16.1.9 Documentation of Statistical Methods

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Study Number CVN058-103

A Placebo-Controlled Study to Evaluate the Effect of a Single Dose of CVN058 on Mismatch Negativity in Subjects with Stable Schizophrenia

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This study will be conducted in compliance with the Protocol, Good Clinical Practice and all other applicable regulatory requirements, including the archiving of essential documents.

Document History

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V2	PPD	07Aug2019	Additions to TOC; revisions to Interim Analyses testing based on data availability
V3	PPD	24SEP2019	Unblinding of interim analysis.
V4	PPD	07May2020	Revisions to TOC to add Exploratory Parameter analyses; Revisions to dose levels per IA results.

Signature Page

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1. Glossary and Abbreviations

Abbreviation	Definition
5-HT	5-hydroxytryptamine (serotonin)
AE	adverse event
ALT	alanine aminotransferase
ATC	Anatomical/Therapeutic/Chemical Class
AST	aspartate aminotransferase
BMI	Body Mass Index
AUC	area under the plasma concentration-time curve
AUC_t	area under the plasma concentration-time curve from 0 to time of last quantifiable concentration
CIAS	cognitive impairment associated with schizophrenia
C_{max}	maximum observed plasma concentration
CNS	central nervous system
CSF	cerebrospinal fluid
C-SSRS	Columbia-Suicide Severity Rating Scale
ECG	Electrocardiogram
EEG	Electroencephalography
F_z	frontal electrodes
F_zC_z	frontocentral electrodes
GABA	γ-aminobutyric acid
GGT	γ-glutamyl transferase
ICH	International Conference on Harmonisation
INR	international normalized ratio
LFT	liver function test
MedDRA	Medical Dictionary for Regulatory Activities
MMN	mismatch negativity
msec	Milliseconds
NFSI	non-fasting spiking interneurons
PANSS	Positive and Negative Syndrome Scale
PD	pharmacodynamic(s)

Abbreviation	Definition
PK	pharmacokinetic(s)
PT	prothrombin time
PTT	partial thromboplastin time
PTE	pretreatment event
P_z	parietal electrodes
P50 Ratio	ratio of amplitude of the auditory evoked potential P50 from the second stimulus (S2) compared to the first stimulus (S1)
QTcB	QT interval with Bazett correction method
QTcF	QT interval with Fridericia correction method
SAE	serious adverse event
SAF	safety population
SAP	statistical analysis plan
$t_{1/2z}$	terminal elimination half-life
CVN058	also known as ENV8058 and TAK-058
TEAE	treatment-emergent adverse event
t_{max}	time to reach Cmax
V_z/F	apparent volume of distribution during the terminal phase
WHODRUG	World Health Organization Drug Dictionary

2. Introduction

2.1. Background

Schizophrenia is a complex psychiatric disorder comprising several clinical features. While the most well-known schizophrenia symptom domains are the so-called positive symptoms, e.g., hallucinations, delusions, disorganization of thought, bizarre behavior, and incongruity of affect, schizophrenics also commonly experience so-called negative symptoms, e.g., avolition (loss of motivation), blunt affect, alogia (reduced speech), and anhedonia (reduced ability to experience pleasure), mood disorders, and cognitive impairment. Cognitive impairment associated with schizophrenia (CIAS) is observed in most cases, usually precedes psychosis, and can involve deficits in a broad range of cognitive domains. Cognitive impairment is considered a core feature of schizophrenia and represents an area of significant unmet medical need.

Cognitive deficits in schizophrenia include problems in speed of processing, attention/vigilance, working memory, verbal learning, visual learning, reasoning and problem solving, and social cognition [1]. These cognitive deficits are believed to underlie much of the significant functional impairments observed in schizophrenia, such as impairment in independent living skills, social functions, vocational functioning, and self-care [2]. Thus, the core nature of CIAS and the associations that have been demonstrated with functional disability suggest that improving cognitive impairment may lead to improved functional outcomes [3]. The cognitive impairments manifest as deficits not only in high-level processes, such as working memory or executive processing, but also as deficits in neurophysiological responses to simple auditory and visual stimuli [4].

Mismatch negativity (MMN) is an established biomarker of cerebral cortical function [5]. This signal is obtained as an evoked potential during an auditory oddball task in which a subject is repeatedly exposed to auditory tones and a small proportion of those tones (the “deviant” stimuli) differ from the others (the “standard” stimuli) in their pitch frequency or duration. Typically, the tones are presented and the evoked potentials are recorded while subjects are engaged on a different task, such as reading. Normally, the occurrence of a deviant stimulus increases the amplitude of the negative component in the evoked potential occurring at around 200 msec. MMN is the difference in amplitude between deviant and standard stimuli responses and is considered to represent an aspect of pre-attentive novelty detection. MMN is consistently reduced in schizophrenia patients relative to healthy individuals, a finding extensively replicated

and with a large effect size (Cohen's d = 1), and this reduction (impairment) in MMN is associated with poor psychosocial functioning. Thus, MMN is potentially a useful biomarker in studies seeking to establish whether candidate therapeutic compounds may have a beneficial effect on CIAS.

2.2. Clinical

Two human clinical studies of CVN058 have been completed (ENV8058_101 and TAK-058- 1002).

