; Panel B: 14 mg and 56 mg) compared with placebo in improving depressive symptoms in subjects with treatment-resistant depression (TRD), as assessed by a change from baseline in the Montgomery-Asberg Depression Rating Scale (MADRS) total score for the combined periods in the double-blind treatment phase.

Secondary Objectives

The secondary objectives are:

1. To evaluate sustained response (≥50% reduction from baseline in MADRS total score) with onset by Day 2 through the end of the double-blind phase (Day 15).
2. To investigate the safety and tolerability of intranasal esketamine in TRD subjects, with special attention to:
    a. Local nasal tolerability, using a nasal tolerability questionnaire and nasal examinations
    b. Effects on heart rate, blood pressure, and blood oxygen saturation (SpO_2)
    c. Effects on suicidal ideation/behavior measured by the Columbia Suicide Severity Rating Scale (C-SSRS);
    d. Effects on alertness and sedation measured by the Modified Observer's Assessment of Alertness/Sedation (MOAA/S) and Clinician Global Assessment of Alertness
    e. Psychosis-like side effects by using a four-item positive symptom subscale of the Brief Psychiatric Rating Scale (BPRS+) consisting of: suspiciousness, hallucinations, unusual thought content, and conceptual disorganization;
    f. Effects on dissociative symptoms using the Clinical Administered Dissociative States Scale (CADSS);
    g. Potential withdrawal symptoms following cessation of intranasal esketamine treatment, as measured by the clinician-administered 20-item Physician Withdrawal Checklist (PWC-20)
    h. Potential treatment-emergent symptoms of cystitis using the Bladder Pain/Interstitial Cystitis Symptom Score (BPIC-SS)
    i. Cognition, using the Cogstate® computerized test battery and the Hopkins Verbal Learning Test-Revised (HVLT-R) (Panel A only)
3. To assess the effect of intranasal esketamine compared to intranasal placebo on:
    a. Depressive symptoms, as assessed by the 16-item Quick Inventory of Depressive Symptomatology- Self Report ($QIDS\text{-}SR_{16}$)

b. Remission, defined as a MADRS score ≤10

c. Response, defined as a ≥50% reduction from baseline in MADRS total score

d. The severity of illness using the Clinical Global Impression - Severity (CGI-S) and the Patient Global Impression - Severity (PGI-S)

e. Symptoms of anxiety as assessed by the Generalized Anxiety Disorder 7-item Scale (GAD-7)

4. To evaluate the pharmacokinetics (PK) of intranasal esketamine in subjects with TRD

Exploratory Objectives

The exploratory objectives are:

1. Subject perspective of global change in major depressive disorder (MDD) from baseline, as measured by the Patient Global Impression of Change (PGI-C)
2. To assess the effect of intranasal esketamine compared to intranasal placebo on depressive symptoms, as assessed by the 9-item Patient Health Questionnaire (PHQ-9)
3. Impact on health status as assessed using the EuroQol-5D, 5-level version (EQ-5D-5L)
4. To evaluate whether pretreatment concentrations of inflammatory and neurotrophic markers, and plasma glycine correlate with the magnitude of clinical change, as measured by the MADRS, following intranasal administration of esketamine.
5. To assess the impact of intranasal esketamine on plasma inflammatory and neurotrophic markers and glutamatergic pathway metabolic markers

1.2. Trial Design

This is a 2-panel, doubly-randomized, double-blind, placebo-controlled, multicenter study conducted in approximately 100 male and female adult subjects with TRD.

Panel A will be conducted in approximately 60 subjects in the United States and Belgium. Panel B will be conducted in approximately 40 Japanese subjects in Japan.

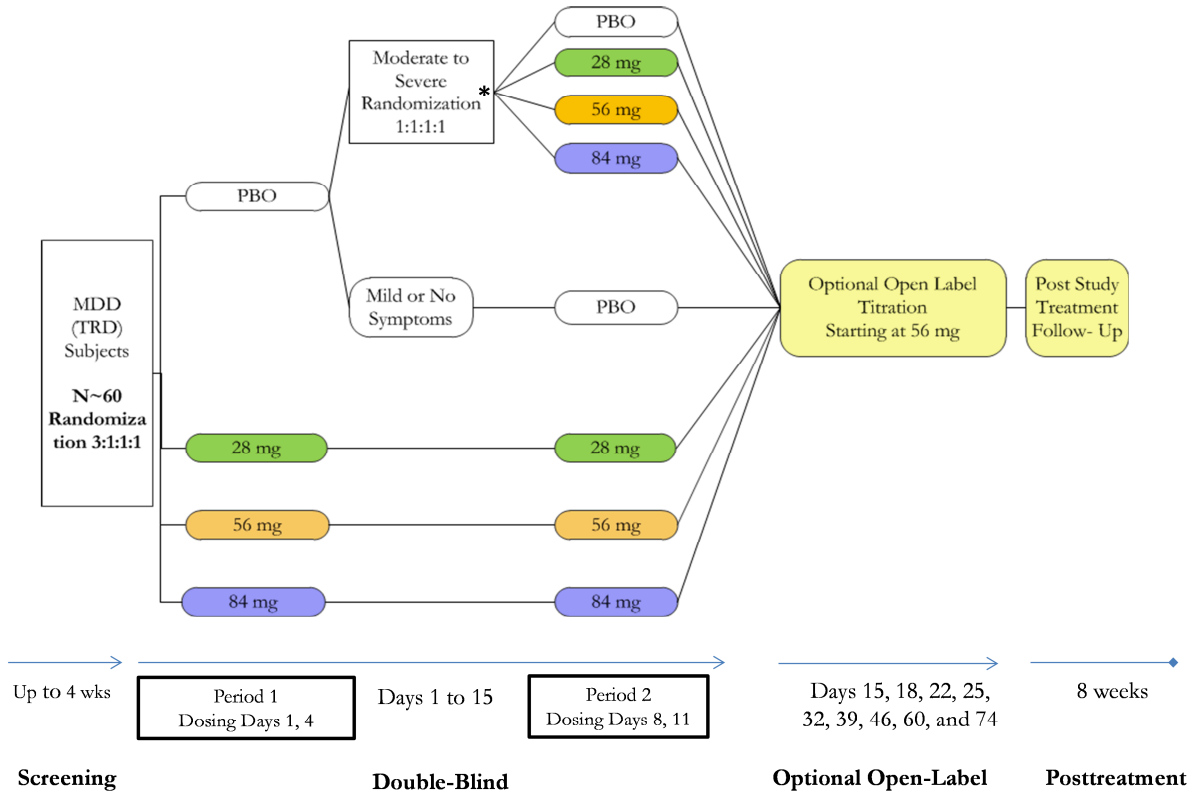
In both panels, each subject will participate in up to 4 phases:

- A screening phase of up to 4 weeks,
- A double-blind treatment phase (Day 1 to Day 15) which includes two 1-week treatment periods (Period 1 and Period 2),
- An optional open-label treatment phase (Panel A: Day 15 to 74; Panel B: Day 15 to 25), and
- An 8-week posttreatment (follow up) phase

The duration of the subject's participation will be approximately 14 to 23 weeks for Panel A and 14 to 16 weeks for Panel B. The end of study will occur when the last subject in the trial completes his/her last study assessment.

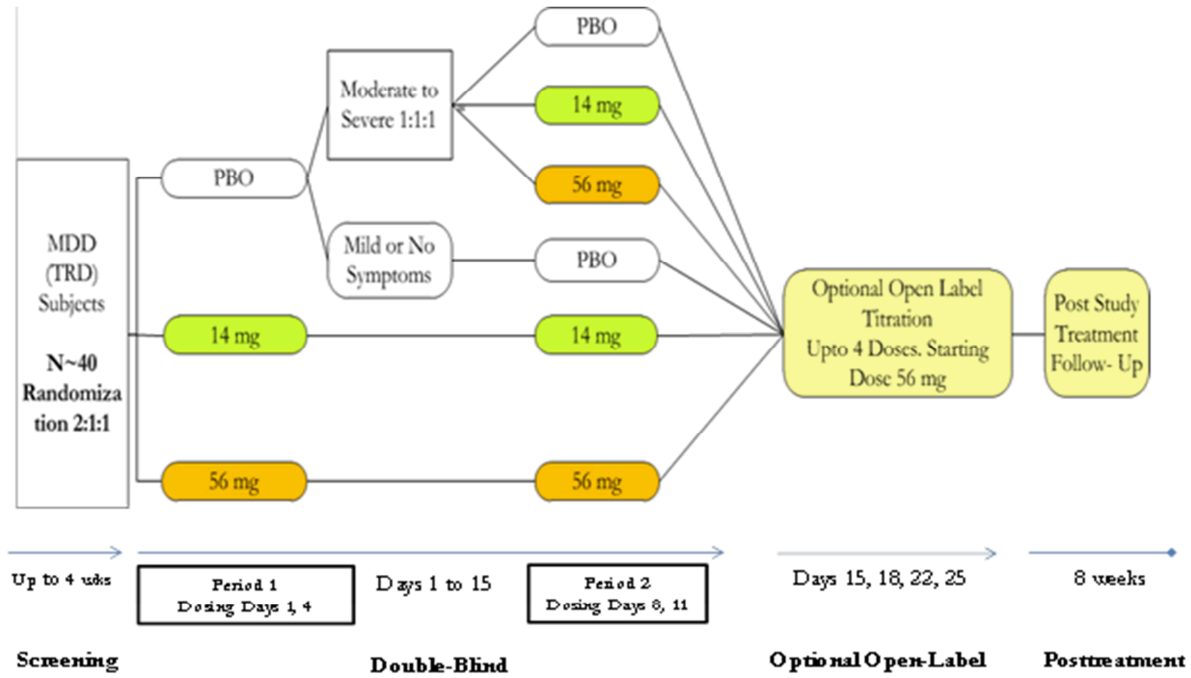
Diagrams of the study design for Panel A and Panel B are provided below (Figure 1 and Figure 2).

Figure 1: Panel A Study Design



* Randomization stratified by QIDS–SR_{16} total score [Moderate = 11 to 16; Severe > 16]

Figure 2: Panel B Study Design



* Randomization stratified by QIDS–SR_{16} total score [Moderate = 11 to 16; Severe > 16]

1.3. Statistical Hypotheses for Trial Objectives

The primary efficacy endpoint for each panel will be the change in MADRS total score from baseline to the 1-week endpoint for the combined periods in the double-blind treatment phase (i.e., Day 1 pre-dose to Day 8 pre-dose and Day 8 pre-dose to Day 15). The null hypothesis that is to be tested to address the primary objective of the trial is that there is no difference between the esketamine dose groups and placebo and no esketamine dose response based on the primary endpoint.

1.4. Sample Size Justification

Panel A

The sample size for Panel A is determined based on the following treatment differences between intranasal esketamine and placebo for the mean change from baseline in MADRS total score: a 9 point treatment difference was assumed for Period 1 (Day 8), a 7 point treatment difference for Period 2 (Day 15) was assumed for subjects with a moderate QIDS-SR_{16} score (QIDS-SR_{16} score = 11 to 16) and a 9 point treatment difference for Period 2 (Day 15) was assumed for subjects with a severe QIDS-SR_{16} score (QIDS-SR_{16} score >16). Based on results of a previous esketamine IV study (ESKETIVRD2001), it is estimated that 40% of placebo subjects at the end of Period 1 (Day 8 pre-dose) will have a moderate QIDS-SR_{16} score and 55% will have a severe QIDS-SR_{16} score. Additional assumptions for the sample size calculation included a standard deviation of 10, 92.5% power for the combined data from both Period 1 and Period 2, an overall 1-sided significance level of 0.05, and a 5% drop-out rate for Period 1. It is calculated that this panel of the doubly-randomized, outcome based design will require 60 subjects to be randomly assigned to treatment on Day 1 in a 3:1:1:1 ratio (30 subjects on placebo and 10 subjects per intranasal esketamine dose group).

Panel B

The sample size for Panel B is determined using the same assumptions for MADRS total score, QIDS-SR_{16}, and drop-out rate as was used for Panel A. Additional assumptions for this panel for the sample size calculation included 90% power for the combined data from both Period 1 and Period 2, and an overall 1-sided significance level of 0.1. It is calculated that this panel of the doubly-randomized, outcome based design will require 40 subjects to be randomly assigned to treatment on Day 1 in a 2:1:1 ratio (20 subjects on placebo and 10 subjects per intranasal esketamine dose group)

1.5. Randomization and Blinding

Panel A

On Day 1 in Period 1, subjects will be randomly assigned to a treatment group based on the first of two computer-generated randomization schedules (Period 1 and Period 2) prepared for the Panel by or under the supervision of the sponsor. The randomization will be balanced by using randomly permuted blocks and will be stratified by study center with an allocation ratio of 3:1:1:1 to placebo and esketamine 28, 56, and 84 mg.

In order to maintain the blind, subjects who have completed Period 1 and continue into Period 2 will not know if they have been re-randomized in Period 2. The randomization will be maintained within the IWRS system and will not be disclosed until after the study has completed and the database has been finalized.

Those subjects who were randomly assigned to treatment with esketamine in Period 1 will continue to receive the same dose of intranasal esketamine in Period 2. Those subjects who were randomly assigned to placebo in Period 1 with a Day 8 (pre-dose) QIDS-SR_{16} score of <11 will continue to receive placebo in Period 2. Subjects who were randomly assigned to placebo in Period 1 with a Day 8 (pre-dose) QIDS-SR_{16} score ≥11 will be re-randomized on Day 8 based on the second of the two computer-generated randomization schedules in a 1:1:1:1 ratio to placebo or 28, 56, or 84 mg of esketamine. The second randomization will be balanced using randomly permuted blocks stratifying by study center and QIDS-SR_{16} [moderate (QIDS-SR_{16} score 11 to 16) or severe (QIDS-SR_{16} score >16)].

Panel B

On Day 1 in Period 1, subjects will be randomly assigned to a treatment group based on the first of two computer-generated randomization schedules (Period 1 and Period 2) prepared for the Panel by or under the supervision of the sponsor. The randomization will be balanced by using randomly permuted blocks and will be stratified by study center with an allocation ratio of 2:1:1 to placebo and esketamine 14 mg and 56 mg.

In order to maintain the blind, subjects who have completed Period 1 and continue into Period 2 will not know if they have been re-randomized in Period 2. The randomization will be maintained within the IWRS system and will not be disclosed until after the study has completed and the database has been finalized.

Those subjects who were randomly assigned to treatment with esketamine in Period 1 will continue to receive the same dose of intranasal esketamine in Period 2. Those subjects who were randomly assigned to placebo in Period 1 with a Day 8 (pre-dose) QIDS-SR_{16} score of <11 will continue to receive placebo in Period 2. Subjects who were randomly assigned to placebo in Period 1 with a Day 8 (pre-dose) QIDS-SR_{16} score ≥11 will be re-randomized on Day 8 based on the second of the two computer-generated randomization schedules in a 1:1:1 ratio to placebo or 14 mg or 56 mg of esketamine. The second randomization will be balanced using randomly permuted blocks stratifying by study center and QIDS-SR_{16} [moderate (QIDS-SR_{16} score 11 to 16) or severe (QIDS-SR_{16} score >16)].

Based on this randomization code, the study drug for the double-blind treatment phase will be packaged and labeled. Unique medication identification numbers will be preprinted on the study drug labels and assigned as subjects qualify for the study and are assigned to treatment.

Central randomization will be implemented in this study. The interactive web response system (IWRS) will assign a unique treatment code, which will dictate the treatment assignment and matching study drug kit for the subject. The requestor must use his or her own user identification

and personal identification number when contacting the IWRS, and will then give the relevant subject details to uniquely identify the subject.

The investigator will not be provided with randomization codes. The codes will be maintained within the IWRS, which has the functionality to allow the investigator to break the blind for an individual subject.

Data that may potentially unblind the treatment assignment (e.g., study drug plasma concentrations, study drug accountability data) will be handled with special care to ensure that the integrity of the blind is maintained and the potential for bias is minimized.

2. GENERAL ANALYSIS DEFINITIONS

2.1. Treatment Groups

Unless specified, for tables summarizing data prior to Day 1 (prior to dosing), the treatment groups are labeled (based on Day 1 randomization) as 'Placebo' and 'Esketamine'.

Planned treatment sequences with respect to Day 1 / Day 8 randomizations are listed below:

Panel A:

- Sequence 1: Placebo /
- Sequence 2: Esketamine 28mg /
- Sequence 3: Esketamine 56mg /
- Sequence 4: Esketamine 84mg /
- Sequence 5: Placebo / Placebo
- Sequence 6: Placebo / Esketamine 28mg
- Sequence 7: Placebo / Esketamine 56mg
- Sequence 8: Placebo / Esketamine 84mg
- Sequence 9: Esketamine 28mg / Esketamine 28mg
- Sequence 10: Esketamine 56mg / Esketamine 56mg
- Sequence 11: Esketamine 84mg / Esketamine 84mg

Panel B:

- Sequence 1: Placebo /
- Sequence 2: Esketamine 14mg /
- Sequence 3: Esketamine 56mg /
- Sequence 4: Placebo / Placebo
- Sequence 5: Placebo / Esketamine 14mg
- Sequence 6: Placebo / Esketamine 56mg
- Sequence 7: Esketamine 14mg / Esketamine 14mg
- Sequence 8: Esketamine 56mg / Esketamine 56mg

Treatment sequences will be labeled as 'Placebo /', 'Esk14 /', 'Esk28 /', 'Esk56 /', 'Esk84 /' 'Placebo / Placebo', 'Placebo / Esk14 ', 'Placebo / Esk28 ', 'Placebo / Esk56 ', 'Placebo / Esk84', 'Esk14/ Esk14', 'Esk28/ Esk28', 'Esk56/ Esk56', and 'Esk84/ Esk84'.

Subjects who receive an incorrect treatment will be analyzed under the treatment randomized.

Tables summarizing data in the OL phase will have columns for 'Pbo/ Esk/ OL Esk', 'Pbo/ Pbo/ OL Esk', 'Esk/ Esk/ OL Esk' and 'Total'.

2.2. Pooling Algorithm

A total of 14 sites in Belgium and US are to enroll subjects in Panel A, and up to 10 sites in Japan are to enroll subjects in Panel B. Actual site enrollment rates are being monitored to avoid gross imbalances across centers. To account for country variability in Panel A, country will be used as a factor in the statistical models to analyze efficacy. Study centers will not be pooled. No pooling of centers will be done for Panel B.

2.3. Analysis Phases

There are 3 analysis phases defined in this study: Double-blind, Open-label and Follow-up (post treatment). Each analysis phase has its own analysis reference start date.

2.3.1. Analysis Phase Start and End Dates

Double-Blind Phase

The analysis reference start date of the DB analysis phase is the date of the first dose of DB medication. The analysis reference end date is the maximum of date of the last visit in the DB treatment phase or early termination date. For randomized subjects who did not receive any medication in the DB period, the reference start date is missing.

Period 1 start date will be the date and time of first administration of study medication. Period 2 start date and time will be the date of first administration of study medication after the second randomization. Period 1 end date will be equal to Period 2 start date or the date of discontinuation if subject does not enter Period 2.

Open-Label Phase

For subjects who enter the OL phase, the analysis reference start date of the OL analysis phase is the date of the first dose of OL medication. This would be the same as the reference end date for the DB analysis phase. The OL analysis reference end date is the date of the last visit in the OL treatment phase.

Follow-Up Phase

The analysis reference start date of the Follow-up (Post-treatment) analysis phase is the day after the end of the preceding period (DB or OL). The analysis reference end date is the maximum of the last follow-up visit date or end of trial date.

2.3.2. Study Reference Start and End Dates

The overall reference start date for the study is defined as the date of the first dose of DB medication (the date is missing for screened subjects who did not receive a dose of DB medication). The overall reference end date for the study is the end of trial date including the last follow-up visit.

2.3.3. Study Day and Relative Days

Study day is calculated relative to the overall reference start date for the study. Relative day is calculated relative to the analysis reference start date of the analysis phase in which the data are captured.

Study day for an event on or after the start of the study is calculated as:

Event date-study start date+1.

Study day for an event prior to the start of the study is calculated as:

Event date-study start date

Relative day for an event on or after a reference start date is calculated as:

Event date – reference start date + 1.

Relative day for an event prior to a reference start date is calculated as:

Event date – reference start date.

2.4. Baseline and End Point

The following baseline values are defined:

- The Periodic Baseline values determined for Period 1 and Period 2 – last observation before receiving the first dose of study drug in the respective Period. If no post-baseline Period 1 measurements are available then Period 2 baseline will be missing.
- Open-Label Baseline – last observation before receiving the first dose of open label study drug.

The DB Phase End Point value will be the final post baseline value assessed during the Double-Blind Phase. In addition, Period 1 End Point and Period 2 End Point value for each study period will be defined as the final post baseline value assessed during the respective Period. The OL Phase End Point value will be the final post baseline value assessed during the Open-Label Phase.

2.5. Visit Windows

As subjects do not always adhere to the protocol visit schedule, the following rules are applied to assign actual visits to protocol visits. Listed below are the visit windows and the target days for each visit. The reference day is Study Day 1 (which is the first day the study drug was taken in the double-blind phase).

If a subject has 2 or more actual visits in one visit window, the visit closest to the target day will be used as the protocol visit for that visit window. The other additional visit(s) will not be used in the summaries or analyses. If 2 actual visits are equidistant from the target day within a visit window, the later visit is used. If a visit window has no scheduled visits but does have unscheduled visits, then the unscheduled visit closest to the scheduled visit will be used.

All assignments will be made in chronological order. Once a visit is assigned to a visit window, it will no longer be used for a later time point except for the end point. Listed below are the visit windows and the target days (if applicable) for each visit defined in the protocol for all phases (Table 1).

Table 1: Analysis Visits

Parameter	Analysis Phase	Analysis Period	Scheduled Day	Time Interval (label on output)	Time Interval (Day)	Target Time Point (Day)
MADRS (2-hour, 24-hour and 7-day recall)	DB	Period 1	1	Baseline (P1)	1 / pre-dose	1
			1	P1 Day 1 (DB): 2H	1	1
			2	P1 Day 2 (DB)	2-3	2
			8	P1 Day 8 (DB)	4-end of P1	8
		Period 2	8	P2 Day 1 (DB): 2H	1	1
			9	P2 Day 2 (DB)	2-3	2
			15	P2 Day 8 (DB)	4-end of DB	8
	OL		18	Day 4 (OL)	2-5	4
			22	Day 8 (OL)	6-9	8
			25	Day 11 (OL)	10-13	11
			32	Day 18 (OL)	14-21	18
			39	Day 25 (OL)	22-28	25
			46	Day 32 (OL)	29-39	32
			60	Day 46 (OL)	40-53	46
			74	Day 60 (OL)	54-end of OL	60
	Follow-up		7	Week 1 (FU)	1-9	7
			14	Week 2 (FU)	10-17	14
			28	Week 4 (FU)	18-32	28
			56	Week 8 (FU)	33-end of FU	56
QIDS-SR_{16}	DB	Period 1	1	Baseline (P1)	1	1
			8	P1 Day 8 (DB)	2- end of P1	8
		Period 2	15	P2 Day 8 (DB)	2-end of DB	8
	OL		18	Day 4 (OL)	2-5	4
			22	Day 8 (OL)	6-9	8
			25	Day 11 (OL)	10-13	11
			32	Day 18 (OL)	14-21	18
			39	Day 25 (OL)	22-28	25
			46	Day 32 (OL)	29-39	32
			60	Day 46 (OL)	40-53	46
			74	Day 60 (OL)	54-end of OL	60
	Follow-up		7	Week 1 (FU)	1-9	7
			14	Week 2 (FU)	10- end of FU	14
CGI-S	DB	Period 1	1	Baseline (P1)	1 / pre-dose	1
			1	P1 Day 1 (DB): 2H	1	1
			2	P1 Day 2 (DB)	2	2
			4	P1 Day 4 (DB): Pre-Dose	3-6 / pre-dose	4
			4	P1 Day 4 (DB): 2H	3-6	4
			8	P1 Day 8 (DB)	7-end of P1 / pre-dose	8
		Period 2	8	P2 Day 1 (DB): 2H	1	1
			9	P2 Day 2 (DB)	2	2
			11	P2 Day 4 (DB): Pre-Dose	3-6 / pre-dose	4
			11	P2 Day 4 (DB): 2H	3-6	4
			15	P2 Day 8 (DB)	7-end of DB	8

Table 1: Analysis Visits

Parameter	Analysis Phase	Analysis Period	Scheduled Day	Time Interval (label on output)	Time Interval (Day)	Target Time Point (Day)
	OL		18	Day 4 (OL)	2-5	4
			22	Day 8 (OL)	6-9	8
			25	Day 11 (OL)	10-13	11
			32	Day 18 (OL)	14-21	18
			39	Day 25 (OL)	22-28	25
			46	Day 32 (OL)	29-39	32
			60	Day 46 (OL)	40-53	46
			74	Day 60 (OL)	54-end of OL	60
	Follow-up		14	Week 2 (FU)	1-17	14
			28	Week 4 (FU)	18-32	28
			56	Week 8 (FU)	33-end of FU	56
PGI-S	DB	Period 1	1	Baseline (P1)	1	1
			1	P1 Day 1 (DB): 2H	1	1
			2	P1 Day 2 (DB)	2	2
			4	P1 Day 4 (DB): Pre-Dose	3-6 / pre-dose	4
			4	P1 Day 4 (DB): 2H	3-6	4
			8	P1 Day 8 (DB)	7-end of P1 / pre-dose	8
		Period 2	8	P2 Day 1 (DB): 2H	1	1
			9	P2 Day 2 (DB)	2	2
			11	P2 Day 4 (DB): Pre-Dose	3-6 / pre-dose	4
			11	P2 Day 4 (DB): 2H	3-6	4
			15	P2 Day 8 (DB)	7-end of DB	8
	OL		18	Day 4 (OL)	2-5	4
			22	Day 8 (OL)	6-9	8
			25	Day 11 (OL)	10-13	11
			32	Day 18 (OL)	14-21	18
			39	Day 25 (OL)	22-28	25
			46	Day 32 (OL)	29-39	32
			60	Day 46 (OL)	40-53	46
			74	Day 60 (OL)	54-end of OL	60
	Follow-up		7	Week 1 (FU)	1-9	7
			14	Week 2 (FU)	10- end of FU	14
PGI-C	DB	Period 1	2	P1 Day 2 (DB)	2-end of P1	2
		Period 2	9	P2 Day 2 (DB)	2-3	2
			15	P2 Day 8 (DB)	4-end of DB	8
PHQ-9	DB	Period 1	1	Baseline	1	1
	OL		15	Day 1 (OL)	1	1
			25	Day 11 (OL)	2-18	11
			39	Day 25 (OL)	19-35	25
			60	Day 46 (OL)	36-53	46
			74	Day 60 (OL)	54-end of OL	60
GAD-7	DB	Period 1	1	Baseline (P1)	1	1
			8	P1 Day 8 (DB)	2-end of P1	8
		Period 2	15	P2 Day 8 (DB)	2-end of DB	8
	OL		22	Day 8 (OL)	2-11	8
			32	Day 18 (OL)	12-24	18
			46	Day 32 (OL)	25-39	32
			74	Day 60 (OL)	40-end of OL	60
	Follow-up		14	Week 2 (FU)	1- end of FU	14

Table 1: Analysis Visits

Parameter	Analysis Phase	Analysis Period	Scheduled Day	Time Interval (label on output)	Time Interval (Day)	Target Time Point (Day)
EQ-5D-5L	DB	Period 1	1	Baseline (P1)	1	1
			4	P1 Day 4 (DB)	2-6	4
			8	P1 Day 8 (DB)	7-end of P1	8
		Period 2	11	P2 Day 4 (DB)	2-6	4
			15	P2 Day 8 (DB)	7-end of DB	8
	OL		18	Day 4 (OL)	2-5	4
			25	Day 11 (OL)	6-17	11
	Follow-up		14	Week 2 (FU)	1- end of FU	14
ECG	DB	Period 1	Screening, 1	Baseline (P1)	≤ 1 / pre-dose	1
			1	P1 Day 1 (DB): 1H	1	1
			4	P1 Day 4 (DB)	2-6 / pre-dose	4
			4	P1 Day 4 (DB): 1H	2-6	4
			8	P1 Day 8 (DB)	7-end of P1 / pre-dose	8
		Period 2	8	Baseline (P2) P2 Day 1 (DB): 1H	1 / pre-dose 1	1
			11	P2 Day 4 (DB)	2-6 / pre-dose	4
			11	P2 Day 4 (DB): 1H	2-6	4
			15	P2 Day 8 (DB)	7-end of DB	8
	OL		15	Baseline (OL) Day 1 (OL): 1H	1 / pre-dose 1	1
			18	Day 4 (OL)	2-5 / pre-dose	4
			18	Day 4 (OL): 1H	2-5	4
			22	Day 8 (OL)	6-9 / pre-dose	8
			22	Day 8 (OL): 1H	6-9	8
			25	Day 11 (OL)	10-13 / pre-dose	11
			25	Day 11 (OL): 1H	10-13	11
			32	Day 18 (OL)	14-21 / pre-dose	18
			32	Day 18 (OL): 1H	14-21	18
			39	Day 25 (OL)	22-28 / pre-dose	25
			39	Day 25 (OL): 1H	22-28	25
			46	Day 32 (OL)	29-39 / pre-dose	32
			46	Day 32 (OL): 1H	29-39	32
			60	Day 46 (OL)	40-53 / pre-dose	46
			60	Day 46 (OL): 1H	40-53	46
			74	Day 60 (OL)	54-end of OL / pre-dose	60
			74	Day 60 (OL): 1H	54-end of OL	60
	Follow-up		14	Week 2 (FU)	1- end of FU	14
Vital Signs (TEMP [pre-dose at each visit] BP, HR, RESP [at each visit, pre-dose, 10M, 40M, 1H and 2H])	DB	Period 1	Screening, 1	Baseline (P1) P1 Day 1 (DB): 10M P1 Day 1 (DB): 40M P1 Day 1 (DB): 1H P1 Day 1 (DB): 2H	≤ 1/ pre-dose 1 1 1 1	1

