NCT #: NCT03569631

Clinical Trial Protocol: BPN14770-CNS-203

Title	A Randomized, Double-blind, Placebo-controlled, 2-period Crossover Study of BPN14770 in Adult Males with Fragile X Syndrome
Substance Identifier	BPN14770
IND Number	127905
Indication	Cognitive Enhancement
Phase	Phase 2
Sponsor	Tetra Discovery Partners, Inc.
Sponsor Contact	[redacted] Tetra Discovery Partners, Inc. 38 Fulton Street West Suite 303 Grand Rapids, MI 49503-2684
Principal Investigator	TBD

Amendment	Revision Date	Reason
N/A	05 January 2018	Original submission
1	18 April 2018	Revisions to address FDA suggestion in May Proceed letter
2	25 June 2018	Inclusion of biomarker sample at baseline, clarify AE/Medical History pre-dose, typographical corrections.

Confidentiality Statement

1 SIGNATURES

1.1 Sponsor Signatures

Protocol Number: BPN14770-CNS-203

Sponsor: Tetra Discovery Partners, Inc.

Sponsor Contact: [redacted]
[redacted] Tetra Discovery Partners, Inc.
38 Fulton Street West, Suite 303
Grand Rapids, MI 4953-2684
Phone: [redacted]

[redacted]

Study Director & Medical Monitor [redacted]
[redacted] Tetra Discovery Partners, Inc.
38 Fulton Street West, Suite 303
Grand Rapids, MI 4953-2684
Phone: [redacted]

[redacted] 6-27-2018
(Date)

1.2 Investigator Signature

I confirm that I have read and that I understand this protocol, the Investigator Brochure, and other product information provided by the Sponsor. I will provide copies of this protocol and access to all information furnished by Tetra Discovery Partners, Inc. to study personnel under my supervision. I will discuss this material with them to ensure they are fully informed about the study product and study procedures. I will let them know that this information is confidential and proprietary to Tetra Discovery Partners, Inc. and that it may not be further disclosed to third parties. I understand that the study may be terminated or enrollment suspended at any time by Tetra Discovery Partners, Inc. with or without cause, or by me if it becomes necessary to protect the best interests of the study subjects.

I agree to conduct this study in accordance with the requirements of this protocol and also protect the rights, safety, privacy, and well-being of study subjects in accordance with the following:

- The ethical principles that have their origin in the Declaration of Helsinki.[1]
- International Conference on Harmonization Guidance for Industry, Good Clinical Practice E6.[2]
- All applicable laws and regulations (21 CFR § 11, 50, 54, 56, and 312 Subpart D), including, without limitation, data privacy laws and regulations.
- Regulatory requirements for reporting serious adverse events defined in this protocol.
- Terms outlined in the Clinical Study Site Agreement.

Investigator:

[redacted]
Rush University Medical Center
1725 W Harrison St. Suite 718
Chicago IL, 60612
[redacted] phone
fax

[redacted]

SYNOPSIS

Name of Sponsor/Company:	Tetra Discovery Partners, Inc.
Name of Investigational Product:	BPN14770
Study Title:	A Randomized, Double-blind, Placebo-controlled, 2-period Crossover Study of BPN14770 in Adult Males with Fragile X Syndrome
Study Number:	BPN14770-CNS-203
Study Phase:	Phase 2

Study Objectives:

In male Fragile X patients aged 18-45 years, inclusive, receiving standard medications:

- To obtain preliminary assessments of the efficacy of BPN14770 25 mg bid
- To evaluate the safety and tolerability of BPN14770 25 mg
- To obtain pharmacokinetic, pharmacodynamic and biomarker data on BPN14770

Exploratory Efficacy Outcome Measures

- NIH Toolbox Cognitive Battery for Intellectual Disabilities (NIH-TCB)
- Test of Attentional Performance (KiTAP)
- Clinical Global Impression Severity – Investigator rated (CGI-S)
- Clinical Global Impression Improvement – Investigator rated (CGI-I)
- Visual Analog Scale (VAS) rating using patient-specific behavioral anchors
- Aberrant Behavior Checklist (ABC)
- Anxiety, Depression, and Mood Scale (ADAMS)
- Vineland-3 Rating Scale
- Event-Related Potentials (ERP)
- Eye Tracking

Safety and Tolerability Endpoints

- Treatment-emergent Adverse events
- Changes in vital signs
- Clinical laboratory evaluations (chemistry, hematology, urinalysis)
- Electrocardiogram (ECG) measurements

Pharmacokinetic Evaluations

- BPN14770 plasma levels obtained at various time points during the study, according to the Schedule of Assessments

Study Design

This is a single-center, Phase 2, randomized, double-blind, placebo-controlled, 2-period crossover study to obtain preliminary assessment of the effects of BPN14770 in patients with Fragile X Syndrome. The study will consist of a Screening period of up to 28 days prior to initial study drug administration, followed by two 12-week double-blind treatment periods. The screening and baseline visits may occur at the same time, provided the results of safety labs can be obtained. No washout period will be utilized between double-blind treatment periods; instead, efficacy and biomarker assessments during the second double-blind period (Period 2) will be obtained no sooner than Week 6 of Period 2 to allow for drug washout. A final follow-up visit or phone contact for safety is planned one week after the conclusion of Period 2.

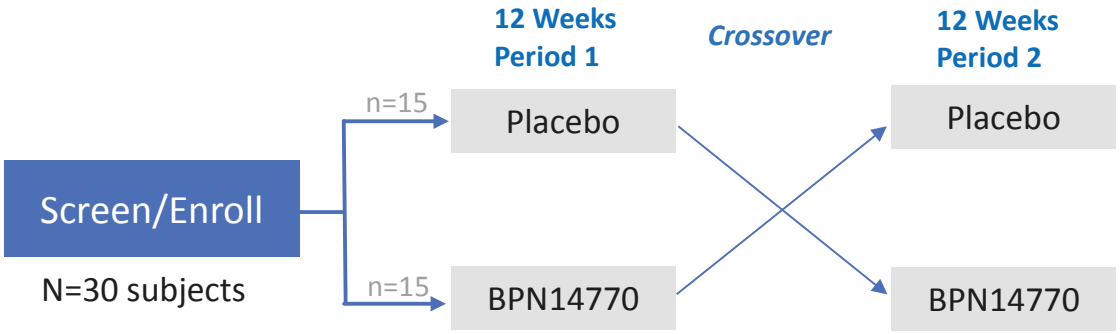
Eligible patients will be randomized in a blinded, balanced (1:1) fashion to receive either BPN14770 25mg bid or

Name of Sponsor/Company:	Tetra Discovery Partners, Inc.
Name of Investigational Product:	BPN14770
Study Title:	A Randomized, Double-blind, Placebo-controlled, 2-period Crossover Study of BPN14770 in Adult Males with Fragile X Syndrome
Study Number:	BPN14770-CNS-203
Study Phase:	Phase 2

matching placebo during Period 1, followed by the opposite treatment during Period 2.

Brief cognitive and behavioral assessments will be performed during each clinic visit. Safety and tolerability assessments throughout the study will include adverse event monitoring, ECGs, vital signs, blood chemistry, hematology, and urinalysis.

Pharmacokinetic and biomarker samples will be collected to at screening and at the end of Period 1 and Period 2. The pharmacokinetic samples are primarily to confirm that study drug is present when expected and to estimate plasma exposure at end of Periods 1 and 2.



Planned Numbers of Subjects

Enrollment of a total of 30 eligible subjects is planned, 15 per treatment sequence.

Study Duration

The total duration of the study for each subject will be up to 29 weeks, including a maximum of 4-week screening period, two 12-week Double-Blind treatment Periods, and a follow-up telephone call approximately 1 week after last treatment.

Name of Sponsor/Company:	Tetra Discovery Partners, Inc.
Name of Investigational Product:	BPN14770
Study Title:	A Randomized, Double-blind, Placebo-controlled, 2-period Crossover Study of BPN14770 in Adult Males with Fragile X Syndrome
Study Number:	BPN14770-CNS-203
Study Phase:	Phase 2

Study Procedures:
The Screening Visit will occur up to 28 days prior to the first study drug administration on Day 1. During screening, patients and their parent/legal authorized guardian, if indicated, will review and sign an Informed Consent/Assent form prior to any study procedures being performed. Following confirmation of a prior diagnosis for Fragile X, patients will have information collected regarding their neurological and medical/surgical history, race/ethnicity, social history (tobacco, alcohol, and/or drug use), and use of prescription and over-the-counter medications. Patients will undergo a full physical exam, have vital signs measured, and 12-lead ECG performed. Height, weight, and BMI will also be collected. Fasting blood samples will be collected for chemistry and hematology, as well as biomarkers and pharmacokinetics. Urine will be collected for urinalysis. The Stanford-Binet test will also be administered at screening, as will an assessment of suicidality risk.

At the Baseline visit (Period 1/Day 1), prior to randomization, patients will receive an abbreviated physical examination including vital signs. However, these will not be repeated if the Screening and Baseline visits occur on the same day. Cognitive and behavioral evaluations will be performed and these measurements will be used as a common set of baseline measurements to which post-treatment assessments will be compared for both treatment Periods.

During both double-blind periods, patients will receive twice-daily treatment with blinded study medication as randomly assigned at baseline. Doses of study medication should be taken in the morning and at night, at least 6 hours apart and at least 30 minutes prior to or 1 hour after meals.

Patients will return to the clinic at the end of weeks 2, 6, and 12 of each Period. Cognitive and behavioral evaluations will be repeated at Weeks 6 and 12 of each Period, with the exception of ERP and eye tracking which will be repeated only at Week 12 of each Period. Additionally, patients will be monitored for adverse events via a telephone call at the end of Week 1 of each Period, and one week following completion of Period 2 or following early discontinuation. During clinic visits, adverse effects will be assessed, and laboratory measures, vital signs, and ECG will be repeated according to the Schedule of Assessments. Suicidality risk with also be evaluated during the treatment periods per the Schedule of Assessments; if a concern is detected, the subject will be referred for further evaluation and treatment.

Subject Inclusion/Exclusion Criteria:
Individuals are eligible for the study if they meet all of the Inclusion and none of the Exclusion criteria. The criteria below will be assessed during Screening, which will be up to 28 days prior to first study drug administration. Continued subject eligibility will be verified on Baseline Day 1.

Subject Inclusion Criteria

1. Subject is male aged 18 to 45 years, inclusive.
2. Subject has Fragile X Syndrome with a molecular genetic confirmation of the full Fragile X Mental Retardation (FMR1) mutation (≥200 CGG repetitions).
3. Current treatment with no more than 3 prescribed psychotropic medications. Anti-epileptic medications are permitted and are not counted as psychotropic medications if they are used for treatment of seizures. Anti-epileptics for other indications, such as the treatment of mood disorders, count towards the limit of permitted medications.
4. Permitted concomitant psychotropic medications must be at a stable dose and dosing regimen for at

Name of Sponsor/Company:	Tetra Discovery Partners, Inc.
Name of Investigational Product:	BPN14770
Study Title:	A Randomized, Double-blind, Placebo-controlled, 2-period Crossover Study of BPN14770 in Adult Males with Fragile X Syndrome
Study Number:	BPN14770-CNS-203
Study Phase:	Phase 2

least 2 weeks prior to Screening and must remain stable during the period between Screening and the commencement of study medication.