CVN058 is orally absorbed and readily passes the blood brain barrier, allowing it to occupy cortical 5-HT3 receptors. In study ENV8058_101, CVN058 was well tolerated up to a dose of 150 mg administered orally as a single dose in aqueous solution. There were no deaths or other serious adverse events (SAEs) reported in this study and no subject discontinued study due to a treatment-emergent adverse event (TEAE). There were no clinically significant laboratory abnormalities, electrocardiogram (ECG) changes or changes in vital signs in the completed single dose study.

The mean C_{max} and area under the plasma concentration–time curve (AUC) values of CVN058 increased less than proportionally with increasing CVN058 dose across the dose range of 5 to 150 mg, but mean C_{max} and AUC values increased in an approximately dose proportional manner across the 5 to 45 mg dose range. The rate of appearance of CVN058 in plasma was rapid with median time to C_{max} (t_{max}) values of 1 to 1.25 hours across the dose range of 5 to 150 mg. The mean terminal elimination half-life (t1/2z) values of CVN058 ranged from approximately 2.8 to 10.5 hours across the cohorts; the lower doses (5 to 30 mg) had lower mean t1/2z values compared to the higher doses (45 to 150 mg). There were no trends with mean apparent clearance values of CVN058 with increasing dose from 5 to 45 mg, but faster apparent clearance was observed at the 75 and 150 mg doses, possibly due to decreased bioavailability (BA) at higher doses. The mean apparent volume of distribution (Vz/F) increased as dose increased. Excretion of unchanged CVN058 in the urine was ≤2.3% of the administered CVN058 dose over the dose range evaluated.

In the multiple-rising dose study (TAK-058-1002), CVN058 was well-tolerated at daily doses of 25, 75, and 150 mg for 7 days, and at a single 300 mg dose, in aqueous solution. There were no deaths or other SAEs reported in this study and no subject discontinued study due to a TEAE. There were no clinically significant laboratory abnormalities, ECG changes or changes in vital signs in the completed single dose study. Following single or multiple doses, the mean C_{max} and

AUC values of CVN058 increased less than proportionally with increasing CVN058 dose across the dose range of 25 to 300 mg. No accumulation was observed following daily administration. CVN058 concentrations in cerebrospinal fluid (CSF) were assessed after daily doses of CVN058 75 mg for 5 days. The CSF concentrations averaged 17.5 ng/mL, or approximately 1% of the concentration in plasma.

The statistical methods to be implemented during the analyses of data collected within the scope of the present study, CVN058-103, will be outlined in this document. The purpose of this plan is to provide specific guidelines from which the statistical analysis will proceed. Any deviations from this plan will be documented in the clinical study report.

2.3. Study Rationale

This study will explore central target engagement and proof of mechanism by measuring auditory evoked potential MMN as a pharmacodynamic (PD) marker of CNS response to the selective 5-HT3 receptor antagonist CVN058. It has been reported that subjects with schizophrenia commonly demonstrate a deficit in neural processing that is manifested as a reduction in MMN and impairment of auditory sensory gating (P50) [8]. Other 5-HT3 receptor antagonists have been reported to improve impairments in auditory evoked potentials in schizophrenic subjects. In a study with ondansetron, a widely-prescribed 5-HT3 receptor antagonist, using P50 auditory gating as the auditory evoked potential measure, subjects were given a single dose of ondansetron (16 mg) or placebo in a double-blind, placebo-controlled, randomized and balanced crossover design [9]. Serial measurements of the P50 evoked potential were done at Baseline and 1, 2, and 3 hours after receipt of placebo or ondansetron. The results of the study indicated that ondansetron significantly enhanced P50 auditory gating in subjects with schizophrenia. Tropisetron, a 5-HT3 antagonist that also has α7 nicotinic cholinergic agonist activity, has similarly been shown to improve P50 auditory gating in subjects with schizophrenia [10].

While the P50 signal is likely a reflection of brainstem and thalamic network activity and is sensitive to antipsychotic treatment, MMN is thought to reflect excitation-inhibition balance in cortical circuits and is not sensitive to antipsychotic treatment [8]. Therefore, to evaluate the potential for CVN058 to improve cognitive function in schizophrenics, MMN will be used as a PD marker. The study will be performed as a 3-way crossover to assess the effect on MMN of CVN058 at two dose levels relative to placebo, with each subject serving as his or her own control to reduce variability. P50 auditory gating will also be measured, as an exploratory endpoint, along with other potentially informative electrophysiological and psychometric parameters.

Medications that might inhibit or mask the effects of CVN058 will not be permitted as concomitant medications. These include serotonin reuptake inhibitors which increase synaptic serotonin levels, as well as drugs that bind to 5-HT3 at clinically relevant concentrations or are reported to inhibit signaling through 5-HT3 non-competitively, including some antipsychotic drugs [11], [12], [13]. Permissible antipsychotic medications are risperidone (Risperdal), haloperidol (Haldol), quetiapine (Seroquel), aripiprazole (Abilify) and paliperidone (Invega).

3. Study Objectives and Endpoints

3.1. Objectives

3.1.1. Primary Objective

- To determine whether improvement in MMN is demonstrated after administration of CVN058 compared to placebo in subjects with schizophrenia.