Table 1: Analysis Visits

Parameter	Analysis Phase	Analysis Period	Scheduled Day	Time Interval (label on output)	Time Interval (Day)	Target Time Point (Day)
			4	P1 Day 4 (DB): Pre-Dose P1 Day 4 (DB): 10M P1 Day 4 (DB): 40M P1 Day 4 (DB): 1H P1 Day 4 (DB): 2H	2-end of P1	4
		Period 2	8	Baseline (P2) P2 Day 1 (DB): 10M P2 Day 1 (DB): 40M P2 Day 1 (DB): 1H P2 Day 1 (DB): 2H	1/ pre-dose 1 1 1 1	1
			11	P2 Day 4 (DB)	2-5	4
			15	P2 Day 8 (DB)	6-end of DB	8
	OL		15	Baseline (OL) Day 1 (OL): 10M Day 1 (OL): 40M Day 1 (OL): 1H Day 1 (OL): 2H	1 / pre-dose 1 1 1 1	1
			18	Day 4 (OL)	2-5	4
			22	Day 8 (OL)	6-9	8
			25	Day 11 (OL)	10-13	11
			32	Day 18 (OL)	14-21	18
			39	Day 25 (OL)	22-28	25
			46	Day 32 (OL)	29-39	32
			60	Day 46 (OL)	40-53	46
			74	Day 60 (OL)	54-end of OL	60
	Follow-up		14	Week 2 (FU)	1- end of FU	14
Weight and BMI	DB	Period 1	Screening, 1	Baseline (P1)	≤ 1	1
			8	P1 Day 8 (DB)	2 -end of P1	8
		Period 2	15	P2 Day 8 (DB)	2-end of DB	8
	OL		18	Day 4 (OL)	2-5	4
			25	Day 11 (OL)	6-end of OL	11
	Follow-up		14	Week 2 (FU)	1- end of FU	14
Pulse oximetry (O2S [at each visit, -5M, 15M, 30M, 45M, 1H])	DB	Period 1	1	Baseline (P1) P1 Day 1 (DB): 15M P1 Day 1 (DB): 30M P1 Day 1 (DB): 45M P1 Day 1 (DB): 1H	1	1
			4	P1 Day 4 (DB)	2-5	4
			8	P1 Day 8 (DB)	6-end of P1	8
		Period 2	11	P2 Day 4 (DB)	2-5	4
			15	P2 Day 8 (DB)	6-end of DB	8
	OL		15	Day 1 (OL)	1	1
			18	Day 4 (OL)	2-5	4
			22	Day 8 (OL)	6-9	8
			25	Day 11 (OL)	10-13	11
			32	Day 18 (OL)	14-21	18
			39	Day 25 (OL)	22-28	25
			46	Day 32 (OL)	29-39	32
			60	Day 46 (OL)	40-53	46
			74	Day 60 (OL)	54-end of OL	60

Table 1: Analysis Visits

Parameter	Analysis Phase	Analysis Period	Scheduled Day	Time Interval (label on output)	Time Interval (Day)	Target Time Point (Day)
Clinical laboratory tests	DB	Period 1	Screening, 1	Baseline (P1)	≤ 1	1
			8	P1 Day 8 (DB)	2-end of P1	8
		Period 2	15	P2 Day 8 (DB)	2-end of DB	8
	OL		25	Day 11 (OL)	2-16	11
			46	Day 32 (OL)	17-39	32
			74	Day 60 (OL)	40-end of OL	60
	Follow-up		14	Week 2 (FU)	1- end of FU	14
Nasal Examination	DB	Period 1	1	Baseline (P1)	1 / pre-dose	1
			1	P1 Day 1 (DB): 2H	1	1
			8	P1 Day 8 (DB)	2-end of P1	8
		Period 2	8	P2 Day 1 (DB): 2H	1	1
			15	P2 Day 8 (DB)	2-end of DB	8
	OL		18	Day 4 (OL)	2-5	4
			25	Day 11 (OL)	6-18	11
	Follow-up		14	Week 2 (FU)	1- end of FU	14
Nasal Tolerability (pre-dose, 2H)	DB	Period 1	1	Baseline (P1)	1 / pre-dose	1
			1	P1 Day 1 (DB): 2H	1	1
			4	P1 Day 4 (DB)	2-6	4
			8	P1 Day 8 (DB)	7-end of P1	8
		Period 2	8	P2 Day 1 (DB)	1	1
			11	P2 Day 4 (DB)	2-5	4
			15	P2 Day 8 (DB)	6-end of DB	8
	OL		15	Day 1 (OL)	1	1
			18	Day 4 (OL)	2-5	4
			22	Day 8 (OL)	6-9	8
			25	Day 11 (OL)	10-13	11
			32	Day 18 (OL)	14-21	18
			39	Day 25 (OL)	22-28	25
			46	Day 32 (OL)	29-39	32
			60	Day 46 (OL)	40-53	46
			74	Day 60 (OL)	54-end of OL	60
BPRS and CADSS (pre-dose, 40M, 2H) MOAA/S (pre-dose and every 5 minutes to 1H) CGAA (1.5H, 2H)	DB	Period 1	1	P1 Day 1 (DB): Pre-Dose	1 / pre-dose	1
			1	P1 Day 1 (DB): 40M	1	1
			1	P1 Day 1 (DB): 2H	1	1
			4	P1 Day 4 (DB): Pre-Dose	2- end of P1	4
			4	P1 Day 4 (DB): 40M	2- end of P1	4
			4	P1 Day 4 (DB): 2H	2-end of P1	4
		Period 2	8	P2 Day 1 (DB)	1	1
			11	P2 Day 4 (DB)	2-end of DB	4
	OL		15	Day 1 (OL)	1	1
			18	Day 4 (OL)	2-5	4
			22	Day 8 (OL)	6-9	8
			25	Day 11 (OL)	10-13	11
			32	Day 18 (OL)	14-21	18
			39	Day 25 (OL)	22-28	25
			46	Day 32 (OL)	29-39	32
			60	Day 46 (OL)	40-53	46
			74	Day 60 (OL)	54-end of OL	60

Table 1: Analysis Visits

Parameter	Analysis Phase	Analysis Period	Scheduled Day	Time Interval (label on output)	Time Interval (Day)	Target Time Point (Day)
C-SSRS	DB	Period 1	<1	Screening	< 1	<1
			1	Baseline (P1)	1	1
			4	P1 Day 4 (DB)	2-6	4
			8	P1 Day 8 (DB)	7-end of P1	8
		Period 2	11	P2 Day 4 (DB)	2-end of DB	4
	OL		15	Day 1 (OL)	1	1
			18	Day 4 (OL)	2-5	4
			22	Day 8 (OL)	6-9	8
			25	Day 11 (OL)	10-13	11
			32	Day 18 (OL)	14-21	18
			39	Day 25 (OL)	22-28	25
			46	Day 32 (OL)	29-39	32
			60	Day 46 (OL)	40-53	46
			74	Day 60 (OL)	54-end of OL	60
	Follow-up		7	Week 1 (FU)	1-9	7
			14	Week 2 (FU)	10- end of FU	14
BPIC-SS	DB	Period 1	1	Baseline (P1)	1	1
		Period 2	15	P2 Day 8 (DB)	2-end of DB	8
	OL		25	Day 11 (OL)	2-16	11
			46	Day 32 (OL)	17-39	32
			74	Day 60 (OL)	40-end of OL	60
	Follow-up		14	Week 2 (FU)	1- end of FU	14
Cogstate and HVLT-R	DB	Period 1	Screening, 1	Baseline (P1)	1	1
	OL		25	Day 11 (OL)	2-16	11
			46	Day 32 (OL)	17-39	32
			74	Day 60 (OL)	40-end of OL	60
	Follow-up		14	Week 2 (FU)	1- end of FU	14

For DB phase, time interval is relative to the first day of Double-Blind treatment within each Period
For OL phase, time interval is relative to the first day of Open-Label treatment
For Follow-up phase, time interval is relative to the first day of Follow-up phase

2.6. Analysis Sets

Subjects will be classified into the following analysis sets: all randomized, intent-to-treat analysis sets and safety analysis sets.

2.6.1. All Randomized Analysis Set

This analysis will include all subjects who were randomized (i.e., subjects who reported a randomization date, or were assigned a randomization number) regardless of whether or not treatment was received. This analysis set will be used for summarizing the overall study completion/withdrawal information.

2.6.2. Efficacy Analysis Set(s)

2.6.2.1. Intent-To-Treat Analysis Sets

For each period of the double-blind phase, an intent-to-treat (ITT) analysis set will be defined:

- The Period 1 ITT analysis set – consists of all randomized subjects who receive at least 1 dose of study medication during Period 1 and have both the baseline and at least one post-baseline MADRS total score within Period 1.
- The Period 2 ITT analysis set – consists of all Period 1 non-responders (QIDS-SR_{16} total score 11 or higher) randomized into Period 2 who receive at least 1 dose of study medication during Period 2 and have both the baseline and at least one post-baseline MADRS total score within Period 2.

The efficacy analyses of data in Period 1 and Period 2 will be based on each respective ITT analysis set.

An ITT (DB) analysis set is defined as all randomized subjects who received at least 1 dose of study medication during the double-blind phase and have both baseline and at least one post-baseline measurement during the 15 day double-blind phase.

An ITT (OL) analysis set is defined as all subjects who received at least 1 dose of study medication during the open-label phase and have both baseline and at least one post-baseline measurement during the open-label phase.

2.6.3. Safety Analysis Sets

Safety analyses for the double-blind phase will be performed on the Safety (DB) analysis set. It will include all randomized subjects who receive at least 1 dose of study drug. Analyses of change from baseline will include only subjects who have both baseline and at least 1 post-baseline observation during the double-blind phase.

In addition, safety analysis sets for each study period will be defined:

- The Period 1 safety analysis set – consists of the randomized subjects who receive at least 1 dose of study medication during Period 1. Analyses of change from baseline will include only subjects who have both baseline and at least 1 postbaseline data during Period 1.
- The Period 2 safety analysis set – consists of all Period 1 placebo non-responders randomized into Period 2 who receive at least 1 dose of study medication during Period 2. Analyses of change from baseline will include only subjects who have both baseline and at least 1 postbaseline data during Period 2.

Safety analyses for the open-label phase will be performed on the Safety (OL) analysis set. It will include all subjects who receive at least 1 dose of study drug during the open-label phase. Analyses of change from baseline will include only subjects who have both baseline and at least 1 post-baseline observation during the open-label phase.

Screen failures and randomized subjects who received no double-blind study medication will be excluded from the safety analysis set. Subjects who received an incorrect treatment will be analyzed under the planned treatment and planned treatment sequence received.

2.7. Definition of Subgroups

Descriptive statistics will be performed for the primary endpoint of MADRS total score change from baseline stratified by the following sub-groups.

- Sex
- Age Group (20-34, 35-54, 55-64 years)
- Country (Panel A only)
- Anxious depression (Yes/No, defined as IDS-C_{30} Items 7+25+26+27+28 $\geq$7)
- Number of antidepressants used in the current episode based on the MGH-ATRQ (1, 2, 3 or more)
- Number of major depressive episodes (<3, 3 or more)
- Family history of alcohol abuse/dependence (Yes/No)
- BDNF genotype (val/val vs met carriers)
- Concomitant use of adjunctive antipsychotics (Yes/No)
- Concomitant use of benzodiazepines (Yes/No)

2.8. Incomplete/Missing Dates for Adverse Events

Treatment-emergent adverse events (AEs) for the double-blind phase are those events with an onset date on or after the start of double-blind study medication, and occurred on or before the end of the double-blind phase. A conservative approach will be used to handle the missing dates for adverse events.

The rules for estimating incomplete AE onset dates will be as follows:

(1) The missing day of the month will be estimated as follows: If the month and year are known and double-blind study medication started during that month then the estimated date is the start date of double-blind study medication. If the month and year are known and double-blind study medication started prior to that month then the estimated date is the 1st day of the month. If the month and year are known and double-blind study medication started after the month, then no estimation will be done, and the AE will not be considered as treatment emergent for the double-blind phase.

(2) If both the day and the month are missing: No estimation will be performed. However, these AEs will be considered treatment emergent for the double-blind phase and will be included in the double-blind treatment summaries, except for the calculation of duration of the AE. Attempts will be made to get at least the month for the adverse events.

For incomplete AE resolution dates, the rules are:

(1) The missing day of the month will be estimated as follows: If the month and year are known and the study medication was stopped before, or during that month, the estimated date is the last day of the month or the end of the double-blind phase, whichever is earlier. If the study medication stopped after that month then the estimated date is the last day of the month.

(2) If both the day and the month are missing: the estimated resolution date is the end of the double-blind phase.

Treatment-emergent adverse events (AEs) for the open-label phase are those events with an onset date on or after the start of OL medication, and occurred on or before the end of the open-label phase. A similar conservative approach that is used for the double-blind phase will be used to handle the missing dates for adverse events.

3. INTERIM ANALYSIS AND DATA MONITORING COMMITTEE REVIEW AND ANALYSIS OF DOUBLE-BLIND PHASE

3.1. Interim Analysis and Data Monitoring Committee Review

A pharmacokinetic/pharmacodynamics analysis was planned after at least 80% of Panel A subjects had completed the double-blind treatment phase. Due to issues in timing it was decided to perform the PK/PD analysis only after 100% of Panel A subjects have completed the double-blind phase, and therefore no unblinded analysis will be performed.

The IWRS randomization data will be reviewed by a Data Review Committee (DRC). The DRC will include experts internal to J&J but who are not directly involved with the study. The DRC is formed to evaluate the percentage of placebo-treated subjects in Period 1 who are eligible for re-randomization at period 2 in order to determine whether an increase in sample size is necessary. Only information for placebo subjects will be reviewed. Details about the review to be performed and the role of DRC are presented in a separate DRC charter.

3.2. Analysis of Double-Blind Phase

For each Panel, after all subjects have completed the double-blind phase of the study, an unblinded analysis will be performed. Some subjects may still be participating in the open label and follow-up phases of the study. This analysis will include the primary efficacy and dose response analysis described in this document, as well as summaries of demographics and baseline characteristics, exposure, adverse events and CADSS assessments.

4. SUBJECT INFORMATION

4.1. Demographics and Baseline Characteristics

Demographic and baseline characteristics (Table 2) and psychiatric history at baseline (Table 3) will be summarized by treatment group for the All Randomized analysis set and the Period 1 and Period 2 ITT analysis sets. The continuous variables will be summarized using descriptive statistics (N, mean, standard deviation [SD], median, minimum, and maximum). The categorical

variables will be summarized using a frequency distribution with the number and percentage of subjects in each category.

Table 2: Demographic Variables and Baseline Characteristics

Continuous Variables:

- Age (years) (informed consent date – date of birth + 1) / 365.25
- Baseline weight (kg)
- Baseline height (cm)
- Baseline BMI (kg/m^2) calculated as Weight (kg)/[Height (m)]^2

Categorical Variables:

- Age (20-34, 35-54, 55-64 years)
- Sex (male, female)
- Race[a] (White, Black or African American, Asian, American Indian or Alaskan native, Native Hawaiian or other Pacific islander, other)
- Ethnicity (Hispanic or Latino, not Hispanic or Latino)
- Baseline BMI (normal: <25 kg/m^2, overweight: 25 kg/m^2 to <30 kg/m^2, obese: ≥30 kg/m^2)

[a] If multiple race categories are indicated, then Race is recorded as "Multiple".

Table 3: Psychiatric History at Baseline Variables

Continuous Variables:

- Baseline MADRS total score
- Baseline IDS-C_{30} total score
- Baseline CGI-S score
- Age when diagnosed with MDD
- Total number of major depressive episodes to date
- Duration of the current episode in weeks

Categorical Variables:

- Baseline CGI-S score
- Antidepressant treatment history (as obtained in the MGH-ATRQ)
- Family history of
  - Depression
  - Anxiety Disorder
  - Bipolar Disorder
  - Schizophrenia
  - Alcohol Abuse/Dependence
  - Substance Abuse

4.2. Disposition Information

The distribution of the number of subjects who are randomized, receive double-blind treatment, and complete the study will be presented by treatment group and period for the double-blind phase of the study. In addition, the distribution of trial termination reasons will be presented. These summaries will be provided by treatment sequence for the All Randomized analysis set and by treatment for the ITT (Period 1) and ITT (Period 2) analysis sets.

The distribution of the number of subjects who enter the open-label phase and complete all open-label visits will be presented by open-label treatment group. The reasons for discontinuing during the open-label phase will be summarized.

A subject will be considered to have completed the double-blind phase if he or she has completed assessments through Day 15. For the open-label treatment phase, a subject will be considered to have completed if he or she has completed assessments through the last planned visit of the open-label phase.

4.3. Extent of Exposure

The total duration in Period 1 and Period 2 is defined as time between the first and the last day of study medication within that Period. For the subjects who enter the optional open-label phase, exposure data recorded on or after the start date of open-label will be summarized separately.

Descriptive statistics (N, mean, SD, median, and range) of total duration will be presented by treatment group for the Period 1 and Period 2 ITT analysis sets. A frequency distribution showing the number of doses received during the double-blind phase (one or two doses per period) will also be provided. The same summary tables will be presented for the 2 week exposure for subjects who received the same treatment for the entire duration of the double-blind phase using the following categories: <1 week, 1 week, <2 weeks, 2 weeks.

A frequency distribution showing the number of doses received during the open-label phase will be provided. The total duration of esketamine exposure across both the double-blind and open-label phases will also be presented using the following categories: <1 week, 1-<2 weeks, 2-<4 weeks, 4-<6 weeks, 6-<8 weeks, 8-10 weeks.

4.4. Protocol Deviations

Deviations that occurred during the study will be tabulated by planned treatment sequence. Major deviations will be tabulated as they are grouped by the Data Management Group prior to the unblinding in the following categories: subject not withdrawn as per protocol, selection criteria not met, excluded concomitant treatment, treatment deviation, non-compliance, regulatory requirement. More categories may be included depending on the nature of the protocol deviation.

4.5. Prior and Concomitant Medications

The number and percent of subjects who receive concomitant therapies will be summarized by treatment sequence by the generic term of the medication for the Safety (DB) analysis set and by OL treatment for the Safety (OL) analysis set.

A similar summary will be presented for antidepressants received prior to study entry according to responses to the MGH-ATRQ for the Safety population.

A table summarizing concomitant use of nervous system medications will also be provided.

5. EFFICACY

Panel A and Panel B will be analyzed separately for efficacy. The same statistical methodology applies to both panels. The efficacy variables for this study are listed in Table 4.

Table 4: Efficacy Variables

Efficacy Variable		Endpoint
MADRS (7-day recall)	• Change in MADRS from Day 1 to Day 8 and Day 8 to Day 15	Primary
	• Sustained response (achieving at least 50% improvement with onset on Day 2 that is maintained to study Day 15)	Secondary
	• Responders (at least 50% reduction)	Secondary
	• Remitters (total score ≤10 and total score ≤12)	Secondary
MADRS (2-hour recall)	• Change in MADRS from Day 1 to 2-hours post dose and Day 8 to 2-hours post dose	Secondary
QIDS-SR_{16} (7-day recall)	• Change in QIDS-SR from Day 1 to Day 8 and Day 8 to Day 15	Secondary
CGI-S	• Change in CGI-S from Day 1 to Day 8 and Day 8 to Day 15	Secondary
PGI-S	• Change in PGI-S from Day 1 to Day 8 and Day 8 to Day 15	Secondary
GAD-7	• Change in GAD-7 from Day 1 to Day 8 and Day 8 to Day 15	Secondary
PGI-C	• PGI-C from Day 2 through Day 15	Exploratory
EQ-5D-5L	• Change in EQ-5D-5L from Day 1 to Day 8 and Day 8 to Day 15	Exploratory
PHQ-9	• Change in PHQ-9 from Day 1 to Day 15 and Day 15 to Days 39, 60 and 74.	Exploratory

5.1. Analysis Specifications

5.1.1. Level of Significance

The overall Type I error rate for testing the overall treatment effect for the primary analysis will be carried out at a 1-sided significance level of 0.05 for Panel A and 0.1 for Panel B.

5.1.2. Data Handling Rules

The last observation carried forward (LOCF) method will be applied to the MADRS total score. For LOCF values within each period, the last post baseline observation in each period will be carried forward as the “End Point” for that period. For LOCF values across the two periods, the last post baseline observation in the DB phase will be carried forward as the “End Point” for Period 2, even if it occurred in Period 1. Besides the observed cases and the end point assessment, the last observation carried forward (LOCF) values for the MADRS total score will be created for intermediate post-baseline time points for each period. These imputed time points will be labeled ‘P1 Day X LOCF’ or ‘P2 Day X LOCF’.

If there are multiple visits within a time window, the last visit will be carried forward. For example, if a subject has a visit on Day 3 for the Day 4 visit and then a final visit on Day 6. The visit on Day 3 will be slotted to the “Day 4” and “Day 4 LOCF” because Day 3 is closer to the target Day 4 than Day 6. The Day 6 visit will be used as “P1 End Point”.

The LOCF approach will be used for missing visit data in the ITT LOCF efficacy analyses. The LOCF calculation will be performed for all scheduled post-baseline time windows in the double-blind phase. If there are missing data for any valid visit windows, the same algorithm will be applied, thus carrying forward the last available observation in chronological order even if not the closest to the target day. LOCF calculation will not be done for the OL phase.

5.1.3. Imputation Methods for Missing Items

Imputation of missing individual item scores will apply to MADRS and is described Section 5.2.1. For all other scales where multiple items are summed to create a total, if any item of the scale is missing on one visit, the total score for that scale at that visit will be left blank.

5.1.4. Change from Baseline

For all efficacy variables changes from baseline will be determined for each period separately, using respective periodical baselines. For subjects who received the same treatment for both periods, changes from baseline will be determined using the Period 1 baseline.

For subjects entering the open-label phase, change from open-label baseline will also be determined.

5.2. Primary Efficacy Endpoint

5.2.1. Definition

The primary efficacy endpoint is the change in MADRS total score from Day 1 to Day 8 in Period 1, and change from Day 8 to Day 15 in Period 2 in the DB phase. The Day 1 and Day 8 MADRS used a 7 day recall period while the other measurements included in this analysis used 24-hour recall. The MADRS consists of 10 items that cover all of the core depressive symptoms (apparent sadness, reported sadness, inner tension, sleep, appetite, concentration, lassitude, interest level, pessimistic thoughts, and suicidal thoughts). For the recall period of 2 hours, the sleep and appetite items will not be assessed (pre-dose scores for these items obtained on the same day will be used). Each item is scored from 0 (item is not present or is normal) to 6 (severe or continuous presence of the symptom). A total score (0 to 60) is calculated by adding the scores of all 10 items. For each item as well as the total score, a higher score represents a more severe condition. If 2 or more items are missing, no imputation will be performed and the total score will be left missing. Otherwise, the total score will be calculated as the sum of the items present multiplied by the ratio of the maximum possible number of items (i.e., 10) to the number of items present.

5.2.2. Analysis Methods

The totality of evidence from the results of the pairwise comparisons of each esketamine IN dose vs. placebo and the comprehensive dose response analyses will be used in the assessment of efficacy.

5.2.2.1. Pairwise Comparisons

ANCOVA

In the first period of the double-blind phase, the esketamine treatment groups will be compared with the placebo group using the primary efficacy endpoint, change from Period 1 baseline to Day 8 in MADRS total score. The comparison will be performed by means of an analysis of covariance (ANCOVA) model, with factors for treatment (placebo, esketamine dose groups), country (for Panel A only), and Period 1 baseline MADRS total score as a continuous covariate. All subjects in the Period 1 ITT analysis set will be included in this analysis. Change from baseline in MADRS total score in Period 2 (Day 8, pre-dose to Day 15) will be analyzed using an ANCOVA model with factors for treatment, country (for Panel A only), Period 2 baseline QIDS-SR_{16} score (moderate or severe), and Period 2 baseline MADRS total score as the continuous covariate. Only data from Period 1 placebo subjects who are re-randomized (moderate and severe QIDS-SR_{16} scores) who continue into Period 2 and have a change value during Period 2 will be included in the analysis of Period 2. Any violations of the normality assumption will be explored for both periods. In Liu et al.[1] a combination test is derived for the doubly randomized design to evaluate the efficacy of the active dose group. The comparison of each intranasal esketamine dose group with placebo will be carried out by a combined test on the weighted test statistics for each period in the double-blind treatment phase:

$$Z = \lambda_1^{1/2} Z_1 + \lambda_2^{1/2} Z_2$$

where Z_1 and Z_2 are the test statistics obtained from treatment comparisons in Periods 1 and 2 respectively, and λ_1 and λ_2 are the weights applied to the corresponding test statistics.

Based on current assumptions for sample size and allocation ratios, the weights are λ_1 = 0.5985084 and λ_2 = 0.4014916 which are based on Chi's technical document[2] (Attachment 1). These same weights will be used for all analyses described in the SAP.

An alternative approach to the weighted combination test will also be provided based on Chi's technical document and the ANCOVA models mentioned above. First, to confirm that the treatment effect is positive in both periods, a consistency test of the interaction of treatment effects will be carried out. A combination test to assess the treatment difference across both periods and the estimate (SE) of this treatment difference will be provided as specified in the technical document.

Descriptive statistics of actual values and changes from baseline by treatment group within each period of the double-blind phase will be provided for observed case and LOCF data. In addition, descriptive statistics of actual values and changes from open-label baseline will be provided for observed case data during the open-label and follow-up phases.

Both observed case data and LOCF mean values and change from baseline over time will be graphically presented for the double-blind phase by period. Observed case values will also be presented over time for the open-label and follow-up phases.

MMRM

To assess the sensitivity of the results of the ANCOVA analysis of the primary endpoint, a mixed-effects model using repeated measures (MMRM) will be performed comparing treatments in change from Period 1 baseline to Day 8 in MADRS total score. The model will include time, treatment, country (for Panel A only), and time-by-treatment interaction as factors and Period 1 baseline MADRS total score as a continuous covariate. An unstructured variance-covariance matrix will be used. In case of convergence problems, simpler variance-covariance structures such as Toeplitz or AR (1) will be considered. The selection of any of these structures will be determined after exploration of the observed correlation structure. The change from Period 2 baseline to Period 2, Day 15 in the MADRS total score in Period 2 will be fit using the same MMRM model as above but with Period 2 baseline MADRS total score as the continuous covariate and including Period 2 baseline QIDS-SR_{16} score (moderate or severe) as a factor. The comparison of intranasal esketamine dose groups with placebo will be carried out by a combined test on the weighted test statistics for each period in the double-blind treatment phase.

Sensitivity Analysis for Missing Data

If the overall missingness is above 15% then sensitivity analyses will be performed to assess the impact of a range of non-ignorable missingness patterns on the robustness of the ANCOVA and MMRM results for the primary efficacy measure. One set of sensitivity analyses will utilize a model-based multiple imputation approach with different imputation parameter values. A range of plausible assumptions for the missing data distribution will be used to produce imputed datasets to evaluate the impact on the primary analysis. Another potential set of sensitivity analyses will use a bootstrap-based framework, by constructing a set of missing data generating models, evaluating the primary analysis method under each of the data generating models and thereby establishing the relationship between selection bias and the missing data impact.

5.2.2.2. Dose Response

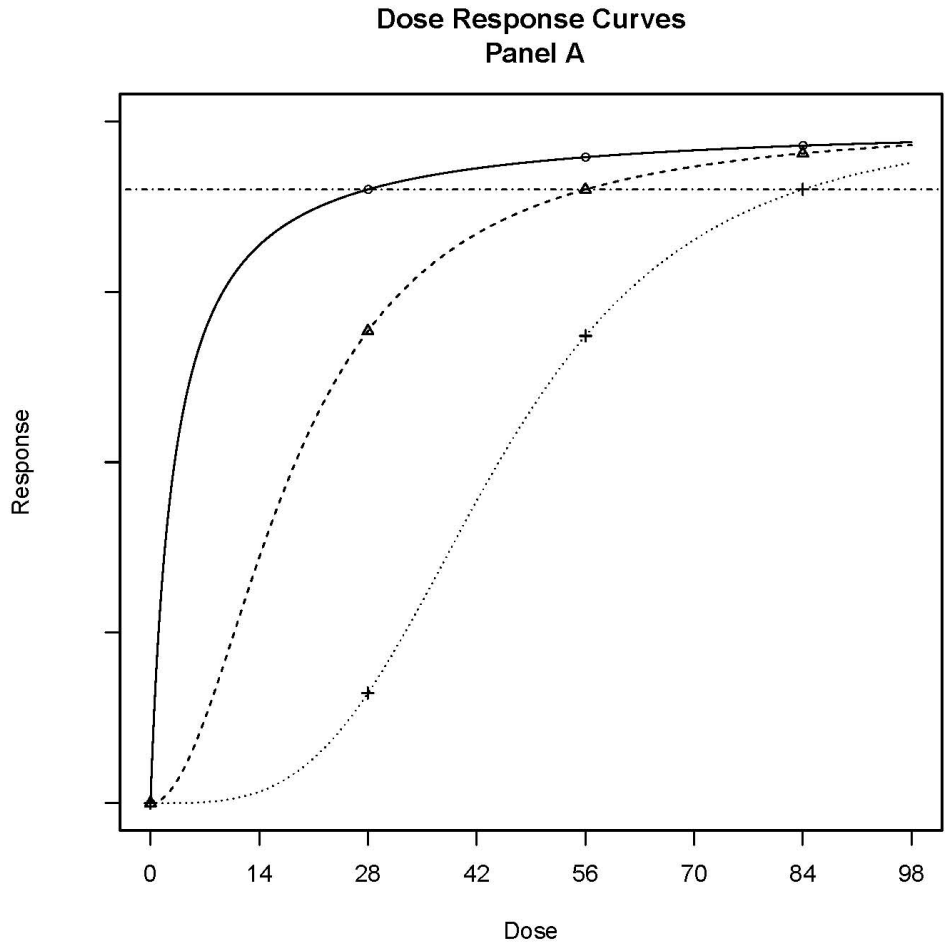
Sigmoid E_{max} Model

In order to provide adequate sensitivity to detect a positive dose response over a range of dose-response patterns, 3 sigmoid E_{max} models are being considered. Under these models, the placebo effect is parameterized to be zero, and therefore the value at a given dosage represents the effect of that dosage compared with placebo.

Panel A

Under the most favorable scenario with the given dose range, the drug is effective at low dosages where ED_{90} is 28 mg and the sigmoid shape parameter is 1. A more realistic scenario may be the one where ED_{90} is 56 mg with 2 for the shape parameter. For the least favorable scenario with the selected dose range, ED_{90} is 84 mg, with 3.5 for the shape parameter. The dose-response curves are shown below in Figure 3.

