Perception Neuroscience, Inc.

Protocol #: PCN-101-21

NCT05414422

A RANDOMIZED, PLACEBO-CONTROLLED, DOUBLE-BLIND STUDY TO ASSESS THE SAFETY AND EFFICACY OF INTRAVENOUS PCN-101 IN TREATMENT RESISTANT DEPRESSION

Version 2.0 (Final)

Document last saved: 14-Nov-2022 11:05 AM

CONTENTS

List of Abbreviations

Abbreviation	Definition
˚C	Celsius
5D-ASC	5-Dimensional Altered States of Consciousness Rating Scale
AE	Adverse event
ANCOVA	Analysis of covariance
BPRS+	Brief Psychiatric Rating Scale - Modified 4 Components
CADSS	Clinician Administered Dissociative States Scale
CFB	Change from baseline
CGI-I	Clinical Global Impression - Improvement
CGI-S	Clinical Global Impression - Severity
CI	Confidence interval
COVID-19	Coronavirus disease 2019
CSSRS	Columbia Suicide Severity Rating Scale
DB	Double-blind
ECG	Electrocardiogram
eCRF	Electronic case report forms
EQ 5D-3L	European Quality 5 Dimensions - 3 Levels
EU	European Union
FAS	Full analysis set
GAD-7	Generalized Anxiety Disorder 7-Item
HAM-D	Hamilton Depression Rating Scale
IV	Intravenous
kg	Kilogram
LOCF	Last observation carried forward
MADRS	Montgomery Åsberg Depression Rating Scale
MAR	Missing-at-random
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligram
MGH-ATRQ	Massachusetts General Hospital Antidepressant Response Questionnaire
MINI	Mini-International Neuropsychiatric Interview
mmHg	Milligrams of mercury
MMRM	Mixed model repeated measures
MOAA/S	Modified Observer’s Assessment of Alertness/Sedation
MNAR	Missing-not-at-random
OR	Odds ratio

Abbreviation	Definition
PPAS	Per-protocol analysis set
PT	Preferred term
Q1	Quartile 1
Q3	Quartile 3
QIDS-SR-16	Quick Inventory of Depressive Symptomatology - 16 Items
QTcF	QT intervals corrected by Fridericia's formula
SAP	Statistical analysis plan
SAS	Safety analysis set
SD	Standard deviation
SOC	System organ class
SpO_2	Oxygen saturation
TEAE	Treatment-emergent AE
TRD	Treatment Resistant Depression
US	United States
VAS	Visual analog scale
WHO	World Health Organization

I. Introduction

A. Background

Perception Neuroscience, Inc., is developing PCN-101 (R-ketamine) for the treatment of treatment resistant depression (TRD). Ketamine is a parenterally administered anesthetic agent that was initially approved in the United States (US) in 1970. Since then, it has seen wide use in both adult and pediatric populations. Intranasally administered S-ketamine was approved in 2019 in the US and European Union (EU) for the treatment of treatment-resistant depression. Evidence from nonclinical depression model studies of subanesthetic doses in rodents suggests that R-ketamine has equal or potentially greater efficacy compared with S-ketamine, and both nonclinical and preliminary clinical studies suggest that R--ketamine may have a more favorable safety profile with a decreased incidence of adverse events (AEs) (eg, dissociative, cognitive impairment, and psychotomimetic effects) compared with S-ketamine. Based on nonclinical studies, R-ketamine may also have less abuse potential than S-ketamine. Overall, the available data support the concept of the use of PCN-101 as a potentially better tolerated, rapidly acting antidepressant compared with ketamine and S-ketamine, which may support the use of PCN-101 outside of a supervised, in-clinic environment.

B. Scope of Document

This statistical analysis plan (SAP) is based on the amended version 4.0 of the protocol dated 19May2022. It will govern the analysis of data for this study. The SAP may be modified until the time of database lock for the final analysis. Any deviations from the SAP, including post- treatment unblinding, will be documented as such in the study report. Supportive reference materials include protocol of the study and the accompanying study data collection documents.

II. Protocol Objectives and Analysis Endpoints

The primary, secondary, safety, and exploratory objectives of the current study are shown along with their corresponding endpoints in Table 1.

Table 1 Objectives and Endpoints

Objectives	Endpoints/Estimands
Primary	
• To determine the efficacy of 2 doses (30 milligram [mg] and 60 mg) of intravenous (IV) PCN-101 compared with placebo in improving depressive symptoms in subjects with TRD as assessed by change	• MADRS change in total score assessed from baseline (predose) to 24 hours after the start of the infusion of PCN-101. • See Section VII.A Primary Estimand for details on estimand definition

from baseline to 24 hours after the start of the infusion of PCN-101 in	
Objectives	**Endpoints/Estimands**
the Montgomery Åsberg Depression Rating Scale (MADRS) total score.	
Secondary	

• To assess the proportion of subjects with a response (defined as ≥ 50% improvement in MADRS total score from predose).	• MADRS total score assessed at 2 and 4 hours, 7 and 14 days after the start of the infusion (Days 8 and 15, respectively). Note, for the 2 hour and 4 hour recall periods, the sleep items are not assessed, and predose scores for these items obtained on the same day will be carried forward. • Proportion of subjects with ≥ 50% improvement in MADRS total score at 24 hours, 7 days and 14 days after start of infusion (Days 8 and 15, respectively).
• To assess the proportion of subjects with remission (defined as MADRS total score ≤ 10).	• Proportion of subjects with a MADRS total score ≤ 10 at 24 hours, 7 days and 14 days after start of infusion (Days 8 and 15, respectively).
• To define changes in Hamilton Depression Rating Scale (HAM-D).	• Changes in HAM-D on Day 8 and Day 15 after start of infusion.
• To define changes in Generalized Anxiety Disorder 7-Item (GAD-7).	• Change from baseline in GAD-7 by visit.
• To assess Clinical Global Impression - Severity (CGI-S) and Clinical Global Impression - Improvement (CGI-I).	• Change from baseline in CGI-S by visit and CGI-I (calculated from predose CGI-S).
• To define changes in Quick Inventory of Depressive Symptomatology - 16 Items (QIDSSR-16)*.	• Change from baseline in QIDS-SR-16-by visit*.
Objectives	**Endpoints/Estimands**