3.1.2. Secondary Objective

- To assess the safety and tolerability of single doses of CVN058 in subjects with schizophrenia.

3.1.3. Exploratory Objectives

- To determine whether improvement in P50 auditory gating, gamma power, and P300 is demonstrated after administration of CVN058 compared to placebo in subjects with schizophrenia.
- [redacted]
- To explore the exposure-response relationship for CVN058 in subjects with schizophrenia.

3.2. Endpoints

3.2.1. Primary Endpoint (PDC Population)

- Mean amplitude of duration MMN at 5 electrodes surrounding frontal (Fz) electrodes following administration of CVN058 compared to placebo.

3.2.2. Secondary Endpoints (SAF Population)

- Percentage of subjects who experience at least one (1) TEAE
- Percentage of subjects who experience at least one (1) clinically significant abnormal laboratory test result

3.2.3. Exploratory Endpoints (PDC Population)

[redacted]

- Pre-stimulus gamma amplitude following administration of CVN058 compared to placebo
- P300 amplitude and latency at average of 5 centroparietal electrodes around parietal electrodes (Pz) following administration of CVN058 compared to placebo
- MMN amplitude to additional deviants (frequency, intensity, location, frequency-modulation) and latency to all deviants following administration of CVN058 compared to placebo

[redacted]

4. Study Design

4.1. Overall Study Design

This is a phase 1, double-blind, placebo-controlled, 3 period cross-over study to evaluate CVN058 target engagement by measuring auditory evoked potential MMN downstream to 5-HT3 as a pharmacodynamic (PD) marker.

4.2. Subjects

The study population will include male and female subjects with schizophrenia, age 18 to 50 years old, inclusive, and who satisfy all entry criteria.

4.3. Study Design Summary

In the initial study design, subjects were randomized to 1 of 6 treatment sequences to receive 1 of 3 dose regimens in each period. Each dose regimen consisted of a single oral administration of CVN058 (15 mg or 150 mg initially) or a matching placebo; study drug to be administered with water in the morning. The sequence will determine the order in which a subject will receive each of the 3 regimens. Discontinued subjects may be replaced so that 20 completed subjects are available for analysis.

After the Interim Analysis, the 15 mg dose in each treatment sequence was replaced with a 75 mg dose under Protocol Amendment 4, applicable to all subjects randomized.

Each treatment period is less than 1 day in duration, with a minimum of 7-day washout, maximum 10-day washout between doses. A follow-up telephone call will occur approximately 7-10 days following the last dose received. Subjects may be inpatients or outpatients at the discretion of the Investigator.

At each testing session, subjects will undergo post dose EEG testing including: MMN, P50, qEEG, gamma power, and P300. Testing sessions will begin approximately 1.5 hours post-dose. Prior to dose and at various time points post-dose, blood samples will be collected for the assessment of plasma concentrations of CVN058 and biomarkers.

Study procedures will be conducted at approximately the same times across Treatment Periods to minimize diurnal effects on outcomes. The effect of CVN058 on MMN as well as on exploratory measures (e.g., P50 auditory gating, gamma power, and P300) will be compared to placebo.

5. Determination of Sample Size

Enrollment of at least 20 subjects with schizophrenia (including schizoaffective disorder) is estimated to provide >80% power to detect a median effect size of Cohen's d=0.5. Discontinued or withdrawn subjects and subjects for whom MMN is not available for all three study periods may be replaced. The sample size chosen of at least 20 completed subjects is considered to be sufficient to explore the changes in MMN between CVN058 and placebo as well as safety and tolerability of CVN058 in this population. There will be approximately three (3) study sites in the United States. The sample size is subject to re-estimation based on preliminary data.

The sample size re-estimation will occur after twelve (12) subjects have completed all study visits. Full details are discussed in Section 8.

6. Analysis Populations

Three subject populations will be defined for this study.

- Safety Set population (SAF): The Safety Set population will consist of all subjects who are randomized and receive study drug. Subjects in this analysis set will be used for demographic, baseline characteristics and safety summaries.
- Pharmacokinetic population (PK): The PK population will consist of all subjects who receive study drug and have at least one (1) measurable plasma concentration of CVN058.
- Pharmacodynamic population (PD): The PD population will consist of all subjects who receive study drug and have measurements in both the placebo-dosed period and at least one (1) CVN058-dosed period for at least one (1) EEG/evoked response endpoint.
- Pharmacodynamic completers population (PDC): The PDC population will consist of all subjects who receive study drug and have values in the placebo-dosed period and both CVN058-dosed periods for at least one (1) EEG/evoked response endpoint.

If any subjects are found to be noncompliant in dosing schedule or with incomplete data, a decision will be made on a case-by-case basis as to their inclusion in the PK and PD endpoint analyses, but data for all subjects will be presented in the subject listings.

7. Statistical Analysis

7.1. General Considerations

7.1.1. Overall

The statistical analyses will be reported using summary tables, figures and listings (TFLs). Numbering for TFLs will be based on the recommended numbering convention provided by the International Council for Harmonisation (ICH). All summary tables will be presented by treatment group. Listing of Screen Failures will also be presented based on Eligibility Criteria (Inclusion/Exclusion) not met. Select baseline tables may also include a total column summary. Tables and listings will be presented in RTF format.