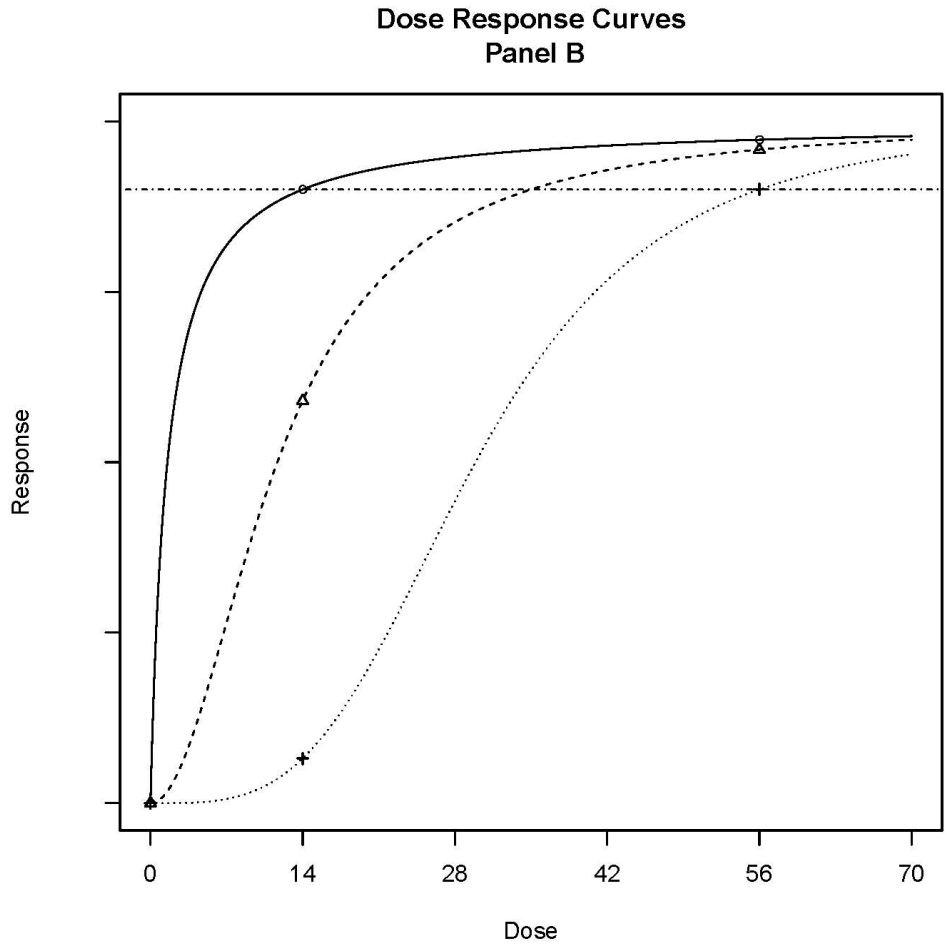
Figure 3: Sigmoid E_{max} Dose Response Curves: Panel A



Panel B

The most favorable scenario with the given dose range, the drug is effective at low dosages where ED_{90} is 14 mg and the sigmoid shape parameter is 1. A more realistic scenario may be the one where ED_{90} is 35 mg with 2 for the shape parameter. For the least favorable scenario with the selected dose range, ED_{90} is 56mg, with 3.5 for the shape parameter. The dose-response curves are shown in the Figure 4 below.

Figure 4: Sigmoid E_{max} Dose Response Curves: Panel B



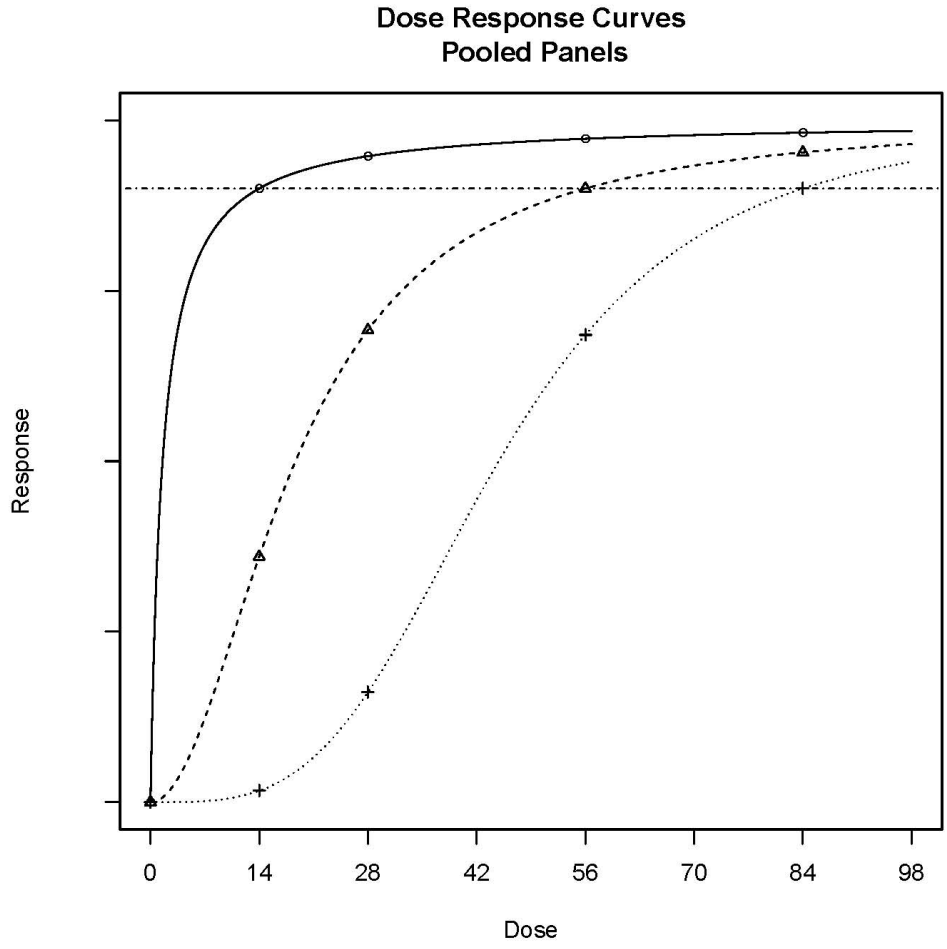
For each Panel, a Period 1 trend test statistic will be derived from an analysis of covariance (ANCOVA) model on the change in MADRS total score, including terms for a linear trend scale, which is derived from a given dose-response curve, a factor for country (in Panel A only), and Period 1 baseline MADRS total score as a continuous covariate. For a given dose-response curve, an optimal linear trend scale will be derived. This gives rise to 3 trend test statistics.

For each Panel, a Period 2 trend test statistic will be derived from an ANCOVA model on the change in MADRS total score in Period 2, including terms for a linear trend scale, which is derived from a given dose-response curve, factors for country (in Panel A only) and Period 2 baseline QIDS-SR_{16} score (moderate or severe), and Period 2 baseline MADRS total score as a continuous covariate. For a given dose-response curve, an optimal linear trend scale will be derived. This gives rise to 3 trend test statistics.

For each period a p-value will be derived from the maximum of the 3 trend test statistics and the multivariate normal distribution of the 3 trend statistics under the null hypothesis of no dose response. The combination test statistic is the weighted sum of the normal inverses of the triple trend test p-values from each of the periods.

Additional dose response analyses will be conducted by pooling data from Panel A and Panel B if it is determined that the two panels can be pooled. The maximum trend test statistics from Period 1 and Period 2 will be derived from the ANCOVA models with an additional factor for panel. For both periods the most favorable scenario with the given dose range, the drug is effective at low dosages where ED_{90} is 14 mg and the sigmoid shape parameter is 1. A more realistic scenario may be the one where ED_{90} is 56 mg with 2 for the shape parameter. For the least favorable scenario with the selected dose range, ED_{90} is 84 mg, with 3.5 for the shape parameter. The dose-response curves shown below in Figure 5 will be used to construct 3 trend test statistics. For each period a p-value will be derived from the maximum of the 3 trend test statistics and the multivariate normal distribution of the 3 trend statistics under the null hypothesis of no dose response. The combination test statistic is the weighted sum of the normal inverses of the triple trend test p-values from each of the periods.

Figure 5: Sigmoid E_{max} Dose Response Curves: Pooled Panel A and Panel B



Best Fit E_{max} model

For each Panel, additional dose response analysis for each period will be the estimation of the best fit sigmoidal E_{max} model based on the observed study efficacy data using the ITT set, and a bootstrapping technique for the calculation of 90% confidence intervals around the estimated treatment effect for a pre-specified set of doses, including the study doses.

The sigmoidal E_{max} model is selected because of its flexibility to capture a wide range of dose-response relationships and the clear interpretation of its parameters. In this model,

$$\mu(dose) = \mu + \tau \frac{dose^{\rho}}{\left(\frac{1}{0.90}-1\right)\times ED_{90}^{\rho} + dose^{\rho}},$$

where μ measures the placebo (dose=0) response and τ measures the maximum effect of the drug.

The maximum likelihood estimates (MLE) of μ, τ, ED_{90}, and ρ are determined using a discretized approach. It can be shown that given a set of values for the ED_{90} and ρ parameters it is possible to identify the conditional MLE's of μ and τ. This is accomplished by computing a trend score for each subject based on the dose received,

$$X(dose; ED_{90}; \rho) = \frac{dose^{\rho}}{\left(\frac{1}{0.90}-1\right)\times ED_{90}^{\rho} + dose^{\rho}}.$$

The trend scores $\underline{X}$ are then used in the ANCOVA model,

$$\underline{Y} = \mu + \tau\underline{X} + \underline{C}\underline{\zeta}^{T} + \underline{e},$$

to identify the conditional MLE's of μ and τ. In the ANCOVA model Y is the vector of observed change in MADRS total score, C is the design matrix representing the covariates, and e is the vector of error terms, which are independent and identically distributed following a normal distribution with mean zero and common standard deviation.

A wide range of ED_{90} and ρ values are considered, and for each pair in the grid search the ANCOVA model will be fit with the corresponding trend scores. The candidate $ED_{0.90}$ values are a sequence from 7 mg [half the lowest dose in this study] to 168 mg [twice the highest dose in this study] with an increment of 3.5 mg [0.25 x lowest dose]. The candidate ρ values are a sequence from 0.50 to 15 with an increment of 0.25. The MLE's are obtained from the ANCOVA model selecting the one associated with the minimum root mean squared error among all candidate models. An estimate of the treatment effect at a particular dose, including those not directly evaluated in the study, is obtained by multiplying $\hat{\tau}_{MLE}$ by the trend score $X\left(dose; ED_{90,MLE}; \rho_{MLE}\right)$.

A single dose response curve will be estimated by the weighted average of the predicted treatment effects at each dose from the best fit sigmoid E_{max} models for each period.

Bootstrapping will be used to construct the confidence intervals for the treatment effect estimates of the selected doses[3]. The original data will be stratified by treatment group, and for each group a random sample equal in size to the original one will be drawn. For each subject, all of their data will be used. The results will comprise the ML estimates of μ, τ, ED_{90}, and ρ for the sigmoidal E_{max} model that best fits the observed data, as well as the treatment effect estimates for the doses in the study. The 90% confidence intervals for the treatment effect associated with these doses will be computed and plotted against the dose level.

MCP-Mod Method

For each panel, additional candidate models for the dose response relationship in each period will be investigated using the MCP-Mod procedure[4]. The set of candidate models includes sigmoid E_{max} models corresponding to the most favorable and least favorable scenarios for the triple trend test, linear and quadratic. The quadratic covers the scenario of a non-monotonic relationship. The dose-response curves are shown below in Figure 6 and Figure 7 for each panelFigure 3.

Figure 6: MCP-Mod Dose Response Curves: Panel A

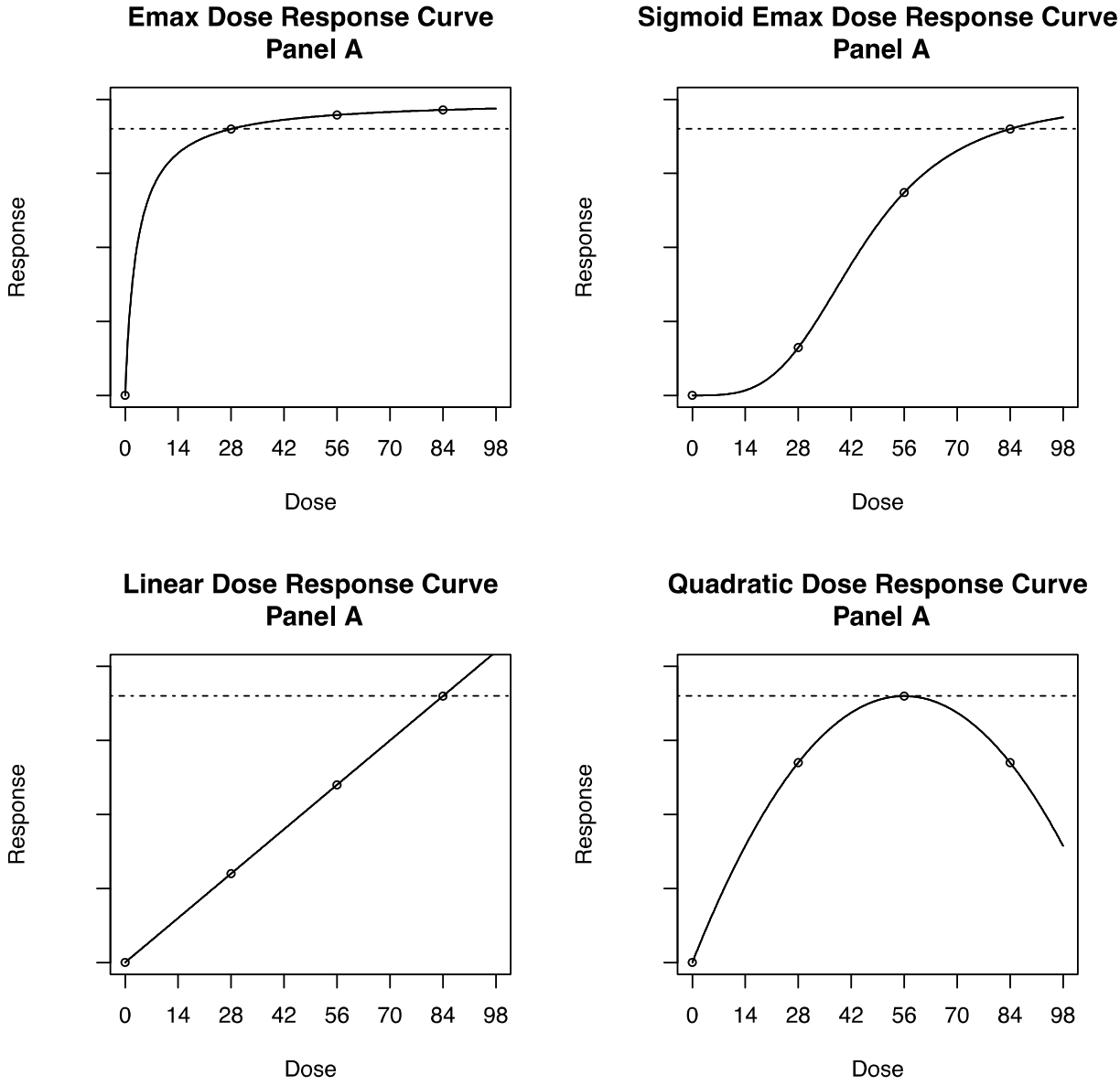
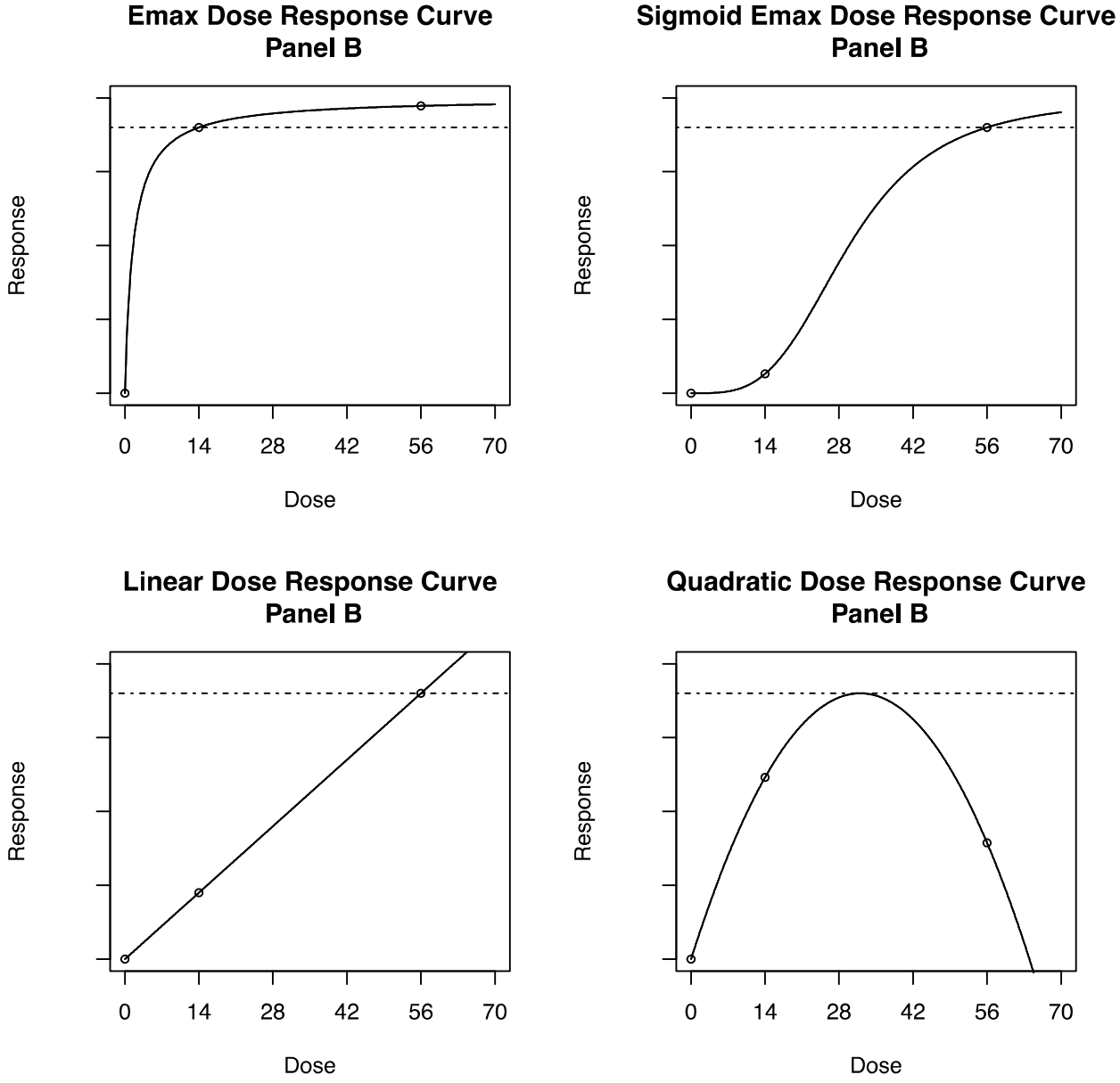


Figure 7: MCP-Mod Dose Response Curves: Panel B



The significance of dose response signal associated with each candidate model will be determined using trend tests with model-specific optimal contrast coefficients, based on the same ANCOVA model described for the triple-trend test. The maximum of the candidate model trend test statistics will be used to evaluate the presence of a dose-response signal, properly accounting for multiplicity. If the maximum test statistic is not significant, no dose-response relationship will be further explored. Otherwise, the model families corresponding to individual candidate models with significant trend test statistics will be fit to the observed data (including the same covariates as in the ANCOVA model as linear terms) and the one with the smallest Akaike Information Criterion (AIC) will be selected to represent the dose response relationship. Using that model a confidence interval for the response at each dose will be computed based on a bootstrap approach. The procedure will be applied to Periods 1 and 2.

A single dose response curve will be estimated by the weighted average of the predicted treatment effects at each dose from the best models for each period, with confidence intervals derived via bootstrapping. The MCP-Mod procedure will be applied to each of Panels A and B as well as to the combined panels, if appropriate.

Spline Model

A nonparametric spline model will also be fit for each of Periods 1 and 2 for the primary efficacy endpoint (change from the corresponding baseline to the end of each Period in the MADRS total score). The spline model for a period has the form

$$Y_i = f(d_i) + c_i + b_i + e_i$$

where i is the subject number, Y_i is the value for the primary efficacy endpoint, d_i is the dose level used in the study period, f is an unknown nonparametric function assumed to be smooth, c_i is the country, b_i is the baseline MADRS total score corresponding to the study period and e_i are independent, zero-mean random errors. The function f is estimated by minimizing the penalized least squares function using the penalty term for the thin-plate smoothing spline method.

The spline model will be used to estimate the response at different dose levels and bootstrap confidence intervals will be computed. The spline models fitted for each of the Periods 1 and 2 will be combined using same weights as for the combination test. These models will be fit separately for Panels A and B as well for the combined panels, if appropriate.

5.3. Major Secondary Endpoints

5.3.1. Sustained Response

5.3.1.1. Definition

Sustained response is defined as at least 50% improvement from baseline in the MADRS total score with onset by Day 2 that is maintained to study Day 15.

5.3.1.2. Analysis Methods

For subjects who remain on the same treatment for the double-blind phase, the summaries will be provided for two groups of subjects 1) those who have completed the double-blind phase using observed case values 2) subjects who have participated in Period 2 (may or may not have completed Period 2) using LOCF values. For both groups of subjects, the adjusted probability for sustained response for the esketamine dose groups (P_{T2}) and placebo (P_{P2}) at Period 2, Day 15 (observed case-completers) and Period 2 Endpoint (LOCF-participated in Period 2) will be estimated using the formulas presented below and in the technical document included in Attachment 2 (Section 1 for the Binary-Binary Case). Subjects who are placebo non-responders in Period 1 who are then randomized to esketamine for Period 2 will not be included for the estimate of the probability of being a sustained responder.

$$\hat{P}_{T2} = \frac{1}{n}\sum_{i=1}^{n} X_{2i} \text{ and } \hat{P}_{P2} = \frac{1}{m}\left(\frac{1}{\alpha}\sum_{i=1}^{\alpha m_n} Y_{2i} + \sum_{m_n+1}^{m} Y_{2i}\right),$$

Where:

n and m are the number of ITT subjects receiving esketamine and placebo, respectively

α is the number of placebo non-responders at the end of period 1 who are re-randomized in Period 2 to stay on placebo/number of placebo non-responders who are re-randomized in Period 2.

X_{2i} and Y_{2i} equal 1 for sustained responder and 0 for non-sustained responder at the end of period 2 for subjects who received esketamine and placebo for both periods of the study.

5.3.2. Responders

5.3.2.1. Definition

The percentage change from baseline at Day X is calculated as 100*(MADRS total score at Day X – Baseline MADRS total score)/(Baseline MADRS total score). Negative percent changes in MADRS total score indicate improvement (e.g., percent change < -50% indicates improvement >50%)

A subject is defined a responder (yes=1 and no=0) at a given time point if the percent improvement in MADRS is ≥50%. Subjects who do not meet such criterion, worsen or discontinue during the DB phase for any reason will be considered as non-responders, i.e. will be assigned a value of 0 (i.e., no).

5.3.2.2. Analysis Methods

The number and percentage of subjects meeting criteria for response will be provided by period at each time point during the DB phase. Response will be determined based on the appropriate period's baseline.

For those subjects who remain on the same treatment for the duration of the double-blind phase, the number and percentage of subjects meeting criteria for response (based on Period 1 baseline) will be provided at double-blind end point. The same adjustment described in Section 5.3.1.2 will be performed.

The cumulative response rate, defined as the percentage of subjects experiencing at least a given value of percent reduction from baseline to Day 8 in MADRS total score will be presented graphically for each period.

Response rates during OL phase will also be summarized.

5.3.3. Remitters

5.3.3.1. Definition

Subjects who have a MADRS total score of ≤10 will be considered remitters. Remission will also be evaluated using a definition of MADRS total score ≤12.

5.3.3.2. Analysis Methods

The number and percentage of subjects meeting criteria for remission will be provided by period at each time point during the DB phase.

For those subjects who remain on the same treatment for the duration of the double-blind phase, the number and percentage of subjects meeting criteria for remission will be provided at double-blind end point. The same adjustment described in Section 5.3.1.2 will be performed.

The number and percentage of subjects meeting criteria for remission will also be provided at each time point during the OL phase.

5.3.4. QIDS-SR$_{16}$

5.3.4.1. Definition

The QIDS-SR$_{16}$ is a self-rated scale that assesses the overall severity of depressive symptoms. The total scores range from 0 to 27. Higher scores indicate greater severity of depression. The total score is obtained by adding the scores for each of the nine symptom domains of the DSM-IV MDD criteria: depressed mood, loss of interest or pleasure, concentration/decision making, self-outlook, suicidal ideation, energy/fatigability, sleep, weight/appetite change, and psychomotor changes. Sixteen items are used to rate the nine criterion domains of major depression: 4 items are used to rate sleep disturbance (early, middle, and late insomnia plus hypersomnia); 2 items are used to rate psychomotor disturbance (agitation and retardation); 4 items are used to rate appetite/weight disturbance (appetite increase or decrease and weight increase or decrease). Only one item is used to rate the remaining 6 domains (depressed mood, decreased interest, decreased energy, worthlessness/guilt, concentration/decision making, and suicidal ideation). Each item is rated 0-3. For symptom domains that require more than one item, the highest score of the item relevant for each domain is taken. For example, if early insomnia is 0, middle insomnia is 1, late insomnia is 3, and hypersomnia is 0, the sleep disturbance domain is rated 3.

5.3.4.2. Analysis Methods

Descriptive statistics of actual values and changes from baseline by treatment group within each period of the double-blind phase will be provided for observed case data. In addition, descriptive statistics of actual values and changes from open-label baseline will be provided for observed case data during the open-label and follow-up phases.

The change from baseline for the QIDS-SR_{16} total score in the double-blind phase will be analyzed using the ANCOVA models similar to those described in Section 5.2.2, except Period 2 will be an ANOVA with factors for treatment, country (for Panel A only) and Period 2 baseline QIDS-SR_{16} score (moderate or severe). The overall comparisons of each esketamine dose group versus placebo will be carried out using the combined test based on the weighted test statistics as described for the primary efficacy endpoint. The same weights used in the primary efficacy analysis will be used for the secondary analyses.

5.3.5. Clinical Global Impression-Severity Scale (CGI-S)

5.3.5.1. Definition

The CGI-S is a physician-rated scale that assesses the severity of mental illness with scores as follows:

1: normal, not at all ill; 2: borderline mentally ill; 3: mildly ill; 4: moderately ill; 5: markedly ill; 6: severely ill; 7: among the most extremely ill patients.

A higher score implies a more severe condition.

5.3.5.2. Analysis Methods

Descriptive statistics of actual values and changes from baseline by treatment group within each period of the double-blind phase will be provided for observed case data. In addition, descriptive statistics of actual values and changes from open-label baseline will be provided for observed case data during the open-label and follow-up phases. Frequency distributions will be provided at each assessment time point during the double-blind, open-label and follow-up phases.

The ranks of the change from baseline for the CGI-S score in the double-blind phase will be analyzed using the ANCOVA models described in Section 5.2.2. The overall comparisons of each esketamine dose group versus placebo will be carried out using the combined test based on the weighted test statistics as described for the primary efficacy endpoint. The same weights used in the primary efficacy analysis will be used for the secondary analyses.

5.3.6. Patient Global Impression-Severity Scale (PGI-S)

5.3.6.1. Definition

The PGI-S is a patient-rated scale that assesses the severity of their illness at the time of assessment, relative to the subject's past experience. It is a 4-point (1 to 4) scale in response to the question 'Considering all aspects of your depression right now would you say your depression is?' with scores as follows: 1: none; 2: mild; 3: moderate; 4: severe. A higher score implies a more severe condition.

5.3.6.2. Analysis Methods

Descriptive statistics of actual values and changes from baseline by treatment group within each period of the double-blind phase will be provided for observed case data. In addition, descriptive statistics of actual values and changes from open-label baseline will be provided for observed

case data during the open-label and follow-up phases. Frequency distributions will be provided at each assessment time point during the double-blind, open-label and follow-up phases.

The ranks of the change from baseline for the PGI-S score in the double-blind phase will be analyzed using the ANCOVA models described in Section 5.2.2. The overall comparisons of each esketamine dose group versus placebo will be carried out using the combined test based on the weighted test statistics as described for the primary efficacy endpoint. The same weights used in the primary efficacy analysis will be used for the secondary analyses.

5.3.7. Generalized Anxiety Disorder (GAD-7)

5.3.7.1. Definition

The GAD-7 is a validated, brief 7-item self-report assessment of anxiety. Each item is scored on a 4-point scale (0-3), with a total score range of 0-21. A higher score indicates more severe anxiety where scores of 5, 10, and 15 represent cut points for mild, moderate, and severe anxiety, respectively.

5.3.7.2. Analysis Methods

Descriptive statistics of actual values and changes from baseline by treatment group within each period of the double-blind phase will be provided for observed case data. A frequency distribution will be provided for GAD-7 severity categories by period for the double-blind phase. In addition, descriptive statistics of actual values and changes from open-label baseline will be provided for observed case data during the open-label and follow-up phases. In addition, a frequency distribution by severity category will be provided at each time point for the open-label and follow-up phases.

The change from baseline for the GAD-7 total score in the double-blind phase will be analyzed using the ANCOVA models described in Section 5.2.2. The overall comparisons of each esketamine dose group versus placebo will be carried out using the combined test based on the weighted test statistics as described for the primary efficacy endpoint. The same weights used in the primary efficacy analysis will be used for the secondary analyses.

5.4. Other Efficacy Variable(s)

5.4.1. Patient Global Impression-Change Scale (PGI-C)

5.4.1.1. Definition

The PGI-C is a 7-point scale that requires the subject to assess how much their illness has improved or worsened relative to a baseline state at the beginning of the intervention. The response options are: 1: very much improved; 2: much improved; 3: improved (just enough to make a difference); 4: no change; 5: worse (just enough to make a difference); 6: much worse; or 7: very much worse. A higher score represents worsening symptoms.

5.4.1.2. Analysis Methods

A frequency distribution will be provided for PGI-C by period at each time point for the double-blind phase. In addition, a frequency distribution will be provided at each time point for the open-label phase.

5.4.2. EuroQol Group; 5 Dimension; 5 Level (EQ-5D-5L)

5.4.2.1. Definition

The EQ-5D-5L is a standardized 2-part instrument for use as a measure of health outcome, primarily designed for self-completion by respondents. It essentially consists of the EQ-5D descriptive system and the EQ visual analogue scale (EQVAS). The EQ-5D-5L descriptive system comprises the following 5 dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Each dimension has 5 levels: no problems, slight problems, moderate problems, severe problems, and extreme problems. The descriptive system can be represented as a health state. The EQ VAS self-rating records the respondent's own assessment of their health status with 0 representing the worst health imagined and 100 representing the best health imagined. Subjects select an answer for each of the 5 dimensions considering the response that best matches their health "today".