5. Anti-epileptic medications must be at a stable dose and dosing regimen for 12 weeks prior to Screening and must remain stable during the period between Screening and the commencement of study medication.
6. Subjects with a history of seizure disorder who are currently receiving treatment with anti-epileptics must have been seizure-free for 3 months preceding Screening, or must be seizure-free for 3 years if not currently receiving anti-epileptics.
7. Behavioral and therapy treatments/interventions must be stable for 4 weeks prior to Screening and must remain stable during the period between Screening and the commencement of study medication, and throughout the study. Minor changes in hours or times of therapy that are not considered clinically significant will not be exclusionary. Changes in therapies provided through a school program, due to school vacations, are allowed.
8. Subject must be willing to practice barrier methods of contraception while on study, if sexually active. Abstinence is also considered a reasonable form of birth control in this study population.
9. Subject has a parent, legal authorized guardian or consistent caregiver.
10. Subject and caregiver are able to attend the clinic regularly and reliably.
11. Subject is able to swallow tablets and capsules.
12. For subjects who are not their own legal guardian, subject's parent/legal authorized guardian is able to understand and sign an informed consent form to participate in the study.
13. If subject is his/her own legal guardian, he/she can understand and sign informed consent to participate in the study.
14. If subject is not their own legal guardian, the subject provides assent for participation in the study, if the subject has the cognitive ability to provide assent.

Subject Exclusion Criteria

1. History of, or current cardiovascular, renal, hepatic, respiratory, gastrointestinal, psychiatric, neurologic, cerebrovascular, or other systemic disease that would place the subject at risk or potentially interfere with the interpretation of the safety, tolerability, or efficacy of the study medication.

   Common diseases such as mild hypertension, well-controlled type 2 diabetes mellitus (hemoglobin A1C [Hgb A1C] <6.5%), etc. are allowed per the investigator's judgment as long as they are stable and controlled by medical therapy that is constant for at least 4 weeks before randomization.
2. Renal impairment, defined as serum creatinine > 1.25 x ULN at screening
3. Hepatic impairment, defined as ALT or AST elevation > 2 x ULN at screening. Note: LFTs may be repeated after 1 week to evaluate return to acceptable limits; if LFTs remain elevated, subject is ineligible to participate.
4. Clinically significant abnormalities, in the investigator's judgment, in safety laboratory tests, vital signs, or ECG, as measured during Screening.

Name of Sponsor/Company:	Tetra Discovery Partners, Inc.
Name of Investigational Product:	BPN14770
Study Title:	A Randomized, Double-blind, Placebo-controlled, 2-period Crossover Study of BPN14770 in Adult Males with Fragile X Syndrome
Study Number:	BPN14770-CNS-203
Study Phase:	Phase 2

5. History of substance abuse within the past year, according to investigator assessment.
6. Significant hearing or visual impairment that may affect the subject's ability to complete the test procedures.
7. Concurrent major psychiatric condition (e.g., Major Depressive Disorder, Schizophrenia or Bipolar Disorder) as diagnosed by the investigator. Subjects with additional diagnosis of Autism Spectrum Disorder or Anxiety Disorder will be allowed.
8. Subject has active diseases that would interfere with participation, such as acquired immunodeficiency disorder, hepatitis C, hepatitis B, or tuberculosis.
9. Subject is planning to commence psychotherapy or cognitive behavior therapy (CBT) during the period of the study or had begun psychotherapy or CBT within 4 weeks prior to Screening.
10. Subject is related to anyone employed by the sponsor, investigator, or study staff.
11. Subject has BMI less than 18 or greater than 36.
12. Subject has participated in another clinical trial within the 30 days preceding Screening.

Investigational Product, Dosage, and Mode of Administration:
In this randomized double-blind, placebo-controlled, 2-period cross-over study, 30 patients will be randomized (1:1) to receive each of the following two treatments (one during each 12 week treatment Period):

- BPN14770 25 mg bid
- Matching Placebo

All study medications will be provided as identical-appearing HPMC capsules in HPE bottles. One capsule is to be taken orally in the morning, and one capsule is to be taken orally at night, with at least 120 mL (4 ounces) of liquid. Doses should be taken at least 6 hours apart, and at least 30 minutes prior to or 1 hour after meals.

Overview of Endpoints

Exploratory Efficacy Outcome Measures

- NIH-TCB: Cognitive battery assessing cognition, administered using an iPad
- KiTAP: Computerized executive battery with assessments of alertness (reaction time), distractibility, go/nogo (impulsiveness), and flexibility
- Clinical Global Impression Severity – Investigator rated (CGI-S) Standardized ranking scale with 7 rankings
- Clinical Global Impression Improvement – Investigator rated (CGI-I): Standardized ranking scale with 7 rankings
- Visual Analog Scale (VAS) – Parent/caregiver-rated assessment of patient-specific behavioral anchors: Domains of daily functioning, anxiety/irritability and language.
- ABC: Parent/caregiver-rated scale with six subscales to assess irritability, social avoidance, lethargy, hyperactivity, inappropriate speech and social avoidance, using ABC-FX factoring system.
- ADAMS: Parent/caregiver rated scale with a total score and five sub-scores to assess manic/hyperactive behavior, depressed mood, social avoidance, general anxiety, and obsessive/compulsive behavior.
- Vineland-3 Adaptive Behavior Scale: Clinician-administered standardized interview yielding adaptive

Name of Sponsor/Company:	Tetra Discovery Partners, Inc.
Name of Investigational Product:	BPN14770
Study Title:	A Randomized, Double-blind, Placebo-controlled, 2-period Crossover Study of BPN14770 in Adult Males with Fragile X Syndrome
Study Number:	BPN14770-CNS-203
Study Phase:	Phase 2

behavior composite score and domain standard scores in domains of: communication (receptive, expressive, and written adaptive language functions), daily living skills (personal, domestic, and community skills), socialization (interpersonal relationships, play and leisure time, and coping abilities), and motor skills (gross and fine motor skills).

- Event-Related Potentials (ERP) - Measure of auditory habituation based on EEG signals
- Eye Tracking – Measure of gaze aversion (social anxiety) and pupilometry (autonomic function)

Safety and Tolerability: The safety variables to be assessed include adverse events; clinical laboratory parameters (chemistry, hematology, and urinalysis); 12-lead electrocardiograms (ECGs); physical examinations; and vital signs (including blood pressure, heart rate, and respiratory rate).

Pharmacokinetics: Plasma BPN14770 concentrations will be obtained at screening and at the final visit during each treatment Period. Samples will be drawn at the time of the clinic visit, with documentation of time of day sample was obtained, and time of day of most recent dose of study medication.

Biomarkers: Pharmacokinetic samples will be retained and may be utilized subsequently for biomarker assessment.

Statistical Considerations

Efficacy Analyses
The primary efficacy population will be the intent to treat (ITT) efficacy population, which will include all randomized subjects who received at least one dose of treatment and returned for at least one follow-up visit. The completers population (CP), defined as all randomized subjects who complete both treatment periods with no significant protocol violations, will be used to evaluate the robustness of the ITT results.

All efficacy parameters will be summarized at each time point collected and standard descriptive statistics provided. Baseline measurements are defined as those obtained on Day 1/Period 1 prior to receipt of any study medication. For cognitive parameters measured at baseline, change from baseline will also be calculated and standard descriptive statistics provided for each time point. Linear mixed models will be used to explore potential treatment effects.

Safety Analyses
The Safety population will include all randomized subjects who received at least one dose of study treatment. Adverse Events (AEs), including clinically meaningful laboratory abnormalities and significant behavioral changes. AE severity and relatedness to treatment will be assessed. AEs will be tabulated for placebo and BPN14770 treatments. Summaries will also be provided by severity and relationship to treatment. Serious Adverse Events (SAEs) will be summarized separately, in a manner similar to that used for Adverse Events.

Sample Size Determination
Given the exploratory nature of this study, a definitive sample size calculation is not possible. However, for the sake of providing an evaluation of the potential power to detect a meaningful difference between treatments, a crossover study having no carryover effects with 80% power, a 5% alpha level, and a common standard deviation of 3 units, a sample size of 30 evaluable patients (15 per sequence group) will allow detection of an effect size of

Name of Sponsor/Company:	Tetra Discovery Partners, Inc.
Name of Investigational Product:	BPN14770
Study Title:	A Randomized, Double-blind, Placebo-controlled, 2-period Crossover Study of BPN14770 in Adult Males with Fragile X Syndrome
Study Number:	BPN14770-CNS-203
Study Phase:	Phase 2
approximately 2.25 units on the ABC Total Score outcome measure. If carryover effects or excessive dropouts allow only for a first Period analysis, a sample size of 15 per group during the first period would allow for detection of an effect size of 3.19 units.	

2 TABLE OF CONTENTS

1 SIGNATURES 2
1.1 Sponsor Signatures 2
1.2 Investigator Signature 3
2 TABLE OF CONTENTS 11
3 LIST OF ABBREVIATIONS 15
4 INTRODUCTION 18
4.1 Background 18
4.2 Rationale 18
4.3 Risk/Benefit 19
4.3.1 Preclinical Pharmacology 19
4.3.2 Clinical Experience 20
4.3.2.1 BPN14770-CNS-101 20
4.3.2.2 BPN14770-CNS-102 20
4.3.2.3 BPN14770-CNS-103 21
4.4 Study Objectives and Endpoints 22
4.4.1 Study Objectives 22
4.4.2 Exploratory Efficacy Outcome Measures 22
4.4.3 Safety and Tolerability Endpoints 22
4.4.4 Pharmacokinetic Endpoints 23
4.4.5 Biomarkers 23
5 INVESTIGATIONAL PLAN 23
5.1 Overall Study Design 23
5.1.1 Dose Selection 24
5.2 Study Duration 24
6 SELECTION AND WITHDRAWAL OF SUBJECTS 24
6.1 Study Population 24
6.2 Subject Inclusion Criteria 24
6.3 Subject Exclusion Criteria 25
6.4 Subject Withdrawal 26
6.5 Replacement of Subjects 27
7 TREATMENT OF SUBJECTS 27
7.1 Treatment Arms 27
7.2 Study Medications 27
7.3 Drug Accountability 28
7.4 Blinding 28
7.5 Assignment of Subjects to Treatment Arms 28