Continuous (quantitative) variable summaries will include the number of subjects (n) with non-missing values, mean, standard deviation (SD), median, minimum, maximum and quartiles. Categorical (qualitative) variables will be summarized by frequencies and percentages of subjects in corresponding categories. The denominator for the percentage calculation will be based upon the total number of subjects in the study population for the treatment or sequence group with non-missing data for the variable of interest.

The minimum and maximum will be reported with the same degree of precision (i.e., the same number of decimal places) as the observed data. Measures of location (mean and median) will be reported to one degree of precision more than the observed data and measures of spread (standard deviation) will be reported to two degrees of precision more than the observed data.

Percentages will be presented to one decimal place unless otherwise specified. Assessments done on unscheduled visits will not be summarized but will be listed.

P-values ≥0.001 will be reported to four decimal places; P-values less than 0.001 will be reported as “<0.001”.

For analysis purposes, repeat laboratory rest results will not be used unless the original laboratory value is missing or indicated as invalid, in which case the first non-missing repeat laboratory value will be used for data analysis.

All collected data will be presented in listings and will be sorted by subject. CDISC-compliant datasets (STDM and ADAM) will also be provided to the sponsor.

7.1.2. Safety Analysis

The Safety Set population will be used for all summaries of Adverse Events (AEs), Pre-Treatment Adverse Events (PTEs), TEAEs, laboratory tests, and vital signs. All safety data will be summarized by treatment.

AEs will be presented in listings. TEAEs will be classified according to system organ class and preferred term and will be tabulated with a breakdown by treatment and by event severity. Similar TEAE tabulations will be performed on those events assessed by the investigator as related to study drug.

Individual results of vital signs will be listed and observed values and changes from Baseline in vital signs will be summarized by treatment.

Physical exam findings, suicidality assessments (C-SSRS), PANSS scores, Diagnostic Screening results and pregnancy test results will be presented in subject-level listings.

7.1.3. Pharmacokinetic Analysis

Blood samples will be collected for the determination of plasma concentration of CVN058, and its metabolite if appropriate, at the following time-points: Pre-dose (within 15 minutes prior to dosing), and a one (1) hour (pre-EEG) and five (5) hours (post-EEG) post-dose. The plasma concentration data (parameters Cmax, tmax and AUC (0 to 5 hrs.) will be summarized over each scheduled sampling interval using descriptive statistics (arithmetic mean, SD, median, minimum and maximum). Individual plasma concentration data versus time will be presented in a data listing.

Descriptive statistics will be used to summarize the plasma PK parameters for CVN058, and its metabolite if appropriate. In addition, geometric mean and percent coefficient of variation will be computed for C_{max} and AUC_t.

7.1.4. Pharmacodynamic Analysis

In general, observed values for the PD parameters (MMN and exploratory PD parameters [P50, qEEG and P300]) in each treatment period will be summarized by treatment and compared to time-matched placebo assessments using summary statistics. Summaries presented will include difference values (CVN058 – placebo).

7.1.4.1 Primary Analysis

The Primary Endpoint analysis will be performed on the PD population and will consist of a comparison of the post dose PD parameter Mean Amplitude of Duration MMN versus the parameter at time-matched placebo period timepoints using Generalized Linear Models with factors for sequence, subject nested within sequence, period, site and treatment. Two-sided 80% confidence intervals will be constructed for the least squares mean differences between CVN058 and placebo in PD parameters at the post-dose time point for each treatment period.

To maintain overall control of alpha (Type I error rate), the Primary Analysis will employ sequential testing, beginning with the higher dose (e.g., CVN058 150 mg) vs. Placebo using alpha = 0.10 and one-sided significance testing. If significant results are obtained from that first comparison, sequential significance testing of the next lower dose or doses (e.g., CVN058 75 mg) vs. Placebo will then be performed. If the first comparison yields a non-significant result, testing of the latter vs. Placebo will be performed to generate a nominal p-value, but that result will not be formally statistically significant regardless of the p-value.

Effect size will be calculated and summarized for comparisons of CVN058 to Placebo using Cohen's d which expresses the difference between the means of two groups as a function of their pooled standard deviation.

Exposure-response relationships for CVN058 will be explored graphically.

7.1.4.2 Exploratory Analyses

Exploratory endpoint analyses will consist of a comparison of each exploratory post-dose PD parameter versus the same parameter at time-matched placebo period timepoints using Generalized Linear Models and including factors for sequence, subject nested within sequence, period, site and treatment. Two-sided 80% confidence intervals will be constructed for the least squares mean differences between CVN058 and placebo in changes of PD parameters at the post-dose time point.

There will be no adjustment to maintain overall control of alpha (Type I error rate) for exploratory endpoints.

Effect sizes will be calculated and summarized for comparisons of CVN058 to placebo using Cohen's d which expresses the difference between the means of two groups as a function of their pooled standard deviation.

Exposure-response relationships for CVN058 will be explored graphically.

7.1.5 Adjustments for Covariates

Randomization for this study will be stratified by the study site in which subjects are enrolled. The model for the primary analysis will include this stratification variable as a main effect. This stratification variable may also be included as a factor for select secondary variables. Baseline values will also be used as a covariate when baseline is available for the endpoint.