Individual scores from the 5 dimensions will be used to obtain a weighted health status index as shown below:

(i) Scores from each dimension will be combined to obtain a 5L profile score: eg, a score of 1 for each dimension will give a 5L profile score of 11111. Dimension scores will be combined in the following order: Mobility, Self-Care, Usual Activities, Pain/Discomfort, Anxiety/Depression

(ii) A spreadsheet containing the Health Status Index for various values of 5L profile scores will be downloaded from the following website: http://www.euroqol.org/fileadmin/user_upload/Documenten/Excel/Crosswalk_5L/EQ-5D-5L_Crosswalk_Value_Sets.xls

(iii) The patient level data will be merged with the spreadsheet data to get the country-specific HSI values. Value sets are available for US and Japan. The value sets for the Netherlands will be used for subjects in Belgium

5.4.2.2. Analysis Methods

Descriptive statistics of actual values and changes from baseline by treatment group will be provided for the individual dimensions, the weighted EQ-5D health status index, and the VAS at each time point for the double-blind, open-label and follow-up phases.

Individual dimension responses will also be summarized at each visit with frequency counts and percentage of subjects by treatment group for the double-blind, open-label and follow-up phases.

5.4.3. Patient Health Questionnaire-9 (PHQ-9)

5.4.3.1. Definition

The PHQ-9 is a 9-item, self-report scale assessing depressive symptoms. Each item is rated on a 4-point scale (0 = Not at all, 1 = Several Days, 2 = More than half the days, and 4 = Nearly every day), with a total score range of 0-27. A higher score indicates greater severity of depression. The recall period is 2 weeks. The scale scores each of the nine symptom domains of the DSM MDD criteria and it has been used both as a screening tool and a measure of response to treatment for depression.

5.4.3.2. Analysis Methods

For the PHQ-9 total score, descriptive statistics of actual values and changes from both Period 1 baseline and open-label baseline will be provided for observed case data during the open-label phase.

6. SAFETY

Safety data from Panels A and B will be pooled for analysis. Selected summaries will be analyzed separately by Panel. In addition selected summaries will be provided by Period in the double-blind phase.

All safety summaries for the double-blind phase will be based on the Safety (DB) analysis set. Safety summaries for the open-label phase will be based on the Safety (OL) analysis set.

6.1. Adverse Events

Adverse events (AEs) are coded using the MedDRA dictionary (version 17.0 or above). Treatment-emergent adverse events (TEAEs) that occurred in each study phase (Double-Blind and Open Label) will be summarized separately by system organ class, preferred term and treatment group.

Treatment-emergent adverse events in different phases and periods of the study are defined as below:

- Treatment-emergent adverse events during Period 1 of the double-blind phase are defined as AEs with onset during Period 1. In other words, treatment-emergent AE during Period 1 should satisfy the condition: Period 1 start date/time ≤ AE onset date/time < Period 1 end date/time. If onset time is missing and AE onset date is the same as Period 1 start date, the AE is defined to be treatment emergent in Period 1.
- Treatment-emergent adverse events during Period 2 of the double-blind phase are defined as AEs with onset during Period 2. Treatment-emergent AE during Period 2 should satisfy the condition: Period 2 start date/time ≤ AE onset date/time < Period 2 end date/time. Since for subjects who enter Period 2 the Period 1 end date is the same as Period 2 start date, an AE with missing onset time and onset date same as Period 2 start date is defined to be treatment emergent during Period 2 (not during Period 1).

- Treatment-emergent adverse events during the DB phase are AEs which are treatment-emergent either during Period 1 or during Period 2 of the double-blind phase.
- Treatment-emergent adverse events during the open-label phase are defined as AEs with onset during OL phase. Treatment-emergent AE during open-label phase should satisfy the condition: (OL phase start date/time ≤ AE onset date/time and AE onset date ≤ OL phase end date). Since for subjects who enter OL phase the Period 2 end date is the same as open label phase start date, an AE with missing onset time and onset date same as OL phase start date is defined to be treatment emergent during the OL phase (not during the DB phase).

AEs occurring in the OL phase will be presented by both treatment received during the double-blind phase and the total esketamine group.

A TEAE is an event that is new in onset or increased in severity following treatment initiation. An event that starts prior to, and ends after the initiation of study medication will be considered treatment-emergent only if the severity increases after the start of medication. Adverse events occurring during the follow-up phase will not be considered treatment-emergent but will be included in listings of all events. AEs will be summarized by severity and relationship to study drug using the preferred term. For the summaries of AEs by severity/relationship to study drug, the observation with the most severe occurrence/closest relationship to study drug will be chosen if there is more than one incident of an adverse event reported during the analysis phase by the subject.

Data from subjects with treatment-emergent AEs that had onset in Period 1 and persisted during Period 2 (AE end date > Period 2 start date or AE end date missing) will be listed by treatment sequence and relationship to the study medication.

Serious AEs (SAEs) and AEs that lead to study discontinuation will be summarized separately by treatment group, system organ class and preferred term. Data listings will also be generated for deaths, SAEs, and discontinuations due to AEs.

Adverse Events of Special Interest

Clinically relevant TEAEs of special interest will be examined separately grouped in the following categories: drug abuse, dependence and withdrawal (SMQ); transient dizziness/vertigo (Dizziness, Dizziness exertional, Dizziness postural, Dizziness procedural, Vertigo, Vertigo labyrinthine, Vertigo positional, Vertigo CNS origin); impaired cognition (Cognitive disorder, Minor cognitive motor disorder); cystitis (Allergic cystitis, Chemical cystitis, Cystitis, Cystitis bacterial, Cystitis erosive, Cystitis escherichia, Cystitis glandularis, Cystitis gonococcal, Cystitis haemorrhagic, Cystitis helminthic, Cystitis interstitial, Cystitis klebsiella, Cystitis noninfective, Cystitis pseudomonal, Cystitis ulcerative, Cystitis viral, Cystitis-like symptom); and anxiety (Anticipatory anxiety, Anxiety).

6.2. Clinical Laboratory Tests

Descriptive statistics (N, mean, median and range) for values and changes from baseline will be provided for the clinical laboratory tests (hematology, chemistry and urinalysis) at each scheduled time point in the double-blind and open-label phases. The Period 1 baseline will be

used to calculated change for the double-blind phase summary. Changes from baseline for the open-label phase will be calculated using both the Period 1 baseline and the open-label baseline.

Clinical laboratory tests that meet the criteria for markedly abnormal will be listed by subject. The incidence of treatment emergent markedly abnormal laboratory values that occurred at any time during the double-blind phase or the open-label phase will be presented. Clinical laboratory test values will be considered "treatment emergent markedly abnormal" (TEMA) using the criteria defined by the Sponsor (Janssen Research & Development, LLC) listed in Attachment 3. The identification of TEMA laboratory values is based on the postbaseline value being out of range while the baseline value is either missing or within the range given in Attachment 3. If post-baseline laboratory results are above the upper limit and the baseline value is below the lower limit, then the post-baseline abnormality will also be considered TEMA. The same applies to the postbaseline value being below the lower limit with the baseline value being above the upper limit. The double-blind phase summary will use the Period 1 baseline as the baseline value to determine markedly abnormal values. Two markedly abnormal summaries will be provided for the open-label phase, one using the Period 1 baseline to determine markedly abnormal values and one using the open-label baseline to determine markedly abnormal values.

6.3. Vital Signs, Weight and BMI

Descriptive statistics for values and changes from baseline at each scheduled time-point during the double-blind and open-label phases will be presented for Systolic Blood Pressure (mmHg), Diastolic Blood Pressure (mmHg), Pulse Rate (beats per minute), Respiratory Rate (breaths per minute), Oxygen Saturation (%), Weight (kg) and BMI.

The proportion of subjects who have a treatment-emergent abnormality, as defined in Table 5 below, will be presented for the double-blind and open-label phases. The double-blind phase summary will use the Period 1 baseline as the baseline value to determine markedly abnormal values. Two markedly abnormal summaries will be provided for the open-label phase, one using the Period 1 baseline to determine markedly abnormal values and one using the open-label baseline to determine markedly abnormal values.

A listing of subjects meeting any of the criteria will also be provided.

Table 5: Treatment-Emergent Abnormality Categories for Vital Signs

	Post-baseline value outside of normal limit if:	
Vital Parameter	Abnormally low	Abnormally high
Pulse (bpm)	A decrease from baseline of ≥ 15 to a value ≤ 50	An increase from baseline of ≥ 15 to a value ≥ 100
Systolic BP (mmHg)	A decrease from baseline of ≥ 20 to a value ≤ 90	An increase from baseline of ≥ 20 to a value ≥ 180
Diastolic BP (mmHg)	A decrease from baseline of ≥ 15 to a value ≤ 50	An increase from baseline of ≥ 15 to a value ≥ 105

BP = blood pressure

A listing of subjects with oxygen saturation less than 92% will be provided.

6.4. Nasal Examination

Targeted nasal examinations (including the upper respiratory tract/throat) will be conducted by a qualified healthcare practitioner. The objective of the examination at Screening is to rule out any subjects with anatomical or medical conditions that may impede drug delivery or absorption.

Subsequent examinations will consist of a visual inspection of the nostrils, nasal mucosa, and throat for nasal erythema, rhinorrhea, rhinitis, capillary/blood vessel disruption and epistaxis and graded as follows: none, mild, moderate, or severe. Any treatment emergent change or worsening from baseline examination will be recorded as an adverse event.

A shift table for changes in rating for each examination will be presented for the double-blind and open-label phase. The Period 1 baseline will be used for the double-blind summary. Two summaries will be provided for the open-label phase, one using the Period 1 baseline and one using the open-label baseline. Changes in findings from the baseline nasal examination (including the upper respiratory tract/throat) will be listed by treatment group.

6.5. Electrocardiogram

The ECG variables that will be analyzed include heart rate, RR, PR, QRS interval, QT interval and QTc intervals. The corrected QT will include the QTcB (Bazett) and QTcF (Fridericia).

Average pre-dose value (defined as the average of ECG results collected at all visits prior to and including Day 1), and maximum post-baseline value will be computed for each ECG parameter using data from both scheduled and unscheduled visits. Average pre-dose will be used as the 'baseline' ECG.

Summary tables for values and changes from average pre-dose will be presented by treatment sequence at each timepoint during the double-blind phase. In addition, summary statistics for values and changes from average pre-dose and open-label baseline will be presented for the open-label phase.

The frequency of treatment-emergent abnormalities will be tabulated and presented for the double-blind and open-label phases. The identification of treatment-emergent abnormal ECG values is based on the post-baseline value (a value occurring after the first study drug administration in each phase) being out of range while the baseline value is either missing or within the limits given in Table 6. If post-baseline ECG results are above the upper limits (abnormally high) and the baseline value is below the lower limits (abnormally low), then the post-baseline abnormality will also be considered treatment-emergent. The same applies to the post-baseline value being below the lower limits (abnormally low) with the baseline value being above the upper limits (abnormally high). The average pre-dose value will be used as baseline for the double-blind summary. Two summaries will be provided for the open-label phase, one using the average pre-dose value and one using the open-label baseline.

Abnormal ranges for the HR, PR, QRS and QT intervals are given in Table 6.

Table 6: Limits for HR, PR, QRS and QT Interval Abnormality

ECG parameter	Abnormally Low	Abnormally High
HR (bpm)	≤ 50	≥ 100
PR interval (msec)	--	≥ 210
QRS interval (msec)	≤ 50	≥ 120
QT interval (msec)	≤ 200	≥ 500

Based on the maximum QTc value for each subject during the double-blind phase and open-label phase (separate for each QTc correction) the incidence of abnormal QTc values and changes from baseline (average pre-dose for the double-blind phase and both average pre-dose and open-label baseline for the open-label phase) will be summarized by treatment group. Criteria for abnormal corrected QT intervals and changes from baseline are given in Table 7 and are derived from the ICH E14 Guidance[5] (the same criteria apply to all QT corrections).

Table 7: Criteria for Abnormal QTc Values and Changes From Baseline

Parameter	Classification	Criteria
Clinically Significant QTc Value	No	≤500
	Yes	>500
QTc change from baseline [a]	No concern	≤30
	Concern	>30 – 60
	Clear concern	> 60
QTc value	Normal	≤450
	> 450 – 480	>450 - ≤480
	> 480 – 500	>480 – ≤500
	> 500	> 500

These criteria are based on ICH E14 Guideline

[a] Baseline is defined as the average pre-dose for the double-blind phase and open-label baseline for the open-label phase.

The proportion of subjects with treatment emergent abnormalities will be presented for each phase. A listing of subjects with abnormalities will also be provided.

6.6. Other Safety Parameters

6.6.1. Columbia Suicide Severity Rating Scale (C-SSRS)

The C-SSRS is a low-burden measure of the spectrum of suicidal ideation and behavior. It is a semi structured clinician-administered questionnaire designed to solicit the occurrence, severity, and frequency of suicide-related ideation and behaviors during the assessment period.

The summaries of the C-SSRS outcomes will be based on the safety analysis set subjects who have at least 1 post-baseline C-SSRS measurement and a pre-treatment C-SSRS assessment (Lifetime assessment at screening).

Using the C-SSRS, potentially suicide-related events will be categorized using the following scores:

Suicidal Ideation (1-5)

1: Wish to be Dead

2: Non-specific Active Suicidal Thoughts

3: Active Suicidal Ideation with Any Methods (Not Plan) without Intent to Act

4: Active Suicidal Ideation with Some Intent to Act, without Specific Plan

5: Active Suicidal Ideation with Specific Plan and Intent

Suicidal Behavior (6-10)

6: Preparatory Acts or Behavior

7: Aborted Attempt

8: Interrupted Attempt

9: Actual Attempt (non-fatal)

10: Completed Suicide

If no events qualify for a score of 1 to 10, a score of 0 will be assigned (0="no event that can be assessed on the basis of C-SSRS").

A frequency distribution at each time point by treatment sequence will be provided for safety analysis set. Shifts from the screening visit to the maximum score during the double-blind phase will be summarized by treatment sequence for Safety analysis set. Shifts from the screening visit to the maximum score during the open-label phase will also be summarized.

The maximum score assigned for each subject will be summarized into one of three broad categories: No suicidal ideation or behavior (0), Suicidal ideation (1-5), Suicidal behavior (6-10). Shifts from the screening visit to the maximum category during the double-blind Phase will be summarized by treatment sequence for safety analysis set. Shifts from the screening visit to the maximum category during the open-label phase will also be summarized.

6.6.2. MOAA/S

The MOAA/S will be used to measure treatment-emergent sedation with correlation to levels of sedation defined by the American Society of Anesthesiologists (ASA) continuum.

The MOAA/S scores range from 0 [No response to painful stimulus; corresponds to ASA continuum for general anesthesia] to 5 [Readily responds to name spoken in normal tone (awake); corresponds to ASA continuum for minimal sedation].

The MOAA/S is measured on each dosing day every 5 minutes from pre-dose to 1 hour post-dose or longer, if necessary, until the subject has a score of 5.

Descriptive statistics of the MOAA/S score and changes from pre-dose will be summarized at each scheduled time point. In addition, the proportion of subjects experiencing sedation (score less than or equal to 3) will be presented by period and treatment group during the double-blind phase and by treatment during the open-label phase.

6.6.3. Clinical Global Assessment of Alertness

The Clinical Global Assessment of Alertness will be used to measure the subject's current level of alertness. The clinician will answer "Yes" or "No" to the question "Is the subject's level of alertness considered to be within the normal range?"

The assessment is measured on each dosing day at 1.5 and 2 hours postdose, repeated every 30 minutes if necessary until the response is 'Yes'.

The proportion of subjects with a response of 'No' at each time point will be presented by period and treatment group during the double-blind phase and by treatment during the open-label phase.

6.6.4. Brief Psychiatric Rating Scale (BPRS)

The Brief Psychiatric Rating is an 18 item rating scale which is used to assess a range of psychotic and affective symptoms rated from both observation of the subject and the subject's own report. Only the four-item positive symptom subscale (consisting of: suspiciousness, hallucinations, unusual thought content and conceptual disorganization) will be used in the study to assess treatment emergent psychotic symptoms. Each symptom is rated on a scale of 0 to 6 as follows: 0: not present, not evident or absent; 1: very mild; 2: mild; 3: moderate; 4: moderate severe; 5: severe; or 6: extreme. A total score will be derived by summing the individual items, with a range of 0 to 24 with a higher score representing a more severe condition.

The BPRS is measured prior to each dose, at 40 minutes and at 2 hours post dose.

Descriptive statistics (N, median, minimum, and maximum) of the total scores at each time point and visit along with change from the pre-dose time point within each visit will be presented.

The proportion of subjects with an increase in BPRS from the pre-dose value at any time during the study will be presented by treatment group. The proportion of subjects with a score of 3 or more at any time during the study will also by presented by treatment group.

Mean change in BPRS from pre-dose value will be presented graphically for each dose day.

6.6.5. Clinician Administered Dissociative States Scale (CADSS)

The Clinician Administered Dissociative States Scale (CADSS) is an instrument for the measurement of present-state dissociative symptoms. The CADSS comprises 23 subjective items and participant's responses are coded on a 5-point scale (0 = "Not at all", 1 = "Mild", 2 = "Moderate", 3 = 'Severe" and 4 = "Extreme"). The CADSS is divided into 3 components using the following scoring method:

Component	Questions	Range
Depersonalization	Sum of 3, 4, 5, 6, 7, 20, 23	0-28
Derealization	Sum of 1, 2, 8, 9, 10, 11, 12, 13, 16, 17, 18, 19, 21	0-52
Amnesia	Sum of 14, 15, 22	0-12
Total Score	Sum of 1 through 23	0-92

For the total score and each component, a higher score represents a more severe condition. The CADSS is measured prior to the start of each dose, at 40 minutes and at 2 hours post dose.

Descriptive statistics (N, median, minimum, and maximum) of the total scores and component scores at each time point and visit along with change from the pre-dose time point within each visit will be presented.

In addition, the proportion of subjects with an increase in CADSS total score from the pre-dose value at any time during the study will be presented by treatment group and period during the double-blind phase and by treatment during the open-label phase.

Mean change in CADSS from pre-dose value will be presented graphically for each dose day.

6.6.6. Nasal Tolerability Questionnaire

Subjects will complete a nasal tolerability questionnaire on every dosing day at pre-dose and again at 2 hours postdose. Subjects will rate as none, mild, moderate or severe the following items: stuffy nose, blocked nose, runny nose, itching nose, crusting discharge in or on nose, dryness of nose, burning sensation in the nose, discomfort of nose, bleeding from the nose, postnasal drip, cough, sore throat, taste disturbance and sneezing.

Frequency distributions will be provided for each of the items at each time point and visit during the study.

6.6.7. Bladder Pain/Interstitial Cystitis Symptom Score (BPIC-SS)

The Bladder Pain/Interstitial Cystitis Symptom Score (BPIC-SS) is a patient-reported measure that was developed by ©Pfizer Ltd to identify an appropriate Bladder Pain Syndrome/Interstitial Cystitis (BPS/IC) population for clinical studies to evaluate new treatments for Bladder Pain Syndrome (BPS).

The BPIC-SS contains 8 questions with a recall period of the past 7 days. Each of the response option check boxes has a number beside it (or below it in the case of question 8). A total score is calculated by adding up the numbers beside the response options chosen by the patient. Questions 1 - 7 are scored from 0 to 4 and Question 8 is scored from 0-10. The range of scores

for the scale is 0 to 38 with higher scores indicating more frequent and bothersome symptoms. The BPIC-SS does not contain domains.

If any items are missing, a total score cannot be calculated.

The BPIC-SS will be assessed at Day 1 pre-dose and at Day 15/ET of the double-blind phase. It will also be assessed during OL on Day 25 (Panel A and Panel B), Day 46 (Panel A only), Day74 (Panel A only) and again at 2 weeks post-treatment.

Descriptive statistics of the scores and changes from pre-dose will be summarized at each scheduled time point. Frequency counts of subjects with a score greater than 18 with negative urinalysis results will be summarized.

6.6.8. Physician Withdrawal Checklist

The PWC-20 will be administered to assess potential withdrawal symptoms following cessation of intranasal esketamine treatment. The PWC-20 is a 20-item simple and accurate method to assess potential development of discontinuation symptoms after stopping of study medication. The PWC-20 is a reliable and sensitive instrument for the assessment of discontinuation symptoms.

Descriptive statistics for the PWC-20 rating scale will be presented. Data listings of subjects with withdrawal symptoms following abrupt cessation of treatment will be presented. Shifts from the end of study visit to follow-up visits at weeks 1 and 2 will also be summarized

6.6.9. Cogstate® Cognitive Test Battery and HVLT-R

The Cogstate® computerized test battery provides assessment of multiple cognitive domains including attention, visual learning and memory, and executive function. The tests use playing card stimuli and a maze task, enabling use in multilingual/multicultural settings.

The HVLT-R, a measure of verbal learning and memory, is a 12-item word list recall test.

These assessments are performed only for subjects in Panel A who have a baseline assessment on Day 1 and subsequently enroll in the optional open-label phase.

Descriptive statistics of each of the cognitive domain scores, the HVLT-R and changes from baseline will be summarized at each scheduled time point.

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ATTACHMENTS

Attachment 1: A New Approach for the Design and Analysis of a Doubly Randomized Delayed Start Design

September 2, 2014

JANSSEN RESEARCH AND DEVELOPMENT, LLC

TECHNICAL DOCUMENT

A New Approach for the Design and Analysis of a Doubly Randomized Delayed Start (DRDS) Design to Reduce the Impact of Placebo Response

George YH Chi

1. Background

It has been known for a long time that in some psychiatric trials such as mood disorder trials, there is a relatively high proportion of subjects that do respond to placebo [Temple (1994)]. This phenomenon often led to failure of these trials to demonstrate a new treatment's effectiveness. The reason is that the potential bias it introduced into these trials under a standard classical randomized double-blind parallel design had the effect of mitigating the overall effect of a new treatment. Temple (1994) had suggested that some kind of enrichment designs should be considered in order to show that a new treatment works. Some trial designs and methods of analysis have since been proposed to address this issue. Fava et al (2003) proposed a sequential parallel design (SPD). However, the SPD design does not involve a re-randomization in the second period. Recently, Liu et al. (2010, 2012) considered a DRDS design with re-randomization of the placebo non-responders in the second period. Chen et al. (2011) considered a SPD design with re-randomization in the second period which they termed a SPD design with re-randomization (SPD-Re). In all of these papers, a combination of the z-statistics from period 1 and period 2 is used that has certain power optimality property. However, there are a couple of issues related to such combination tests. First, it is not obvious how to interpret their combination tests, since it is not exactly clear what the combination test is estimating because the coefficients themselves are functions of the unknown treatment effects from period 1 and period 2 and are possibly non-linear. Secondly, the rejection of the global null hypothesis by the combination test alone is not sufficient to conclude that the new treatment is effective for the target population. It is the purpose of this paper to propose a new approach to the design and analysis of a DRDS or SPD-Re trial so that the above issues can be reasonably addressed and the evidence from a DRDS or SPD-Re trial can provide the strength of evidence required to establish the effectiveness of the treatment for the target population.

In this paper, the basic doubly randomized delayed start design (DRDS) as proposed by Liu et al (2012) is considered where the placebo non-responders from the first period as shown in Figure 1 will be re-randomized in the second period. However, here a new and more rigorous approach is proposed for assessing the treatment effect for the target population by taking into account the totality of evidence from the two periods in a more comprehensive way. In Tamura and Huang (2007) and Liu et al. (2012), an optimal combination test was proposed to test the global null hypothesis. However, it is not clear what this combined statistic is actually estimating and hence it is not clear how to interpret the combination test. In addition, the rejection of the global null hypothesis by the combination test only implies that the treatment effects from period 1 and period 2 cannot be both negative or zero, and it does not rule out the possibility that the treatment effects from period 1 and period 2 can be of opposite sign, particularly if the treatment effect from period 1 is negative and that from period 2 is positive. Furthermore, even if the

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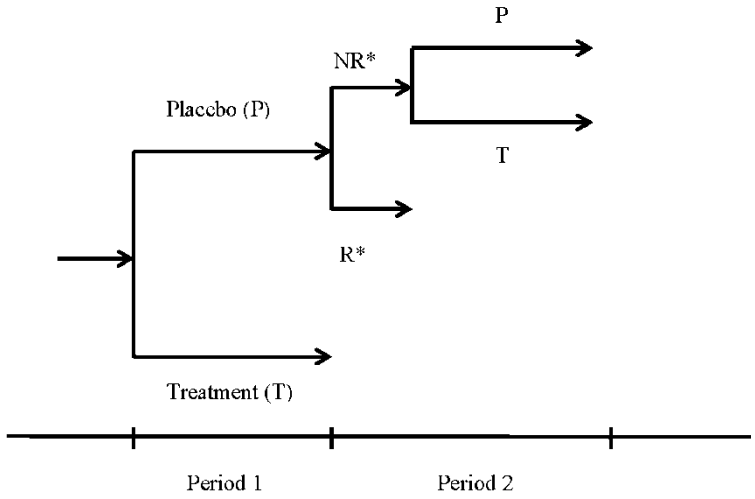
treatment effects from period 1 and period 2 are both positive, it would be helpful to have some measure of their consistency.

In this paper, a simpler combination test is proposed for testing the global null hypothesis that affords a clearer interpretation of what its combined statistic is actually estimating. In addition, a second test, termed a consistency test, is proposed for testing a consistency null hypothesis. Upon the rejection of both the global null hypothesis and the consistency null hypothesis by their respective tests, one can then conclude that the treatment is effective for the target population. Then, the combined statistic is shown to provide an unbiased estimate of the latent treatment effect which is the treatment effect for the target population that is defined with appropriate adjustment to compensate for the presence of placebo responders in the population. Associated confidence interval for the latent treatment effect can also be provided. Similarly, the consistency test can then also provide an estimate of the consistency of the treatment effects from period 1 and period 2 as well as its associated confidence interval.

FIGURE 1

A Basic DRDS Design for Assessing Treatment Effect in Psychiatric Trials

with High Placebo Responders



* NR = Placebo non-responders, R = Placebo responders

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2. A New Approach to the Design and Analysis of the DRDS Design

2.1 The DRDS Design for Comparing a Treatment to Placebo

In a basic DRDS design as illustrated in Figure 1, it is of interest to compare a new treatment T to a placebo P in two successive periods relative to a primary endpoint X of interest. For the purpose of this paper, it will be assumed that X is a continuous variable. Assume that a response criterion has been defined in terms of this primary endpoint X. In Period 1, a traditional parallel randomized double-blind treatment period is used to compare T to P to obtain an estimate $\hat{\Delta}_1 = \hat{X}_{1,T} - \hat{X}_{1,P}$ for the treatment effect $\Delta_1 = \mu_{1,T} - \mu_{1,P}$, where $\mu_{1,T}$ and $\mu_{1,P}$ represent the mean change from baseline of the variable X for the treatment T and placebo P group respectively in period 1. Then, in period 2, only the placebo non-responders from period 1 will be re-randomized to T and P to obtain an estimate of the treatment effect Δ_2 for this enriched population. Due to the presence of placebo responders among the subjects in period 1, the treatment effect Δ_1 has been diluted. The concept of a latent treatment effect is introduced and defined in the Appendix. The latent treatment effect is defined in an objective manner based on the DRDS design to compensate for the presence of placebo responders by reducing the degree of dilution. Thus, the actual treatment effect Δ_1 to be estimated for period 1 is equal to the latent treatment effect Δ_L^* minus a positive bias term β_1 attributed to the presence of placebo responders, i.e., $\Delta_1 = \Delta_L^* - \beta_1$, $\beta_1 > 0$. If there were no placebo responders or if the placebo responders only represent a very small fraction of the target population, then β_1 would be effectively equal to 0.

If the placebo responders represent a relatively high fraction of the target population, then re-randomization of the placebo non-responders in period 2 to treatment and placebo would be meaningful. Similarly, in period 2, one can obtain an estimate $\hat{\Delta}_2 = \hat{X}_{2,T} - \hat{X}_{2,P}$ for the treatment effect $\Delta_2 = \mu_{2,T} - \mu_{2,P}$, where $\mu_{2,T}$ and $\mu_{2,P}$ represent the change from period 2 baseline of the variable X for the treatment T and placebo P respectively in period 2. Now in period 2, due to the nature of the enrichment, the actual treatment effect Δ_2 to be estimated in period 2 is equal to the latent treatment effect Δ_L^* plus a positive bias term β_2 attributed to the exclusion of the period 1 placebo responders and placebo dropouts from this period, i.e., $\Delta_2 = \Delta_L^* + \beta_2$, $\beta_2 > 0$.

Now, in a DRDS design, neither period 1 nor period 2 alone is likely to show significant treatment effect. The former is due to the presence of placebo responders in period 1 and the latter is due to the much smaller sample size in period 2. More importantly, neither period alone can provide an unbiased estimate of the latent treatment effect Δ_L^*. In this paper, a more direct procedure is proposed to provide an unbiased estimate of the latent treatment effect Δ_L^* based on the DRDS design. The estimate is derived from a combined statistic $\hat{\Delta} = \alpha_1 \hat{\Delta}_1 + \alpha_2 \hat{\Delta}_2$, where $\alpha_1 + \alpha_2 = 1$ and α_i, i=1, 2 are simply weights defined in terms of the sample sizes from period 1 and period 2 that are favoring period 1. Then, under certain reasonable assumptions given in the theorem in the Appendix, the theorem shows that the combined statistic provides an unbiased estimate for the latent treatment effect $E(\hat{\Delta}) = \Delta_L^*$.

Now, using the test derived from the combined statistic $\hat{\Delta}$ alone to test the global null hypothesis as was done by previous authors referenced earlier would not provide sufficient evidence that the treatment is effective for the target population. It is necessary to consider another statistic to test the consistency of the treatment effects from both period 1 and period 2. Once the treatment has been shown to be effective and the consistency of the treatment effects Δ_1 and Δ_2 across the two periods has been established, then

one can obtain an estimate of the latent treatment effect Δ_L^* and its confidence interval from the combination test and also an estimate of the consistency and its confidence interval from the consistency test. These test statistics and their properties will be discussed in more details in the subsequent sections.

2.2 The Specifics of the DRDS Design Elements and Parameters

Let N_1 represent the total number of subjects to be randomized to the treatment T and placebo P at the baseline of period 1. Let r_1 denote the allocation ratio of P to T, i.e., $r_1 = n_{1,P}/n_{1,T}$, where $n_{1,P}$ = the number of subjects to be randomized to P and $n_{1,T}$ = the number of subjects to be randomized to T. Let π represent the expected percent of placebo subjects who either drop out early or who complete the double-blind treatment period 1 and meet the responder criterion. Thus, at the end of period 1, the expected number of placebo subjects who had completed the double-blind treatment period 1 and who were non-responders is given by $N_2 = n_{1,T}\, r_1(1-\pi)$. In period 2, the N_2 placebo non-responders will be re-randomized to T and P. Let r_2 denote the allocation ratio of placebo to treatment in period 2. Then, the number of placebo non-responders randomized to treatment T in period 2 is given by $n_{2,T} = N_2/(1+r_2) = n_{1,T}\, r_1(1-\pi)/(1+r_2)$. The corresponding expected number of placebo non-responder to be randomized to placebo is given by $n_{2,P} = n_{2,T}\, r_2 = n_{1,T}\, r_1(1-\pi)r_2/(1+r_2)$.