• To define changes in European Quality 5 Dimensions - 3 Levels (EQ 5D-3L).	• Change from baseline in EQ 5D-3L by visit.
Safety	
• To determine the safety and tolerability of 2 doses of PCN-101 administered IV in subjects with TRD compared with placebo.	• Adverse events (AEs) • Columbia Suicide Severity Rating Scale (C-SSRS) • Clinical laboratory parameters • Vital signs and oxygen saturation (SpO_2) • Physical Examination • 12-lead electrocardiogram (ECG) • Concomitant Therapies • Modified Observer's Assessment of Alertness/Sedation (MOAA/S) • Clinician Administered Dissociative States Scale (CADSS) • Brief Psychiatric Rating Scale - Modified 4 Components (BPRS+) • 5-Dimensional Altered States of Consciousness Rating Scale (5D-ASC)

* An abbreviated version (ie, QIDS-SDR-14) is only done once at Visit 2 'discharge' timepoint. For this timepoint, the decreased/increased weight items score from Day 1 predose will be carried forward to Visit 2 in the calculation of the total score at Visit 2 'discharge'.

III. Study Design

A. **Design Overview**

This is a Phase 2a, double-blind, randomized, placebo-controlled, multicenter study comprised of 3 phases: screening (up to 2 weeks), [Day -15 to Day -2]), In-Clinic

Treatment (Day -1 to Day 2; including double-blind treatment [Day 1]), and post-treatment follow-up (7 and 14 days after infusion, on Days 8 and 15, respectively). The study consists of 3 arms: placebo, PCN-101 Solution for Injection 30 mg, and PCN-101 Solution for Injection 60 mg. A total of 93 adult subjects with TRD will be randomly allocated in equal cohorts of 31 subjects/arm to the 3 arms of the study in a blinded manner. Randomization will be stratified by region (US, EU).

Subjects will be randomized within 14 days of screening (Visit 1). Subjects will be admitted to the clinic the evening prior to study treatment administration (Day -1, Visit 2) and undergo baseline testing to ensure continued study eligibility. Study treatments will be infused IV over 40 minutes the next morning (Day 1, Visit 2). Starting immediately prior to dosing, subjects will be monitored closely for safety. Additionally, the subjects' alertness, mood, and other psychological parameters will be assessed by clinician- and patient completed scales and questionnaires. Subjects will be discharged no earlier than 24 hours postinfusion and after the final in clinic assessments have been completed (Day 2, Visit 2). Subjects will be asked to return to the clinic approximately 6 days (Day 8, Visit 3) and 13 days (Day 15, Visit 4) after discharge to assess the safety and tolerability of the study treatments and to determine the durability of the antidepressant effect.

During the coronavirus disease 2019 (COVID-19) pandemic, the specific guidance from local public health and other competent authorities regarding the protection of individuals' welfare must be applied. If subjects cannot attend follow up visits in person because of restrictions arising as a consequence of the COVID-19 pandemic, the Investigator must discuss with the Medical Monitor potential mitigation.

B. Study Population

Eligibility for participation in the study will be based on the inclusion/exclusion criteria. Specifically, candidates must meet all entry criteria at Screening or at the time point specified in the individual eligibility criteria listed in the protocol. Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

C. Sample Size Predictions

The intent of the primary efficacy analysis is to demonstrate superiority of at least 1 therapeutic dose of PCN-101 Solution for Injection (30 mg or 60 mg) versus placebo based on the change in the MADRS total score from predose to 24 hours postdose.

For the primary analysis, a sample size of 93 randomized subjects (31:31:31) will provide 80% power to detect an 8-point difference between each PCN-101 Solution for Injection dose and placebo in the mean change from baseline MADRS total score at 24 hours postdose, using a t-test with $\alpha = 0.05$ (2 tailed) and assuming a common standard deviation (SD) of 11.

Approximately 20 study centers in the EU and US are expected to participate in this study. The enrollment of 93 subjects is planned. Subjects who do not receive a dose of study treatment on Day 1 (Visit 2) will be replaced.

D. Treatment Randomization

Two doses (30 mg and 60 mg) of IV PCN-101 will be compared with placebo to determine the most effective dose in improving depressive symptoms in subjects with TRD.

Randomization will be used to minimize the bias in the assignment of subjects to treatment arms and to increase the likelihood that known and unknown subject characteristics are evenly balanced across treatment arms. Randomization will be stratified by region (US, EU). This will improve the validity of the statistical comparisons among treatment arms across different geographical regions. Blinded treatment will be used to reduce any potential bias during the collection of data and the analysis of clinical endpoints.

E. Assessment Schedule

The various assessments that will be conducted during this study are summarized in the protocol.

IV. Interventions

A. Clinical Trial Material

The investigational medical products are PCN-101 (30 mg and 60 mg) and Placebo.

V. General Analytical Considerations

A. Data Sources

All reported study data will be recorded on the electronic case report forms (eCRF) using Medidata Rave EDC. During the data collection process, automated quality assurance programs will be used to identify missing data, out-of-range data, and other data inconsistencies.

B. Definition of Baseline

Unless otherwise indicated, the baseline is defined as the most recent non-missing and evaluable observation of the investigated parameter (including any unscheduled or repeat observations), prior to study drug dosing. Details are provided within the respective analysis sections of this SAP.

A change from baseline will be calculated as the post-baseline measurement minus the baseline measurement. If either the baseline or post-baseline value is missing, then the change from baseline value will be considered missing.

C. Missing Data

1. Primary and Secondary Endpoints

In general, the missing data which is observed from this study will be assumed to be missing-at-random (MAR).

Since subjects are confined to clinic at the time of the primary endpoint, it is anticipated that missing data will be infrequent. No imputation will be applied to primary analysis.

Baseline values will not be carried forward.

2. Imputation of Partial Dates

Partial dates are allowed in selected eCRFs including but not limited to AE onset and resolution dates, concomitant therapy start and stop dates, and medical history dates. An entry for the year is required in the eCRF system for each of these dates. Only the month and day may be entered as unknown. Dates from these forms will be reported in listings as collected. Every effort will be made to query missing dates.

A set of imputation rules will be implemented for the determination of whether an AE is treatment-emergent, or a therapy is concomitant. For the imputation methods of missing start or end dates in the classification of treatment-emergence or concomitant therapy, refer to Section XI.A Missing or Partial Date Imputation.