7.1.6 Testing

Unless noted otherwise, all statistical tests will be one-sided with a significance level of $\alpha = 0.10$. Tests will be declared statistically significant if the calculated p-value is < 0.10.

The analyses described in this plan are considered a priori, in that they have been defined prior to database lock. Any additional analysis performed after database lock will be considered post-hoc and exploratory. Post-hoc analyses will be labeled as such on the output and identified in the CSR.

All analyses and tabulations will be performed using SAS® version 9.4. Upon completion, all SAS programs will be validated by an independent programmer. The validation process will be used to confirm that statistically valid methods have been implemented and that all data manipulations and calculations are accurate. Checks will be made to ensure accuracy, consistency with this plan, consistency with tables, and consistency between tables and corresponding data listings.

7.1.7 Baseline Values

Unless otherwise noted, baseline is defined as the Period 1 – Day 1 (pre-dose) value recorded prior to the first study treatment. However, the baseline value for clinical laboratory assessments is defined as Visit 1 - Screening Day-28 for Hematology, Serum Chemistry, Urinalysis and Coagulation. If a baseline value is missing, the last non-missing value prior to the first study

treatment will be used. When applicable, unscheduled visits will be used in the determination of baseline values.

7.1.8 Handling of Missing Data

In general, with the exception of the primary and secondary variables, missing values are not considered for percentage calculations, unless stated otherwise. In these cases, footnotes will specify the percentage denominator definition.

8. Sample-size Re-assessment and Futility Analysis

8.1. Sample-size Re-assessment

Sample size re-estimation may occur when MMN data from all 3 periods are available for 12 patients, or when 20 patients have been dosed and are expected to complete the study, whichever is sooner. To ensure adequate study power, sample size may be increased up to a total of 40 patients.

The original study SAP describes procedures for a blinded interim analysis and sample size re-assessment. Upon review of the outputs from the blinded interim analysis (which included data only from the first 12 subjects, who had already completed the study), the Sponsor determined that an unblinded analysis of those interim data is necessary to properly inform decisions about study continuation and dose selection. Unblinding of the interim analysis data was thus introduced in Protocol Amendment 4, and the SAP has been modified accordingly.

The unblinded sample size re-estimation procedure will be performed utilizing the following steps:

1) Based on treatment allocations, only subjects with evaluable MMN data from all three study periods will be included in the sample size re-estimation.
2) Sample size re-estimation calculation will be based on the specifications of approximately 80% power and alpha=0.10 utilizing SAS Proc GLMPOWER for Analysis of Variance and the unblinded group values by treatment assignment (placebo, CVN058 15 mg, CVN058 150 mg) and standard deviation values from the twelve (12) study subjects.

8.2. Interim Futility Analysis

As noted above, the original SAP describes procedures for a blinded interim analysis, while unblinding of the interim analysis was introduced in Protocol Amendment 4. This version (3.0) of the SAP has been modified accordingly to incorporate changes that correspond to the updates in Protocol Amendment 4.

An unblinded interim analysis for futility will be performed when twelve (12) subjects have completed all study visits. An analysis structured identically to the Primary Analysis, unblinded to treatment, will be performed using data only from the first 12 subjects, who have already completed the study. This analysis will utilize the O'Brien-Fleming group-sequential stopping boundary to control alpha (α). SAS Proc SEQTEST will be employed to perform the conditional power analysis for one interim analysis. Study stoppage for futility may occur if conditional power for both active treatment arms is very low.

However, multiple stopping rules may be implemented if preliminary results indicate that the study may yield beneficial results based on other outcome variables. Additional outcome variables to assess in determining study stoppage include, but are not limited to:

- Early phase (120 msec - 200 msec) MMN
- Late phase (200 msec - 260 msec) MMN
- Cohen's d for MMN
- Differences in the secondary endpoints when comparing treatment assignments, utilizing appropriate testing (test selection dependent upon frequency counts) for comparisons of proportions.

Additionally, prior to study stoppage, an assessment will be performed to calculate the conditional power values associated with protocol-permissible increases in the sample size (i.e., up to N=40), based on the observed unblinded data at the time of the interim analysis.

9. Background Characteristics

9.1. Subject Disposition

Study completion and reasons for discontinuation for all randomized subjects in the study will be summarized for each treatment group. Discontinuations by reason will be tabulated for each treatment group. All randomized subjects and study completion status will be presented in a listing.

9.2. Protocol Deviations

All major protocol deviations will be listed.

9.3. Demographic and Baseline Characteristics

Continuous demographic variables (e.g., age, height, weight) will be summarized by descriptive statistics for each treatment group. Qualitative demographic characteristics (e.g., gender, race, ethnicity) will be summarized by counts and percentages for each treatment group. Demographics will also be presented in a subject listing.

9.4. Prior and Concomitant Medications

Per protocol, medications that might inhibit or mask the effects of CVN058 are not permitted as concomitant medications. These include serotonin reuptake inhibitors which increase synaptic serotonin levels, as well as drugs that bind to 5-HT3 receptors at clinically relevant concentrations or are reported to inhibit signaling through 5-HT3 non-competitively, including some antipsychotic drugs [14], [15], [16]. Permissible antipsychotic medications are risperidone (Risperdal), haloperidol (Haldol), quetiapine (Seroquel), aripiprazole (Abilify) and paliperidone (Invega).