Let $X_{1,T} \sim N(\mu_{1,T}, \sigma_{1,T}^2)$ and $X_{1,P} \sim N(\mu_{1,P}, \sigma_{1,P}^2)$ be normally distributed random variables. Let $\Delta_1 = \mu_{1,T} - \mu_{1,P}$ denote the treatment effect from period 1. Similarly, let $X_{2,T} \sim N(\mu_{2,T}, \sigma_{2,T}^2)$ and $X_{2,P} \sim N(\mu_{2,P}, \sigma_{2,P}^2)$ be normally distributed and $\Delta_2 = \mu_{2,T} - \mu_{2,P}$, denote the treatment effect from period 2.

It is important to note that due to the presence of relatively high proportion of placebo responders in period 1, the test for the treatment effect Δ_1 in period 1 is not expected to be statistically significant unless the sample size is very large. In addition, as noted earlier the treatment effect Δ_1 is not the latent treatment effect Δ unless there are few or no placebo responders. On the other hand, despite the enrichment of the cohort in period 2 by excluding the placebo responders from period 1, the test for the treatment effect Δ_2 in period 2 is also not expected to be statistically significant by itself due to a much smaller sample size on account of the exclusion of placebo responders and dropouts from period 2. In addition, the treatment effect Δ_2 is not the latent treatment effect Δ unless there are no placebo responders in period 1. Therefore, an unbiased estimate of the latent treatment effect Δ is needed and this is discussed next.

2.3 The Combination Test

Continuing with the design specifics discussed above, let $\hat{\Delta}_i = (\hat{\mu}_{i,T} - \hat{\mu}_{i,P})$, where $\hat{\mu}_{i,T}$ and $\hat{\mu}_{i,P}$ represent the observed change from baseline for T and P where $\hat{\mu}_{i,T} \sim N\left(\mu_{i,T}, \frac{\sigma_{i,T}^2}{n_{i,T}}\right)$, and $\hat{\mu}_{i,P} \sim N\left(\mu_{i,P}, \frac{\sigma_{i,P}^2}{n_{i,P}}\right)$ for period i, i =1, 2. Assume that $\sigma_{i,T}^2 = \sigma_{i,P}^2 = \sigma_i^2$, for period i, i =1,2. Then, $(\hat{\Delta}_1, \hat{\Delta}_2)$ is bivariate normal

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where $\hat{\Delta}_1 \sim N\left(\Delta_1, \frac{\sigma_1^2}{n_{1,T} R_1}\right)$ and $\hat{\Delta}_2 \sim N\left(\Delta_2, \frac{\sigma_2^2}{n_{2,T} R_2}\right)$ with correlation $corr\left(\hat{\Delta}_1, \hat{\Delta}_1\right) = \rho_{1,2}$. where $R_i = \frac{r_i}{1+r_i}, i = 1,2$ represents the proportion of placebo subjects in the respective period.

Let

$$\alpha_1 = \frac{\sqrt{n_{1,T} R_1}}{\sqrt{n_{1,T} R_1} + \sqrt{n_{2,T} R_2}} \quad and \quad \alpha_2 = \frac{\sqrt{n_{2,T} R_2}}{\sqrt{n_{1,T} R_1} + \sqrt{n_{2,T} R_2}} \tag{1}$$

Then, $\alpha_1 + \alpha_2 = 1$

Now consider the following weighted linear combination of the statistics $\hat{\Delta}_1$ from period 1 and $\hat{\Delta}_2$ from period 2:

$$\hat{\Delta} = \alpha_1 \hat{\Delta}_1 + \alpha_2 \hat{\Delta}_2 \tag{2}$$

or equivalently,

$$\hat{\Delta} = \frac{\sigma_1}{\sqrt{n_{1,T} R_1} + \sqrt{n_{2,T} R_2}} \hat{U}_1 + \frac{\sigma_2}{\sqrt{n_{1,T} R_1} + \sqrt{n_{2,T} R_2}} \hat{U}_2 \tag{3}$$

where $\hat{U}_1 = \frac{\hat{\Delta}_1}{\frac{\sigma_1}{\sqrt{n_{1,T} R_1}}} \sim N\left(\frac{\Delta_1}{\frac{\sigma_1}{\sqrt{n_{1,T} R_1}}}, 1\right)$ and $\hat{U}_2 = \frac{\hat{\Delta}_2}{\frac{\sigma_2}{\sqrt{n_{2,T} R_2}}} \sim N\left(\frac{\Delta_2}{\frac{\sigma_2}{\sqrt{n_{2,T} R_2}}}, 1\right)$.

Then, one has

$$E\left(\hat{\Delta}\right) = \left(\frac{\sqrt{n_{1,T} R_1}}{\sqrt{n_{1,T} R_1} + \sqrt{n_{2,T} R_2}}\right) \Delta_1 + \left(\frac{\sqrt{n_{2,T} R_2}}{\sqrt{n_{1,T} R_1} + \sqrt{n_{2,T} R_2}}\right) \Delta_2 \tag{4}$$

Or equivalently,

$$E\left(\hat{\Delta}\right) = \left(\frac{\sigma_1}{\sqrt{n_{1,T} R_1} + \sqrt{n_{2,T} R_2}}\right) U_1 + \left(\frac{\sigma_2}{\sqrt{n_{1,T} R_1} + \sqrt{n_{2,T} R_2}}\right) U_2 \tag{5}$$

where $U_1 = \frac{\Delta_1}{\frac{\sigma_1}{\sqrt{n_{1,T} R_1}}}$ and $\hat{U}_2 = \frac{\Delta_2}{\frac{\sigma_2}{\sqrt{n_{2,T} R_2}}}$ and

$$Var\left(\hat{\Delta}\right) = \left(\frac{1}{\sqrt{n_{1,T} R_1} + \sqrt{n_{2,T} R_2}}\right)^2 \left(\sigma_1^2 + 2\rho_{1,2}\sigma_1\sigma_2 + \sigma_2^2\right) \tag{6}$$

and $\rho_{1,2}$ is the correlation between the two estimators $\hat{\Delta}_1$ and $\hat{\Delta}_2$.

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Then, let

$$\hat{Z} = \frac{\hat{\Delta} - E(\hat{\Delta})}{\sqrt{Var(\hat{\Delta})}} = \frac{\hat{\Delta} - \left[\left(\frac{\sqrt{n_{1,T}R_1}}{\sqrt{n_{1,T}R_1} + \sqrt{n_{2,T}R_2}}\right)\Delta_1 + \left(\frac{\sqrt{n_{2,T}R_2}}{\sqrt{n_{1,T}R_1} + \sqrt{n_{2,T}R_2}}\right)\Delta_2\right]}{\frac{\sqrt{\sigma_1^2 + 2\rho_{1,2}\sigma_1\sigma_2 + \sigma_2^2}}{\sqrt{n_{1,T}R_1} + \sqrt{n_{2,T}R_2}}} \quad (7)$$

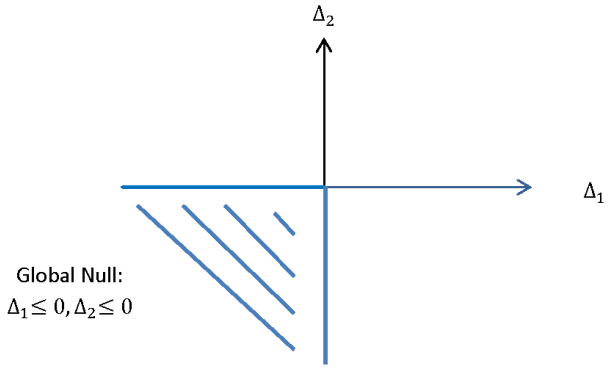
Now consider the following global null which is illustrated in Figure 2:

$$H_{o,G}: \Delta_1 \leq 0 \ and \ \Delta_2 \leq 0 \quad vs. \quad H_{a,G}: \Delta_1 > 0 \ or \ \Delta_2 > 0 \quad (8)$$

The alternative hypothesis is represented by the complementary region in the parameter space in Figure 2 which is simply the region covered by the first, second and fourth quadrant minus the two negative axes.

FIGURE 2

Region of the Parameter Space for the Global Null Hypothesis



At the point (0,0) on the boundary of the global null, the treatment effect is assumed to be zero in both period 1 and period 2. Now assume that the correlation between the outcome measurement *X* from period 1 and the outcome measure *X* from period 2 is constant across the placebo non-responders from period 1 who were re-randomized in period 2 to treatment, and similarly for placebo non-responders from period 1 who were re-randomized in period 2 to placebo. Then, under the global null when the treatment effect Δ_2 is assumed to be equal to zero, the two constant correlations should be the same. Now with a re-randomization ratio of $r_1 = 1$ in period 2, one can show that the correlation between the treatment effect

estimate $\hat{\Delta}_1$ from period 1 and the treatment effect estimate $\hat{\Delta}_2$ from period 2 is zero. This was essentially shown in Chen et al (2011) under the further assumption that the variance across the treatment groups be constant in both period 1 and period 2. However, this latter assumption is not necessary but not unreasonable based on the HDRS17 Anxiety and Somatization score data from a phase 2 study shown in TABLE 1 that will be used for illustration later. In the proposed method to be developed below, the assumption of a constant correlation between treatment measures from both periods for all re-randomized placebo responders across treatment groups in period 2 will be assumed. Under this assumption, the correlation between the treatment effect estimates from period 1 and period 2 will be zero, i.e., $corr(\hat{\Delta}_1, \hat{\Delta}_2) = \rho_{1,2} = 0$.

TABLE 1

HDRS17 Anxiety and Somatization Data from a Phase 2 Study

Period 1

$n_{1,T}$	$\hat{\Delta}_{1,T}$	$s.e.(\hat{\Delta}_{1,T})$	$\hat{\sigma}_{1,T}$	$n_{1,P}$	$\hat{\Delta}_{1,P}$	s. $e.(\hat{\Delta}_{1,P})$	$\hat{\sigma}_{1,P}$
61	3.5	0.31	2.42	58	3.1	0.31	2.36

Period 2

$n_{2,T}$	$\hat{\Delta}_{2,T}$	$s.e.(\hat{\Delta}_{2,T})$	$\hat{\sigma}_{2,T}$	$n_{2,P}$	$\hat{\Delta}_{2,P}$	s. $e.(\hat{\Delta}_{2,P})$	$\hat{\sigma}_{2,P}$
11	4.0	0.59	1.96	11	2.9	0.61	2.02

Thus, with the above assumption, at the point (0, 0) on the boundary of the global null, the correlation $\rho_{1,2} = 0$, and the statistic

$$\hat{Z}_o = \frac{\hat{\Delta}}{\frac{\sqrt{\sigma_1^2 + \sigma_2^2}}{\sqrt{n_{1,T}R_1} + \sqrt{n_{2,T}R_2}}} \sim N(0,1) \qquad (9)$$

can be used to test the global null hypothesis given in (8).

When the variances σ_1^2 *and* σ_2^2 for $\hat{\Delta}_1$ and $\hat{\Delta}_2$ in period 1 and period 2 are considered unknown as is the case, then in the test statistic $\hat{Z}_o$ given by Eqn. (9), they may be substituted by their respective pooled sample variances given by

$$\hat{\sigma}_1^2 = \frac{(n_{1,T}-1)\,\hat{S}_{1,T}^2 + (n_{1,P}-1)\,\hat{S}_{1,P}^2}{(n_{1,T} + n_{1,P} - 2)} \text{ and } \hat{\sigma}_2^2 = \frac{(n_{2,T}-1)\,\hat{S}_{2,T}^2 + (n_{2,P}-1)\,\hat{S}_{2,P}^2}{(n_{2,T} + n_{2,P} - 2)} \qquad (10)$$

where

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$$\hat{S}_{1,T}^2 = \frac{1}{(n_{1,T}-1)}\sum_{i=1}^{n_{1,T}}\left(X_{1,T,i} - \bar{X}_{1,T}\right)^2,\ \hat{S}_{1,P}^2 = \frac{1}{(n_{1,P}-1)}\sum_{i=1}^{n_{1,P}}\left(X_{1,P,i} - \bar{X}_{1,P}\right)^2$$

$$\hat{S}_{2,T}^2 = \frac{1}{(n_{2,T}-1)}\sum_{i=1}^{n_{2,T}}\left(X_{2,T,i} - \bar{X}_{2,T}\right)^2,\ \hat{S}_{2,P}^2 = \frac{1}{(n_{2,P}-1)}\sum_{i=1}^{n_{2,P}}\left(X_{2,P,i} - \bar{X}_{2,P}\right)^2$$

The expected value of the combined test $\hat{\Delta} = \alpha_1\hat{\Delta}_1 + \alpha_2\hat{\Delta}_2$ as given by (4) provides a close estimate of the latent treatment effect. It is a weighted average of the estimated treatment effects $\hat{\Delta}_1$ and $\hat{\Delta}_2$ from period 1 and period 2 respectively, where the treatment effect Δ_1 underrepresents the latent treatment effect from period 1, while Δ_2 overrepresents the latent treatment effect from period 2, and the weights are proportional to the sample sizes in the two periods with more weight assigned to the period 1 estimate $\hat{\Delta}_1$.

2.3.1 The Type I Error, Power and Sample Size Calculation for the Combination Global Test

The type I error rate for the combination test $\hat{Z}$ for testing the global null hypothesis is given by

$$\alpha = P\left(\hat{Z} > c_\alpha \middle| H_{o,G}\right) = P\left(\hat{Z}_o > c_\alpha\right) = P\left(\frac{\hat{\Delta}}{\frac{\sqrt{\sigma_1^2+\sigma_2^2}}{\sqrt{n_{1,T}R_1}+\sqrt{n_{2,T}R_2}}} > c_\alpha\right) \quad (11)$$

Equivalently, it is given by

$$\alpha = P\left(\sigma_1\hat{U}_1 + \sigma_2\hat{U}_2 > c_\alpha\sqrt{\sigma_1^2+\sigma_2^2}\right) \quad (12)$$

where $\hat{U}_1 = \frac{\hat{\Delta}_1}{\frac{\sigma_1}{\sqrt{n_{1,T}R_1}}} \sim N(0,1)$ and $\hat{U}_2 = \frac{\hat{\Delta}_2}{\frac{\sigma_2}{\sqrt{n_{2,T}R_2}}} \sim N(0,1)$ at the global null (0,0).

The equivalent expression in Eqn. (12) also allows one to evaluate the type I error rate through the standard bivariate normal distribution.

The power of the combination test at a specified alternative (Δ_1, Δ_2) in first quadrant with a correlation of $\rho_{1,2}$ is given by

$$1-\beta = P\left(\hat{Z}_o > c_\alpha \middle| (\Delta_1,\Delta_2;\rho_{1,2})\ in\ 1st\ Quadrant\right) = P\left(\hat{Z}_o > c_\alpha \middle| (\Delta_1,\Delta_2;\rho_{1,2})\ in\ 1st\ Quadrant\right)$$

$$= P\left(\frac{\alpha_1\hat{\Delta}_1 + \alpha_2\hat{\Delta}_2}{\frac{\sqrt{\sigma_1^2+\sigma_2^2}}{\sqrt{n_{1,T}R_1}+\sqrt{n_{2,T}R_2}}} > c_\alpha \middle| (\Delta_1,\Delta_2;\rho_{1,2})\ in\ 1st\ Quadrant\right)$$

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$$= P\left(\hat{Z}_a > \frac{c_\alpha\sqrt{\sigma_1^2+\sigma_2^2} - [\sigma_1 U_1 + \sigma_2 U_2]}{\sqrt{\sigma_1^2 + 2\rho_{1,2}\sigma_1\sigma_2 + \sigma_2^2}} | (\Delta_1, \Delta_2; \rho_{1,2})\right), \quad (13)$$

$$where\ \hat{Z}_a = \frac{(\sigma_1(\hat{U}_1 - U_1) + \sigma_2(\hat{U}_2 - U_2))}{\sqrt{\sigma_1^2 + 2\rho_{1,2}\sigma_1\sigma_2 + \sigma_2^2}} = \frac{(\sigma_1\hat{V}_1 + \sigma_2\hat{V}_2)}{\sqrt{\sigma_1^2 + 2\rho_{1,2}\sigma_1\sigma_2 + \sigma_2^2}} \sim N(0,1)$$

Equation (13) can be equivalently evaluated by the bivariate normal distribution of $(\hat{V}_1, \hat{V}_2)$ where $\hat{V}_i - (\hat{U}_i - U_i) \sim N(0,1)$, i=1,2 with correlation $corr(\hat{V}_1, \hat{V}_2) = \rho_{1,2}$ as follows:

$$1 - \beta = P\left(\hat{V}_2 > \frac{c_\alpha\sqrt{\sigma_1^2+\sigma_2^2} - [\sigma_1 U_1 + \sigma_2 U_2] - \sigma_1\hat{V}_1}{\sigma_2} | (\hat{V}_1, \hat{V}_2) \sim BN(0,0,\rho_{1,2})\right) \quad (14)$$

In the third from the last column in TABLE 5 are the powers for selected values of the DRDS design parameters based on the same HRDS17 Anxiety and Somatization score data.

Now from Eqn.(13), the sample size formula is given by

$$\frac{c_\alpha\sqrt{\sigma_1^2+\sigma_2^2} - \left|\sqrt{n_{1,T}R_1}\Delta_1 + \sqrt{n_{2,T}R_2}\Delta_2\right|}{\sqrt{\sigma_1^2 + 2\rho_{1,2}\sigma_1\sigma_2 + \sigma_2^2}} = -c_{1-\beta}$$

Based on the DRDS design parameter γ, one has $n_{2,T} = n_{1,T}\gamma$, and the sample size formula is then given by

$$n_{1,T} = \left(c_\alpha + c_{1-\beta}\sqrt{1 + \frac{2\rho_{1,2}\sigma_1\sigma_2}{(\sigma_1^2+\sigma_2^2)}}\right)^2 \frac{(\sigma_1^2+\sigma_2^2)}{\left(\sqrt{R_1}\Delta_1 + \sqrt{R_2}\Delta_2\right)^2} \quad (15)$$

The sample size given in Eqn.(15) can also be evaluated through the bivariate normal distribution using Eqn. (14) by substituting $U_1 = \sqrt{n_{1,T}R_1}(\Delta_1/\sigma_1)$ and $U_2 = \sqrt{n_{2,T}R_2}(\Delta_2/\sigma_2) = \sqrt{n_{1,T}\gamma R_2}(\Delta_2/\sigma_2)$.

TABLE 2 presents the power and sample size for selected scenarios and DRDS design parameter values based on the HDRS17 Anxiety and Somatization data.

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TABLE 2
Selected Powers and Sample Sizes at one-sided $\alpha = 0.025$ for the Combination Test $\hat{Z}_o$ for Selected Design Parameter Values based on the HDRS17 Anxiety & Somatization Score Data

$\rho_{1,2}$	c_α	1- β	Δ_1	σ_1	Δ_2	σ_2	r_1	π	γ	r_2	n_{1T}	n_{1P}	N_1	n_{2T}	$n2_P$	N2
0.00	1.96	80%	0.40	2.40	1.10	2.00	2	0.60	0.40	1	114	228	342	46	46	92
		85%									131	262	393	52	52	104
		90%									153	306	459	61	61	122
0.50		80%									130	260	390	52	52	104
		85%									152	304	454	61	61	104
		90%									181	362	543	72	72	144
0.75		80%									137	274	411	55	55	110
		85%									161	322	483	64	64	128
		90%									194	388	582	78	78	156

2.4 The Consistency Test

In the preceding section, if the combination test rejects the global null hypothesis, can one then conclude that the treatment is effective for the target population? The answer is 'No', since the rejection of the global null hypothesis by the combination test $\hat{Z}_o$ simply implies that the true treatment effects for period 1 and period 2 may be located somewhere in the first, second or fourth quadrant of the $\Delta_1 \times \Delta_2$ parameter space, even though it is less likely to fall in the fourth quadrant in light of the enrichment in period 2 of the DRDS design. If the true treatment effect pair (Δ_1, Δ_2) lies in the second quadrant or on the positive Δ_2- axis, then it would be difficult to argue that the treatment is effective for the intended target population since it suggests that the treatment is ineffective in period 1. Therefore, one would need to show that the treatment effect pair (Δ_1, Δ_2) is consistent by ruling out qualitative interaction and showing that it is located in the positive first quadrant. This leads to the idea of a consistency test to show that the treatment effect pair (Δ_1, Δ_2) is consistent. The rejection of the consistency null hypothesis by this consistency test together with the rejection of the global null hypothesis by the combination test then should allow one to infer that the treatment effect pair (Δ_1, Δ_2) is located in the positive first quadrant. Once this is established, then the combination test can be used to provide an estimate for the latent treatment effect for the target population along with its associated 95% confidence interval. The consistency test can then also be used to provide an estimate of the consistency and its associated 90% confidence interval.

The consistency null hypothesis and its alternative hypothesis is defined as follows:

$$H_{o,C}: \{(\Delta_1, \Delta_2) | \Delta_1 \Delta_2 < 0\} \cup \{(0,0)\} \quad vs. \quad H_{a,C}: \{(\Delta_1, \Delta_2) | \Delta_1 \Delta_2 \geq 0\} \backslash \{(0,0)\} \qquad (16)$$

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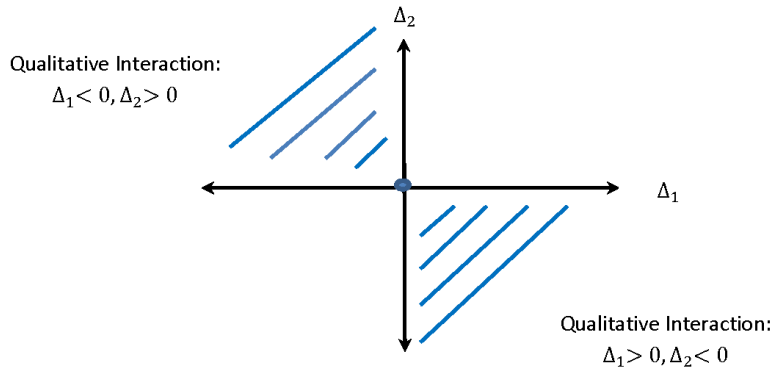
The consistency null hypothesis defined in Eqn. (16) is illustrated in Figure 3 as the shaded open region in the second and fourth quadrant (excluding the axes) but including the origin (0,0). The alternative hypothesis is represented by the region in the first and third quadrants including the two axes minus the origin (0,0).

FIGURE 3

Region of the Paramter Space for the Consistency Null Hypothesis



Now consider the following statistic for testing the consistency null hypothesis defined in (16).

Let

$$\hat{\Lambda} = \hat{\Delta}_1\hat{\Delta}_2$$

Then, one has

$$E(\hat{\Lambda}) = E(\hat{\Delta}_1\hat{\Delta}_2) = \rho_{1,2}\frac{\sigma_1}{\sqrt{n_{1,T}R_1}}\frac{\sigma_2}{\sqrt{n_{2,T}R_2}} + \Delta_1\Delta_2 \qquad (17)$$

and

$$Var(\hat{\Lambda}) = Var(\hat{\Delta}_1\hat{\Delta}_2)$$

$$= (1+\rho_{1,2}^2)\left(\frac{\sigma_1}{\sqrt{n_{1,T}R_1}}\right)^2\left(\frac{\sigma_2}{\sqrt{n_{2,T}R_2}}\right)^2 + 2\rho_{1,2}\Delta_1\Delta_2\left(\frac{\sigma_1}{\sqrt{n_{1,T}R_1}}\right)\left(\frac{\sigma_2}{\sqrt{n_{2,T}R_2}}\right) + \Delta_1^2\left(\frac{\sigma_2}{\sqrt{n_{2,T}R_2}}\right)^2$$
$$+ \Delta_2^2\left(\frac{\sigma_1}{\sqrt{n_{1,T}R_1}}\right)^2 \qquad (18)$$

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Let

$$\widehat{W} = \frac{\widehat{\Lambda} - E(\widehat{\Lambda})}{\sqrt{Var(\widehat{\Lambda})}}$$

$$= \frac{\hat{\Delta}_1\hat{\Delta}_2 - (\rho_{1,2}\frac{\sigma_1}{\sqrt{n_{1,T}R_1}}\frac{\sigma_2}{\sqrt{n_{2,T}R_2}} + \Delta_1\Delta_2)}{\sqrt{(1+\rho_{1,2}^2)\left(\frac{\sigma_1}{\sqrt{n_{1,T}R_1}}\right)^2\left(\frac{\sigma_2}{\sqrt{n_{2,T}R_2}}\right)^2 + 2\rho_{1,2}\Delta_1\Delta_2\left(\frac{\sigma_1}{\sqrt{n_{1,T}R_1}}\right)\left(\frac{\sigma_2}{\sqrt{n_{2,T}R_2}}\right) + \Delta_1^2\left(\frac{\sigma_2}{\sqrt{n_{2,T}R_2}}\right)^2 + \Delta_2^2\left(\frac{\sigma_1}{\sqrt{n_{1,T}R_1}}\right)^2}} \quad (19)$$

Then, the statistic $\widehat{W}$ at the point (0,0) on the boundary of the consistency null where it achieves its maximum type I error rate is given by

$$\widehat{W}_o = (\widehat{W}|H_{o,C}) = \left(\frac{\widehat{\Lambda} - E(\widehat{\Lambda})}{\sqrt{Var(\widehat{\Lambda})}}|H_{o,C}\right) \quad (20)$$

since under the earlier assumption, asymptotically the correlation $\rho_{1,2} = 0$ at the point (0,0).

Therefore. $\widehat{W}_o$ given by Eqn. (20) which is simply the product $\widehat{U}_1$ and $\widehat{U}_2$ is a natural test statistic for the consistency null hypothesis as defined in (16) as one can see from below.

Note that Eqn. (19) can be equivalently written as

$$\widehat{W} = \frac{\widehat{\Lambda} - E(\widehat{\Lambda})}{\sqrt{Var(\widehat{\Lambda})}} = \frac{\widehat{U}_1\widehat{U}_2 - (\rho_{1,2} + U_1U_2)}{\sqrt{(1+\rho_{1,2}^2) + 2\rho_{1,2}U_1U_2 + U_1^2 + U_2^2}} \quad (21)$$

where

$$E(\widehat{U}_1\widehat{U}_2) = (\rho_{1,2} + U_1U_2) = \xi$$

$$Var(\widehat{U}_1\widehat{U}_2) = (1+\rho_{1,2}^2) + 2\rho_{1,2}U_1U_2 + U_1^2 + U_2^2$$

and thus under the consistency null, Eqn. (20) is equivalent to

$$\widehat{W}_o = (\widehat{W}|H_{o,C}) = \widehat{U}_1\widehat{U}_2 \quad (22)$$

where

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NOTE: When the global null and the consistency null hypotheses are rejected by Z_o and $\widehat{W}_o$ respectively, then one may infer that (Δ_1, Δ_2) lies in the positive first quadrant and the correlation $\rho_{1,2}$ is likely to be positive on account of the nature of the DRDS design. Hence, $E(\hat{U}_1\hat{U}_2) = \xi = \rho_{1,2} + U_1U_2 > 0$ can be viewed as a measure of consistency, where U_1U_2 provides consistency information on the relative location of (Δ_1, Δ_2) while $\rho_{1,2}$ provides consistency information on how the observed pair $(\hat{U}_1, \hat{U}_2)$ varies together relative to their respective means. Thus, when both tests Z_o and $\widehat{W}_o$ reject their respective nulls, then the product moment $E(\hat{U}_1\hat{U}_2) = \xi = \rho_{1,2} + U_1U_2 > 0$ provides a natural measure of consistency of (Δ_1, Δ_2), where U_1 and U_2 are simply the normalized version of Δ_1 and Δ_2 respectively.

2.4.1 Type I Error Rate Calculation for the Consistency Test

Although not normally distributed, the distribution of $\widehat{W} = \hat{U}_1\hat{U}_2$ at the point (0, 0) on the boundary of the consistency null, i.e., the distribution $\widehat{W}_o$ given in (22), can be derived from the joint distribution of two independent standard normal distributions. One can find the type I error, the critical value, the power and sample size from the distribution of $\widehat{W}_o = (\hat{U}_1\hat{U}_2|(\Delta_1, \Delta_2) = (0,0))$ through numerical integration.

$$\alpha = P(\widehat{W} > c_{\alpha,W} \,|\, H_{o,G} \;\&\; H_{o,C}) = P(\hat{U}_1\hat{U}_2 > c_{\alpha,W}|(\Delta_1, \Delta_2) = (0,0)) = P(\widehat{W}_o > c_{\alpha,W})$$

Hence, the type I error is given by

$$\alpha = P\left(\hat{U}_2 < \frac{c_{\alpha,W}}{\hat{U}_1}, \hat{U}_1 < 0 \,\middle|\, H_{o,C}\right) + P\left(\hat{U}_2 > \frac{c_{\alpha,W}}{\hat{U}_1}, \hat{U}_1 > 0 \,\middle|\, H_{o,C}\right)$$

$$= P\left(\hat{U}_2 < \frac{c_{\alpha,W}}{\hat{U}_1}, \hat{U}_1 < 0 \middle| (U_1, U_2) = (0,0)\right) + P\left(\hat{U}_2 > \frac{c_{\alpha,W}}{\hat{U}_1}, \hat{U}_1 > 0 \,\middle|\, (U_1, U_2) = (0,0)\right)$$

$$= \int_{-\infty}^{0} \varphi(z_1)\Phi\left(\frac{c_{\alpha,W}}{z_1}\right)dz_1 + \int_{0}^{\infty} \varphi(z_1)\left[1 - \Phi\left(\frac{c_{\alpha,W}}{z_1}\right)\right]dz_1$$

$$= \frac{1}{2} + \int_{-\infty}^{0} \varphi(z_1)\Phi\left(\frac{c_{\alpha,W}}{z_1}\right)dz_1 - \int_{0}^{\infty} \varphi(z_1)\Phi\left(\frac{c_{\alpha,W}}{z_1}\right)dz_1 \qquad (23)$$

where φ and Φ represent the standard normal density and cumulative distribution functions. $c_{\alpha,W}$ is the critical value corresponding to the significance level α derived from the distribution of $\widehat{W}_o = (\hat{U}_1\hat{U}_2|(\Delta_1, \Delta_2) = (0,0))$ which is not normally distributed and has a heavy tail distribution. TABLE 3 below provides a list of critical values $c_{\alpha,W}$ for selected significance level α based on the distribution of $\widehat{W}_o$.