D. Timing of Interim Analysis and Sample Size Reassessment

Not applicable.

E. Test Sizes

Statistical hypothesis testing will be performed based on the comparison of PCN-101 60 mg and 30 mg versus placebo, in two-sided tests with a significance level $\alpha=0.05$. The 95% of CI of the mean treatment difference will be two-sided.

F. Multiple Comparisons

No multiple comparison adjustments will be made for analyses.

G. Analysis Populations

Analysis sets are defined as described in Table 2:

Table 2 Description of Analysis Sets

Analysis set	Description
Screened Analysis Set	All subjects who signed the informed consent form.
Full Analysis Set (FAS)	The FAS will consist of all randomized subjects who receive any amount of study treatment and have at least 1 postbaseline assessment available. This population will serve as the basis for efficacy analysis. Subjects will be analyzed according to their randomized treatment.
Safety Analysis Set (SAS)	The SAS will consist of all randomized subjects who receive any amount of study treatment. This analysis set will be used for all summaries of subject disposition, demographic and baseline data, and safety information including AE incidence. Subjects will be analyzed according to the treatment they actually received.

H. Subgroups of Analysis Populations

Data from subgroups of subjects in the defined analysis populations will be analyzed as specified below. These analyses will comprise descriptive summaries; the goal will be to identify signals of additional effects that analyses of data from the defined populations do not take into account. Such analyses will be considered exploratory and other subgroups may be considered. They may not be sufficient for drawing conclusions; hence, these analyses will not involve hypothesis testing.

- Age Group (18-44, 45-65 years))
- Sex (Male, Female)
- Region (United States or Europe)
- Duration of depression (<5 years, 5-10 years, > 10 years)
- Duration of current episode of depression (weeks)
- Number of previous treatment failures in the current episode (<3, >=3)
- Baseline MADRS Score (<35, ≥35)
- Baseline medication
    - Selective Serotonin reuptake inhibitor (SSRI)

- Serotonin and norepinephrine reuptake inhibitor (SNRI)
- Antidepressant with adjunctive medications

I. Data Display Characteristics

Data displays produced for this study will include three types—summary tables, data listings, and figures. Unless stated otherwise, data listings will be produced for all recorded data. Summary tables will be produced as specified in the following sections. Additional data listings will be produced for outcome measures that involve extensive procedures to derive the analyzed outcomes. Figures will be produced when specified in sections to follow.

Data listings will simply list the data recorded on the CRF or derived for each subject. They will be ordered by treatment, site, subject number, and time of assessment. When expedient, additional levels of ordering hierarchy may reflect subsets of assessments within subject. Data listings will not display subject initials.

Summary tables will display summary statistics calculated for each of the treatment groups, unless described otherwise in the following sections. Tables may have either of two general layouts. When the greatest interest is in direct comparison of one treatment group with the others at particular times, different columns of a summary table will display the statistics for the different treatment groups.

The summary statistics displayed will be a function of the type of data associated with the summarized assessment. Unless stated otherwise in relevant sections to follow, continuous data will be summarized with the number of non-missing values, arithmetic mean, standard deviation, minimum, quartile 1 (Q1), median, quartile 2 (Q3), and maximum. Categorical data will be summarized with the number of nonmissing values and the numbers of values equal to each of the possible values. Percentages of subjects with each of the possible values will be calculated from the number of subjects in the corresponding analysis population, unless stated otherwise. Some continuous variables may also be grouped into categorical levels and evaluated in frequency tables.

A subset or all of the following tabular column headers will be used in presenting data for the study:

- PCN-101 60 mg
- PCN-101 30 mg
- Placebo

Efficacy analysis will be implemented according to the planned randomization treatment schedule. Safety analysis will be implemented according to the actual dosed treatment.

VI. Subject Accountability

Unless otherwise indicated, the subject characteristics will be presented by treatment groups for the FAS. The same analysis will be repeated for the SAS if the composition of subjects within these analysis sets are different from the FAS.

A. Disposition

Subject disposition events (screened, randomized, completed treatment, completed the study, early discontinuation from the study along with the reason for discontinuation) will be summarized using frequency counts and percentages by treatment groups.

The duration of the study will be up to 29 days per subject, which includes a screening period, an in-clinic visit, and 2 follow-up visits. A subject will be considered to have completed the study if he/she has completed all phases of the study including the second follow-up visit.

All disposition data will be presented in a listing format for the FAS. Subjects who failed screening will be presented in a listing format for the Screening Analysis Set.

B. Protocol Deviations

Major protocol deviations will be reviewed and determined prior to the unblinding of this study.

All deviation data will be presented in a listing format for the FAS.

C. Subject Characteristics

The subject characteristics defined below will be presented in summary tables using the most recent data collected prior to study drug dosing. No formal statistical comparisons will be performed.

All subject characteristic data will be listed by subject for the FAS.

Data collected for the following subject characteristics at Baseline will be summarized by treatment group. Race and ethnicity were not planned to be collected at the start of the study as the trial was originally held in Europe only, with local regulations preventing the collection of this data. With expansion of enrolment to US sites, it was determined by the Sponsor that it would be non-critical to include race and ethnicity in the analysis for this proof of concept study. As a result, no race or ethnicity data is collected in the study.

- Age (years)
- Age Category (18 to 44 years and 45 to 65 years
- Sex (Male, Female)

- Region (US or EU)
- Baseline MADRS total score
- Baseline MADRS Score (<35, ≥35)
- Baseline HAM-D total score
- Body Mass Index (kg/m^2)
- Duration of depression (years)
- Duration of depression (<5 years, 5 to 10 years, >10 years)
- Total number of depressive episodes to date
- Duration of current episode
- Family history of psychiatric disorder (yes, no, unknown) and subcategories
- Number of previous treatment failures in the current episode (<3, >=3)
- Baseline medication*
  - Selective Serotonin reuptake inhibitor (SSRI)
  - Serotonin and norepinephrine reuptake inhibitor (SNRI)
  - Antidepressant with adjunctive medications

*Baseline medication is defined as medication taken on Day 1.

D. Medical History

Medical history will be coded using Medical Dictionary for Regulatory Activities (MedDRA) version 23.0 or higher. System Organ Class (SOC) and Preferred Term (PT) will be summarized by number and percentage of subjects having at least one occurrence of the event for the FAS only. All Medical history information be listed by subject.