All medications taken during the course of the study with a start date or an end date on or after the date of the first study treatment or marked as ongoing will be considered concomitant. Medications stopped prior to the date of the first study treatment will be considered prior medications.

If start and/or end dates for medications are missing, the dates will be imputed as described below.

Imputing partial or missing start dates:

- If the year is unknown, the start date will not be imputed. The data will remain missing.
- If the month is unknown, and the year is the same as the first treatment date of the study, and the corresponding stop date is not prior to the first treatment date of the study, then impute the month and day of the date to be equal to the first dose month and day. Otherwise, impute the month as January.
- If the day is unknown and the month and year are the same as the first treatment date of the study, and the corresponding stop date is not prior to the first treatment date of the study, then impute the day to be equal to the day of the first treatment. Otherwise, impute the day as '01'.

Imputing partial or missing end dates:

- If the year is unknown, the end date will not be imputed. The data will remain missing.
- If the month is unknown, impute the month as December.
- If the day is unknown, impute the day to be the last day of the month.

If an imputed stop date is greater than the last medication date or study completion/discontinuation date, then the imputed stop date will be set equal to the maximum of these two dates (last medication date or completion/discontinuation date).

The number and percentage of subjects using each concomitant medication will be summarized for each dose group according to the World Health Organization Drug Dictionary (WHODRUG) (Version 01 March 2018) Anatomic Therapeutic Class (ATC) Level II preferred term. Subjects with multiple use of a concomitant medication during a treatment period will be counted only once for a given drug class or Preferred Term (PT) for the treatment period. Medication summaries will be based on the Safety Population (SAF) and presented in summary tables and a subject listing.

9.5. Medical History

Medical history (prior and ongoing medical illness) will be coded using the Medical Dictionary for Regulatory Activities (MedDRA, Version 21.0) coding dictionary. Medical history to be obtained will include determining whether the subject has any significant conditions or diseases relevant to the disease under study that stopped at or prior to signing of informed consent. Ongoing

conditions are considered concurrent medical conditions. Medication history information to be obtained includes any medication relevant to eligibility criteria stopped at or within 45 days prior to signing of informed consent.

Prior and ongoing medical conditions will be summarized by presenting for each group the number and percentage of subjects having any condition. Prior and ongoing conditions will be presented in separate Medical History tables.

Subject data listing of all medical history will also be presented.

10. Adjustments for Covariates

Randomization for this study will be stratified by the study site in which subjects are enrolled. The model for the primary analysis will include this stratification variable as a main effect. This stratification variable may also be included as a factor for select secondary variables. Baseline values will also be used as a covariate when baseline is available for the endpoint.

11. Safety Analysis

All safety analysis will be based on the SAF population. Analysis using the SAF population will be based on the actual treatment received.

11.1. Pre-treatment Events and Adverse Events

All Pre-treatment Events (PTEs) will be presented in a subject-level listing.

Adverse events and medical conditions will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) (Version 21.0). Treatment-emergent adverse events (TEAEs) will be defined as any event with onset during or after the first dose of study treatment (active or placebo).

Treatment-emergent adverse events will be summarized for each treatment group by presenting the number and percentage of subjects having any treatment-emergent AE, having an AE in each body system, and having each individual AE. (Note: In any given category [e.g., body system] a subject will only be counted once.)

Serious TEAEs, TEAEs by severity and TEAEs by relationship to study drug will also be summarized by treatment group.

All AEs will be presented in a subject-level listing.

11.2. Laboratory Data

The summary statistics of raw data (hematology, chemistry, coagulation and applicable urinalysis) and change from baseline values (means, medians, standard deviations, min, and max) will be presented. For urinalysis parameters that are categorical, the number and percentage of subjects falling under each category of the test will be presented. Clinical laboratory tests to be performed are:

Category	Parameters
Hematology	RBC, WBC with differential (percentage and absolute), Hemoglobin, Hematocrit, Platelets, Prothrombin Time (PT), International Normalized Ratio (INR), Partial Thromboplastin Time (PTT)
Serum Chemistry	ALT, Albumin, Alkaline phosphatase, AST, Total bilirubin, Direct bilirubin, Total protein, Creatinine, Blood urea nitrogen, Creatine kinase, GGT, Potassium, Sodium, Glucose, Chloride, Bicarbonate, Calcium
Urinalysis	pH, Specific gravity, Protein, Glucose, Blood, Nitrite Microscopic Analysis (only if positive dipstick results): . RBC/high power field . WBC/high power field . Epithelial cells, casts etc.

11.3. Vital Signs and Body Measurements

Data from vital signs and body measurements (see below) will be listed. Observed values and change from baseline will be summarized by treatment group. Variables to be summarized are:

Variable
Heart Rate
Diastolic Blood Pressure

Systolic Blood Pressure
Respiratory Rate
Temperature
Weight

11.4. ECG Assessment

Data from ECG measurements (heart rate, PR interval, RR interval, QRS interval, QT interval, QTcF and QTcB intervals) will be presented in subject-level listings.