In light of the proposed procedure of testing both the global null hypothesis by the combination test $\hat{Z}_o$ and the consistency null hypothesis by the consistency test $\widehat{W}_o$, a rejection of the global null hypothesis by the test $\hat{Z}_o$ implies that (Δ_1, Δ_2) does not lie in the third quadrant which effectively reduces the

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nominal α level of the consistency test $\widehat{W}_o$ by half. For this reason, it is suggested that the type I error rate for the consistency test $\widehat{W}_o$ be held at the one-sided significance level of $\alpha = 0.05$ corresponding to a critical value of $c_{0.05,W} = 1.60$. This yields an effective significance level of $\alpha = 0.025$ for the consistency test $\widehat{W}_o$ under the joint testing procedure. This is the significance level that is later used in the calculations to generate various sample size and power for the consistency test $\widehat{W}_o$.

TABLE 3

Critical Values for the Consistency Test $\widehat{W}_o$ at Selected Significance Level α

α	$c_{\alpha,W}$
0.0125	2.79
0.025	2.18
0.05	1.60
0.10	1.03
0.20	0.52

2.4.2 Power Derivation for the Consistency Test

The power for the consistency test $\widehat{W}_o$ is derived for a special alternative (Δ_1, Δ_2) in the positive 1st quadrant with positive correlation $corr(\hat{\Delta}_1, \hat{\Delta}_2) = \rho_{1,2} > 0$ as follows:

$$\beta = P(\widehat{W}_o < c_{\alpha,W} \mid \Delta_1 > 0 \,\&\, \Delta_2 > 0, \rho_{1,2} > 0), where\ \widehat{W}_o = (\hat{U}_1 \hat{U}_2 | (U_1, U_2) = (0,0))$$

Under the special alternative,

$$\hat{U}_1 = \frac{\hat{\Delta}_1}{\frac{\sigma_1}{\sqrt{n_{1,T}\, R_1}}} \sim N\left(\frac{\Delta_1}{\frac{\sigma_1}{\sqrt{n_{1,T}\, R_1}}}, 1\right) = N(U_1, 1)\ and\ \hat{U}_2 = \frac{\hat{\Delta}_2}{\frac{\sigma_2}{\sqrt{n_{2,T}\, R_2}}} \sim N\left(\frac{\Delta_2}{\frac{\sigma_2}{\sqrt{n_{2,T}\, R_2}}}, 1\right) = N(U_2, 1)$$

where

$$U_1 = \frac{\Delta_1}{\frac{\sigma_1}{\sqrt{n_{1,T}\, R_1}}}\ \ and\ \ U_2 = \frac{\Delta_2}{\frac{\sigma_2}{\sqrt{n_{2,T}\, R_2}}}$$

one has,

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$$1-\beta = P\left(\hat{U}_1\hat{U}_2 > c_{\alpha,W} \,\middle|\, \Delta_1 > 0 \;\&\; \Delta_2 > 0, \rho_{1,2} > 0\right)$$

$$= P\left(\hat{V}_1\hat{V}_2 + U_1\hat{V}_2 + U_2\hat{V}_1 > c_{\alpha,W} - U_1U_2 \,\middle|\, U_1 > 0 \;\&\; U_2 > 0, \rho_{1,2} > 0\right)$$

where $\hat{V}_1 = \left(\hat{U}_1 - U_1\right) \sim N(0,1)$, $\hat{V}_2 = \left(\hat{U}_2 - U_2\right) \sim N(0,1)$ and $corr(\hat{V}_1, \hat{V}_2) = \rho_{1,2}$. Hence,

$$1-\beta = P\left(\hat{V}_2 < \frac{c_{\alpha,W} - U_1U_2 - U_2\hat{V}_1}{U_1 + \hat{V}_1}, \hat{V}_1 < -U_1 \,\middle|\, U_1 > 0 \;\&\; U_2 > 0, \rho_{1,2} > 0\right) +$$

$$P\left(\hat{V}_2 > \frac{c_{\alpha,W} - U_1U_2 - U_2\hat{V}_1}{U_1 + \hat{V}_1}, \hat{V}_1 > -U_1 \,\middle|\, U_1 > 0 \;\&\; U_2 > 0, \rho_{1,2} > 0\right)$$

$$= \int_{-\infty}^{-U_1} \varphi_{\hat{V}_1}(x) \int_{-\infty}^{\frac{c_{\alpha,W} - U_1U_2 - U_2x - \rho_{1,2}x(x+U_1)}{(x+U_1)\sqrt{1-\rho_{1,2}^2}}} \varphi(y)\, dydx +$$

$$\int_{-U_1}^{\infty} \varphi_{\hat{V}_1}(x) \int_{\frac{c_{\alpha,W} - U_1U_2 - U_2x - \rho_{1,2}x(x+U_1)}{(x+U_1)\sqrt{1-\rho_{1,2}^2}}}^{\infty} \varphi(y)\, dydx \qquad (24)$$

In the second from the last column of TABLE 5, powers for the consistency test $\hat{W}_o$ at some selected values of the DRDS design parameters and sample sizes are provided based on the HRDS17 Anxiety and Somatization score data.

2.4.3 Sample Size Derivation for the Consistency Test

Now from the above power function, if one substitutes the expressions

$$U_1 = \frac{\Delta_1}{\frac{\sigma_1}{\sqrt{n_{1,T}\, R_1}}} \;\; and \;\; U_2 = \frac{\Delta_2}{\frac{\sigma_2}{\sqrt{n_{2,T}\, R_2}}}$$

for U_1 and U_2 in Eqn. (24), and recall the relation $n_{2,T} = n_{1,T}\,\gamma$, where γ is the design parameter, then one can solve for sample size $n_{1,T}$ implicitly from Eqn. (24) for a given power $1-\beta$.

TABLE 4 provides sample sizes for selected power and design parameter values for the consistency test based on the HRDS17 Anxiety and Somatization score data.

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TABLE 4
Selected Powers and Sample Sizes at one-sided $\alpha = 0.05$ for the Consistency Test $\widehat{W}_o$ for Selected Design Parameter Values based on the HDRS17 Anxiety & Somatization Score Data

$\rho_{1,2}$	c_α	$1-\beta$	Δ_1	σ_1	Δ_2	σ_2	r_1	π	γ	r_2	n_{1T}	n_{1P}	N_1	n_{2T}	$n2_P$	N2
0.00	1.60	80%	0.40	2.40	1.10	2.00	2	0.60	0.40	1	132	264	396	53	53	106
		85%									154	308	462	62	62	124
		90%									186	372	558	74	74	148
0.50		80%									137	274	411	55	55	110
		85%									163	326	489	66	66	132
		90%									200	400	600	80	80	160
0.75		80%									139	278	417	56	56	112
		85%									167	334	501	67	67	134
		90%									206	412	618	82	82	164

2.5 The Joint Test ($\hat{Z}_o > c_{0.025}$, $\widehat{W}_o > c_{0.05,W}$)

The joint test given by ($\hat{Z}_o > c_{0.025}$, $\widehat{W}_o > c_{0.05,W}$) is necessarily a more stringent requirement than a single combination test, especially when $\hat{Z}_o$ and $\widehat{W}_o$ are asymptotically independent. It is analogous to testing two co-primary endpoints, albeit they are often correlated. But as discussed previously, the test of the global null hypothesis by the combination test alone is not sufficient to establish the effectiveness of the treatment for the target population in period 1.

It is proposed that in order to establish the effectiveness of the treatment for the target population in period 1, the joint testing of the global null hypothesis by the combination test $\hat{Z}_o$ at $\alpha = 0.025$ and the consistency null hypothesis by the consistency test $\widehat{W}_o$ at $\alpha = 0.05$ should be performed. When both the global null and the consistency null have been rejected by their respective tests $\hat{Z}_o$ and $\widehat{W}_o$, then one may conclude that the treatment effect pair (Δ_1, Δ_2) is located in the first quadrant and the treatment effects for both period 1 and period 2 are positive and consistent. The combination test can then provide an estimate of the latent treatment effect and its associated 95% confidence interval given by $\hat{\Delta} = \alpha_1\hat{\Delta}_1 + \alpha_2\hat{\Delta}_2$ and $(\alpha_1\hat{\Delta}_1 + \alpha_2\hat{\Delta}_2) \pm 1.96 \frac{\sqrt{\hat{\sigma}_1^2+\hat{\sigma}_2^2}}{\sqrt{n_{1,T}R_1}+\sqrt{n_{2,T}R_2}}$, where $\hat{\sigma}_1^2$ and $\hat{\sigma}_2^2$ are the estimates given in Eqn. (10).

The consistency test can then also provide an estimate of the consistency and its associated 90% confidence interval given by $\widehat{W}_o = \hat{U}_1\hat{U}_2$ and $(\hat{U}_1\hat{U}_2) \pm 1.60$.

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The power of the joint test ($\hat{Z}_o > c_{0.025}$, $\hat{W}_o > c_{0.05,W}$) is given in the last column of TABLE 5. As expected, the power will be much smaller. In fact, its power is asymptotically equal to the product of the power of the combination test $\hat{Z}_o > c_{0.025}$ and that of the consistency test $\hat{W}_o > c_{0.05,W}$, due to the fact that the two tests $\hat{Z}_o$ and $\hat{W}_o$ are asymptotically independent. Nonetheless, the power of the joint test is still greater than the power for the traditional parallel design for period 1 alone resulting in up to roughly a 30% reduction in sample size required due to the fact that the traditional parallel design is testing a smaller treatment effect Δ_1 while the combined test is essentially testing the latent treatment effect $\alpha_1\Delta_1 + \alpha_2\Delta_2 > \Delta_1$.

TABLE 5

Powers for the Combination Test $\hat{Z}_o$, the Consistency Test $\hat{W}_o$ and their Joint Test at Selected Values of the DRDS Design based on the HDRS17 Anxiety and Somatization Score Data

Δ_1	σ_1	Δ_2	σ_2	N_1	$r1$	n_{1T}	n_{1P}	π	γ	$r2$	n_{2T}	$\rho_{1,2}$	c_α	c_W	$P(\hat{Z}_o > c_\alpha \mid H_a)$	$P(\hat{W}_o > c_W \mid H_a)$	$P(\hat{Z}_o > c_\alpha, \hat{W}_o > c_W \mid H_a)$
0.00	2.40	0.00	2.00	240	2	80	160	0.60	0.40	1	32	0.00	1.96	1.60	0.02499	0.0497	0.00124
0.40	2.40	1.10	2.00	240	2	80	160	0.60	0.40	1	32	0.00			0.6493	0.6166	0.3814
												0.50			0.6263	0.6227	0.3725
												0.75			0.6144	0.6298	0.3707
				300		100	200				40	0.00			0.7454	0.6993	0.5069
												0.50			0.7055	0.6974	0.4774
												0.75			0.6917	0.6993	0.4689
				360		120	240				48	0.00			0.8186	0.7677	0.6164
												0.50			0.7719	0.7574	0.5719
												0.75			0.7550	0.7557	0.5580
				420		140	280				56	0.00			0.8729	0.8204	0.7068
												0.50			0.8247	0.8056	0.6538
												0.75			0.8065	0.8015	0.6361
				480		160	320				64	0.00			0.9122	0.8608	0.7783
												0.50			0.8662	0.8441	0.7228
												0.75			0.8478	0.8387	0.7030

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TABLE 6 provides the sample sizes required for some selected powers and values for the DRDS design parameters based on the HDRS17 Anxiety and Somatization score data. As expected, the sample size requirement is much more demanding.

TABLE 6

Selected Powers and Sample Sizes for the Joint Test ($\hat{Z}_o > c_{0.025}$, $\hat{W}_o > c_{0.05,W}$) for Selected Design Parameter Values based on the HDRS17 Anxiety & Somatization Score Data

$\rho_{1,2}$	$C_{0.025}$	$C_{0.05,W}$	$1-\beta$	Δ_1	σ_1	Δ_2	σ_2	r_1	π	γ	r_2	n_{1T}	n_{1P}	N_1	n_{2T}	$n2_P$	$N2$
0.00	1.96	1.60	80%	0.40	2.40	1.10	2.00	2	0.60	0.40	1	167	334	501	67	67	134
			85%									187	374	561	75	75	150
			90%									216	432	748	86	86	172
0.50			80%									188	376	564	75	75	150
			85%									213	426	639	85	85	170
			90%									246	492	738	98	98	196
0.75			80%									197	394	591	79	79	158
			85%									223	446	669	89	89	178
			90%									260	520	780	104	104	208

3. Example: HDRS17 Anxiety and Somatization Score Data

This is a doubly-randomized, placebo controlled (DRDS) early phase 2 study designed to assess the efficacy of an antidepressant in alleviating anxiety symptoms that was recently completed. A summary of the DRDS design features and the study results for HDRS17 Anxiety and Somatization score are given in Table 1 in the main text. It should be noted that due to the exploratory nature of the study, the significance level of this study was set at 1-sided $\alpha = 0.20$ and the sample size was determined based on an 90% power. However, for the purpose of illustration, the concept and methodology discussed in this Technical Document will be applied retrospectively to analyze the study data.

The results of the application of the present methods described in the main text of this Technical Document are given below with notations as used in the main text.

$\hat{\Delta}_1 = 0.40$ (s.e.0.438), $\hat{\Delta}_2 = 1.10$ (s.e.0.863), $\hat{\sigma}_1 = 2.41, \hat{\sigma}_2 = 2.01$, $\alpha_1 = 0.699$, $\alpha_2 = 0.301$,

$\alpha_{NR} \approx 0.379$. $\alpha_1^* = \alpha_2 + \alpha_{NR}(1-\alpha_2) = 0.566$, $\alpha_2^* = 0.434$

For the Combination Test:

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$\hat{\Delta} = \alpha_1\hat{\Delta}_1 + \alpha_2\hat{\Delta}_2 = 0.61\ (s.e.\ 0.40)$

Combination Test: $\hat{Z} = 1.52,\ p = 0.065$

60% CI for the latent treatment effect Δ_L^* = (0.27, 0.95) [Based on the design 1-sided $\alpha = 0.20$]

95% CI for the latent treatment effect Δ_L^* = (-0.18, 1.40)

Under the assumptions (1) – (3) of Theorem in the Appendix, one has for the apparent treatment effect Δ_1 from Period 1 that

$$E(\hat{\Delta}_1) = \Delta_1 = 0.40 \approx \Delta^* = \alpha_{NR}^*\Delta_{NR}^* + \alpha_R^*\Delta_R^*$$

while for the latent treatment effect Δ_L^*, one has

$$\Delta_L^* = \alpha_1^*\Delta_{NR}^* + \alpha_2^*\Delta_R^*$$

Therefore, from the above study data, the latent treatment effect estimate given by the combined statistic is

$$E(\hat{\Delta}) = E(\alpha_1\hat{\Delta}_1 + \alpha_2\hat{\Delta}_2) = \Delta = 0.61 \approx \Delta_L^* = \alpha_1^*\Delta_{NR}^* + \alpha_2^*\Delta_R^*$$

Thus, one can see that the latent treatment effect of 0.61 is obtained as a result of compensating for the presence of placebo responders by increasing the weight $\alpha_{NR}^* = 0.379$ placed on Δ_{NR}^* in Δ^* to the weight $\alpha_1^* = 0.566$ placed on Δ_{NR}^* in Δ_L^* by an amount

$$(\alpha_1^* - \alpha_{NR}^*) = (0.566 - 0.379) = 0.187$$

which represents an 18.7% increase in the weight placed on Δ_{NR}^* (which is essentially equal to Δ_2 the treatment effect in Period 2 under assumption (3) of the Theorem in the Appendix) to compensate for the dilution due to the presence of placebo responders.

For the Consistency Test:

$\hat{\Lambda} = 0.44\ (s.e.\ 0.38)$

$\hat{U}_1 = 0.905$ (s.e. 0.438), $\hat{U}_2 = 1.283$ (s.e. 0.863)

$\hat{W}_o = 1.16,\ 0.05 < p < 0.10$

60% CI for the consistency measure U_1U_2 – (0.62, 1.70) [Based on the design 1-sided $\alpha = 0.20$]

90% CI for the consistency measure U_1U_2 – (-0.44, 2.76)

Discussion: The study as designed is obviously underpowered. However, it does provide a good illustration of the application of the methods described in this document. For Period 1, the estimate of the apparent overall treatment effect $\hat{\Delta}_1$ = 0.40 (s.e.0.438) will not be significant due to the lack of power as

well as the presence of placebo responders. Whereas, for Period 2, the treatment effect estimate $\hat{\Delta}_2 = 1.10$ (s.e.0.863) is also not significant due to the lack of power despite a large positive treatment effect which is biased due to the enriched population. But the combined statistic $\hat{\Delta} = \alpha_1\hat{\Delta}_1 + \alpha_2\hat{\Delta}_2 = 0.61\ (s.e.0.40)$, both the 60% CI and the 95% CI suggest a positive trend despite the lack of power at the significance level of $\alpha = 0.025$. As discussed earlier in this appendix, the combined statistic $\hat{\Delta} = 0.61$ provides an estimate of the latent treatment effect [as defined by Eqn. (A-2) in the Appendix] which represents an adjustment in the apparent overall treatment effect estimate given by $\hat{\Delta}_1 = 0.40$ for the presence of placebo responders. The latent treatment effect reduces the degree of dilution of the apparent overall treatment effect due to the presence of placebo responders. In this example, the estimate of the latent treatment effect is $\hat{\Delta} = 0.61$ which shows an increment of 0.21 from the apparent overall treatment effect estimate given by $\hat{\Delta}_1 = 0.40$ as a compensation for the presence of placebo responders.

The consistency measure gives a consistency estimate of $\widehat{W} = \hat{U}_1 \times \hat{U}_2 = 0.905 \times 1.283 = 1.16$ with a 90% CI of (-0.438, 2.762) or $0.05 < p < 0.10$. Despite the lack of power, the result suggests that the Period 1 apparent treatment effect estimate of $\hat{\Delta}_1 = 0.40$ and the Period 2 treatment effect estimate of $\hat{\Delta}_2 = 1.10$ are fairly consistent as suggested by the pair of normalized statistics $(\hat{U}_1, \hat{U}_2) = (0.905, 1.283)$ which is close to the 45-degree line defined by $U_1 = U_2$. This is because when the effects estimates $\hat{\Delta}_1$ and $\hat{\Delta}_2$ are too disparate, i.e., if the pair $(\hat{U}_1, \hat{U}_2)$ deviates too much from the 45-degree line $U_1 = U_2$, then one would have less confidence that the Period 1 and Period 2 results are consistent and the consistency test would not tend to be significant. In this example, if the study has been properly powered, the consistent test would be expected to be significant showing a strong consistency.

4. Summary Discussion

In psychiatric trials, the presence of a relatively high proportion of placebo responders has caused many trials using a traditional parallel randomized trial to fail because the observed treatment effect has been diluted. Various authors have proposed a sequential design [Fava et al (2003) and Liu et al (2012)] in an attempt to resolve this problem. They also proposed a combination test with certain power optimality criteron to test for the treatment effect. In this paper, a joint testing of the global null hypothesis and the consistency null hypothesis by the simpler combination test $\hat{Z}_o$ and the consistency test $\widehat{W}_o$ respectively is proposed for establishing the effectiveness of the treatment for the target population based on a DRDS design. A joint test is thought to be needed because a combination test alone will not provide the strength of evidence required to establish the effectiveness of the treatment for the intended population. The combination test $\hat{Z}_o$ proposed in this paper is a weighted average of the treatment effect estimates from both periods with the weights defined in terms of the sample sizes from the two periods that favor the period 1 treatment effect Δ_1. The combined statistic $\hat{\Delta} = \alpha_1\hat{\Delta}_1 + \alpha_2\hat{\Delta}_2$ affords a more direct interpretation and provides an unbiased estimate of the latent treatment effect under some reasonable assumptions. It provides an estimate of the true treatment effect that is much less biased than the treatment estimate from either period alone. Furthermore, the combination test results in sample size reduction due to the fact that it is testing for a latent treatment Δ_L^* that is greater than the treatment effect Δ_1 to be detected in a traditional parallel design.

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However, the rejection of the global null hypothesis by the combination test $\hat{Z}_o$ only allows one to infer that the treatment effect pair (Δ_1, Δ_2) may lie in either the first, second or fourth quadrant of the parameter space $\Delta_1 \times \Delta_2$. What one would like to be able to do is to rule out the possibility that the treatment effect pair (Δ_1, Δ_2) may lie in the second and fourth quadrants which would imply qualitative interaction. For example, it would be difficult to argue that the treatment is effective if (Δ_1, Δ_2) falls in the second quadrant or on the positive Δ_2 - axis. To address this problem, a consistency hypothesis and a consistent test $\widehat{W}_o$ are defined. If the consistency null hypothesis is also rejected by the consistency test $\widehat{W}_o$, then one may infer that the treatment effect pair (Δ_1, Δ_2) falls in the positive first quadrant. Thus, one may conclude that the treatment is effective in both periods. In addition, the combination test can then provide an estimate of the latent treatment effect with its associated 95% confidence interval and the consistency test can provide an estimate of the consistency of the pair of treatment effect (Δ_1, Δ_2) and its associated 90% confidence interval. A one-sided significance level of $\alpha = 0.05$ is suggested for the consistency test because the simultaneous rejection of the global null hypothesis essentially eliminates the likelihood that the treatment effect pair (Δ_1, Δ_2) is located in the third quadrant which is within part of the region in the parameter space $\Delta_1 \times \Delta_2$ that is defined by the alternative consistency hypothesis thus yielding an effective one-sided significance level of α = 0.025.

The joint test $(\hat{Z}_o, \widehat{W}_o)$ is expected to be much more stringent than the combination test alone since the pair $\hat{Z}_o$ and $\widehat{W}_o$ is asymptotically independent. It is akin to testing two co-primary endpoints, albeit they are often correlated. This is shown by the power and sample size requirements. But the consistency test is necessary in order to provide the definitive evidence required for establishing the effectiveness of the treatment for the target population based on a DRDS design.

After both the global null hypothesis and the consistency null hypothesis have been rejected at the one-sided significance level of α = 0.025 and α = 0.05 respectively, one can then derive the estimate of the latent treatment effect Δ_L^* and its 95% CI which provides a better estimate of the real treatment effect after compensating for the presence of placebo responders. The consistency test and the consistency measure also shows that the apparent treatment effects Δ_1 and Δ_2 are consistent, the treatment has an effect $\Delta_1 > 0$ despite the presence of placebo responders. The combined statistic $\hat{\Delta} = \alpha_1\hat{\Delta}_1 + \alpha_2\hat{\Delta}_2$ then provides an estimate of the latency treatment effect and its 90% CI.

The method developed in this paper can also be extended to cover endpoints with binary outcomes under Bernoulli distributions as well as time-to-event endpoints under survival distributions. These extensions will be investigated.

The consistency test and the consistency measure can also be applied to support effectiveness claim for pre-specified subgroup.

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Appendix I: The Latent Treatment Effect

The Problem

One of the key problems encountered in the design and analysis of clinical trial with high placebo response rate is how to define the concept of latent treatment effect. Under a traditional randomized parallel placebo control trial, the treatment difference is only apparent, since the impact of the presence of a high proportion of placebo responders is already inherent in this relative difference. This treatment difference will be called an apparent treatment difference because the real treatment difference cannot be directly observed. To fix idea, let us suppose that one can pre-identify who are the placebo responders and who are the placebo non-responders in the target population. Then, one can randomize subjects within the placebo non-responder subgroup and placebo responder subgroup. Let Δ_{NR}^{*} denote the treatment effect for the placebo non-responder subgroup, Δ_{R}^{*} denote the apparent treatment effect for the placebo responder subgroup, α_{NR}^{*} denote the proportion of placebo non-responders and $\alpha_{R}^{*} = (1 - \alpha_{NR}^{*})$ denote the proportion of placebo responders. The treatment effect Δ_{NR}^{*} for the placebo non-responder subgroup is *real* due to the absence of placebo responders. The treatment effect Δ_{R}^{*} for the placebo responder subgroup is *apparent* due to the dilution effect of the placebo responders. Then, the overall treatment effect for the target population is defined by $\Delta^{*} = \alpha_{NR}^{*}\Delta_{NR}^{*} + (1 - \alpha_{NR}^{*})\Delta_{R}^{*}$ which is a weighted average of Δ_{NR}^{*} and Δ_{R}^{*}. The overall treatment effect Δ^{*} for the target population is also *apparent*, since it inherits the dilution effect of the presence of placebo responders from Δ_{R}^{*}, although the degree of dilution is less due to the weighted average with the real effect from Δ_{NR}^{*}. If $\hat{\Delta}_{NR}^{*}$ and $\hat{\Delta}_{R}^{*}$ denote the observed estimate of Δ_{NR}^{*} and Δ_{R}^{*} respectively, then, $\hat{\Delta}^{*} = \alpha_{NR}^{*}\hat{\Delta}_{NR}^{*} + (1 - \alpha_{NR}^{*})\hat{\Delta}_{R}^{*}$ provides an unbiased estimate of the apparent treatment effect Δ^{*}.

Now, if there are no placebo responders, then $\alpha_{R}^{*} = 0$, and the overall treatment effect $\Delta^{*} = \Delta_{NR}^{*}$ then represents the real treatment effect. On the other hand, if there are no placebo non-responders, then $\alpha_{NR}^{*} = 0$ and $\Delta^{*} = \Delta_{R}^{*}$. In this case, the overall treatment effect $\Delta^{*} = \Delta_{R}^{*}$ is only apparent reflecting the full impact of dilution from the placebo responders (In this case, placebo essentially plays the role of an effective control as in an active control trial). However, in general, one has $0 < \alpha_{NR}^{*} < 1$. In this case,

$$\Delta^{*} = \alpha_{NR}^{*}\Delta_{NR}^{*} + (1 - \alpha_{NR}^{*})\Delta_{R}^{*} \qquad \text{(A-1)}$$

represents the apparent overall treatment effect with its degree of dilution determined by the proportion of placebo non-responders. So, the problem is how can one compensate in an objective way for this dilution of the real treatment effect? Clearly, if more weight is placed on Δ_{NR}^{*}, then the degree of dilution will be less. How can one increase the weight on Δ_{NR}^{*} in an objective and natural manner to compensate for the impact of dilution? Furthermore, in reality, in a randomized parallel placebo control trial, one cannot identify at the baseline who are the placebo responders and who are not, and hence stratified randomization within the placebo non-responders and placebo responders is not possible. Thus, one cannot derive the unbiased estimates $\hat{\Delta}_{NR}^{*}$ and $\hat{\Delta}_{R}^{*}$ for Δ_{NR}^{*} and Δ_{R}^{*} respectively. In the next section, it is

shown how these problems may be addressed within the framework of a DRDS design under certain reasonable assumptions.

The Latent Treatment Effect

Let us consider the DRDS design as depicted in Figure 1 in the main text. All the design parameters associated with the DRDS design as described earlier will be retained here.

Again let Δ^*_{NR} and Δ^*_R denote the treatment effect for the placebo non-responders and the apparent treatment effect for the placebo responders respectively as before.

Definition: The *latent treatment effect* of T relative to P denoted by Δ^*_L is defined under the DRDS design as the weighted linear combination of the treatment effects Δ^*_{NR} for the placebo non-responders and the apparent treatment effect Δ^*_R for the placebo responders given by:

$$\Delta^*_L(\alpha^*_{NR}) = \alpha^*_1\Delta^*_{NR} + \alpha^*_2\Delta^*_R \qquad \text{(A-2)}$$

where

$$\alpha^*_1 = (\alpha_2 + \alpha^*_{NR} - \alpha_2\alpha^*_{NR}) \text{ and } \alpha^*_2 = 1 - (\alpha_2 + \alpha^*_{NR} - \alpha_2\alpha^*_{NR})$$

$$0 < \alpha^*_1,\ \alpha^*_2 < 1\ ,\ \alpha^*_1 + \alpha^*_2 = 1$$

where α^*_{NR} = the proportion of placebo non-responders in the target population and $\alpha_2 = \frac{\sqrt{n_{2,T}R_2}}{\sqrt{n_{1,T}R_1}+\sqrt{n_{2,T}R_2}}$ is as defined in Eqn. (1) based on the DRDS design parameters in the main text.

Remark: Upon comparing the latent treatment effect $\Delta^*_L(\alpha^*_{NR})$ as defined in (A-2) to the apparent overall treatment effect Δ^* as defined in (A-1), one observes that both the latent treatment effect $\Delta^*_L(\alpha^*_{NR})$ and the apparent overall treatment effect Δ^* are weighted combination of Δ^*_{NR} and Δ^*_R. Furthermore, one notes that $\alpha^*_1 = (\alpha_2 + \alpha^*_{NR} - \alpha_2\alpha^*_{NR}) = \alpha_2 + (1-\alpha_2)\alpha^*_{NR} > \alpha^*_{NR}$. Hence, the latent treatment effect $\Delta^*_L(\alpha^*_{NR}) = \alpha^*_1\Delta^*_{NR} + \alpha^*_2\Delta^*_R$ reduces the impact of the dilution due to the presence of placebo responders by placing more weight on Δ^*_{NR} than the apparent overall treatment effect Δ^*. Therefore, the latent treatment effect reduces the degree of dilution due to the presence of placebo responders.

Note: When there are no or few placebo responders, then $\alpha^*_{NR} \approx 1$ and therefore $\alpha^*_1 \approx 1$ and $\alpha^*_2 \approx 0$. Thus, the latent treatment effect is the real treatment effect, $\Delta^*_L(1) = \Delta^*_{NR} = \Delta_2 = \Delta^*$, since the entire target population consists of only placebo non-responders and there is no impact from placebo responders. But if all placebo are responders, then $\alpha^*_{NR} = 0$ and $\alpha^*_1 = \alpha_2 = 0$ and $\alpha^*_2 = 1$ and the latent treatment effect is the apparent treatment effect, $\Delta^*_L(0) = \Delta^*_R = \Delta_1 = \Delta^*$, since the entire target population consists of placebo responders, and there is no compensation for the dilution.

Now, in general, for $0 < \alpha^*_{NR} < 1$, the latent treatment effect $\Delta^*_L(\alpha^*_{NR}) = \alpha^*_1\Delta^*_{NR} + \alpha^*_2\Delta^*_R$ and the apparent treatment effect $\Delta^* = \alpha^*_{NR}\Delta^*_{NR} + (1-\alpha^*_{NR})\Delta^*_R$ are different.