E. Population Inclusions

A hierarchical table of the populations in which Screened Analysis Set subjects were included will summarize the relationship of the analysis populations. Given population definitions, the Screen Analysis Set will be treated as the parent analysis set. The numbers and percentages of Screened Analysis Set included in the FAS, SAS and PPAS will be presented for each treatment group. The numbers and percentages of FAS subjects included in the SAS and PPAS population will be displayed for each treatment group. This table will be produced for subjects in both US and Europe regions, and as an overall.

VII. Efficacy Analyses

The analysis of the efficacy data obtained from the subjects in this clinical study will be performed on the FAS primarily.

All efficacy analyses will be performed based on a subject's randomized treatment group.

A. Primary Estimand

The primary estimand is defined as follows

- Population: Subjects in the FAS and analyzed according to their randomized treatment. The analysis population includes all randomized subjects who receive any amount of study treatment and have at least 1 postbaseline assessment available.
- Variable: Change in total MADRS score from baseline at 24 hours after the start of infusion.
- Intercurrent event: Incidents where a subject does not receive the full treatment infusion will be considered an intercurrent event. The intercurrent event will however be handled using a treatment policy where all observed values will be used regardless of occurrence of an intercurrent event. No imputation is performed in the primary efficacy analysis.
- Population-level summary: Difference in mean change from baseline between each PCN-101 group versus placebo

Additional methodology will be specified for sensitivity and supplementary analyses for assessing the robustness of results. These sensitivity or supplementary analyses will explore different methods for handling intercurrent events and different assumptions for missing data.

B. Primary Efficacy Endpoints

The primary efficacy endpoint will be based on the change in total MADRS score from baseline at 24 hours after start of infusion.

The MADRS is a ten-item diagnostic questionnaire administered by the study staff to measure the overall severity of depressive symptoms.

The total score is computed as the sum of scores across all 10 items. Higher MADRS score indicates more severe depression. Each item yields a score of 0 to 6, hence the total score ranges from 0 to 60.

$$CMADRS_{total,24hr} = MADRS_{total,24\ hr} - MADRS_{total,Baseline}$$

where

- $CMADRS_{total,24hr}$: Change from baseline in total score MADRS at 24 hours after the start of infusion
- $MADRS_{total,24hr}$: Total score MADRS at 24 hours after the start of infusion

- $MADRS_{total,Baseline}$: Total score MADRS at Baseline

C. Secondary Efficacy Estimand

All secondary efficacy estimands are defined using the same population, intercurrent event strategy, and population level summary (difference in means or proportions between each randomized treatment and placebo, as appropriate) as described in Section VII.A Primary Estimand.

D. Secondary Efficacy Endpoint

Change from baseline in MADRS total score at 2 hours, 4 hours, 7 days, and 14 days after the start of the infusion

The change from baseline MADRS total score will be analyzed for all analysis timepoints.

Since the sleep items will not be assessed at 2 hours and 4 hours after the start of infusion, and the predose scores for these items obtained on the same day will be carried forward.

Proportion of subjects with at least 50% improvement (i.e. reduction) in MADRS total score at 24 hours, 7 days, and 14 days after start of infusion

A subject is considered a responder at a timepoint if a ≥50% improvement in MADRS total score (from baseline) is achieved at that timepoint. Any subject who is assessed but not considered as responder at a timepoint will be assumed to be a non-responder at that timepoint.

The proportion of subjects who meet this improvement criterion will be analyzed across different timepoints.

Subjects with MADRS total score ≤10 at 24 hours, 7 days, and 14 days after start of infusion will be considered to be in remission

The proportion of subjects who meet this criterion of MADRS total score ≤ 10 will be analyzed across different timepoints. Any subject who is assessed but not considered as remitter at a timepoint will be assumed to be a non-remitter at that timepoint.

Change in QIDS-SR-16 score from baseline by analysis visit

The QIDS-SR-16 is a 16-item self-assessed questionnaire which measures depression symptoms. Each item contributes a score of 0 to 3.

Item #6 and #7 measures decreased and increased appetite respectively, hence at least 1 of these 2 items should be reported as 0. Similarly, item #8 and #9 measures

decreased and increased weights respectively, hence at least 1 of these 2 items should be reported as 0.

An abbreviated version (ie, QIDS-SDR-14) is only done once at Visit 2 ‘discharge’ timepoint. For this timepoint, the decreased/increased weight items score from Day 1 predose will be carried forward to Visit 2 in the calculation of the total score at Visit 2 ‘discharge’.

As a result, arithmetically, the maximum (most symptomatic) total score would be 42, while the minimum (least symptomatic) total score would be 0.

The change from baseline QIDS-SR-16 score will be analyzed by timepoint.

Change in GAD-7 score from baseline by analysis visit

The GAD-7 is an initial screening tool for generalized anxiety disorder. It consists of 7 questions pertaining to the different anxiety disorder problems over a 2 week period prior to the assessment. Each item contributes a score 0 to 3, where a score of 0 indicates ‘Not at all’ and 3 indicates ‘Nearly every day’ for each of the 7 items.

The GAD-7 total score defined as the summation of scores across all questions. Arithmetically, the maximum (most anxiety) total score would be 21, while the minimum (no anxiety) total score would be 0.

The change from baseline GAD-7 score will be analyzed by timepoint.

Change in EQ-5D-3L index from baseline by analysis visit

The EQ-5D-3L is a questionnaire that essentially consists of 2 pages - the EQ-5D descriptive system and a visual analog scale (VAS). The descriptive system is comprised of 5 dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Each dimension has 3 levels: no problems, some problems, and extreme problems. The subject will be asked to indicate his/her health state by marking the box for the most appropriate statement in each of the 5 dimensions. The VAS records the subject’s self-rated health on a vertical VAS where the endpoints are labeled “best imaginable health state” and “worst imaginable health state.” This information will be used as a quantitative measure of health outcome.

The change from baseline EQ-5D-3L descriptive system and VAS will be analyzed by timepoint.