11.5. Additional Assessment Items

Physical Examination, Urine Pregnancy, C-SSRS, PANSS and qEEG results will be presented in subject-level listings.

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12. Planned Tables, Listings and Figures

12.1. Planned Tables

Table Number	Table Title
14.1.1.1	Subject Disposition by Treatment Sequence and Overall (All Subjects)
14.1.1.2	Subject Disposition by Dose Level and Overall (All Subjects)
14.1.2.1	Demographics and Baseline Characteristics by Treatment Sequence and Overall (All Subjects)
14.1.2.2	Demographics and Baseline Characteristics by Dose Level and Overall (All Subjects)
14.1.3.1	Medical History by Dose Level and Overall (All Subjects): Reported at Baseline
14.1.3.2	Medical History by Dose Level and Overall (All Subjects): Ongoing
14.2.1.1	Primary Analysis: MMN Improvement (CVN058 vs. Placebo), Summary Statistics and Modeling Summary of Amplitude of Duration MMN (PD) by Dose Level
14.2.2.1	Secondary Analysis: Summary of Subject Adverse Events (SAF) by Most Recently Administered Dose Level and Overall
14.2.2.2	Treatment-Emergent Adverse Events by SOC and PT (SAF) by Most Recently Administered Dose Level and Overall
14.2.2.3	Serious Treatment-Emergent Adverse Events by SOC and PT (SAF) by Most Recently Administered Dose Level and Overall
14.2.2.4	Treatment-Emergent Adverse Events by Severity, SOC and PT (SAF) by Most Recently Administered Dose Level and Overall
14.2.2.5	Treatment-Emergent Adverse Events by Relationship to Study Drug (SAF) by Most Recently Administered Dose Level and Overall
14.2.3.1	Secondary Analysis: Summary of Clinically Significant Abnormal Laboratory Test Results (SAF) by Most Recently Administered Dose Level and Overall
14.2.3.2	Summary of Hematology Laboratory Data (SAF) Overall
14.2.3.3	Summary of Chemistry Laboratory Data (SAF) Overall
14.2.3.4	Summary of Urinalysis Laboratory Data (Continuous Variables) (SAF) Overall
14.2.3.5	Summary of Urinalysis Laboratory Data (Categorical Variables) (SAF) Overall
14.2.4	Summary of Vital Signs and Body Measurements (SAF) by Most Recently Administered Dose Level and Overall
14.2.5.1	Pharmacokinetic Analysis: Summary at Scheduled Sampling Intervals (PK) by Dose Level
14.2.5.2	Pharmacokinetic Analysis: Summary of PK Parameters (PK) by Dose Level
14.2.5.3.1	Pharmacodynamic Analysis: Summary of P50 Ratio (S2/S1) (PD) by Dose Level
14.2.5.3.2	Pharmacodynamic Analysis: Summary of P50 Differences (S2-S1) (PD) by Dose Level
14.2.5.3.3	Pharmacodynamic Analysis: Summary of Pre-stimulus Gamma Amplitude (PD) by Dose Level
14.2.5.3.4	Pharmacodynamic Analysis: Summary of P300 Amplitude (PD) by Dose Level
14.2.5.3.5	Pharmacodynamic Analysis: Summary of P300 Latency (PD) by Dose Level
14.2.5.3.6	Pharmacodynamic Analysis: Summary of Amplitude of Frequency MMN (PD) by Dose Level

Table Number	Table Title
14.2.5.3.7	Pharmacodynamic Analysis: Summary of Amplitude of Intensity MMN (PD) by Dose Level
14.2.5.3.8	Pharmacodynamic Analysis: Summary of Amplitude of Location MMN (PD) by Dose Level
14.2.5.3.9	Pharmacodynamic Analysis: Summary of Amplitude of Frequency-Modulation MMN (PD) by Dose Level
14.2.5.3.10	Pharmacodynamic Analysis: Summary of Latency to Peak Amplitude of Frequency MMN (PD) by Dose Level
14.2.5.3.11	Pharmacodynamic Analysis: Summary of Latency to Peak Amplitude of Intensity MMN (PD) by Dose Level
14.2.5.3.12	Pharmacodynamic Analysis: Summary of Latency to Peak Amplitude of Location MMN (PD) by Dose Level
14.2.5.3.13	Pharmacodynamic Analysis: Summary of Latency to Peak Amplitude of Frequency-Modulation MMN (PD) by Dose Level
14.2.5.4.1	Pharmacodynamic Analysis: ANCOVA Modeling (PD) of P50 Ratio (S2/S1)
14.2.5.4.2	Pharmacodynamic Analysis: ANCOVA Modeling (PD) of P50 Differences (S2-S1)
14.2.5.4.3	Pharmacodynamic Analysis: ANCOVA Modeling (PD) of Pre-stimulus Gamma Amplitude
14.2.5.4.4	Pharmacodynamic Analysis: ANCOVA Modeling (PD) of P300 Amplitude
14.2.5.4.5	Pharmacodynamic Analysis: ANCOVA Modeling (PD) of P300 Latency
14.2.5.4.6	Pharmacodynamic Analysis: ANCOVA Modeling (PD) of Amplitude of Frequency MMN
14.2.5.4.7	Pharmacodynamic Analysis: ANCOVA Modeling (PD) of Amplitude of Intensity MMN
14.2.5.4.8	Pharmacodynamic Analysis: ANCOVA Modeling (PD) of Amplitude of Location MMN
14.2.5.4.9	Pharmacodynamic Analysis: ANCOVA Modeling (PD) of Frequency-Modulation MMN