7.6 Study Drug Administration 29
7.7 Prior and Concomitant Medications 29
7.8 Other Therapeutic Treatments 29
7.9 Dietary Guidelines 29
7.10 Lifestyle Guidelines 29
8 STUDY PROCEDURES AND ASSESSMENTS 29
8.1 Informed Consent 29
8.2 Medical /Surgical History 30
8.3 Demographics and Social History 30
8.4 Stanford Binet Intelligence Scale 30
8.5 Physical Examination (Full and Abbreviated) 30
8.6 Height, Weight, Body Mass Index 31
8.7 Vital Signs 31
8.8 Electrocardiograms 31
8.9 Laboratory Assessments 31
8.10 Management of Abnormal Clinical Laboratory Tests 32
8.11 Suicidality Assessment 32
8.12 Additional Safety Measures 32
8.13 Exploratory Efficacy Assessments 32
8.13.1 Descriptions of Efficacy Assessment Instruments 32
8.13.1.1 NIH-TCB 33
8.13.1.2 Test of Attentional Performance (KiTAP) 33
8.13.1.3 Clinical Global Impression 33
8.13.1.4 VAS Rating Scale 34
8.13.1.5 Aberrant Behavior Checklist (ABC) 34
8.13.1.6 Anxiety Depression and Mood Scale (ADAMS) 35
8.13.1.7 Vineland-3 Rating Scale 35
8.13.1.8 Event-Related Potentials (ERP) 35
8.13.1.9 Eye Tracking 36
8.13.2 Timing of Efficacy Assessments 36
8.13.2.1 Baseline (Period 1/Day 1) 36
8.13.2.2 Week 6 (Periods 1 and 2) 36
8.13.2.3 Week 12 (Periods 1 and 2) 36
8.14 Pharmacokinetic Assessment 37
8.14.1 Plasma Pharmacokinetic Samples 37
8.14.2 Processing and Shipment of Pharmacokinetic Samples 37
8.14.3 Analytical Method 38

8.14.4 Plasma Pharmacokinetic Parameters 38
8.15 Biomarker Assessments 38
9 EVALUATION AND REPORTING OF ADVERSE EVENTS 38
9.1 Adverse Events 38
9.2 Definitions 39
9.2.1 Adverse Drug Reaction 39
9.2.2 Unexpected Adverse Event/ Unexpected Adverse Drug Reaction 39
9.2.3 Serious Adverse Events 39
9.3 Assessment of Adverse Events by the Investigator 40
9.3.1 Causality/Relatedness 40
9.3.2 Severity 41
9.3.3 Adverse Event Monitoring and Follow-up 41
9.3.4 Reporting Serious Adverse Events 42
10 STUDY PROCEDURES AND ASSESSMENTS BY VISIT 43
10.1 Schedule of Assessments 43
10.2 Screening (Day -28 to Day -1) 43
10.3 Baseline (Period 1/Day 1) 43
10.3.1 Prior to Randomization 44
10.3.2 Week 1 (Periods 1 and 2) 44
10.3.3 Week 2 (Periods 1 and 2) 44
10.3.4 Week 6 (Periods 1 and 2) 45
10.3.5 Week 12 (Periods 1 and 2) 45
10.3.6 Week 13 (Period 2, Final Contact at End of Study) 46
10.3.7 Early Termination 46
11 PLANNED STATISTICAL METHODS 47
11.1 Sample Size 47
11.2 Demographics 47
11.3 Analysis Populations 47
11.4 Efficacy: Cognitive Testing Analysis 47
11.5 Safety Analysis 48
11.6 Pharmacokinetic Analysis 48
11.7 Biomarker Analysis 48
12 DATA MANAGEMENT 48
12.1 Data Handling 48
12.2 Computer Systems 48
12.3 Data Entry 48
12.4 Data Validation 49

13 INVESTIGATOR REQUIREMENTS AND QUALITY CONTROL 49
13.1 Ethical Conduct of the Study 49
13.2 Institutional Review Board (IRB) 49
13.3 Informed Consent 50
13.4 Study Monitoring 50
13.5 Disclosure of Information 51
13.6 Record Storage and Retention 51
13.7 Protocol Amendments and Deviations 52
13.8 Access to Source Documentation 52
13.9 Financial Disclosure 52
13.10 Publication Policy 52
14 ADMINISTRATIVE INFORMATION 53
14.1 Sponsor 53
14.2 Biological Specimens 53
15 REFERENCES 54
16 APPENDICES 56
16.1 Schedule of Assessments 56
16.2 Clinical Laboratory Analytes 58

3 LIST OF ABBREVIATIONS

ABC (-FX)	Aberrant Behavior Checklist (Fragile X specific factoring system)
ADAMS	Anxiety, Depression, and Mood Scale
AE(s)	Adverse event(s)
ADR	Adverse drug reaction
ALT	Alanine transaminase
API	Active pharmaceutical ingredient
bid	Twice daily
BMI	Body mass index
BP	Blood pressure
BPM	Beats per minute
BUN	Blood urea nitrogen
°C	Degrees centigrade
cAMP	3'-5'- cyclic adenosine monophosphate
CBT	Cognitive Behavioral Therapy
CDR	Clinical Dementia Rating
CDR-SB	Clinical Dementia Rating – Sum of Boxes
CFR	Code of Federal Regulations
CGI-I	Clinical Global Impression Improvement – Investigator rated
CGG	CGG trinucleotide repeating codon
CGI-S	Clinical Global Impression Severity – Investigator rated
C_{max}	Maximum plasma concentration
CNS	Central nervous system
CP	Completers population
CREB	cAMP response element binding
CRF	Case report form
DCCS	Dimensional change card sort (test)
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic acid
EEG	Electroencephalogram
EH	Extraction ratio
ERP	Event-Related Potentials
FDA	Food and Drug Administration

FMR1	Fragile X mental retardation
FXS	Fragile X Syndrome
gm	Gram
GCP	Good Clinical Practice
GI	gastrointestinal
hERG	human Ether-à-go-go-Related Gene
HDPE	High density polyethylene
HgbA1C	Hemoglobin A1C
HPMC	Hydroxypropyl methylcellulose
ICF	Informed consent form
ICH	International Conference on Harmonization
IND	Investigational New Drug
IRB	Institutional Review Board
ITI	Inter-trial interval
ITT	Intent to treat (population)
kg	kilogram
KiTAP	Test of Attentional Performance
MAD	Multiple ascending dose
MED	Minimum effective dose
mg	Milligram
mL	Milliliter
msec	Millisecond
MTD	Maximum tolerated dose
NAM	Negative allosteric modulator
µ	Micro
ng	Nanogram
NIH-TCB	NIH Toolbox Cognitive Battery for Intellectual Disabilities
NOAEL	No observed adverse effect level
PDE4	phosphodiesterase type-4
PDE4D	phosphodiesterase type-4 subtype D
PDE4D-NAM	phosphodiesterase type-4D-negative allosteric modulator
PK	Pharmacokinetic
PO	By mouth (per os)

QD	Once daily
QTc	Corrected QT interval
RBC	Red blood cell
ROI	Region of interest
SAD	Single ascending dose
SAE	Serious adverse event
SD	Standard deviation
SOP	Standard operating procedure
T_{max}	Time to maximum concentration
VAS	Visual analog scale

4 INTRODUCTION

4.1 Background

Tetra Discovery Partners, Inc. is a central nervous system (CNS) biotechnology company that uses structure-based drug design to discover negative allosteric modulators (NAMs) of phosphodiesterase-4 (PDE4) subtypes for neurological and psychiatric diseases.

Tetra has developed a mechanistically novel class of drugs to address cognitive impairment across multiple psychiatric and neurological indications. These new drugs selectively target subtypes of phosphodiesterase type-4D (PDE4D).[3,4] The PDE4D enzyme subtype is a highly validated CNS target for improving cognition.

4.2 Rationale

Fragile X syndrome (FXS) is a disorder in which affected individuals display intellectual disability as well as symptoms typical of autism spectrum disorder, due to silencing of the X-linked, fragile-X mental retardation-1 (*FMR1*) gene. Dysregulation of cAMP metabolism is a consistent finding in patients and in preclinical models of FXS. BPN14770, a prototypic phosphodiesterase-4D negative allosteric modulator (PDE4D-NAM) Daily treatment of adult male *fmr1* C57Bl6 knock-out mice with BPN14770 for 14 days reduced hyperarousal, improved social interaction, and improved natural behaviors such as nesting and marble burying as well as dendritic spine morphology. There was no decrement in behavioral scores in control mice treated with BPN14770. The behavioral benefit of BPN14770 persisted two weeks after washout of the drug. Thus, BPN14770 may be useful for the treatment of FXS with potential expansion to the treatment of autism spectrum disorder.[5]

A total of 136 subjects have received BPN14770 either as a single dose or as a multiple dose regimen. Of these subjects, 106 (78%) were ≤ 55 years of age and 30 (22%) were ≥60 years of age.

The single dose escalation study (-101) in 24 subjects ≤ 55years of age determined that a single 100mg dose was the maximum tolerated dose of BPN14770, based on the occurrence of nausea and vomiting in the majority of subjects at this dose. There were no notable adverse events in other body systems, no observed changes in vital signs or electrocardiogram, no adverse laboratory findings, and no serious adverse events were recorded. The multiple dose escalation study assessed 15 mg, 30 mg, and 50 mg doses given BID to 18 subjects ≤45 years of age and doses of 10 mg, 20 mg, and 40 mg given BID to 30 subjects ≥ 60 years of age. All of these doses were well tolerated. An additional cohort of 6 subjects ≤45 years of age received 75mg once daily in order to compare the safety and PK profiles to the 100mg MTD determined in the single dose study. The 75 mg dose was associated with GI adverse experiences, confirming the findings at 100 mg in the single ascending dose study. The BPN14770 doses assessed in the scopolamine challenge study were 10 mg and 50 mg QD. Of the 38 subjects enrolled, 37 received at least one of the two doses of BPN14770; one subject withdrew from the crossover study prior to a dosing cohort containing BPN14770.

In the multiple ascending dose study (-102), BPN14770 administered twice daily to healthy elderly subjects was associated with improvement in complex attention/working memory, delayed recall of verbal information, and delayed recall of visual information (10 and 20 mg doses). No beneficial or detrimental effects were observed on other cognitive tasks studied. The 40-mg BID dose was not associated with improvement in any cognitive domain, possibly because of a higher frequency of adverse events, primarily headaches, at this dose level. Therefore, a daily dose of 25 mg bid was chosen for this clinical study in FXS.

4.3 Risk/Benefit

4.3.1 Preclinical Pharmacology

BPN14770 is a first-in-class, subtype selective, phosphodiesterase type-4D-negative allosteric modulator (PDE4D-NAM). The unique mechanism of action and subtype selectivity distinguishes BPN14770 from the two approved PDE4 inhibitors, roflumilast (Daliresp™) and apremilast (Otezla™). BPN14770 does not bind to the active site of the PDE4 enzyme and, unlike roflumilast and apremilast, does not cause emesis in ferrets, cynomolgous monkeys or marmosets; furthermore, it has not been found to cause mesenteric vasculopathy in rats or dogs in toxicological studies of 3 months duration. Thus, BPN14770 presents a unique procognitive and preclinical safety profile.