Change in CGI-S (and CGI-I) score from baseline at 24 hours after start of infusion

The CGI is the general name for two scales: the CGI-S and CGI-I. The CGI-S answers the question “Considering your total clinical experience with this particular case, how ill is the subject at this time?” on a 7-point scale:

- 0 = Not Assessed
- 1 = Normal, not at all ill impaired
- 2 = Borderline ill
- 3 = Mildly ill
- 4 = Moderately ill
- 5 = Markedly ill
- 6 = Severely ill
- 7 = Among the most extremely ill patients

CGI-I answers the question “Compared to her/his condition at admission to the project, how much has he/she changed?” on a 7-point scale and considers the response from the CGIC-S at baseline during the evaluation by the investigator:

- 0 = Not assessed
- 1 = Very much improved
- 2 = Much improved
- 3 = Minimally improved
- 4 = No change
- 5 = Minimally worse
- 6 = Much worse
- 7 = Very much worse

The change from baseline CGI-S scores will be analyzed by timepoint. Since the baseline CGI-S score will be used as a basis in the evaluation of CGI-I by the investigator, the CGI-I is evaluated once during the study.

Change in HAM-D score from baseline by analysis visit

The HAM-D is a 17-item assessment tool pertaining to symptoms of depression experience over the 1 week period prior to assessment.

Classification of symptoms which may be difficult to obtain can be scored as:

- 0 - absent;
- 1 - doubtful or trivial
- 2 - present

Classification of symptoms where more detail can be obtained can be expanded to:

- 0 - absent;

- 1 - mild;
- 2 - moderate;
- 3 - severe;
- 4 - incapacitating.

The HAM-D total score is defined as the summation of scores across the 17 items. Arithmetically, the maximum (most severe) total score would be 52, while the minimum (least severe) total score would be 0.

The change from baseline HAM-D total score will be analyzed by timepoint.

E. Handling of Missing Item Efficacy Endpoint

It is theoretically possible that an individual item score of a questionnaire or assessment scale is left missing, which would lead to an incomplete calculation of the total score. As a result, the following rules will be used to re-calculate total scores if an individual item score(s) is not reported.

Within a single analysis timepoint per patient, if ≥25% of items used to calculate a total score for a given scale are missing, the total scale score for that patient and timepoint will be considered missing.

If <25% of items are missing, the total score will be calculated (prorated) as the arithmetic mean of non-missing items multiplied by the total expected number of items and rounded up to the nearest integer.

Additionally, separate sensitivity analyses will be performed with missing MADRS data imputed with a multiple imputation method. The multiple imputation (MI) method assumes a missing-at-random (MAR) data missing pattern. Imputation will be performed using the Fully Conditional Specifications parametric regression model. A total of 50 imputations will be generated using PROC MI. For each of the 50 datasets, missing MADRS total score will be imputed using the regression model, as implemented in the PROC MI with the following variables considered (as given on the VAR statement): Treatment, Region, Baseline MADRS and each subsequent post-baseline MADRS score. Response status (Yes, No) and revised change from baseline values will be derived in each MI dataset based on the newly imputed data. The seed to be used in this MI model is 60773.

Example SAS code for PROC MI is as follows:

```
PROC MI DATA=MI NIMPUTE=50 SEED=&SEED. OUT=MI1 ROUND=1 MINIMUM=0;
      CLASS TRTP REGION1;
      VAR TRTP REGION1 ATPTN20 ATPTN40 ATPTN50 ATPTN70 ATPTN80
ATPTN90;
      FCS REG (ATPTN20 = TRTP REGION1);
```

```
      FCS REG (ATPTN40 = TRTP REGION1 ATPTN20);
      FCS REG (ATPTN50 = TRTP REGION1 ATPTN20 ATPTN40 );
      FCS REG (ATPTN70 = TRTP REGION1 ATPTN20 ATPTN40 ATPTN50) ;
      FCS REG (ATPTN80 = TRTP REGION1 ATPTN20 ATPTN40 ATPTN50
ATPTN70 );
      FCS REG (ATPTN90 = TRTP REGION1 ATPTN20 ATPTN40 ATPTN50
ATPTN70 ATPTN80);
      RUN;
```

Note: TRTP = randomized treatment group, REGION1 = region (US/Europe). Note: Each of the ATPTNxx variables represent the MADRS score at each analysis timepoint (Pre-dose, 2 hours, 4 hours, 24 hours, Day 8 and Day 15).

The imputation models may be modified based on the actual data if there is an issue in model convergence. If the missingness at the MADRS 24 hour timepoint is >5%, additional analyses exploring missing-not-at-random (MNAR) assumptions will be performed.

F. Primary Efficacy Outcome Analysis

The primary objective endpoint of change from baseline MADRS total score at 24 hours post start of infusion will be tested with a 1-sided test of equivalence in means at 0.025 significance level, a mixed model repeated measures (MMRM) model will be used to analyze the change from baseline values using fixed effects of treatment group, analysis visit, region and treatment group-by-visit interaction. The baseline value will be included as a covariate. An unstructured covariance matrix will be used to model the within-patient variance-covariance errors. A random effect of subject will be included. If the unstructured covariance structure matrix results in a lack of convergence, the heterogenous first-order autoregressive covariance structure will be used as the first alternative, followed by the compound symmetry covariance structure. The Kenward-Roger approximation will be used to estimate the denominator degrees of freedom. Any missing data are assumed to be missing at random. . The hypothesis is defined as follows:

$$H_0: \Delta_{\text{active}} - \Delta_{\text{placebo}} = 0 \qquad vs \qquad H_1: \Delta_{\text{active}} - \Delta_{\text{placebo}} < 0$$

where

- Δ_{active}: Mean change from baseline MADRS total score at 24 hours after start of infusion (Test)
- Δ_{placebo}: Mean change from baseline MADRS total score at 24 hours after start of infusion (Reference)

Model-based adjusted means for each treatment group, pairwise adjusted differences with placebo (reference), 95% CI for the differences and the associated p-values will be presented at each analysis visit. Each PCN-101 dose will be compared with placebo. An example of the SAS code is provided below:

```
proc mixed data=data;   class USUBJID
AVISITN TRTPN SITEID;
  model CHG = BASE REGION1 AVISITN TRTPN AVISITN*TRTPN/s cl ddfm=kr;
repeated AVISITN / TYPE=UN SUB=USUBJID;   lsmeans AVISITN * TRTPN /
diff; run;
```

Since subjects are confined to the clinic at the time of the primary endpoint, it is anticipated that missing data will be infrequent. No imputation will be applied for this primary efficacy analysis but missing data assumptions will be explored in sensitivity analyses.