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Remark: It should be noted that $\Delta^* = \alpha_{NR}^*\Delta_{NR}^* + (1-\alpha_{NR}^*)\Delta_R^*$ in Eqn. (A-1) is called the apparent treatment effect because it reflects the impact of the presence of placebo responders through the second term $(1-\alpha_{NR}^*)\Delta_R^*$ where the treatment effect Δ_R^* among the placebo responders has been diluted.

The latent treatment effect $\Delta_L^*(\alpha_{NR}^*) = \alpha_1^*\Delta_{NR}^* + \alpha_2^*\Delta_R^*$ as defined in Eqn. (A-2) implicitly reduces the impact of the presence of the placebo responders through the weights α_1^* and α_2^* assigned to Δ_{NR}^* and Δ_R^* in an objective manner. Observe that $\alpha_2^* = 1-(\alpha_2+\alpha_{NR}^*-\alpha_2\alpha_{NR}^*) = 1-[\alpha_2+(1-\alpha_2)\alpha_{NR}^*] > (1-\alpha_{NR}^*) = \alpha_R^*$. Thus, compared to the apparent treatment effect $\Delta^* = \alpha_{NR}^*\Delta_{NR}^* + (1-\alpha_{NR}^*)\Delta_R^*$, the latent treatment effect $\Delta_L^*(\alpha_{NR}^*) = \alpha_1^*\Delta_{NR}^* + \alpha_2^*\Delta_R^*$ effectively decreases the weight from $(1-\alpha_{NR}^*) = \alpha_R^*$ assigned to Δ_R^* and increases the weight α_1^* assigned to Δ_{NR}^* and thereby reduces the impact of the placebo responders as compared to the apparent treatment effect $\Delta^* = \alpha_{NR}^*\Delta_{NR}^* + (1-\alpha_{NR}^*)\Delta_R^*$. This compensatory effect arises naturally within the context of the DRDS design as one shall see in the next theorem. In addition, having just defined the latent treatment effect $\Delta_L^*(\alpha_{NR}^*)$, one would also want to know how one can estimate this latent treatment effect. This will be done through the DRDS design as shown in the next theorem.

Theorem: Given the DRDS design, the combined statistic $\hat{\Delta} = \alpha_1\hat{\Delta}_1 + \alpha_2\hat{\Delta}_2$ provides an unbiased estimate of the latent treatment effect $\Delta_L^*(\alpha_{NR}^*) = \alpha_1^*\Delta_{NR}^* + \alpha_2^*\Delta_R^*$ under the following assumptions: (1) the randomization in Period 1 maintains the proportion, α_{NR}^*, of placebo non-responders across the treatment and placebo arms, (2) the randomization in Period 1 also achieves balance for all key prognostic factors between the treatment and placebo for the placebo non-responders and placebo responders, and (3) the proportion, α_{NR}^*, of placebo non-responders is also maintained among the placebo dropouts, if any, in Period 1.

Proof: Consider the DRDS design as discussed earlier in the main text and let

$$n_{1,T} = \textit{the number of treated subjects in Period } 1$$

$$n_{T,NR} = \textit{the number of treated subjects who are placebo non} - \textit{responders in Period } 1$$

$$n_{T,R} = \textit{the number of treated subjects who are placebo responders in Period } 1$$

Then, $n_{1,T} = n_{T,NR} + n_{T,R}$.

Similarly, let

$$n_{1,P} = \textit{the number of placebo subjects in Period } 1$$

$$n_{P,NR} = \textit{the number of placebo subjects who are placebo non} - \textit{responders in Period } 1$$

$$n_{P,R} = \textit{the number of placebo subjects who are placebo responders in Period } 1$$

Then, $n_{1,P} = n_{P,NR} + n_{P,R}$ and $\alpha_{NR}^* = \frac{n_{2,T}+n_{2,P}}{n_{1,P}}$ = the proportion of placebo non-responders in Period 1.

Now, consider ordering the observed values for the response variable in the treatment group first for the placebo non-responders, followed by the placebo responders. Then, do the same for the placebo group as follows:

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$X_{T,1}, X_{T,2}, \ldots, X_{T,n_{T,NR}}$ denote the observed values for treated subjects in Period 1 who are placebo non-responders.

$X_{T,n_{T,NR}+1}, X_{T,n_{T,NR}+2}, \ldots, X_{T,n_{T,n_{T,NR}+n_{T,R}}}$ denote the observed values for treated subjects in Period 1 who are placebo non-responders.

$X_{P,1}, X_{P,2}, \ldots, X_{P,n_{P,NR}}$ denote the observed values for placebo subjects in Period 1 who are placebo non-responders.

$X_{P,n_{P,NR}+1}, X_{P,n_{P,NR}+2}, \ldots, X_{P,n_{P,n_{P,NR}+n_{P,R}}}$ denote the observed values for placebo subjects in Period 1 who are placebo non-responders.

Then, the observed treatment difference between the treatment and placebo groups in Period 1 is given by:

$$\hat{\Delta}_1 = \hat{X}_{1,T} - \hat{X}_{1,P} = \frac{1}{n_{1,T}}\sum_{i=1}^{n_{1,T}} X_{T,i} - \frac{1}{n_{1,P}}\sum_{i=1}^{n_{1,P}} X_{P,i}$$

$$= \frac{1}{n_{1,T}}\left[\sum_{i=1}^{n_{T,NR}} X_{T,i} + \sum_{i=n_{T,NR}+1}^{n_{T,NR}+n_{T,R}} X_{T,i}\right] - \frac{1}{n_{1,P}}\left[\sum_{i=1}^{n_{P,NR}} X_{P,i} + \sum_{i=n_{P,NR}+1}^{n_{P,NR}+n_{P,R}} X_{P,i}\right]$$

$$= \left[\frac{1}{n_{1,T}}\sum_{i=1}^{n_{T,NR}} X_{T,i} - \frac{1}{n_{1,P}}\sum_{i=1}^{n_{P,NR}} X_{P,i}\right] + \left[\frac{1}{n_{1,T}}\sum_{i=n_{T,NR}+1}^{n_{T,NR}+n_{T,R}} X_{T,i} - \frac{1}{n_{1,P}}\sum_{i=n_{P,NR}+1}^{n_{P,NR}+n_{P,R}} X_{P,i}\right]$$

$$= \left[\frac{n_{T,NR}}{n_{1,T}}\left(\frac{1}{n_{T,NR}}\sum_{i=1}^{n_{T,NR}} X_{T,i}\right) - \frac{n_{P,NR}}{n_{1,P}}\left(\frac{1}{n_{P,NR}}\sum_{i=1}^{n_{P,NR}} X_{P,i}\right)\right] + \left[\frac{n_{T,R}}{n_{1,T}}\left(\frac{1}{n_{T,R}}\sum_{i=n_{T,NR}+1}^{n_{T,NR}+n_{T,R}} X_{T,i}\right) - \frac{n_{P,R}}{n_{1,P}}\left(\frac{1}{n_{P,R}}\sum_{i=n_{P,NR}+1}^{n_{P,NR}+n_{P,R}} X_{P,i}\right)\right]$$

$$= \left[\frac{n_{T,NR}}{n_{1,T}}\left(\frac{1}{n_{T,NR}}\sum_{i=1}^{n_{T,NR}} X_{T,i}\right) - \frac{n_{P,NR}}{n_{1,P}}\left(\frac{1}{n_{P,NR}}\sum_{i=1}^{n_{P,NR}} X_{P,i}\right)\right] + \left[\frac{n_{T,R}}{n_{1,T}}\left(\frac{1}{n_{T,R}}\sum_{i=n_{T,NR}+1}^{n_{T,NR}+n_{T,R}} X_{T,i}\right) - \frac{n_{P,R}}{n_{1,P}}\left(\frac{1}{n_{P,R}}\sum_{i=n_{P,NR}+1}^{n_{P,NR}+n_{P,R}} X_{P,i}\right)\right]$$

Let

$$\alpha^*_{T,NR} = \frac{n_{T,NR}}{n_{1,T}} \text{ and } \alpha^*_{T,R} = \frac{n_{T,R}}{n_{1,T}}, \text{ then } \alpha^*_{T,NR} + \alpha^*_{T,R} = 1 \text{ and } \alpha^*_{T,R} = 1 - \alpha^*_{T,NR}$$

Similarly,

$$\alpha^*_{P,NR} = \frac{n_{P,NR}}{n_{1,P}} \text{ and } \alpha^*_{P,R} = \frac{n_{P,R}}{n_{1,P}}, \text{ then } \alpha^*_{P,NR} + \alpha^*_{P,R} = 1 \text{ and } \alpha^*_{P,R} = 1 - \alpha^*_{P,NR}$$

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Note that since subjects are randomized in Period 1, under assumption (1), one can expect that

$$\alpha^*_{T,NR} = \alpha^*_{P,NR} = \alpha^*_{NR}\,,\ \alpha^*_{T,R} = \alpha^*_{P,R} = \alpha^*_R \text{ where } \alpha^*_{NR} + \alpha^*_R = 1$$

Hence, we have

$$\hat{\Delta}_1 = \left[\frac{n_{T,NR}}{n_{1,T}}\left(\frac{1}{n_{T,NR}}\sum_{i=1}^{n_{T,NR}} X_{T,i}\right) - \frac{n_{P,NR}}{n_{1,P}}\left(\frac{1}{n_{P,NR}}\sum_{i=1}^{n_{P,NR}} X_{P,i}\right)\right] + \left[\frac{n_{T,R}}{n_{1,T}}\left(\frac{1}{n_{T,R}}\sum_{i=n_{T,NR}+1}^{n_{T,NR}+n_{T,R}} X_{T,i}\right) - \frac{n_{P,R}}{n_{1,P}}\left(\frac{1}{n_{P,R}}\sum_{i=n_{P,NR}+1}^{n_{P,NR}+n_{P,R}} X_{P,i}\right)\right]$$

$$= \left[\alpha^*_{NR}\left(\frac{1}{n_{T,NR}}\sum_{i=1}^{n_{T,NR}} X_{T,i}\right) - \alpha^*_{NR}\left(\frac{1}{n_{P,NR}}\sum_{i=1}^{n_{P,NR}} X_{P,i}\right)\right] + \left[\alpha^*_R\left(\frac{1}{n_{T,R}}\sum_{i=n_{T,NR}+1}^{n_{T,NR}+n_{T,R}} X_{T,i}\right) - \alpha^*_R\left(\frac{1}{n_{P,R}}\sum_{i=n_{P,NR}+1}^{n_{P,NR}+n_{P,R}} X_{P,i}\right)\right]$$

$$= \alpha^*_{NR}\left[\left(\frac{1}{n_{T,NR}}\sum_{i=1}^{n_{T,NR}} X_{T,i}\right) - \left(\frac{1}{n_{P,NR}}\sum_{i=1}^{n_{P,NR}} X_{P,i}\right)\right] + \alpha^*_R\left[\left(\frac{1}{n_{T,R}}\sum_{i=n_{T,NR}+1}^{n_{T,NR}+n_{T,R}} X_{T,i}\right) - \left(\frac{1}{n_{P,R}}\sum_{i=n_{P,NR}+1}^{n_{P,NR}+n_{P,R}} X_{P,i}\right)\right]$$

$$= \alpha^*_{NR}\left|\hat{\Delta}_{NR}\right| + \alpha^*_R\left|\hat{\Delta}_R\right|,$$

where

$\hat{\Delta}_{NR} = \left[\left(\frac{1}{n_{T,NR}}\sum_{i=1}^{n_{T,NR}} X_{T,i}\right) - \left(\frac{1}{n_{P,NR}}\sum_{i=1}^{n_{P,NR}} X_{P,i}\right)\right]$ = observed treatment effect among the placebo non-responders

$\hat{\Delta}_R = \left[\left(\frac{1}{n_{T,R}}\sum_{i=n_{T,NR}+1}^{n_{T,NR}+n_{T,R}} X_{T,i}\right) - \left(\frac{1}{n_{P,R}}\sum_{i=n_{P,NR}+1}^{n_{P,NR}+n_{P,R}} X_{P,i}\right)\right]$ = observed treatment effect among the placebo responders

Since $\hat{\Delta}_{NR}$ and $\hat{\Delta}_R$ are not derived from stratified randomization, therefore, generally, one may not expect $\hat{\Delta}_{NR}$ and $\hat{\Delta}_R$ to be unbiased estimator of Δ^*_{NR} and Δ^*_R respectively as is true with the estimator $\hat{\Delta}^*_{NR}$ and $\hat{\Delta}^*_R$ defined earlier under stratified randomization. However, under assumptions (1) and (2), one may assume that $E(\hat{\Delta}_{NR}) = \Delta^*_{NR}$ and $E(\hat{\Delta}_R) = \Delta^*_R$ hold true.

Therefore, under assumptions (1) and (2), one may expect that

$$E(\hat{\Delta}_1) = \alpha^*_{NR}E(\hat{\Delta}_{NR}) + \alpha^*_R E(\hat{\Delta}_R) = \alpha^*_{NR}\Delta^*_{NR} + \alpha^*_R\Delta^*_R = \Delta^* \qquad \text{(A-3)}$$

which is the apparent overall treatment effect. As mentioned before, due to the presence of placebo responders, Δ^* reflects the dilution caused by the presence of placebo responders in the second term of Eqn. (A-3).

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Now, it follows that the combined statistic

$$\hat{\Delta} = \alpha_1\hat{\Delta}_1 + \alpha_2\hat{\Delta}_2$$

can be viewed as providing an unbiased estimate for the latent treatment effect $\Delta_L^*(\alpha_{NR}^*)$ which provides a weighted adjustment for the impact of the presence of placebo responders in Δ^*. To see this, recall that the weights α_1 and α_2 defined previously by Eqn. (1) in the main text are defined by.

$$\alpha_1 = \frac{\sqrt{n_{1,T}R_1}}{\sqrt{n_{1,T}R_1} + \sqrt{n_{2,T}R_2}} \text{ and } \alpha_2 = \frac{\sqrt{n_{2,T}R_2}}{\sqrt{n_{1,T}R_1} + \sqrt{n_{2,T}R_2}}$$

Hence, it follows that the combined statistic

$$\begin{aligned}\hat{\Delta} &= \alpha_1\left(\alpha_{NR}^*\hat{\Delta}_{NR} + \alpha_R^*\hat{\Delta}_R\right) + \alpha_2\hat{\Delta}_2 \\ &= \left(\alpha_1\alpha_{NR}^*\hat{\Delta}_{NR} + \alpha_1\alpha_R^*\hat{\Delta}_R\right) + \alpha_2\hat{\Delta}_2 \\ &= \left(\alpha_1\alpha_{NR}^*\hat{\Delta}_{NR} + \alpha_2\hat{\Delta}_2\right) + \alpha_1\alpha_R^*\hat{\Delta}_R\end{aligned}$$

Then,

$$E(\hat{\Delta}) = \left[\alpha_1\alpha_{NR}^*E(\hat{\Delta}_{NR}) + \alpha_2E(\hat{\Delta}_2)\right] + \alpha_1\alpha_R^*E(\hat{\Delta}_R)$$

and it follows from assumptions (1) and (2) that $E(\hat{\Delta}_{NR}) = E(\hat{\Delta}_{NR}^*) = \Delta_{NR}^*$ and $E(\hat{\Delta}_R) = E(\hat{\Delta}_R^*) = \Delta_R^*$, and it further follows from assumption (3) that $E(\hat{\Delta}_2) = E(\hat{\Delta}_{NR}^*) = \Delta_{NR}^*$.

Hence, under assumptions (1) (3), one has

$$\begin{aligned}E(\hat{\Delta}) &= \left[\alpha_1\alpha_{NR}^*E(\hat{\Delta}_{NR}^*) + \alpha_2E(\hat{\Delta}_{NR}^*)\right] + \alpha_1\alpha_R^*E(\hat{\Delta}_R^*) \\ &= (\alpha_1\alpha_{NR}^* + \alpha_2)\Delta_{NR}^* + \alpha_1\alpha_R^*\Delta_R^* \\ &= \left((1-\alpha_2)\alpha_{NR}^* + \alpha_2\right)\Delta_{NR}^* + (1-\alpha_2)(1-\alpha_{NR}^*)\Delta_R^* \\ &= (\alpha_2 + \alpha_{NR}^* - \alpha_2\alpha_{NR}^*)\Delta_{NR}^* + (1-(\alpha_2 + \alpha_{NR}^* - \alpha_2\alpha_{NR}^*))\Delta_R^* \\ &= \alpha_1^*\Delta_{NR}^* + (1-\alpha_1^*)\Delta_R^* \\ &= \alpha_1^*\Delta_{NR}^* + \alpha_2^*\Delta_R^* \\ &= \Delta_L^*(\alpha_{NR}^*)\end{aligned}$$

Thus, under the assumptions (1) – (3), the combined statistic $\hat{\Delta}$ provides an unbiased estimate of the latent treatment effect $\Delta_L^*(\alpha_{NR}^*)$.

End of proof of theorem.

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Remark: As discussed earlier in the text, it is of interest to derive the biases β_1 and β_2 in Δ_1 and Δ_2 respectively relative to the latent treatment effect $\Delta_L^*(\alpha_{NR}^*)$. The bias β_1 due to presence of placebo in Period 1 and the bias β_2 due to the enrichment in Period 2 are derived below.

Deriving the bias $\boldsymbol{\beta_1}$:

From the above theorem, one has

$$E(\hat{\Delta}) = \alpha_1\Delta_1 + \alpha_2\Delta_2$$
$$= \alpha_1^*\Delta_{NR}^* + \alpha_2^*\Delta_R^*$$
$$= \Delta_L^*(\hat{\alpha_{NR}^*})$$

Hence,

$$\Delta_L^*(\alpha_{NR}^*) = \alpha_1\Delta_1 + \alpha_2\Delta_2$$
$$= \alpha_1\Delta_1 + (\Delta_1 - \Delta_1) + \alpha_2\Delta_2$$
$$= \Delta_1 - (1-\alpha_1)\Delta_1 + \alpha_2\Delta_2$$

It follows that

$$\Delta_1 = \Delta_L^*(\alpha_{NR}^*) + [(1-\alpha_1)\Delta_1 - \alpha_2\Delta_2]$$
$$= \Delta_L^*(\alpha_{NR}^*) + [\alpha_2\Delta_1 - \alpha_2\Delta_2]$$
$$= \Delta_L^*(\alpha_{NR}^*) + \alpha_2(\Delta_1 - \Delta_2)$$
$$= \Delta_L^*(\alpha_{NR}^*) + \beta_1$$

where

$$\beta_1 = \alpha_2(\Delta_1 - \Delta_2) < 0$$

Thus, the bias β_1 can be estimated by

$$\hat{\beta_1} = \alpha_2(\hat{\Delta}_1 - \hat{\Delta}_2)$$

$$var(\hat{\beta_1}) = \alpha_2^2\big(var(\hat{\Delta}_1) + var(\hat{\Delta}_2)\big)$$

Deriving the bias $\boldsymbol{\beta_2}$:

Similarly, one has

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$$\Delta_L^*(\hat{\alpha}_{NR}) = \alpha_1\Delta_1 + \alpha_2\Delta_2$$
$$= \alpha_1\Delta_1 + \alpha_2\Delta_2 + (\Delta_2 - \Delta_2)$$
$$= \alpha_1\Delta_1 + \alpha_2\Delta_2 + (\Delta_2 - \Delta_2)$$

It follows that

$$\Delta_2 = \Delta_L^*(\alpha_{NR}^*) - (\alpha_1\Delta_1 + \alpha_2\Delta_2 - \Delta_2)$$
$$= \Delta_L^*(\alpha_{NR}^*) + [(1-\alpha_2)\Delta_2 - \alpha_1\Delta_1]$$
$$= \Delta_L^*(\alpha_{NR}^*) + [\alpha_1\Delta_2 - \alpha_1\Delta_1]$$
$$= \Delta_L^*(\alpha_{NR}^*) + \alpha_1(\Delta_2 - \Delta_1)$$
$$= \Delta_L^*(\alpha_{NR}^*) + \beta_2$$

where

$$\beta_2 = \alpha_1(\Delta_2 - \Delta_1) > 0$$

Thus, the bias β_1 can be estimated by

$$\hat{\beta}_2 = \alpha_1(\hat{\Delta}_2 - \hat{\Delta}_1)$$

$$var(\hat{\beta}_2) = \alpha_1^2\big(var(\hat{\Delta}_2) + var(\hat{\Delta}_1)\big)$$

Attachment 2: Unbiased Estimator for the Doubly-Randomized Delayed-Start Design

Unbiased Estimator for the Doubly-Randomized Delayed-Start Design

1 Binary-Binary Case

1.1 Model and Results

Assume that the endpoints for period 1 and period 2 are both binary (responder or nonresponder). Suppose at the beginning of period 1, n subjects were randomized to receive treatment and m subjects were randomized to receive placebo. Let X_{1i} $(i = 1, ..., n)$ and Y_{1i} $(i = 1, ..., m)$ be the period 1 endpoints for the n subjects receiving treatment and the m subjects receiving placebo respectively. Let X_{1i} or Y_{1i} equals to 1 if the subject is a responder and X_{1i} or Y_{1i} equals to 0 if the subject is a nonresponder. Let n_n and m_n be the number of nonresponders at the end of period 1 for the treatment group and placebo group respectively. Without loss of generality, assume that subjects $1, ..., n_n$ in the treatment group and subjects $1, ..., m_n$ in the placebo group are the nonresponders in period 1. All the subjects in the treatment group continue to receive treatment in period 2. Assume their period 2 endpoints are X_{2i}, $i = 1, ..., n_n$ for the previous nonresponders and $i = n_n + 1, ..., n$ for the previous responders. The subjects in the placebo group who are period 1 nonresponders are re-randomized at the beginning of period 2 to either continue to receive placebo or to switch to receiving treatment. Let α be the proportion of placebo nonresponders who are re-randomized to stay on placebo (and such that αm_n is an integer). Without loss of generality, assume that subjects $1, ..., \alpha m_n$ in the placebo group continued on placebo in period 2. For the subjects that received two periods of placebo, assume the period 2 endpoints are Y_{2i}, $i = 1, ..., \alpha m_n$ for the previous nonresponders and $i = m_n + 1, ..., m$ for the previous responders. For the placebo nonresponders who were re-randomized to receive treatment, their responses at the end of period 2 would not be considered. Similar to the period 1 endpoint, Let X_{2i} or Y_{2i} equals to 1 if the subject is a responder and X_{2i} or Y_{2i} equals to 0 if the subject is a nonresponder at the end of period 2.

Let P_{T1} and P_{P1} be the probabilities of being a responder at the end of period 1 for subjects receiving treatment and placebo respectively. That is,

$$P_{T1} = P(X_{1i} = 1), \quad P_{P1} = P(Y_{1i} = 1).$$

By assumption, n_n and m_n both follow binomial distributions:

$$n_n \sim Bin(n, 1 - P_{T1}), \quad m_n \sim Bin(m, 1 - P_{P1}).$$

Let P_{Tr} be the probability that a treatment subject is a responder at the end of period 2, conditioning on the fact that he was also a responder at the end of period 1; and let P_{Tn} be the probability that a treatment subject is a responder at the end of period 2, conditioning on the fact that he was a nonresponder at the end of period 1. That is,

$$P_{Tr} = P(X_{2i} = 1 \mid X_{1i} = 1), \quad P_{Tn} = P(X_{2i} = 1 \mid X_{1i} = 0).$$

Similarly, define P_{Pr} and P_{Pn} for the placebo group as,

$$P_{Pr} = P(Y_{2i} = 1 \mid Y_{1i} = 1), \quad P_{Pn} = P(Y_{2i} = 1 \mid Y_{1i} = 0).$$

Let P_{T2} and P_{P2} be the marginal probabilities of being a responder at the end of period 2, regardless of period 1 results, for subjects receiving treatment and placebo respectively. That is,

$$P_{T2} = P(X_{2i} = 1), \quad P_{P2} = P(Y_{2i} = 1).$$

Unbiased estimators for P_{T2} and P_{P2} are:

$$\hat{P}_{T2} = \frac{1}{n}\sum_{i=1}^{n} X_{2i} \text{ and } \hat{P}_{P2} = \frac{1}{m}\Big(\frac{1}{\alpha}\sum_{i=1}^{\alpha m_n} Y_{2i} + \sum_{m_n+1}^{m} Y_{2i}\Big),$$

and the variances are

$$var(\hat{P}_{T2}) = \frac{1}{n}P_{T2}(1 - P_{T2}),$$

$$var(\hat{P}_{P2}) = \frac{1}{m}P_{P2}(1 - P_{P2}) + \frac{1}{m}(\frac{1}{\alpha} - 1)(1 - P_{P1})P_{Pn}(1 - P_{Pn}).$$

1.2 Proofs and Derivations

The unbiasedness and variance of $\hat{P}_{T2}$ are straightforward, since P_{T2} is the marginal probability at the end of period 2, and all n subjects in the treatment group received two periods of treatment.

To show the unbiasedness of $\hat{P}_{P2}$:

$$E\Big(\sum_{m_n+1}^{m} Y_{2i}\Big) = E\Big[E\Big(\sum_{m_n+1}^{m} Y_{2i} \mid Y_{1(m_n+1)}, ..., Y_{1m} = 1\Big)\Big]$$
$$= E\Big[(m - m_n)P_{Pr}\Big] = mP_{P1}P_{Pr},$$

$$E\Big(\sum_{i=1}^{\alpha m_n} Y_{2i}\Big) = E\Big[E\Big(\sum_{i=1}^{\alpha m_n} Y_{2i} \mid Y_{11}, ..., Y_{1\alpha m_n} = 0\Big)\Big]$$
$$= E\Big[\alpha m_n P_{Pn}\Big] = \alpha m(1 - P_{P1})P_{Pn},$$

so

$$
\begin{aligned}
E\left(\hat{P}_{P2}\right) &= E\Big(\frac{1}{m}\big(\frac{1}{\alpha}\sum_{i=1}^{\alpha m_n} Y_{2i} + \sum_{m_n+1}^{m} Y_{2i}\big)\Big) \\
&= \frac{1}{m}\Big(\frac{1}{\alpha}(\alpha m(1-P_{P1})P_{Pn}) + mP_{P1}P_{Pr}\Big) \\
&= (1-P_{P1})P_{Pn} + P_{P1}P_{Pr} \\
&= P(Y_{1i}=0)P(Y_{2i}=1 \mid Y_{1i}=0) + P(Y_{1i}=1)P(Y_{2i}=1 \mid Y_{1i}=1) \\
&= P(Y_{2i}=1) = P_{P2}.
\end{aligned}
$$

In the proof above, notice that the subject id's are arbitrary, and we have assumed earlier that subjects $1, ..., m_n$ are the nonresponders at the end of period 1, and that subjects $1, ..., \alpha m_n$ were randomized to continue on placebo in period 2. So what matters here is the number m_n, which is a binomial random variable dependent on P_{P1}.

Next, to derive the variance of $\hat{P}_{P2}$:

$$
\begin{aligned}
var(\hat{P}_{P2}) &= var\Big(\frac{1}{m}\big(\frac{1}{\alpha}\sum_{i=1}^{\alpha m_n} Y_{2i} + \sum_{m_n+1}^{m} Y_{2i}\big)\Big) \\
&= \frac{1}{m^2}\Bigg[E\Big(var\Big(\frac{1}{\alpha}\sum_{i=1}^{\alpha m_n} Y_{2i} + \sum_{m_n+1}^{m} Y_{2i} \mid Y_{11}, ..., Y_{1m_n}=0,\ Y_{1(m_n+1)}, ..., Y_{1m}=1\Big)\Big) \\
&\qquad + var\Big(E\Big(\frac{1}{\alpha}\sum_{i=1}^{\alpha m_n} Y_{2i} + \sum_{m_n+1}^{m} Y_{2i} \mid Y_{11}, ..., Y_{1m_n}=0,\ Y_{1(m_n+1)}, ..., Y_{1m}=1\Big)\Big)\Bigg] \\
&= \frac{1}{m^2}\Big[E\Big(\frac{1}{\alpha^2}\alpha m_n P_{Pn}(1-P_{Pn}) + (m-m_n)P_{Pr}(1-P_{Pr})\Big) + var\Big(\frac{1}{\alpha}\alpha m_n P_{Pn} + (m-m_n)P_{Pr}\Big)\Big] \\
&= \frac{1}{m}\Big[\frac{1}{\alpha}(1-P_{P1})P_{Pn}(1-P_{Pn}) + P_{P1}P_{Pr}(1-P_{Pr}) + (P_{Pr}-P_{Pn})^2 P_{P1}(1-P_{P1})\Big] \\
&= \frac{1}{m}\Bigg[\frac{1}{\alpha}P(Y_{1i}=0,Y_{2i}=1)(1-P_{Pn}) + P(Y_{1i}=1,Y_{2i}=1)(1-P_{Pr}) \\
&\qquad + P(Y_{1i}=1,Y_{2i}=1)(1-P_{P1})P_{Pr} + P(Y_{1i}=0,Y_{2i}=1)P_{P1}P_{Pn} \\
&\qquad - 2P(Y_{1i}=1,Y_{2i}=1)P(Y_{1i}=0,Y_{2i}=1)\Bigg] \\
&= \frac{1}{m}\Bigg[P(Y_{1i}=1,Y_{2i}=1) - P(Y_{1i}=1,Y_{2i}=1)^2 + P(Y_{1i}=0,Y_{2i}=1) - P(Y_{1i}=0,Y_{2i}=1)^2 \\
&\qquad + (\frac{1}{\alpha}-1)P(Y_{1i}=0,Y_{2i}=1)(1-P_{Pn}) - 2P(Y_{1i}=0,Y_{2i}=1)P(Y_{1i}=1,Y_{2i}=1)\Bigg] \\
&= \frac{1}{m}P_{P2}(1-P_{P2}) + \frac{1}{m}(\frac{1}{\alpha}-1)(1-P_{P1})P_{Pn}(1-P_{Pn}).
\end{aligned}
$$

3

2 Binary-Continuous Case

2.1 Model and Results

Assume that the endpoint for period 1 is binary (responder or nonresponder) and the endpoint for period 2 is continuous. Suppose at the beginning of period 1, n subjects were randomized to receive treatment and m subjects were randomized to receive placebo. Let X_{1i} $(i = 1, ..., n)$ and Y_{1i} $(i = 1, ..., m)$ be the responses at the end of period 1 for the n subjects receiving treatment and the m subjects receiving placebo respectively. Let X_{1i} or Y_{1i} equals to 1 if the subject is a responder and X_{1i} or Y_{1i} equals to 0 if the subject is a nonresponder. Let n_n and m_n be the number of nonresponders at the end of period 1 for the treatment group and placebo group respectively. Without loss of generality, assume that subjects $1, ..., n_n$ in the treatment group and subjects $1, ..., m_n$ in the placebo group are the nonresponders. All the subjects in the treatment group continue to receive treatment in period 2. Assume their responses at the end of period 2 are X_{2i}, $i = 1, ..., n_n$ for the nonresponders and $i = n_n+1, ..., n$ for the responders. The subjects in the placebo group who are nonresponders are re-randomized at the beginning of period 2 to either continue to receive placebo or to switch to receiving treatment. Let α be the proportion of placebo nonresponders who are re-randomized to stay on placebo (and such that αm_n is an integer). Without loss of generality, assume that subjects $1, ..., \alpha m_n$ in the placebo group continued on placebo in period 2. For the subjects that received two periods of placebo, assume their responses at the end of period 2 are Y_{2i}, $i = 1, ..., \alpha m_n$ for the nonresponders and $i = m_n + 1, ..., m$ for the responders. For the placebo nonresponders who were re-randomized to receive treatment, their responses at the end of period 2 would not be considered.