Clearance of BPN14770 by hepatocytes from human, rat, dog and monkey, expressed as hepatic extraction ratio (EH), was 16%, 8.2%, 17%, and 12%, respectively. Qualitative metabolite profiling in human, rat, dog, and monkey hepatocytes showed a single metabolite in human (<1% parent). BPN14770 was highly bound to plasma proteins in all species tested. The fraction unbound (free) was 0.5, 0.4, 0.2, and 0.5%, for mouse, rat, dog, and human, respectively.

BPN14770 is highly bioavailable in rats, mice and dogs (F% = 100), distributes to brain (B/P = 0.32-0.48), and has a plasma $t_{½}$ of 4.8 hours in rats, 10.9 hours in mice, and 11 hours in dogs. The multi-species pharmacokinetic data suggest that BPN14770 will have adequate half-life for once daily oral administration in humans. In bile duct cannulated rats, BPN14770 was eliminated as a conjugate through the bile. Only the unchanged drug was detected in plasma, and no BPN14770 was detected in urine.

BPN14770 did not affect cardiovascular function in beagle dogs at doses up to 100 mg/kg, nor did the compound affect respiratory function or exert neuropharmacologic effects in rats at doses up to 60 mg/kg. In the in vitro human Ether-à-go-go-Related Gene (hERG) assay, an increase in current of 12% and 33% was seen at concentrations of 10 and 30 µM, respectively. No IC_{50} could be calculated due to this minor increase in current.

In rat toxicology studies of 28-days and 13-weeks, BPN14770 was well tolerated up to 60 mg/kg with no deaths on study, and no adverse hematology, clinical chemistry, liver weight or gross necropsy findings. There were no microscopic findings. Unlike the PDE4 inhibitors roflumilast and apremilast, BPN14770 did not cause mesenteric vasculopathy at the doses studied.

In dog toxicology studies of 28-days and 13-weeks, the no observed adverse effect level (NOAEL) for BPN14770 was 30 mg/kg. At 100 mg/kg in the 28-day study, BPN14770 caused inappetance and weight loss and in female dogs an approximately 30 msec increase in QTc with a concomitant increase in heart rate of 10 bpm. There were no changes in hematology or ophthalmology. Macroscopic observations included pale livers in two of eight animals at 100

mg/kg which correlated with mild alanine transaminase (ALT) elevation only in those two animals. Microscopic changes of periportal hepatocellular cytoplasmic vacuolation were observed in the livers of one male and two female dogs at 100 mg/kg. No liver changes were observed in recovery dogs suggesting that these changes, if present, were reversible.

4.3.2 Clinical Experience

4.3.2.1 BPN14770-CNS-101

A first-in-human (FIH), single ascending dose (SAD) trial (BPN14770-CNS-101) in 24 healthy subjects at doses ranging from 5 mg to 100 mg has been completed. The results from this study indicate single doses of BPN14770, in the range of 5 mg to 100 mg, were safe and generally well tolerated. The maximum tolerated dose (MTD) was determined to be 100 mg, after 4 of 6 subjects in this dose group experienced nausea and, of those, two experienced vomiting.

The most commonly reported treatment-emergent, drug-related adverse events were nausea and vomiting. No safety signals were noted in the results of the other safety analyses. No deaths or other serious adverse events were reported. No subjects withdrew from the study due to adverse events.

In the SAD trial, detectable plasma BPN14770 concentrations were measured after dosing (at all 4 dose levels) starting between 0.5 hour and 1 hour after dosing, continuing through at least 48 hours after dosing. Drug absorption was variable but moderately rapid with median time to maximum concentration (T_{max}) values ranging from 1.5 to 3 hours. The plasma pharmacokinetics of BPN14770 after oral administration appeared to be linear for single doses ranging from 5 to 100 mg; although a slight trend for a greater than proportional increase in exposure was observed in the 100-mg group as compared to the lower dose groups. The apparent terminal elimination half-life of BPN14770 was consistent among dose groups and averaged between 11 and 13 hours. Based on the preclinical models, the concentrations associated with doses as low as 15 mg were potentially adequate to produce cognitive improvement after once- or twice- per day dosing.

4.3.2.2 BPN14770-CNS-102

In the multiple ascending dose (MAD) trial (BPN14770-CNS-102), 76 healthy young and elderly subjects were administered multiple doses of BPN14770 twice daily (every 12 hours) for 8 days. Young subjects (≤45 years; n=18 treated, 7 placebo) received twice-daily doses of 15 mg, 30 mg, 50 mg or placebo for 8 days. Elderly subjects (≥60 years; n=30 treated, 15 placebo) received twice-daily doses of 10 mg, 20 mg, and 40 mg for 8 days. Twice-daily dosing was selected to ensure adequate BPN14770 levels in the brain and plasma throughout the day and night. An additional cohort of 6 young subjects received a once-daily dose of 75 mg for 8 days to obtain additional safety and pharmacokinetic (PK) data at a dose level close to the MTD determined in the SAD study.

No deaths or serious adverse events were reported, and no subjects in either the young or elderly cohorts withdrew from the study due to adverse events. All treatment-emergent adverse events resolved without sequelae by the follow-up visit.

All study-drug related adverse events that occurred in more than 1 young subject were in the 75-mg QD dose group and consisted of nausea (reported by 4 of 6 subjects, vomiting (reported by 2 of 6 subjects who also experienced nausea), and decreased appetite (reported by 3 of 6 subjects). Across the young subject cohorts, all but 2 of the reported adverse events were mild in severity; moderate nausea and vomiting were reported in 1 of 6 subjects (16.7%) in the 75-mg QD dose group on Day 2 of multiple dosing. These events were considered by the Investigator as probably related to study treatment, resolved without sequelae within 13 hours of onset, and did not recur upon subsequent dosing. Among the young subject dose groups, no clinically significant effects of multiple oral doses of BPN14770 (15 mg, 30 mg, 50 mg, or 75 mg) were observed on biochemistry, hematology, or urinalysis; vital signs; or ECGs.

Among the elderly subject cohorts, the percentage of subjects who experienced at least one treatment-emergent adverse event was similar between the 10-mg BID, 20-mg BID, and placebo groups (ranging from 30% to 50%) and was higher for the 40-mg BID dose group (90%). Adverse events reported in 2 or more elderly subjects within a dose group were all reported by subjects in the 40-mg dose group and consisted of diarrhea, increased alanine and aspartate aminotransferase, hot flush (each reported at a frequency rate of 20%) and headache (reported at a frequency rate of 40%). Among the elderly subject dose groups, no clinically significant effects of multiple oral doses of BPN14770 (10 mg, 20 mg, or 40 mg) were observed on biochemistry, hematology, or urinalysis; vital signs; or ECGs.

With respect to cognitive testing, BPN14770 10 mg given twice daily was associated with improvement in complex attention/working memory and delayed recall of both visual and verbal information. Treatment with BPN14770 20 mg twice daily was associated with similar benefits as the 10 mg twice daily regimen, excluding improvement in delayed recall of visual information. Neither the 10- or 20-mg BID regimens was associated with improvement in psychomotor function, visual, or verbal learning, or executive function. The 40-mg BID regimen was not associated with improvement in any cognitive domain.

Pharmacokinetic analyses found that steady-state appeared to be reached after 3 to 4 days of BID or QD dosing in both young and elderly subjects. Following BID dosing of BPN14770 in healthy young subjects, Cmax and AUC12 appeared to increase in a greater than dose-proportional fashion from 15 mg to either 30 mg or 50 mg. A moderate degree of accumulation (up to approximately 5-fold) was observed after BID dosing in healthy young subjects, with less accumulation (approximately 2-fold) after QD dosing.

In healthy young subjects, mean peak to trough fluctuation was similar among BID regimens, ranging from 171% to 179%, and was 337% for the QD regimen. In healthy elderly subjects, mean peak to trough fluctuation was similar among BID regimens, ranging from 156% to 249%.

4.3.2.3 BPN14770-CNS-103

The third Phase 1 trial of BPN14770 (BPN14770-CNS-103) was a randomized, double-blind, placebo-controlled, 6-period crossover study to evaluate the effects of BPN14770 10 and 50 mg in reversing scopolamine-induced cognitive impairment in healthy volunteers. A positive control, donepezil 10 mg, was included, and additivity of BPN14770 50 mg to donepezil 10 mg in

reversing scopolamine effects was also evaluated. A total of 38 subjects were enrolled into the study.

The Cogstate Computerized Battery Test results suggest that, as expected, a 0.5 mg dose of scopolamine induced a decline in cognitive function, while a 10-mg dose of donepezil ameliorated this decline. Doses of 10 or 50 mg of BPN14770 did not produce a systematic reversal of the scopolamine-induced cognitive decline, administered alone or with a 10-mg dose of donepezil.

BPN14770 was safe and generally well tolerated when administered with scopolamine in healthy subjects. The incidence of nausea and vomiting increased several fold when either BPN14770 50 mg or donepezil was administered with scopolamine, or when both BPN14770 and donepezil were administered with scopolamine, compared to BPN14770 10 mg, placebo, or scopolamine alone.

4.4 Study Objectives and Endpoints

4.4.1 Study Objectives

In male Fragile X patients aged 18-45 years, inclusive, receiving standard medications:

- To obtain a preliminary assessment of the efficacy of BPN14770 25 mg bid
- To evaluate the safety and tolerability of BPN14770 25 mg
- To obtain pharmacokinetic, pharmacodynamic, and biomarker data on BPN14770

4.4.2 Exploratory Efficacy Outcome Measures

The following instruments will be used to assess the exploratory efficacy endpoints:

- NIH Toolbox Cognitive Battery Modified for Intellectual Disabilities (NIH-TCB)
- Test of Attentional Performance (KiTAP)
- Clinical Global Impression Severity – Investigator rated (CGI-S)
- Clinical Global Impression Improvement – Investigator rated (CGI-I)
- VAS Rating Scale using patient-specific behavioral anchors
- Aberrant Behavior Checklist (ABC)
- Anxiety, Depression, and Mood Scale (ADAMS)
- Vineland-3 Rating Scale
- Event-Related Potentials (ERP)
- Eye Tracking

4.4.3 Safety and Tolerability Endpoints

The following safety assessments will be conducted during the study.

- Treatment-emergent Adverse events
- Changes in vital signs
- Clinical laboratory evaluations (chemistry, hematology, urinalysis)
- Changes in electrocardiogram (ECG) measurements

4.4.4 Pharmacokinetic Endpoints

Plasma BPN14770 concentrations will be measured to verify that study drug is present when expected and to estimate plasma exposure after 12 weeks of treatment.

4.4.5 Biomarkers

Retained plasma samples may be used to explore biomarkers potentially associated with FXS.

5 INVESTIGATIONAL PLAN

5.1 Overall Study Design

This is a single center, Phase 2, randomized, double-blind, placebo-controlled, 2-period crossover study to obtain preliminary assessment of the effects of BPN14770 in patients with Fragile X Syndrome. As schematic display of the study design is shown in Figure 1.