The described analysis will be performed on the FAS.

G. Primary Efficacy Sensitivity Analysis

Sensitivity analyses reviewing missing data assumptions will be performed using the same MMRM model specifications as described in Section VII.F Primary Efficacy Outcome Analysis. One analysis will be performed for only the data collected from subjects who were treatment compliant and a separate analysis will be performed with those who completed the study.

In addition, sensitivity analysis exploring the MAR assumptions with multiple imputation of MADRS related endpoints will be performed as described in Section VII.E Handling of Missing Item Efficacy Endpoint. Analysis of each primary and secondary MADRS endpoints will be performed for each of the MI datasets. The analyzed results from the 50 imputed datasets will be combined based on Rubin's rules (Rubin, 1987) using PROC MIANAYLZE in SAS to estimate the pooled analysis results.

H. Secondary Efficacy Analyses

Change from baseline in MADRS total score

Summaries of actual values and changes from baseline score will be presented by treatment group for all analysis timepoints (including 24 hours after start of infusion), beginning with the Baseline visit for the FAS.

Proportion of subjects with ≥50% improvement in MADRS total score

For the responder analysis based on a ≥50% improvement in MADRS total score at each analysis visit, the proportion of responders will be calculated using frequency counts and percentages, presented for each treatment group.

The response rate between each PCN-101 dose versus Placebo at each analysis visit will be compared using a logistic regression using fixed effects of treatment group, analysis visit, region and treatment group-by-visit interaction. The baseline value will be included as a covariate. Odds ratios (ORs), corresponding two-sided 95% CI and p--values will be derived.

Proportion of subjects with a MADRS total score ≤ 10

The same analysis will be performed as described for the endpoint of proportion of subjects with ≥50% improvement in MADRS total score.

Change from baseline in QIDS-SR-16, GAD-7 and EQ-5D-3L

The MMRM model with the same specification as detailed in Change from baseline in MADRS total score will be used to analyze the following secondary efficacy measures for the FAS. Missing data will not be imputed.

- Change from baseline in QIDS-SR-16 by visit
- Change from baseline in GAD-7 by visit
- Change from baseline in EQ-5D-3L VAS by visit

The adjusted means for each treatment group, pairwise adjusted differences between each treatment group, 95% CI for the differences will be presented at each visit.

Summaries of actual values and changes from baseline scores will be presented by treatment group for each analysis timepoint.

For EQ-5D-3L, frequency counts and percentages of each response to the 5 domains descriptive system will be presented for each treatment group for all analysis visits.

Clinician Global Impression-Severity (CGI-S) and Clinician Global ImpressionChange (CGI-I)

Summaries of actual values and changes from baseline CGI-S scores will be presented by treatment group for each analysis timepoint. Only the actual values of CGI-I will be presented by treatment group for each analysis timepoint.

The observed CGI-S and CGI-I will also be summarized categorically by the number and percentage of subjects reporting each score at each assessment.

The CGI-S and CGI-I scores will be analyzed using the same MMRM model specifications as outlined in the primary efficacy analysis. Since CGI-I cannot be

performed at baseline, the baseline CGI-S score will be used as a covariate in the CGI-I MMRM model.

Model-based point estimates for the difference in adjusted means, 95% CIs and p-values will be reported. Each PCN-101 dose will be compared with placebo. .

I. Secondary Sensitivity Analyses

All inferential statistical analyses as described in Section VII.H Secondary Efficacy Analyses will be performed with only the data collected from subjects who completed the study. The secondary sensitivity analyses include:

- MMRM model for
    - Change from baseline in QIDS-SR-16
    - Change from baseline in GAD-7
    - Change from baseline in EQ-5D-3L VAS
- Logistic Regression for
    - Proportion of subjects with ≥50% improvement in MADRS total score
    - Proportion of subjects with a MADRS total score ≤ 10

The secondary MADRS endpoints will also be analyzed using the multiple imputation approach. The estimates and standard errors of the log (odds ratio) based on the 50 imputed datasets will be combined by applying Rubin's rules to report a pooled result. The combined odds ratio and associated 95% CI and the resulting p-value will be provided.

J. Overview of Inferential Efficacy Analyses

The following Table 3 shows an overview of the types of inferential analyses that will be performed for each efficacy endpoint.

Table 3 Overview of Inferential Efficacy Analyses

Endpoint	Main	Sensitivity
Primary: Change from baseline MADRS total score	MMRM (observed cases [OC], FAS)	MMRM (MI, complete cases, and treatment compliant cases, FAS)

Secondary: ≥50% improvement in MADRS total score	Logistic Regression (OC, FAS)	Logistic Regression (MI, complete cases and treatment compliant cases, FAS)
MADRS total score ≤ 10	Logistic Regression (OC, FAS)	Logistic Regression (MI, complete cases and treatment compliant cases, FAS)
Change from baseline in QIDS-SR-16, GAD-7 and EQ-5D-3L	MMRM (OC, FAS)	MMRM (complete cases and treatment compliant cases, FAS)
CGI-S, CGI-I score	MMRM (OC, FAS)	MMRM (complete cases and treatment compliant case, FAS)

K. Exploratory Analyses

All descriptive statistics analyses which are performed on the FAS as detailed in Section VII Efficacy Analyses for the primary and secondary endpoints will be repeated based on the subgroup categories as detailed in Section V.H Subgroups of Analysis Populations.

L. Pharmacokinetics

Not applicable.

VIII. Safety Analyses

All safety related analyses will be performed on the SAS except for the listings of SAEs and AEs leading to death, which will be presented for the FAS.

A. Treatment Exposure and Compliance

Study treatment non-compliance is defined as having an infusion duration with a deviation of more than 5 minutes from the 40 minute infusion time (ie, <35 or >45 minutes of infusion duration).

Exposure to PCN-101 or Placebo will be summarized with descriptive statistics for the following exposure measures.

1. Duration of Treatment (minutes)
    - If not interrupted, defined as the time of end of infusion – the time of start of infusion+1
    - If interrupted, defined as the (time of end of infusion – the time of start of infusion+1) - (infusion restart time – infusion stop time+1)

Details of the infusion drug administration will be listed by subject.