12.2. Planned Listings

Listing Number	Listing Title
16.2.1.1	Subject Disposition
16.2.1.2	Subject Screen Failures
16.2.2	Protocol Deviations
16.2.3	Demographics
16.2.4.1	Medical History
16.2.4.2	Prior and Concomitant Medications
16.2.5.1	Primary Endpoints: MMN
16.2.5.2	Exploratory Endpoints: P50, qEEG and P300
16.2.5.3	Audiometric Screening and Tone Matching Test
16.2.6.1	Pre-treatment Events
16.2.6.2	Adverse Events
16.2.6.3	Serious Adverse Events
16.2.7.1	Hematology Laboratory Parameters
16.2.7.2	Chemistry Laboratory Parameters
16.2.7.3	Urinalysis Laboratory Parameters
16.2.7.4	Urine Pregnancy Test
16.2.8.1	Physical Exam
16.2.8.2	Vital Signs
16.2.8.3	ECG
16.2.9.1	C-SSRS
16.2.9.2	PANSS
16.2.10	Pharmacokinetics
16.2.11	Eligibility Criteria
16.2.12	Study Drug Administration

12.3. Planned Figures

Figure Number	Figure Title
16.3.1.1	Box-plot: Amplitude of Duration MMN (CVN058 versus Placebo)
16.3.1.2	Box-plot: Change (CVN058 - Placebo) in Amplitude of Duration MMN
16.3.2.1	Box-plot: P50 Ratio (S2/S1) (CVN058 versus Placebo)
16.3.2.2	Box-plot: P50 Difference (S2-S1) (CVN058 versus Placebo)
16.3.3	Box-plot: Pre-stimulus Gamma Amplitude (CVN058 versus Placebo)
16.3.4.1	Box-plot: P300 Amplitude (CVN058 versus Placebo)
16.3.4.2	Box-plot: P300 Latency (CVN058 versus Placebo)
16.3.5.1.1	Box-plot: Amplitude of Frequency MMN (CVN058 versus Placebo)
16.3.5.1.2	Box-plot: Amplitude of Intensity MMN (CVN058 versus Placebo)
16.3.5.1.3	Box-plot: Amplitude of Location MMN (CVN058 versus Placebo)
16.3.5.1.4	Box-plot: Amplitude of Frequency-Modulation MMN (CVN058 versus Placebo)
16.3.5.2.1	Box-plot: Latency to Peak Amplitude of Frequency MMN (CVN058 versus Placebo)
16.3.5.2.2	Box-plot: Latency to Peak Amplitude of Intensity MMN (CVN058 versus Placebo)
16.3.5.2.3	Box-plot: Latency to Peak Amplitude of Location MMN
16.3.5.2.4	Box-plot: Latency to Peak Amplitude of Frequency-Modulation MMN
16.3.6.1	Pharmacokinetic Relationships: Scatterplot of Individual CVN058 Concentration Values Versus Time by Dose with Calculations of Summary Statistics
16.3.6.4.1.1	Pharmacokinetic Relationships: Scatterplot of Change from Placebo in Amplitude of Duration MMN versus CVN058 Concentration at 1-Hour Timepoint
16.3.6.4.1.2	Pharmacokinetic Relationships: Scatterplot of Change from Placebo in Amplitude of Duration MMN versus CVN058 Concentration at 5-Hour Timepoint
16.3.6.4.2.1	Pharmacokinetic Relationships: Scatterplot of Change from Placebo in P50 Ratio (S2/S1) versus CVN058 Concentration at 1-Hour Timepoint
16.3.6.4.2.2	Pharmacokinetic Relationships: Scatterplot of Change from Placebo in P50 Ratio (S2/S1) versus CVN058 Concentration at 5-Hour Timepoint
16.3.6.4.3.1	Pharmacokinetic Relationships: Scatterplot of Change from Placebo in P50 Difference (S2-S1) versus CVN058 Concentration at 1-Hour Timepoint
16.3.6.4.3.2	Pharmacokinetic Relationships: Scatterplot of Change from Placebo in P50 Difference (S2-S1) versus CVN058 Concentration at 5-Hour Timepoint
16.3.6.4.4.1	Pharmacokinetic Relationships: Scatterplot of Change from Placebo in Pre-Stimulus Gamma Amplitude versus CVN058 Concentration at 1-Hour Timepoint
16.3.6.4.4.2	Pharmacokinetic Relationships: Scatterplot of Change from Placebo in Pre-Stimulus Gamma Amplitude versus CVN058 Concentration at 5-Hour Timepoint
16.3.6.4.5.1	Pharmacokinetic Relationships: Scatterplot of Change from Placebo in P300 Amplitude versus CVN058 Concentration at 1-Hour Timepoint
16.3.6.4.5.2	Pharmacokinetic Relationships: Scatterplot of Change from Placebo in P300 Amplitude versus CVN058 Concentration at 5-Hour Timepoint