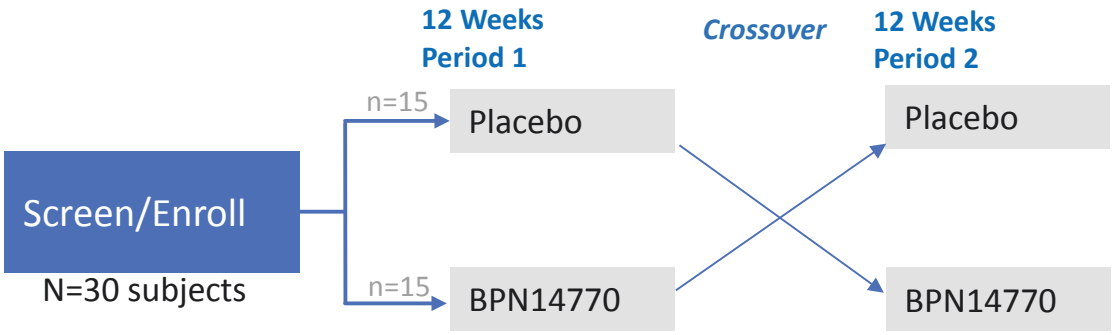
The study will consist of a Screening period of up to 28 days prior to initial study drug administration, followed by two 12-week double-blind treatment periods. The screening and baseline visits may occur at the same time, provided the results of safety labs can be obtained. No washout period will be utilized between double-blind treatment periods; instead, efficacy and biomarker assessments during the second double-blind period (Period 2) will be obtained after a minimum of 6 weeks to allow for drug washout. A final follow-up phone contact for safety is planned one week after the conclusion of Period 2.

Eligible patients will be randomized in a blinded, balanced (1:1) fashion to receive either BPN14770 25mg bid or matching placebo during Period 1, followed by the opposite treatment during Period 2.

Brief cognitive and behavioral assessments will be performed during each clinic visit. Safety and tolerability assessments throughout the study will include adverse event monitoring, ECGs, vital signs, blood chemistry, hematology, and urinalysis.

Pharmacokinetic samples will be collected at screening and at end of Periods 1 and 2 (Week 12to confirm that study drug is present when expected and to estimate plasma exposure.). Biomarker samples will be collected at the time the pharmacokinetics samples are obtained.

Figure 1 Study Design Schematic



5.1.1 Dose Selection

The dose of 25 mg BPN14770 to be taken twice daily was chosen for testing in this study.

Based on pharmacokinetic data from study BPN14770-CNS-102 (MAD), average plasma concentrations of BPH14770 at 10 mg dosing are projected to be approximately 60 ng/ml. Therefore, the lower dose of 10 mg has the potential to demonstrate efficacy in this study, although given the uncertainties of translation from preclinical models, it may be below the effective range. On the other hand, the 25 mg dose should be above that needed for efficacy, with projected average plasma concentrations over 200 ng/ml. The twice-daily dose of 25mg selected for this study falls well within the tolerance limits defined by the SAD and MAD studies, as noted above.

5.2 Study Duration

The total duration of the study for each subject will be up to 29 weeks, including a maximum of a 4-week screening period, two 12-week double-blind Treatment Periods, and a follow-up call approximately 1 week after last treatment.

6 SELECTION AND WITHDRAWAL OF SUBJECTS

6.1 Study Population

Individuals are eligible for the study if they meet all of the inclusion and none of the exclusion criteria. The criteria below will be assessed at the Screening visit which should within 28 days prior to first study drug administration. The Screening and Baseline Visits may be combined if site procedures allow.

6.2 Subject Inclusion Criteria

1. Subject is male aged 18 to 45 years, inclusive.

2. Subject has Fragile X Syndrome with a molecular genetic confirmation of the full Fragile X Mental Retardation (FMR1) mutation (≥200 CGG repetitions).
3. Current treatment with no more than 3 prescribed psychotropic medications. Anti-epileptic medications are permitted and are not counted as psychotropic medications if they are used for treatment of seizures. Anti-epileptics for other indications, such as the treatment of mood disorders, count towards the limit of permitted medications.
4. Permitted concomitant psychotropic medications must be at a stable dose and dosing regimen for at least 2 weeks prior to Screening and must remain stable during the period between Screening and the commencement of study medication.
5. Anti-epileptic medications must be at a stable dose and dosing regimen for 12 weeks prior to Screening and must remain stable during the period between Screening and the commencement of study medication.
6. Subjects with a history of seizure disorder who are currently receiving treatment with anti-epileptics must have been seizure-free for 3 months preceding Screening, or must be seizure-free for 3 years if not currently receiving anti-epileptics.
7. Behavioral and therapy treatments/interventions must be stable for 4 weeks prior to Screening and must remain stable during the period between Screening and the commencement of study medication, and throughout the study. Minor changes in hours or times of therapy that are not considered clinically significant will not be exclusionary. Changes in therapies provided through a school program, due to school vacations, are allowed.
8. Subject must be willing to practice barrier methods of contraception while on study, if sexually active. Abstinence is also considered a reasonable form of birth control in this study population.
9. Subject has a parent, legal authorized guardian or consistent caregiver.
10. Subject and caregiver are able to attend the clinic regularly and reliably.
11. Subject is able to swallow tablets and capsules.
12. For subjects who are not their own legal guardian, subject's parent/legal authorized guardian is able to understand and sign an informed consent form to participate in the study.
13. If subject is his/her own legal guardian, he/she can understand and sign informed consent to participate in the study.
14. If subject is not their own legal guardian, the subject provides assent for participation in the study, if the subject has the cognitive ability to provide assent.

6.3 Subject Exclusion Criteria

The following Exclusions apply to findings during Screening or at Baseline (Day 1):

1. History of, or current cardiovascular, renal, hepatic, respiratory, gastrointestinal,

psychiatric, neurologic, cerebrovascular, or other systemic disease that would place the subject at risk or potentially interfere with the interpretation of the safety, tolerability, or efficacy of the study medication.

Common diseases such as mild hypertension, well-controlled type 2 diabetes mellitus (hemoglobin A1C [HgbA1C] <6.5%), etc. are allowed per the investigator's judgment as long as they are stable and controlled by medical therapy that is constant for at least 4 weeks before randomization.

2. Renal impairment, defined as serum creatinine > 1.25 x ULN at screening.
3. Hepatic impairment, defined as ALT or AST elevation > 2 x ULN at screening. Note: LFTs may be repeated after 1 week to evaluate return to acceptable limits; if LFTs remain elevated, subject is ineligible to participate.
4. Clinically significant abnormalities, in the investigator's judgment, in safety laboratory tests, vital signs, or ECG, as measured during Screening.
5. History of substance abuse within the past year, according to investigator assessment.
6. Significant hearing or visual impairment that may affect the subject's ability to complete the test procedures.
7. Concurrent major psychiatric condition (e.g., Major Depressive Disorder, Schizophrenia or Bipolar Disorder) as diagnosed by the investigator. Subjects with additional diagnosis of Autism Spectrum Disorder or Anxiety Disorder will be allowed.
8. Subject has known or suspected human immune deficiency virus-positive status or has diseases such as acquired immunodeficiency disorder, hepatitis C, hepatitis B, or tuberculosis.
9. Subject is planning to commence psychotherapy or cognitive behavior therapy (CBT) during the period of the study or had begun psychotherapy or CBT within 4 weeks prior to Screening.
10. Subject is related to anyone employed by the sponsor, investigator, or study staff.
11. Subject has BMI less than 18 or greater than 36.
12. Subject has participated in another clinical trial within the 30 days preceding Screening.

6.4 Subject Withdrawal

All subjects have the right to withdraw from this study at any time. In addition, it is the right of the Investigator to remove subjects from the study as a result of adverse reactions, a protocol violation, or non-compliance, or any other reason. Subjects must be discontinued for the following reasons:

- Subject withdraws consent or requests discontinuation from the study for any reason;
- Occurrence of a medical condition or circumstance that exposes the subject to substantial risk and/or does not allow the subject to adhere to the requirements of the protocol as determined by the Investigator;

- Any serious adverse event (SAE), clinical significant adverse event, severe laboratory abnormality, concomitant illness, or other medical condition which indicates to the Investigator that continued participation is not in the best interest of the subject;
- Requirement for prohibited concomitant medication;
- Termination of the study by the Sponsor or the regulatory authority.

Should a subject withdraw after administration of BPN14770 (or placebo), or should the Investigator decide to withdraw the subject, all efforts will be made to complete and report the protocol-stipulated observations up to the time of withdrawal. Week 12 procedures (see Section 10.3.5 and Section 16.1 Schedule of Study Assessments) will be completed at the time of the subject's withdrawal and an explanation provided as to why the subject is withdrawing or being withdrawn from the study.

In a case of subjects lost to follow-up, attempts to contact the subject must be made and documented in the subject's medical records.

The Investigator must document the primary reason for discontinuation of a study subject on the appropriate case report form (CRF). If the reason for withdrawal is a clinical AE, monitoring will continue until the AE resolves or the Investigator assesses the AE as chronic and stabilized.

6.5 Replacement of Subjects

Subjects who do not meet eligibility criteria at Baseline will not be randomized and will be considered screen failures. Subjects who withdraw from the study for any reason will not be replaced.

7 TREATMENT OF SUBJECTS

7.1 Treatment Arms

In this randomized double-blind, placebo-controlled, 2-period cross-over study, 30 patients will be randomized (1:1) to receive each of the following two treatments (one during each 12 week Treatment Period):

- BPN14770 25 mg bid
- Matching Placebo

7.2 Study Medications

The 25 mg BPN14770 capsules and placebo capsules intended for use in this Phase 2 clinical trial are manufactured by Catalent, Inc. and will be packaged, labeled, and shipped for clinical use to sites by Sherpa Clinical Packaging.

The 25 mg BPN14770 capsules are manufactured using a wet granulation process. The appropriate amount of granulation is blended with additional excipients to provide a homogeneous blend. The blend is then filled into hydroxypropyl methylcellulose (HPMC), size 1, opaque white/opaque white capsules. The capsules are packaged as 30 count in 60 cc, white,

high-density polyethylene (HDPE), round bottles with 33 mm, white polypropylene ribbed caps. The container-closure system is labeled with a study-specific label and should be stored at controlled room temperature (20ºC – 25ºC).

7.3 Drug Accountability

The Investigator or designated study personnel is responsible for keeping accurate records of the study drugs (and other components) used in this study. These records should include documentation of receipt, inventory, and disposition to subject. The records should include dates, quantities, batch/serial numbers, expiration dates (if applicable), and any unique code numbers assigned (if applicable).

The study monitor will review study drug records periodically during the conduct of the study.

At the end of the study, all partial and empty containers must be returned to the Sponsor or, if requested by the Sponsor, destroyed at the site according to standard operating procedure (SOP). Records of destruction of study drug at the site must include bottle identifying information and number of capsules in each bottle. All documentation is to be filed in the Pharmacy Manual.