B. Adverse Events

Adverse events (AEs) will be documented on the AE eCRF and monitored continuously throughout the study. Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA), version 23.0 or higher, associating lower-level terms with preferred terms and system organ classes by the primary hierarchy. All adverse events will be included in the listings.

Treatment-emergent AEs (TEAEs) are defined as AEs that first occurred or worsened in severity after the start of infusion but no later than the second follow-up visit.

The tables will display counts and percentages of subjects who reported at least one TEAE in each system organ class represented in the AE data. Within each system organ class, the tables will display the counts and percentages of subjects reporting at least one TEAE, as designated by the preferred terms.

If a subject has multiple occurrences of an adverse event, the worst severity grade and the highest level of relationship to study drug for a given AE will be used in summary tables.

Adverse events that have missing onset dates will be considered as treatment emergent unless the stop date is known to be prior to the first administration of the study medication. If the adverse event onset date is partial, the date will be correlated as far as possible with the date of study medication. Adverse events will be assumed to be treatment-emergent, unless there is clear evidence (through comparison of partial dates) to suggest that the adverse event started prior to the

first dose of study medication. Detailed imputation rules for dates can be found in Section V.C.2 Partial Dates.

An overall summary of AE table will summarize the number of subjects who meet the following criteria for:

1. Number of subjects with any AEs
2. Number of subjects with any TEAEs
3. Number of subjects with any TEAEs by maximum severity
4. Number of subjects with any Treatment-emergent SAEs
5. Number of subjects with any Treatment-emergent SAEs by maximum severity
6. Number of subjects with any TEAEs related to study drug (possibly, probably, definitely related or unknown)
7. Number of subjects with any SAEs related to study drug (possibly, probably, definitely related or unknown)
8. Number of subjects with any TEAEs resulting in death
9. Number of subjects with any severe TEAEs related to study drug (possibly, probably, definitely related or unknown)
10. Number of subjects with any TEAEs resulting in the permanent discontinuation of study treatment
11. Number of subjects with any treatment-emergent SAEs resulting in the permanent discontinuation of study treatment
12. Number of subjects with any TEAEs resulting in the discontinuation from study
13. Number of subjects with any TEAEs of COVID-19

Treatment-emergent AEs will be tabulated according to the MedDRA system organ class and preferred term and will include the following categories. Analysis marked with and asterisk (*) will also be tabulated based on the preferred term in descending frequency only.

1. All TEAEs *
2. TEAEs related to study drug (possibly, probably, definitely related or unknown)
3. Treatment-emergent SAEs
4. Treatment-emergent SAEs related to study drug (possibly, probably, definitely related or unknown)
5. Severe TEAEs

6. Severe TEAEs related to study drug (possibly, probably, definitely related or unknown)
7. TEAEs resulting in the permanent discontinuation of study treatment
8. TEAEs by maximum severity grade

The following AE listings (including both TEAEs and non-TEAEs) will be produced. AEs recorded at any time of the study (ie, not necessarily TEAEs only) will be included.

9. All AEs
10. All SAEs
11. Severe SAEs
12. AEs leading to discontinuation of study treatment 13. AEs leading to death

C. C-SSRS

The C-SSRS is a questionnaire used for suicide assessment developed by multiple institutions, including Columbia University. The scale is evidence-supported and is part of a national and international public health initiative involving the assessment of suicidality.

The number of subjects who reported “Yes” to each of the questions relating to suicidal ideation and suicidal behavior at any post-baseline timepoint will be summarized by treatment group.

All C-SSRS assessments details will be listed.

D. Clinical Laboratory Results

Laboratory values will be presented in tables and data listings using System International (SI) units.

For chemistry and hematology parameters, summaries of actual values and changes from baseline will be presented by treatment group for each scheduled assessment timepoint, beginning with the Baseline visit. For this analysis, any lab collection performed on unscheduled analysis visits will not be summarized, with the exception of the inclusion of unscheduled pre-dose assessments used in the classification of baseline, as described in Section V.B Definition of Baseline.

For chemistry and hematology parameters, shift tables summarizing the number and percentages of patients who were normal at baseline, but who became abnormal subsequently, will also be displayed. For this analysis, any lab collection performed on unscheduled analysis visits will be included for analysis.

All laboratory data (including and not limited to chemistry, hematology, urinalysis, serology, pregnancy, urine alcohol, SARS-CoV-2) will be listed, including normal ranges and fasting status, if applicable.

For chemistry, hematology, and urinalysis, any laboratory abnormalities will be listed separately. The abnormal laboratory listing will provide the entire profile for each subject and parameter with an abnormal post-baseline result.

E. Vital Signs and Pulse Oximetry

For vital signs (systolic and diastolic blood pressure, tympanic/temporal temperature, pulse, respiratory rate) and pulse oximetry, summaries of actual values and changes from baseline will be presented by treatment group for each scheduled assessment timepoint, beginning with the Baseline visit. For this analysis, any lab collection performed on unscheduled analysis visits will not be summarized, with the exception of the inclusion of unscheduled pre-dose assessments used in the classification of baseline, as described in Section V.B Definition of Baseline.

Vital signs will consist of three (3) blood pressure measurements (recorded at intervals of at least 1 minute). The arithmetic mean of the three (3) blood pressure readings will be recorded in the CRF and used for this analysis.

Temperature data collected via tympanic or temporal methods will be pooled for analysis.

The incidence of clinically relevant abnormality will be summarized by treatment. For this analysis, any unscheduled post-baseline records will be included for assessment. A listing of clinically relevant abnormality vital signs will be displayed.

Table 4 Potential Clinically Relevant Abnormalities in Vital Signs

Parameter	Unit	Low	High
Systolic blood pressure	mmHg	≤ 90 or CFB ≤ -20	≥ 180 or CFB ≥ 20
Diastolic blood pressure	mmHg	≤ 50 or CFB ≤ -15	≥ 105 or CFB ≥ 15
Pulse	beats/min	≤ 50 or CFB ≤ -15	≥ 120 or CFB ≥ 15
Temperature	˚C	CFB ≤ -2	≥ 38.3 or CFB ≥ 1.1
Respiratory Rate	breaths/min	<8	Not applicable
Oxygen Saturation	%	< 90%	Not applicable

CFB = change from Baseline.