By assumption, n_n and m_n both follow binomial distributions. Let P_T and P_P be the probability of being a responder at the end of period 1 for subjects receiving treatment and placebo respectively. Then $n_n \sim Bin(n, 1 - P_T)$ and $m_n \sim Bin(m, 1 - P_P)$. Assume that the conditional distributions of the responses at the end of period 2 conditioning on whether the subject was a responder or nonresponder at the end of period 1 are normal with respective parameters. That is, assume that

$$X_{2i} \mid X_{1i} = 0 \sim N(\mu_{Tn}, \sigma^2_{Tn}),\ X_{2i} \mid X_{1i} = 1 \sim N(\mu_{Tr}, \sigma^2_{Tr});$$

$$Y_{2i} \mid Y_{1i} = 0 \sim N(\mu_{Pn}, \sigma^2_{Pn}),\ Y_{2i} \mid Y_{1i} = 1 \sim N(\mu_{Pr}, \sigma^2_{Pr}).$$

Let μ_{T2} and μ_{P2} be the marginal means of X_{2i} and Y_{2i} respectively, and let $\delta_2 = \mu_{T2} - \mu_{P2}$ be the difference between the marginal mean responses of treatment and placebo at the end of period 2. An unbiased estimator of δ_2 is

$$\hat{\delta}_2 = \frac{1}{n}\sum_{i=1}^{n} X_{2i} - \frac{1}{m}\Big(\frac{1}{\alpha}\sum_{i=1}^{\alpha m_n} Y_{2i} + \sum_{i=m_n+1}^{m} Y_{2i}\Big),$$

where $\hat{\mu}_{T2} = \frac{1}{n}\sum_{i=1}^{n} X_{2i}$ and $\hat{\mu}_{P2} = \frac{1}{m}\left(\frac{1}{\alpha}\sum_{i=1}^{\alpha m_n} Y_{2i} + \sum_{i=m_n+1}^{m} Y_{2i}\right)$ are unbiased estimators of μ_{T2} and μ_{P2} respectively, and

$$var(\hat{\delta}_2) = \frac{1}{n}\left(P_T\sigma^2_{Tr} + (1-P_T)\sigma^2_{Tn} + P_T(1-P_T)(\mu_{Tr}-\mu_{Tn})^2\right) + \frac{1}{m}\left(P_P\sigma^2_{Pr} + \frac{1}{\alpha}(1-P_P)\sigma^2_{Pn} + P_P(1-P_P)(\mu_{Pr}-\mu_{Pn})^2\right).$$

2.2 Proofs and Derivations

$$\begin{aligned}
E\left(\hat{\mu}_{T2}\right) &= E\left(\frac{1}{n}\sum_{i=1}^{n} X_{2i}\right) \\
&= \frac{1}{n}E\left(E\left(\sum_{i=1}^{n_n} X_{2i} + \sum_{i=n_n+1}^{n} X_{2i} \mid X_{11},...,X_{1n_n}=0,\ X_{1(n_n+1)},...,X_{1n}=1\right)\right) \\
&= \frac{1}{n}E\left(\sum_{i=1}^{n_n} E\left(X_{2i} \mid X_{1i}=0\right) + \sum_{i=n_n+1}^{n} E\left(X_{2i} \mid X_{1i}=1\right)\right) \\
&= \frac{1}{n}E\left(n_n\mu_{Tn} + (n-n_n)\mu_{Tr}\right) \\
&= \frac{1}{n}\left(E(n_n)\mu_{Tn} + E(n-n_n)\mu_{Tr}\right) \\
&= \frac{1}{n}\left(n(1-P_T)\mu_{Tn} + nP_T\mu_{Tr}\right) \\
&= \mu_{Tn}(1-P_T) + \mu_{Tr}P_T \\
&= \mu_{T2}.
\end{aligned}$$

The last step above follows from the assumptions of the conditional distributions of the period 2 endpoints, and the fact that μ_{T2} is defined as the marginal mean.

Following similar steps,

$$
\begin{aligned}
E\left(\hat{\mu}_{T2}\right) &= E\left(\frac{1}{m}\left(\frac{1}{\alpha}\sum_{i=1}^{\alpha m_n} Y_{2i} + \sum_{i=m_n+1}^{m} Y_{2i}\right)\right) \\
&= \frac{1}{m}E\left(E\left(\frac{1}{\alpha}\sum_{i=1}^{\alpha m_n} Y_{2i} + \sum_{i=m_n+1}^{m} Y_{2i} \mid Y_{11},...,Y_{1m_n}=0,\ Y_{1(m_n+1)},...,Y_{1m}=1\right)\right) \\
&= \frac{1}{m}E\left(\frac{1}{\alpha}\sum_{i=1}^{\alpha m_n} E\left(Y_{2i} \mid Y_{1i}=0\right) + \sum_{i=m_n+1}^{m} E\left(Y_{2i} \mid Y_{1i}=1\right)\right) \\
&= \frac{1}{m}E\left(\frac{1}{\alpha}\alpha m_n \mu_{Pn} + (m-m_n)\mu_{Pr}\right) \\
&= \frac{1}{m}\left(E(m_n)\mu_{Pn} + E(m-m_n)\mu_{Pr}\right) \\
&= \frac{1}{m}\left(m(1-P_P)\mu_{Pn} + mP_P\mu_{Pr}\right) \\
&= \mu_{Pn}(1-P_P) + \mu_{Pr}P_P \\
&= \mu_{P2}.
\end{aligned}
$$

Thus we have proved that $\hat{\mu}_{T2}$ and $\hat{\mu}_{P2}$ are unbiased estimators of μ_{T2} and μ_{P2} respectively. The unbiasedness of $\hat{\delta}_2$ follows immediately.

$$
\begin{aligned}
var\left(\hat{\mu}_{T2}\right) &= \frac{1}{n^2}var\left(\sum_{i=1}^{n_n} X_{2i} + \sum_{i=n_n+1}^{n} X_{2i}\right) \\
&= \frac{1}{n^2}\left[var\left(E\left(\sum_{i=1}^{n_n} X_{2i} + \sum_{i=n_n+1}^{n} X_{2i} \mid X_{11},...,X_{1n_n}=0,\ X_{1(n_n+1)},...,X_{1n}=1\right)\right)\right. \\
&\quad \left. + E\left(var\left(\sum_{i=1}^{n_n} X_{2i} + \sum_{i=n_n+1}^{n} X_{2i} \mid X_{11},...,X_{1n_n}=0,\ X_{1(n_n+1)},...,X_{1n}=1\right)\right)\right] \\
&= \frac{1}{n^2}\left[var\left(n_n\mu_{Tn} + (n-n_n)\mu_{Tr}\right) + E\left(n_n\sigma^2_{Tn} + (n-n_n)\sigma^2_{Tr}\right)\right] \\
&= \frac{1}{n^2}\left[var\left(n_n(\mu_{Tn}-\mu_{Tr})\right) + n(1-P_T)\sigma^2_{Tn} + nP_T\sigma^2_{Tr}\right] \\
&= \frac{1}{n}\left[P_T(1-P_T)(\mu_{Tr}-\mu_{Tn})^2 + (1-P_T)\sigma^2_{Tn} + P_T\sigma^2_{Tr}\right]
\end{aligned}
$$

$$
\begin{aligned}
var\left(\hat{\mu}_{P2}\right) &= \frac{1}{m^2} var\Big(\frac{1}{\alpha}\sum_{i=1}^{\alpha m_n} Y_{2i} + \sum_{i=m_n+1}^{m} Y_{2i}\Big) \\
&= \frac{1}{m^2}\Bigg[var\Bigg(E\Big(\frac{1}{\alpha}\sum_{i=1}^{\alpha m_n} Y_{2i} + \sum_{i=m_n+1}^{m} Y_{2i} \mid Y_{11},...,Y_{1m_n}=0,\ Y_{1(m_n+1)},...,Y_{1m}=1\Big)\Bigg) \\
&\quad + E\Bigg(var\Big(\frac{1}{\alpha}\sum_{i=1}^{\alpha m_n} Y_{2i} + \sum_{i=m_n+1}^{m} Y_{2i} \mid Y_{11},...,Y_{1m_n}=0,\ Y_{1(m_n+1)},...,Y_{1m}=1\Big)\Bigg)\Bigg] \\
&= \frac{1}{m^2}\Big[var\big(m_n\mu_{Pn} + (m-m_n)\mu_{Pr}\big) + E\big(\frac{1}{\alpha}m_n\sigma^2_{Pn} + (m-m_n)\sigma^2_{Pr}\big)\Big] \\
&= \frac{1}{m^2}\Big[var\big(m_n(\mu_{Pn}-\mu_{Pr})\big) + \frac{1}{\alpha}m(1-P_P)\sigma^2_{Pn} + mP_P\sigma^2_{Pr}\Big] \\
&= \frac{1}{m}\Big[P_P(1-P_P)(\mu_{Pr}-\mu_{Pn})^2 + \frac{1}{\alpha}(1-P_P)\sigma^2_{Pn} + P_P\sigma^2_{Pr}\Big]
\end{aligned}
$$

The variance of $\hat{\delta}_2$ follows immediately by taking the sum of $var(\hat{\mu}_{T2})$ and $var(\hat{\mu}_{P2})$.

3 Continuous-Continuous Case

3.1 Model and Results

Assume we have continuous endpoints for both periods. Suppose at the beginning of period 1, n subjects were randomized to receive treatment and m subjects were randomized to receive placebo. Let X_{1i} $(i = 1, ..., n)$ and Y_{1i} $(i = 1, ..., m)$ be the responses at the end of period 1 for the n subjects receiving treatment and the m subjects receiving placebo respectively. Subjects are classified as responders if their response for period 1 (X_{1i} or Y_{1i}) is larger than predefined constant C, and they are classified as nonresponders if otherwise. Suppose there are n_n and m_n nonresponders at the end of period 1 for the treatment group and placebo group respectively. Without loss of generality, assume that subjects $1, ..., n_n$ in the treatment group and subjects $1, ..., m_n$ in the placebo group are the nonresponders. All the subjects in the treatment group continue to receive treatment in period 2. Assume their responses at the end of period 2 are X_{2i}, $i = 1, ..., n_n$ for the nonresponders and $i = n_n+1, ..., n$ for the responders. The subjects in the placebo group who are nonresponders are re-randomized at the beginning of period 2 to either continue to receive placebo or to switch to receiving treatment. Let α be the proportion of placebo nonresponders who are re-randomized to stay on placebo (and such that αm_n is an integer). Without loss of generality, assume that subjects $1, ..., \alpha m_n$ in the placebo group continued on placebo in period 2. For the subjects that received two periods of placebo, assume their responses at the end of period 2 are Y_{2i}, $i = 1, ..., \alpha m_n$ for the nonresponders and $i = m_n + 1, ..., m$ for the

responders. For the placebo nonresponders who were re-randomized to receive treatment, their responses at the end of period 2 would not be considered.

Now, assume that X_{1i} and Y_{1i} are both normally distributed with means μ_{T1} and μ_{P1}, variances σ^2_{T1} and σ^2_{P1} respectively. Assume that the conditional distributions of the responses at the end of period 2 conditioning on whether the subject was a responder or nonresponder at the end of period 1 are normal with respective parameters. That is, assume that

$$X_{2i} \mid X_{1i} \le C \sim N(\mu_{Tn}, \sigma^2_{Tn}),\ X_{2i} \mid X_{1i} > C \sim N(\mu_{Tr}, \sigma^2_{Tr});$$

$$Y_{2i} \mid Y_{1i} \le C \sim N(\mu_{Pn}, \sigma^2_{Pn}),\ Y_{2i} \mid Y_{1i} > C \sim N(\mu_{Pr}, \sigma^2_{Pr}).$$

Let $P_T = P(X_{1i} > C)$ and $P_P = P(Y_{1i} > C)$ be the probability of being a responder at the end of period 1 for subjects receiving treatment and placebo respectively. Let μ_{T2} and μ_{P2} be the marginal means of X_{2i} and Y_{2i} respectively, and let $\delta_2 = \mu_{T2} - \mu_{P2}$ be the difference between the marginal mean responses of treatment and placebo at the end of period 2. An unbiased estimator of δ_2 is

$$\hat{\delta_2} = \frac{1}{n}\sum_{i=1}^{n} X_{2i} - \frac{1}{m}\Big(\frac{1}{\alpha}\sum_{i=1}^{\alpha m_n} Y_{2i} + \sum_{i=m_n+1}^{m} Y_{2i}\Big),$$

and

$$\begin{aligned} var(\hat{\delta_2}) = &\frac{1}{n}\Big(P_T\sigma^2_{Tr} + (1-P_T)\sigma^2_{Tn} + P_T(1-P_T)(\mu_{Tr}-\mu_{Tn})^2\Big) \\ &+ \frac{1}{m}\Big(P_P\sigma^2_{Pr} + \frac{1}{\alpha}(1-P_P)\sigma^2_{Pn} + P_P(1-P_P)(\mu_{Pr}-\mu_{Pn})^2\Big). \end{aligned}$$

Let $\delta_1 = \mu_{T1} - \mu_{P1}$ be the difference between the mean responses of treatment and placebo at the end of period 1. The unbiased estimator of δ_1 is very straight forward:

$$\hat{\delta_1} = \frac{1}{n}\sum_{i=1}^{n} X_{1i} - \frac{1}{m}\sum_{i=1}^{m} Y_{1i},$$

and

$$var(\hat{\delta_1}) = \frac{1}{n}\sigma^2_{T1} + \frac{1}{m}\sigma^2_{P1}.$$

In addition, we have

$$cov(\hat{\delta_1}, \hat{\delta_2}) = \frac{1}{n}\sigma_{T1}(\mu_{Tr}-\mu_{Tn})\phi\Big(\frac{C-\mu_{T1}}{\sigma_{T1}}\Big) + \frac{1}{m}\sigma_{P1}(\mu_{Pr}-\mu_{Pn})\phi\Big(\frac{C-\mu_{P1}}{\sigma_{P1}}\Big),$$

where $\phi(\cdot)$ is the density function of the standard normal distribution.

3.2 Proofs and Derivations

The unbiasedness of the estimators and the variances follow directly from the binary-continuous case. Below is the derivation for the covariance.

$$cov\Big(\hat{\delta}_1, \hat{\delta}_2\Big) = cov\Big(\hat{\mu}_{T1} - \hat{\mu}_{P1}, \hat{\mu}_{T2} - \hat{\mu}_{P2}\Big) = cov\Big(\hat{\mu}_{T1}, \hat{\mu}_{T2}\Big) + cov\Big(\hat{\mu}_{P1}, \hat{\mu}_{P2}\Big).$$

$$cov\Big(\hat{\mu}_{T1}, \hat{\mu}_{T2}\Big) = cov\Big(\frac{1}{n}\sum_{i=1}^{n} X_{1i}, \frac{1}{n}\sum_{i=1}^{n} X_{2i}\Big) = \frac{1}{n^2}\sum_{i=1}^{n} cov(X_{1i}, X_{2i}) = \frac{1}{n} cov(X_{1i}, X_{2i}).$$

For convenience, we drop the subscript i in the following derivation.

$$\begin{aligned}
cov(X_1, X_2) &= E\Big[cov\Big(X_1, X_2 \mid I_{\{X_1>C\}}\Big)\Big] + cov\Big[E\Big(X_1 \mid I_{\{X_1>C\}}\Big), E\Big(X_2 \mid I_{\{X_1>C\}}\Big)\Big] \\
&= cov\Big(X_1, X_2 \mid X_1 > C\Big)P(X_1 > C) + cov\Big(X_1, X_2 \mid X_1 \le C\Big)P(X_1 \le C) \\
&\quad + E\Big[\Big(E(X_1 \mid I_{\{X_1>C\}}) - E(X_1)\Big)\Big(E(X_2 \mid I_{\{X_1>C\}}) - E(X_2)\Big)\Big] \\
&= 0 + 0 + P(X_1 > C)\Big[\big(E(X_1 \mid X_1 > C) - \mu_{T1}\big)\big(\mu_{Tr} - \mu_{T2}\big)\Big] \\
&\quad + P(X_1 \le C)\Big[\big(E(X_1 \mid X_1 \le C) - \mu_{T1}\big)\big(\mu_{Tn} - \mu_{T2}\big)\Big] \\
&= P(X_1 \le C)P(X_1 > C)\Big[E(X_1 \mid X_1 > C) - E(X_1 \mid X_1 \le C)\Big]\big(\mu_{Tr} - \mu_{Tn}\big) \\
&= \sigma_{T1}\big(\mu_{Tr} - \mu_{Tn}\big)\phi\Big(\frac{C - \mu_{T1}}{\sigma_{T1}}\Big).
\end{aligned}$$

It follows that

$$cov\Big(\hat{\mu}_{T1}, \hat{\mu}_{T2}\Big) = \frac{1}{n}\sigma_{T1}\big(\mu_{Tr} - \mu_{Tn}\big)\phi\Big(\frac{C - \mu_{T1}}{\sigma_{T1}}\Big)$$

In the derivation above, we used the facts that $\mu_{T1} = P(X_1 > C)E(X_1|X_1 > C) + P(X_1 \le C)E(X_1|X_1 \le C)$ and $\mu_{T2} = P(X_1 > C)\mu_{Tr} + P(X_1 \le C)\mu_{Tn}$. We also directly used formulas for the conditional means of a truncated normal distribution.

For $cov(\hat{\mu}_{P1}, \hat{\mu}_{P2})$, notice that

$$\begin{aligned}
E\Big(\hat{\mu}_{P2} \mid I_{\{Y_{1i}>C\}}, i = 1, ..., m\Big) &= E\Big[\frac{1}{m}\Big(\frac{1}{\alpha}\sum_{i=1}^{\alpha m_n} Y_{2i} + \sum_{i=m_n+1}^{m} Y_{2i}\Big) \mid I_{\{Y_{1i}>C\}}, i = 1, ..., m\Big] \\
&= E\Big[\frac{1}{m}\Big(\sum_{i=1}^{m_n} Y_{2i} + \sum_{i=m_n+1}^{m} Y_{2i}\Big) \mid I_{\{Y_{1i}>C\}}, i = 1, ..., m\Big] \\
&= E\Big[\frac{1}{m}\sum_{i=1}^{m} Y_{2i} \mid I_{\{Y_{1i}>C\}}, i = 1, ..., m\Big].
\end{aligned}$$

So we have

$$\begin{aligned} cov(\hat{\mu}_{P1}, \hat{\mu}_{P2}) &= E\Big[cov\Big(\hat{\mu}_{P1}, \hat{\mu}_{P2} \mid I_{\{Y_{1i}>C\}}, i = 1, ..., m\Big)\Big] \\ &\quad + cov\Big[E\Big(\hat{\mu}_{P1} \mid I_{\{Y_{1i}>C\}}, i = 1, ..., m\Big), E\Big(\hat{\mu}_{P2} \mid I_{\{Y_{1i}>C\}}, i = 1, ..., m\Big)\Big] \\ &= 0 + cov\Big[E\Big(\frac{1}{m}\sum_{i=1}^{m} Y_{1i} \mid I_{\{Y_{1i}>C\}}, i = 1, ..., m\Big), E\Big(\frac{1}{m}\sum_{i=1}^{m} Y_{2i} \mid I_{\{Y_{1i}>C\}}, i = 1, ..., m\Big)\Big] \\ &= cov\Big(\frac{1}{m}\sum_{i=1}^{m} Y_{1i}, \frac{1}{m}\sum_{i=1}^{m} Y_{2i}\Big) \\ &= \frac{1}{m} cov(Y_{1i}, Y_{2i}). \end{aligned}$$

Following the same derivation as $cov(X_{1i}, X_{2i})$, we have

$$cov(Y_{1i}, Y_{2i}) = \sigma_{P1}(\mu_{Pr} - \mu_{Pn})\phi\Big(\frac{C - \mu_{P1}}{\sigma_{P1}}\Big),$$

and

$$cov(\hat{\mu}_{P1}, \hat{\mu}_{P2}) = \frac{1}{m}\sigma_{P1}(\mu_{Pr} - \mu_{Pn})\phi\Big(\frac{C - \mu_{P1}}{\sigma_{P1}}\Big).$$

The formula for $cov\Big(\hat{\delta}_1, \hat{\delta}_2\Big)$ follows immediately by taking the sum of $cov(\hat{\mu}_{T1}, \hat{\mu}_{T2})$ and $cov(\hat{\mu}_{P1}, \hat{\mu}_{P2})$.

4 Adaptive Testing

4.1 Model and Results

Assume $\delta_2 = r\delta_1 = r\delta$, for a given constant r. We wish to test for $H_0 : \delta = 0$ against $H_a : \delta > 0$. By assumption, $\delta = \lambda\delta_1 + (1 - \lambda)\frac{1}{r}\delta_2$, for any λ. Let $\hat{\delta} = \lambda\hat{\delta}_1 + (1 - \lambda)\frac{1}{r}\hat{\delta}_2$, where $\hat{\delta}_1$ and $\hat{\delta}_2$ are defined previously. It is easy to show that $\hat{\delta}$ is unbiased, and

$$var(\hat{\delta}) = \lambda^2 var(\hat{\delta}_1) + \frac{2}{r}\lambda(1 - \lambda)cov(\hat{\delta}_1, \hat{\delta}_2) + \frac{(1 - \lambda)^2}{r^2} var(\hat{\delta}_2).$$

To maximize the power of testing $H_a : \delta > 0$, we choose λ that minimizes $var(\hat{\delta})$. We rewrite $var(\hat{\delta})$ as a quadratic function of λ:

$$var(\hat{\delta}) = \Big[var(\hat{\delta}_1) - \frac{2}{r}cov(\hat{\delta}_1, \hat{\delta}_2) + \frac{1}{r^2}var(\hat{\delta}_2)\Big]\lambda^2 + \Big[\frac{2}{r}cov(\hat{\delta}_1, \hat{\delta}_2) - \frac{2}{r^2}var(\hat{\delta}_2)\Big]\lambda + \frac{1}{r^2}var(\hat{\delta}_2).$$

It can be easily shown that the minimum variance is achieved when

$$\lambda = \lambda^* = \frac{var(\hat{\delta}_2) - cov(\hat{\delta}_1, \hat{\delta}_2)r}{var(\hat{\delta}_1)r^2 - 2cov(\hat{\delta}_1, \hat{\delta}_2)r + var(\hat{\delta}_2)},$$

and the corresponding optimal $\hat{\delta}$ and $var(\hat{\delta})$ are

$$\hat{\delta} = \frac{\left[var(\hat{\delta}_2) - cov(\hat{\delta}_1, \hat{\delta}_2)r\right]\hat{\delta}_1 + \left[var(\hat{\delta}_1)r - cov(\hat{\delta}_1, \hat{\delta}_2)\right]\hat{\delta}_2}{var(\hat{\delta}_1)r^2 - 2cov(\hat{\delta}_1, \hat{\delta}_2)r + var(\hat{\delta}_2)},$$

$$var(\hat{\delta}) = \frac{var(\hat{\delta}_1)var(\hat{\delta}_2) - cov(\hat{\delta}_1, \hat{\delta}_2)^2}{var(\hat{\delta}_1)r^2 - 2cov(\hat{\delta}_1, \hat{\delta}_2)r + var(\hat{\delta}_2)}.$$

Let Z be the Wald statistic for testing $H_a : \delta > 0$, computed from the optimal $\hat{\delta}$ and $var(\hat{\delta})$, then

$$Z = \frac{\hat{\delta}}{\sqrt{var(\hat{\delta})}} = \frac{\left[var(\hat{\delta}_2) - cov(\hat{\delta}_1, \hat{\delta}_2)r\right]\hat{\delta}_1 + \left[var(\hat{\delta}_1)r - cov(\hat{\delta}_1, \hat{\delta}_2)\right]\hat{\delta}_2}{\sqrt{var(\hat{\delta}_1)var(\hat{\delta}_2) - cov(\hat{\delta}_1, \hat{\delta}_2)^2}\sqrt{var(\hat{\delta}_1)r^2 - 2cov(\hat{\delta}_1, \hat{\delta}_2)r + var(\hat{\delta}_2)}}.$$

In reality, we do not know the value of the constant r. Suppose we have a vector of possible values of r: $(r_1, r_2, ..., r_k)$. Let Z_i be the optimal Wald statistic derived above for corresponding r_i. Assume that $(Z_1, Z_2, ..., Z_k)$ follows a multivariate normal distribution under the null. Marginally, $Z_i \sim N(0, 1)$ under the null. And it can be shown that

$$cov(Z_i, Z_j) = \frac{var(\hat{\delta}_2) + var(\hat{\delta}_1)r_i r_j - cov(\hat{\delta}_1, \hat{\delta}_2)(r_i + r_j)}{\sqrt{var(\hat{\delta}_1)r_i^2 - 2cov(\hat{\delta}_1, \hat{\delta}_2)r_i + var(\hat{\delta}_2)}\sqrt{var(\hat{\delta}_1)r_j^2 - 2cov(\hat{\delta}_1, \hat{\delta}_2)r_j + var(\hat{\delta}_2)}}.$$

Let $Z^\star = max(Z_1, ..., Z_k)$. Let $F^\star(x)$ be the distribution function of $Z^\star$ under the null, that is $F^\star(x) = P(Z^\star \leq x) = P(Z_1 \leq x, ..., Z_k \leq x)$, which is known under the assumed multivariate normal distribution of $(Z_1, Z_2, ..., Z_k)$.

We define our adaptive test statistic $\mathbb{Z}$ as

$$\mathbb{Z} = \Phi^{-1}\left(F^\star(Z^\star)\right).$$

A one-sided p-value for testing $H_a : \delta > 0$ can be obtained by comparing $\mathbb{Z}$ to the standard normal distribution. That is,

$$p = 1 - \Phi(\mathbb{Z}) \ \left(= 1 - F^\star(Z^\star) = P_{null}(max(Z_1, ..., Z_k) > Z^\star) \right).$$

4.2 Proofs and Derivations

Below is the derivation of the covariance structure of $(Z_1, Z_2, ..., Z_k)$. For simplicity of notation, let $v_1 = var(\hat{\delta}_1)$, $v_2 = var(\hat{\delta}_2)$ and $c = cov(\hat{\delta}_1, \hat{\delta}_2)$.

$$cov(Z_i, Z_j) = \frac{1}{v_1 v_2 - c^2}\frac{1}{\sqrt{v_1 r_i^2 - 2cr_i + v_2}}\frac{1}{\sqrt{v_1 r_j^2 - 2cr_j + v_2}}$$
$$\times cov\Big((v_2 - cr_i)\hat{\delta}_1 + (v_1 r_i - c)\hat{\delta}_2, (v_2 - cr_j)\hat{\delta}_1 + (v_1 r_j - c)\hat{\delta}_2\Big).$$

$$
\begin{aligned}
&cov\Big((v_2 - cr_i)\hat{\delta}_1 + (v_1 r_i - c)\hat{\delta}_2, (v_2 - cr_j)\hat{\delta}_1 + (v_1 r_j - c)\hat{\delta}_2\Big) \\
&= (v_2 - cr_i)(v_2 - cr_j)var(\hat{\delta}_1) + (v_1 r_i - c)(v_1 r_j - c)var(\hat{\delta}_2) \\
&\quad + \Big((v_2 - cr_i)(v_1 r_j - c) + (v_1 r_i - c)(v_2 - cr_j)\Big)cov(\hat{\delta}_1, \hat{\delta}_2) \\
&= v_2^2 v_1 + r_i r_j v_1^2 v_2 - r_j c v_1 v_2 - r_i c v_1 v_2 - r_i r_j c^2 v_1 + r_j c^3 - c^2 v_2 + r_i c^3 \\
&= (v_1 v_2 - c^2)(v_2 + v_1 r_i r_j - c(r_i + r_j)).
\end{aligned}
$$

So

$$
cov(Z_i, Z_j) = \frac{v_2 + v_1 r_i r_j - c(r_i + r_j)}{\sqrt{v_1 r_i^2 - 2cr_i + v_2}\sqrt{v_1 r_j^2 - 2cr_j + v_2}}.
$$

Attachment 3: Criteria of Markedly Abnormal Laboratory Values

	Markedly Abnormal Limits	
Laboratory Parameter	Low	High
Albumin [g/L]	24	60
Alkaline phosphatase [U/L]	N/A	250
Alanine transaminase (SGPT) [U/L]	N/A	200
Aspartate transaminase (SGOT) [U/L]	N/A	250
Bicarbonate [mmol/L]	15.1	34.9
Blood urea nitrogen [mmol/L]	N/A	17.9
Calcium [mmol/L]	1.5	3
Chloride [mmol/L]	94	112
Cholesterol [mmol/L]	N/A	7.8
Creatine kinase (U/L)	N/A	990
Creatinine [µmol/L]	N/A	265.2
Gamma glutamyl transferase [U/L]	N/A	300
Glucose [mmol/L]	2.2	16.7
Lactate Dehydrogenase[U/L]	N/A	500
Phosphate [mmol/L]	0.7	2.6
Potassium [mmol/L]	3.0	5.8
Sodium [mmol/L]	125	155
Bilirubin, total [µmol/L]	N/A	51.3
Protein, total [g/L]	50	N/A
Urate [µmol/L]	89.2	594.8
Urine pH	N/A	6.5
Hematocrit [fraction] - female	0.28	0.5
- male	0.24	0.55
Hemoglobin [g/L]	80	190
Neutrophils, segmented [%]	30	90
Monocytes [%]	N/A	20
Eosinophils [%]	N/A	10
Basophils [%]	N/A	6
Lymphocytes [%]	10	60
Platelet count [$x10^9$/L]	100	600
Red blood cell count [$x10^{12}$/L] -- female	3.0	5.5
-- male	3.0	6.4
White blood cell count [$x10^9$/L]	2.5	15.0

Note: The same limits apply to both males and females unless gender is indicated;
N/A = Not applicable.