In accordance with Good Pharmacy Practices, gloves should always be worn by study personnel when handling study drug.

7.4 Blinding

This is a randomized, double-blind study, meaning that neither site staff nor subject will know what the subject is receiving.

Randomization codes will be provided to the site pharmacy for use in case of an emergency code break requirement. Confirmation of receipt of the randomization code will be required by the Sponsor. The site pharmacy will be responsible for maintaining the blind, consistent with protocol design, throughout the study, except in the case of an emergency code break.

The Investigator will be required to notify the Sponsor in the event of any breaking of the blind for any reason. Blinding is not to be broken during the study unless considered necessary by the Investigator for emergency situations for reasons of subject safety. The Investigator should contact the Medical Monitor before breaking the blind. When the blind is broken, the reason must be fully documented.

The subjects, Investigator, and all other study personnel involved with subject assessments will remain blinded to the actual treatment assignments of the subjects. The Investigator will be ultimately responsible for ensuring that the integrity of the blind is maintained throughout the study.

7.5 Assignment of Subjects to Treatment Arms

A randomization schedule will be generated by a statistician unassociated with the study execution prior to the start of the study. Subjects will be randomized on Day 1and receive their first bottle of medication that same day.

7.6 Study Drug Administration

One capsule is to be taken orally in the morning, and one capsule is to be taken orally at night, with at least 120 mL (4 ounces) of liquid. Doses should be taken at least 6 hours apart, and at least 30 minutes prior to or 1 hour after meals.

7.7 Prior and Concomitant Medications

A prior medication is defined as any psychotropic or anti-epileptic medication taken by the subject for more than 28 days during the 6 months prior to first dose of study drug (Day 1), which was subsequently discontinued within that same 6 month period.

A concomitant medication is defined as any medication taken 14 days prior to the first dose of study drug (Day 1) and during the study through the Week 13 Follow-up Visit should be recorded with indication, daily dose, and start and stop dates of administration.

Current treatment with no more than 3 prescribed psychotropic medications is allowed. Anti-epileptic medications are permitted and are not counted as psychotropic medications if they are used for treatment of seizures. Anti-epileptics for other indications, such as the treatment of mood disorders, count towards the limit of three permitted medications. Permitted concomitant psychotropic medications must be at a stable dose and dosing regimen for at least 2 weeks prior to the commencement of study medication.

Anti-epileptic medications must be at a stable dose and dosing regimen for 12 weeks prior to Screening and must remain stable during the period between Screening and the commencement of study medication.

7.8 Other Therapeutic Treatments

Behavioral and therapy treatments/interventions must be stable for 4 weeks prior to Screening and must remain stable during the period between Screening and the commencement of study medication, and throughout the study.

7.9 Dietary Guidelines

There are no dietary restrictions. Subjects should follow their usual eating behaviors.

7.10 Lifestyle Guidelines

There no lifestyle restrictions or guidelines associated with this study.

8 STUDY PROCEDURES AND ASSESSMENTS

8.1 Informed Consent

Informed consent must be obtained prior to the subject entering into the study and before any protocol-directed procedures are performed.

8.2 Medical /Surgical History

Medical/Surgical history will be recorded at the Screening Visit as specified in Section 10.2. Subject eligibility will be evaluated to determine all inclusion and none of the exclusion criteria are met. The Investigator will inquire with the subject on Period 1/Day 1 (Baseline, prior to randomization) to determine if there have been any changes in the subjects's health affecting eligibility or requiring an update to their medical and surgical history.

8.3 Demographics and Social History

Demographics (sex, ethnicity, race) and social history (tobacco, alcohol, and/or drug use) will be recorded at the Screening Visit as specified in the Schedule of Assessments.

8.4 Stanford Binet Intelligence Scale

The Stanford Binet Intelligence Scale will be administered at Screening in order to support characterization of the study cohort and to determine whether cognitive and behavioral responses are dependent on baseline level of cognitive functioning. The scale will be scored according to the Hessl z-deviation method to prevent floor effects.[6] If the site has performed a Stanford-Binet assessment on a subject in the 6 months prior to screening, the result of that assessment may be used.

8.5 Physical Examination (Full and Abbreviated)

The full physical examination will be conducted at the Screening as specified in the Schedule of Assessments and will include:

- General appearance
- Skin
- Eyes, ears, nose, and throat (EENT)
- Head/neck
- Extremities
- Musculoskeletal examination
- Respiratory examination
- Cardiovascular assessment including rhythm and presence of cardiac abnormalities
- Abdominal examination
- Basic neurologic examination
- Any additional assessments necessary to establish baseline status or evaluate symptoms or adverse experiences

An abbreviated physical examination will be conducted at Period 1/Day 1 (if not the same day as Screening) and at Period 2/Week 12 visit and will include:

- Any changes noted since the initial physical examination
- General appearance
- Skin
- Extremities
- Respiratory examination

- Cardiovascular assessment including rhythm and presence of cardiac abnormalities
- Abdominal examination

Significant findings prior to the start of dosing will be recorded on the Medical History page of the CRF. Only changes from baseline physical examination findings that meet the definition of a treatment-emergent AE will be recorded as an AE.

8.6 Height, Weight, Body Mass Index

Body weight and height are to be measured at Screening. Body weight will be repeated at the Period 1/Week12 and Period 2/Week 12 visits as specified in the Schedule of Assessments. Subjects will wear indoor clothing and remove their shoes prior to the measurements. Body Mass Index (BMI) will be calculated and recorded.

8.7 Vital Signs

Vital signs (pulse rate, blood pressure, respiration rate, and temperature) will be measured at Screening and during the Treatment Visits, as specified in the Schedule of Assessments.

Blood pressure and heart rate will be measured using a calibrated, fully automated machine with a cuff that is appropriate to the size of the upper arm. If a fully automated machine is not available, blood pressure may be measured manually. For each subject, measurement on the same arm (right or left) using the same method (either automated or manual) should occur throughout the study. All blood pressure measurements should be obtained with the subject in the sitting position, after the subject is at rest for 5 minutes.

Additional vital signs measurements may be performed as deemed medically necessary by the Investigator.

8.8 Electrocardiograms

Single 12-lead ECGs will be performed at every clinic visit except the Week 1 visit during each Period as specified in the Schedule of Assessments.

ECGs should be obtained after the subject has been resting comfortably in a supine position for approximately 5 minutes. ECGs will be collected prior to blood collection whenever possible.

ECGs will be assessed locally for eligibility determinations and safety. All ECG traces will be kept as source data.

For purposes of qualifying any given subject for study participation based on Inclusion/Exclusion Criteria, clinically significant out-of-range results may be repeated once.

8.9 Laboratory Assessments

Appendix 16.2 Clinical Laboratory Analytes provides a list of the clinical laboratory tests that will be performed according to the collection schedule provided in the Schedule of Assessments.

Blood samples for chemistry and hematology and urine samples will be collected in accordance with acceptable laboratory procedures.

Since a single-site study, an accredited local laboratory will be used to analyze the clinical laboratory samples.

8.10 Management of Abnormal Clinical Laboratory Tests

It is the Investigator's responsibility to review the results of all lab tests as they become available and to document their review by signing and dating the lab report. For each lab test outside of the laboratory normal range, the Investigator must ascertain if this is a clinically significant change from baseline for the individual subject. This determination does not necessarily need to be made the first time an abnormal lab is observed. The Investigator may repeat the lab test or request additional tests to verify the results of the original lab test.

All clinically significant laboratory abnormalities occurring during the study that were not present at baseline should be followed and evaluated with additional tests if necessary until determination of the underlying cause or adverse event resolution.

8.11 Suicidality Assessment

Suicidality will be assessed at Screening and at Weeks 1, 2, 6, and 12 of each period. If a concern is detected, it is the responsibility of the Investigator to refer the subject for further evaluation and treatment.

A straightforward, caregiver-based assessment tool will be used, as more complex, subject-completed instruments are not effective in this patient population. The caregiver will be asked three questions addressing whether the subject has wished for death or to go to sleep and not awaken, whether a suicide attempt has been made, and whether the subject has engaged in non-suicidal, self-injurious behavior since the last visit. If the answer to any of the three questions is "Yes," the Investigator will further evaluate the suicidal risk of the subject.

8.12 Additional Safety Measures

Subjects and caregivers will be instructed to inform the study physician and/or research personnel of any AEs, including significant behavioral changes not within the typical variation for the subject, that occur at any time during the study.

Procedures will be completed as specified in this protocol unless contraindicated due to a reported AE.

If, in the judgement of the Investigator, additional testing is required to assess an adverse event or otherwise clinically significant event, this testing should be discussed first with the sponsor unless needed urgently.

8.13 Exploratory Efficacy Assessments

8.13.1 Descriptions of Efficacy Assessment Instruments

Several assessment tools for cognitive and behavioral measures will be utilized in this exploratory study. Raw scores (not standardized) will be collected for all assessments, except for the Vineland -3 composite where age-equivalent scores will also be collected.

8.13.1.1 NIH-TCB

The NIH-TCB, a component of the NIH Toolbox for Assessment of Neurological and Behavioral Function, was developed by a team of more than 300 scientists from nearly 100 academic institutions as part of the NIH Blueprint for Neuroscience Research to standardize evaluations in specific clinical populations for investigations of neurological development and change, disease recovery, and therapeutic interventions.[7,8,9] The NIH-TCB is a battery of extensively validated computer-administered cognitive tests with utility across childhood and adolescence, early adulthood, and old age. The NIH-TCB assessments were designed to minimize floor and ceiling effects which often are present in testing batteries designed for the general population. Downward extensions of many of the NIH-TCB tests have been created to allow feasibility down to a mental age of 3. Therefore, there is good reason to believe that the assessments are appropriate for individuals with intellectual disabilities. In fact, a pilot study has been published showing good feasibility and test-retest reliability in FXS and Down syndrome, and the instructions and testing protocol has been adjusted (without changing construct validity) for individuals with intellectual disabilities based on issues identified in the pilot study.[10] Further validation of the adjusted measure is ongoing.

The NIH-TCB includes 7 evaluations: Dimensional change card sort test (DCCS, measure of cognitive flexibility), Flanker inhibitory control and attention test (measure of inhibition and visual attention), Picture sequence memory test (measures episodic memory), List sorting working memory test (measures immediate recall and sequencing of different visually and orally presented stimuli), Pattern comparison test (measures processing speed), Oral reading recognition test (measures recognition of letters and words), Picture vocabulary test (measures receptive vocabulary).