All vital signs data will be listed

F. Physical Examination

All physical examination results will be listed by subject.

G. Electrocardiograms

12-Lead ECGs will be obtained using an ECG machine that automatically calculates the parameters for analysis. If abnormal, the ECG needs to be repeated.

An overall interpretation of the ECG results, PR intervals, RR intervals, QT uncorrected intervals, QT intervals corrected by Fridericia's formula (QTcF) will be listed by subject.

In the case where a second (repeat) measurement was taken for a particular timepoint, only the second (repeat) measure will be included for analysis and the first measure will not be analyzed.

For the following 12-lead ECG parameters, summaries of values and its changes from baseline will be presented by treatment group for each scheduled assessment timepoint, beginning with the Baseline visit.

- Heart rate
- PR interval
- QRS interval
- QT interval (uncorrected)
- QT interval corrected with Fridericia's formula (QTcF)

A separate summary table will provide the number and percentage of subjects meeting the following criteria post-baseline. For this analysis, any ECG performed on unscheduled analysis visits will be included for analysis. However, any initial measurement which was superseded by a repeat measurement within the same analysis timepoint would NOT be included for this analysis.

- Absolute QTcF interval prolongation:
  - QTcF interval ≥ 450 msec
  - QTcF interval ≥ 480 msec
  - QTcF interval ≥ 500 msec
- Change from baseline in QTcF interval:
  - QTcF interval increase from baseline ≥ 30 msec

- QTcF interval increase from baseline ≥ 60 msec

In the case where a subject reports qualifying records (ie, ignoring superseded initial readings) of both Abnormality with Clinical Significance and Abnormality without Clinical Significance at different analysis timepoints in a post-baseline period, the most severe record (ie, abnormality with clinical significance) will be selected for summarization.

A listing of all ECG measures will be presented.

H. Prior and Concomitant Therapy

Concomitant therapy is defined as any therapy with a start date after or on the date of the study treatment and continuing after the study treatment (ie, including up to the second follow-up visit or ongoing). Any therapy with a start date after the second follow-up visit will not be considered a concomitant therapy.

Any therapy which started prior to the infusion of study treatment is defined as a *prior* therapy. For the imputation of missing therapy dates in the categorization of prior vs concomitant status, refer to Section XI.A Missing or Partial Date Imputation.

Concomitant medications will be coded using World Health Organization (WHO) Drug version September 2020 or higher coding dictionary and will be summarized by the anatomical main class (1st level) of each coded medication and, within that, the preferred name of the coded medication.

Subjects are counted only once in each therapeutic class category. Prior and concomitant medications will be summarized separately and tabulated for each treatment group using frequencies and percentages. Percentages will be based on the number of subjects in each treatment group. Prior and concomitant medications and therapies will be listed by subject.

I. Assessments of Alertness/Sedations and other Safety Rating Scales

For the following rating scales, summaries of actual values (total scores) and changes from baseline will be presented by treatment group for each scheduled assessment timepoint, beginning with the Baseline visit.

A listing displaying the scores for these scales (individual items and total) will be provided by subject.

- Modified Observer's Assessment of Alertness/Sedation (MOAA/S)
- Clinician-Administered Dissociative States Scale (CADSS)
- Brief Psychiatric Rating Scale - Modified 4 Components (BPRS+)

The 5-Dimensional Altered States of Consciousness Rating Scale (5D-ASC) is only assessed post-baseline and for only one (1) time during the study. A listing displaying the details of the 5D-ASC will be provided by subject.

J. Others

Any other assessments which were not described in any previous sections will be listed based on the FAS. This includes (and not limited to) the following:

- Non-compliance in inclusion or exclusion criteria
- Massachusetts General Hospital Antidepressant Response Questionnaire (MGH-ATRQ)
- Mini-International Neuropsychiatric Interview (MINI)

IX. Interim Analysis

No interim analysis is planned.

X. References

Lee S, Lee DK. What is the proper way to apply the multiple comparison test? [published correction appears in Korean J Anesthesiol. 2020 Dec;73(6):572]. Korean J Anesthesiol. 2018;71(5):353-360. doi:10.4097/kja.d.18.00242

XI. Appendices

A. Missing or Partial Date Imputation

For records with missing AE onset/resolution date, the following procedure will be employed for use in determining whether the AE is treatment emergent:

- AE onset dates with missing day and non-missing month will be assumed to occur on the first day of the non-missing month, except for AEs occurring in the month and year of first dosing, in which case the date will be imputed using the date of first dosing.
- AE onset dates with missing day and month will be assumed to occur on the first day of the non-missing year (ie, January 1), except for AEs occurring in the year of first dosing, in which case the date will be imputed using the date of the first dosing.
- AEs that are not ongoing and have a resolution date with missing day and non-missing month, they will be assumed to occur on the last day of the

month, except when the end of study date or date of death is prior to the last day of the month. The date will be imputed as end of study date, or death date, whichever happens the earliest.

- AEs that are not ongoing and have a resolution date with end date month missing, the imputed end date should be set to the last day of the year 31DECYYYY, except when the end of study date or date of death is prior to the last day of the year. The date will be imputed as end of study date, or death date, whichever happens the earliest.
- If subjects died during the study and experience AEs at the time of death, the AEs that lead to death will use death date as the AE end date, otherwise, AEs that did not lead to death will be reported as ongoing.

For records with a missing therapy start and/or stop date, the following procedure will be employed for use in determining whether the therapy is prior or concomitant:

- Therapy start dates with a missing day and non-missing month will be assumed to occur on the first day of the non-missing month.
- Therapy start dates with missing day and month will be assumed to occur on the first day of the non-missing year (ie, January 1), except for therapies occurring in the year of first dosing, in which case the date will be the date of first dosing.
- Therapies that are not ongoing and have a stop date with a missing day and non-missing month will be assumed to occur on the earlier of the last day of the non-missing month or the date of death (if applicable).
- Therapies that are not ongoing and have a stop date with missing day and month will be assumed to occur on the earlier of the last day of the nonmissing year (ie, December 31) or the date of death (if applicable).
- Therapies that are not ongoing but do not have a stop date will be assumed to occur on the earlier of the last day of the non-missing year (ie, December 31) or the date of death (if applicable)