8.13.1.2 Test of Attentional Performance (KiTAP)

The KiTAP is a computerized executive function battery that consists of eight nonverbal subtests measuring different basal as well as higher-order components of attention and executive functioning.[11] Each subtest can be assessed separately. Four subtests will be utilized in this study: alertness, distractibility, go-nogo, and flexibility. To ensure optimal motivation and compliance, all subtests are designed in the form of short games with an enchanted castle theme. The four subtests chosen for use in this study have been utilized in FXS populations and shown to be feasible, reproducible with evidence of clinical validity when scores are compared with standardized behavioral questionnaires addressing relevant functions. The KiTAP has shown evidence of response to pharmacological intervention in clinical trials of AFQ056 and metadoxine in FXS.[12,13]

8.13.1.3 Clinical Global Impression

The CGI-I (and CGI-S) are gold standard global measures of severity and change with treatment in placebo-controlled pharmacotherapy trials in developmental disabilities and have been used extensively in prior clinical trials in FXS. [14,15]

8.13.1.3.1 Clinical Global Impression Severity: Investigator Rated (CGI-S)

The Clinical Global Impression−Severity (CGI-S) is a global measure to provide a clinical judgment of a subject's overall condition based on a trained clinician's assessment of cognition, behavior and activities of daily living.[16] The assessment of severity will be made with a 7-point scale: 1, not ill; 2, very mild; 3, mild; 4, moderate; 5, marked; 6, severe; 7, extremely severe. The comparison will be made with respect to the overall experience of the clinician with individuals of the same age and sex.

8.13.1.3.2 Clinical Global Impression Improvement: Investigator –Rated (CGI-I)

The Clinical Global Impression – Improvement (CGI-I) assessment is a 7-point scale that requires the clinician to assess how much the subject's illness has improved or worsened relative to a baseline state at the beginning of the intervention, and rated as: 1, very much improved; 2, much improved; 3, minimally improved; 4, no change; 5, minimally worse; 6, much worse; or 7, very much worse. [16]

Completion of this scale requires the clinician to rate how much the subject's illness (including behavioral and adaptive functioning) has improved or worsened relative to a baseline state. The CGI-I must be administered by the same rater for a given subject at all applicable visits throughout the trial.

8.13.1.4 VAS Rating Scale

In an attempt to measure the level of behavioral difficulty experienced by the parent/caregiver with respect to the child with FXS, the VAS will allow parents to mark on a visual line measuring 10 cm with one side marked "worst behavior" and the other side marked "best behavior." The caregiver will rate the subject's behavior with respect to three domains: daily functioning, anxiety/irritability and language; with guidance form the investigator, the caregiver will select a behavior within each domain that will be assessed throughout the study. The caregiver marks are measured in milliimeter distance so that improvements or worsening of behavior over the treatment period can be evaluated. The VAS has been successfully used in multiple clinical trials conducted in the FXS population.[17] The caregiver completing the assessment should remain the same at all applicable visits throughout the trial.

8.13.1.5 Aberrant Behavior Checklist (ABC)

The Aberrant Behavior Checklist- Community Edition (ABC) is a 58-item parent/caregiver rating scale used to assess behaviors across five dimensions or subscales: irritability, hyperactivity, lethargy/withdrawal, stereotypy, and inappropriate speech.[18] Items are evaluated on a four-point Likert scale ranging from 0 (not at all a problem) to 3 (the problem is severe in degree). This scale has been used extensively in FXS in clinical trials and other projects. The ABC will be scored using the FXS-specific factoring system (ABC-FX). The caregiver completing the assessment should remain the same at all applicable visits throughout the trial.

8.13.1.6 Anxiety Depression and Mood Scale (ADAMS)

The ADAMS (Anxiety, Depression, and Mood Scale) is a 28-item behavior-based informant instrument rated by the parent/caregiver and designed to assess anxiety, depression and mood disorders in individuals with intellectual disability.[19] Items are rated on a scale of 0 ("behavior has not occurred, or is not a problem") to 3 ("behavior occurs a lot, or is a severe problem"). The scale is composed of 5 factors which address: Manic/Hyperactive Behavior, Depressed Mood, Social Avoidance, General Anxiety and Obsessive/Compulsive Behavior. A caregiver identified upon enrollment of subject should have intimate knowledge of the subject's situation and level of impairment to be able to provide accurate information as required to complete the ADAMS. The caregiver completing the assessment should remain the same at all applicable visits throughout the trial.

8.13.1.7 Vineland-3 Rating Scale

The Vineland Adaptive Behavior Scales, Third Edition (Vineland-3)[20] assesses adaptive behavior in five domains, each with subdomains. The following three domains will be assessed in this study, as most applicable to the FXS population:

1. Communication
    - Subdomains: Receptive, Expressive, Written
2. Daily Living Skills
    - Subdomains: Personal, Domestic, Community
3. Socialization
    - Subdomains: Interpersonal Relationships, Play and Leisure, Coping Skills

The Vineland-3 will be administered as a standardized interview to a parent/caregiver by trained study staff trained and authorized to perform the interview. The Vineland-3 must be administered by the same rater for a given subject at all applicable visits throughout the trial. The caregiver completing the assessment with the study staff member should also remain the same at all applicable visits throughout the trial. If the site has performed a Vineland-3 assessment on the subject in the 6 months prior to screening, the results of that assessment may be used for the baseline (Day 1) assessment in this study.

8.13.1.8 Event-Related Potentials (ERP)

Event-Related Potentials (ERPs) enable extraction of neural responses associated with specific sensory, cognitive, or motor events from an overall EEG. [21,22]Auditory stimuli are presented and EEG events assessed in relation to timing of the stimuli. For this study, an ERP protocol developed to measure auditory habituation to trains of repeated stimuli, resting state delta power, and CHIRP responses in FXS will be used.[23] This protocol has shown feasibility and clinical validity based on comparison to sensory hypersensitivity on the Sensory Profile in FXS. The EEGs will be read by [redacted] who developed the protocol and has extensive experience in ERP evaluation.

8.13.1.9 Eye Tracking

Eye tracking has been successfully used to assess social gaze in FXS.[24] Testing will be conducted in a quiet room with the lights turned off. The eye tracker (Tobii) will be calibrated for each subject at the beginning of each session. Following calibration, subjects will view pictures shown on the screen. Each assessment begins with presentation of a scrambled face image for 1 s followed immediately by its matched face image for 3 s. An inter-trial interval (ITI) containing a uniform grey screen is shown for 0.5, 1, or 2 s, randomly determined. The order of face presentation is pseudorandomized and each eye tracking session lasts approximately 6 min. Measurements include looking time to the eye region of interest (ROI), and number of fixations to the eye ROI, as well as pupil dilatation by pupilometry. These measurements have been shown to have good test-retest reproducibility. All measurements are analyzed offline by a trained specialist.

8.13.2 Timing of Efficacy Assessments

8.13.2.1 Baseline (Period 1/Day 1)

The following exploratory efficacy assessments will be performed at Baseline (Period 1/ Day 1, prior to randomization):

- NIH Toolbox Cognitive Battery for Intellectual Disabilities (NIH-TCB)
- Test of Attentional Performance (KiTAP)
- Clinical Global Impression Severity – Investigator rated (CGI-S)
- VAS Rating Scale using patient-specific behavioral anchors
- Aberrant Behavior Checklist (ABC)
- Anxiety, Depression, and Mood Scale (ADAMS)
- Vineland-3 Rating Scale
- Event-Related Potentials (ERP)
- Eye Tracking

8.13.2.2 Week 6 (Periods 1 and 2)

The following cognitive tests will be assessed at Week 6 during each Period:

- NIH Toolbox Cognitive Battery for Intellectual Disabilities (NIH-TCB)
- Test of Attentional Performance (KiTAP)
- Clinical Global Impression Severity – Investigator rated (CGI-S)
- Clinical Global Impression Improvement – Investigator rated (CGI-I)
- Visual Analog Scale (VAS) assessment of patient-specific behavioral anchors)

8.13.2.3 Week 12 (Periods 1 and 2)

The full battery of cognitive tests will be performed at Week 12 during each Period:

- NIH Toolbox Cognitive Battery for Intellectual Disabilities (NIH-TCB)
- Test of Attentional Performance (KiTAP)
- Clinical Global Impression Severity – Investigator rated (CGI-S)

- Clinical Global Impression Improvement – Investigator rated (CGI-I)
- Visual Analog Scale (VAS) assessment of patient-specific behavioral anchors
- Aberrant Behavior Checklist (ABC)
- Anxiety, Depression, and Mood Scale (ADAMS)
- Event-Related Potentials (ERP)
- Eye Tracking
- Vineland-3 Rating Scale

8.14 Pharmacokinetic Assessment

8.14.1 Plasma Pharmacokinetic Samples

Blood samples for BPN14770 concentrations will be drawn at screening and during the clinic visit at Week12 during each Period. Samples will be drawn at the time of the clinic visit, with documentation of time of day testing was performed, and time of day of most recent dose of study medication.

Pharmacokinetic samples will be retained and may also be utilized subsequently for biomarker assessment. Blood samples (1 × 4 mL) will be collected in Vacutainer tubes containing K_2-EDTA as a preservative.

8.14.2 Processing and Shipment of Pharmacokinetic Samples

Blood samples will be kept on wet ice from the time of collection and throughout processing until frozen.

Blood samples will be centrifuged at 1500 x g (gravity) for 10 minutes at 4°C. The resulting plasma samples will be harvested and transferred into appropriately labeled polypropylene screw-cap tubes. Pharmacokinetic samples will be placed in a freezer at -80°C within 60 minutes of the blood draw. Samples will remain frozen until assayed. A more detailed description of plasma sample preparation requirements may be provided by the analytical laboratory and will supersede those provided in this protocol. Appropriate documentation will be placed in the study master file at the Clinical Site.

The samples will be transferred to the analytical laboratory after completion of the study or at mutually agreed upon time points during the clinical conduct of the study. Prior to shipment, the samples will be appropriately packed in a Styrofoam cooler containing dry ice. Sufficient dry ice will be added to ensure that the samples will remain frozen for at least 72 hours for remote shipments. The shipment will be accompanied by documentation containing the following information: name of the study drug product, protocol number, number of subjects, and number of samples included in the shipment.

All frozen pharmacokinetic samples will be transferred with accompanying documentation to:

Senior Research Analyst/Investigator
CMIC, Inc.
2860 Forbs Avenue
Hoffman Estates, IL 60192-3702
Telephone: 847.945.0407

Pharmacokinetic samples at CMIC, Inc. will be stored in a freezer at -80°C.

8.14.3 Analytical Method

Plasma samples will be analyzed for BPN14770 using a validated assay.[25]

Samples will only be analyzed for subjects whose treatment included administration of BPN14770. Samples will not be analyzed when matching placebo for BPN14770 was administered.

8.14.4 Plasma Pharmacokinetic Parameters

BPN14770 plasma levels will be presented in tabular form by Period at Week 12, and a listing of all subjects' data will be provided.

8.15 Biomarker Assessments

Biomarker samples will be collected concurrently with the pharmacokinetic samples at screening and at the Week 12 visits at the end of each Period. Samples will be processed in the same manner as the pharmacokinetic samples (see Section 8.14.2). Biomarker samples will be shipped to the sponsor for analysis.

9 EVALUATION AND REPORTING OF ADVERSE EVENTS

9.1 Adverse Events

An adverse event is defined as any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product, which does not necessarily have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and/or unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational medicinal product, whether or not related to the investigational medicinal product. All adverse events, including observed or volunteered problems, complaints, or symptoms, are to be recorded on the appropriate CRF.

Adverse events, which include abnormal and clinically significant clinical laboratory test variables, will be monitored and documented from the time of first dose until study participation is complete (the Follow-up Visit). Subjects should be instructed to report any adverse event that they experience to the Investigator. Any medical condition already present prior to the subject taking the first dose of study treatment will be reported in the medical history. Should any changes in the subject's health occur between consent and first dose of study treatment, , the Investigator will make corresponding updates to medical history.

Beginning with the first dose of study drug, the Investigator will make an assessment for adverse events at each visit and record all adverse events, non-serious and serious, on the appropriate adverse event CRF. Any pre-existing medical condition or signs or symptoms that change adversely in severity, frequency, or seriousness (outside of the range of typical variation of the symptom over time in the past for the patient) after the subject takes the first dose of study drug and through the Follow-up Visit should be reported as an adverse event.

Wherever possible, a specific disease or syndrome rather than individual associated signs and symptoms should be identified by the Investigator and recorded on the CRF. However, if an observed or reported sign or symptom is not considered a component of a specific disease or syndrome by the Investigator, it should be recorded as a separate adverse event on the CRF. Additionally, the condition that led to a medical or surgical procedure (e.g., surgery, endoscopy, tooth extraction, or transfusion) should be recorded as an adverse event, not the procedure. Concomitant procedures should be recorded as such on the appropriate CRF.

Clinically significant abnormal laboratory values or other examinations (e.g., ECG) that are detected after the first dose of study drug and worsen during the study should be reported as adverse events. An abnormal laboratory result that is not verified by repeat testing does not necessitate reporting as an adverse event. The Investigator will exercise his or her medical, scientific, and clinical judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant. Clinically significant abnormal laboratory values occurring during the clinical study will be followed until repeat tests return to normal, stabilize, or are no longer clinically significant. Any abnormal test that is determined to be an error does not require reporting as an adverse event.

Treatment of any adverse reactions will be evaluated and managed by a physician.

9.2 Definitions

9.2.1 Adverse Drug Reaction

For adverse events with a causal relationship to study drug, follow-up by the Investigator will be required until the event or its sequelae resolve or stabilize to a level acceptable to the Investigator.

9.2.2 Unexpected Adverse Event/ Unexpected Adverse Drug Reaction

An unexpected AE/unexpected suspected adverse reaction is an AE or suspected adverse reaction that is not listed in the Investigator Brochure or is not listed at the specificity or severity that has been observed; or, if an Investigator Brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended.

9.2.3 Serious Adverse Events

An adverse event or adverse reaction is considered serious if, in the view of either the Investigator or Sponsor, it results in any of the following outcomes:

- Death;
- A life-threatening adverse event;
- NOTE: An adverse event or adverse reaction is considered “life-threatening” if, in view of either the Investigator or Sponsor, its occurrence places the subject at immediate risk of death. It does not include an event that, had it occurred in a more severe form, might have caused death.

- Requires hospitalization or prolongation of existing hospitalizations;
  NOTE: Any hospital admission with at least one overnight stay will be considered an inpatient hospitalization. An emergency room visit without hospital admission will not be recorded as a SAE under this criterion, nor will hospitalization for a procedure scheduled or planned before signing of informed consent. However, unexpected complications and/or prolongation of hospitalization that occur during elective surgery should be recorded as adverse events and assessed for seriousness. Admission to the hospital for social or situational reasons (i.e., no place to stay, live too far away to come for hospital visits) will not be considered inpatient hospitalizations.
- A persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions;
- A congenital anomaly/birth defect; or
- An important medical event.
  NOTE: Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalizations, or the development of drug dependency or drug abuse.

9.3 Assessment of Adverse Events by the Investigator

9.3.1 Causality/Relatedness

The relationship of an adverse event to the administration of the study drug is to be assessed by the Investigator according to the following definitions:

Not Related (unlikely related, unrelated, not related, no relation) – The time course between the administration of study drug and the occurrence or worsening of the adverse event rules out a causal relationship and/or another cause (e.g., medical history, concomitant drugs, therapies, and complications) is suspected.

Related (possibly related, related) – The time course between the administration of study drug and the occurrence or worsening of the adverse event is consistent with a causal relationship and no other cause (e.g., medical history, concomitant drugs, therapies, and complications) can be identified.

The definition implies a reasonable possibility of a causal relationship between the event and the study drug. This means that there are facts (evidence) or arguments to suggest a causal relationship. The following factors should also be considered:

- The temporal sequence from study drug administration - The event should occur after the study drug is given. The length of time from study drug exposure to event should be evaluated in the clinical context of the event.

- Underlying, concomitant diseases (medical history) - Each report should be evaluated in the context of the natural history and course of the disease being treated and any other disease the subject may have.

- Concomitant drug - The other drugs the subject is taking or the treatment the subject receives should be examined to determine whether any of them might be recognized to cause the event in question.

- Known response pattern for this class of study drug - Clinical and/or preclinical data may indicate whether a particular response is likely to be a class effect.

- Exposure to physical and/or mental stresses - The exposure to stress might induce adverse changes in the recipient and provide a logical and better explanation for the event.

- The pharmacology and PK of the study drug - The known pharmacologic properties (absorption, distribution, metabolism, and excretion) of the study drug should be considered.

9.3.2 Severity

The Investigator is responsible for assessing the severity (intensity) of each adverse event as mild, moderate, or severe according to the following definitions:

Mild – An event that is easily tolerated and generally not interfering with normal daily activities.

Moderate – An event that is sufficiently discomforting to interfere with normal daily activities.

Severe – An event that is incapacitating with inability to work or perform normal daily activities.

It should be noted that a severe AE need not be serious and that a serious adverse event (SAE) need not, by definition, be severe.

9.3.3 Adverse Event Monitoring and Follow-up

Subjects who experience AEs will be monitored with relevant clinical assessments and laboratory tests, as determined by the Investigator. In accordance with good medical practice, all AEs must be followed to satisfactory resolution or stabilization of the event(s), or, if a chronic condition, until fully characterized. All follow-up results are to be reported to the Sponsor. Any actions taken and follow-up results must be recorded either on the appropriate page of the CRF or in a follow-up letter to the Sponsor, as well as in the subject's source documentation. Follow-up laboratory results should be filed with the subject's source documentation.

For all AEs that require the subject to be discontinued from the study, relevant clinical assessments and laboratory tests must be repeated at appropriate intervals until final resolution or stabilization of the event(s).

Subjects who have an ongoing SAE at study completion or study withdrawal must be followed until the event resolves or the event or sequela stabilizes. Subjects who have non-serious AEs that are ongoing at study completion or study withdrawal and are considered at least possibly

related to study treatment must be followed until resolution or for 30 days after the last dose of study drug, whichever comes first.

9.3.4 Reporting Serious Adverse Events

From the time of informed consent until 30 days following the last administration of study drug the Investigator or designee will notify the sponsor contact within 24 hours after the SAE detection, observation, or report of occurrence (regardless of the relationship to study treatment). The Sponsor contact information for SAE reporting is provided below:

[redacted]
Tetra Discovery Partners, Inc.
Email: [redacted]
Office: [redacted]
24-hour contact number (Cell): [redacted]

and

[redacted]
Consulting for Tetra Discovery Partners, Inc.
Email: [redacted]
Office: [redacted]
24-hour contact number (Cell): [redacted]

The SAE report must contain the following information:

- Study name/number (for EU also the EudraCT number)
- Study drug
- Investigator details (name, phone, fax, e-mail)
- Subject number (randomization number)
- Subject initials
- Subject demographics (age, date of birth, sex, weight)
- Clinical event
  - Description
  - Date of onset
  - Treatment (drug, dose, dosage form)
  - AE relationship to study drug
  - Action taken regarding study drug in direct relationship to the AE
- If the AE was fatal or life-threatening
- Cause of death (whether or not the death was related to study drug)
- Autopsy findings (if available)

The Investigator must continue to follow the subject until the SAE has subsided or until the condition becomes chronic in nature, stabilizes (in the case of persistent impairment), or the subject dies. Within 24 hours of receipt of follow-up information, the Investigator must update the Sponsor and submit any supporting documentation (e.g., subject discharge summary or autopsy reports).

Any new SAE that occurs within one month after the study period and is considered to be possibly related to the investigational product should be recorded and reported immediately to the Sponsor.

All serious event reporting will adhere to Code of Federal Regulations (CFR), specifically 21 CFR 312.32 (7- and 15-day alerts) for Investigational New Drugs (IND). The clinical research site will be responsible for reporting SAEs to the Institutional Review Board (IRB) per FDA regulations. The Sponsor will be responsible for reporting and processing any SAEs to the FDA or other applicable regulatory agency.

10 STUDY PROCEDURES AND ASSESSMENTS BY VISIT

10.1 Schedule of Assessments

The study timetable in Section 16.1 shows the schedule of planned study procedures. Every effort should be made to adhere to this procedure schedule.

10.2 Screening (Day -28 to Day -1)

The study will consist of a Screening period of 28 days or less. Screening and Baseline visits may be combined.

Each potential study participant will have the following assessments completed by the Investigator or designee up to 28 days prior to the first dose of study medication:

- Obtain informed consent;
- Obtain medical/surgical history;
- Obtain demographics and social history (tobacco, alcohol, and/or drug use);
- Obtain prior/concomitant medication use;
- Conduct full physical exam;
- Obtain height and weight;
- Obtain vital signs;
- Conduct single ECG;
- Draw fasting blood samples for Chemistry and Hematology and for PK and biomarkers (following a minimum 8-hour fast);
- Collect urine for safety urinalysis;
- Assess suicidality;
- Complete Stanford-Binet assessment;
    - If the site has performed a Stanford-Binet test on the subject in the previous 6 months, the results of that assessment may be used for Screening in this study.
- Evaluate inclusion and exclusion criteria.

10.3 Baseline (Period 1/Day 1)

Subjects will return to the clinic for the Period 1/Day 1 Visit (Baseline) following successful screening. If clinic processes allow, the Screening and Baseline visits may be combined; in this case, it is not necessary to repeat vital signs nor to conduct the abbreviated physical exam.

10.3.1 Prior to Randomization

The following assessments will be completed:

- Conduct abbreviated physical exam (not to be repeated if the Screening and Baseline visits occur on the same day);
- Obtain vital signs (not to be repeated if the Screening and Baseline visits occur on the same day);
- Record any updates to medical/surgical history;
- Record any updates to prior/concomitant medication use;
- Re-evaluate inclusion and exclusion criteria based on preceding activities;
- Verify continued eligibility;
- Complete baseline cognitive assessments:
    - NIH Toolbox Cognitive Battery for Intellectual Disabilities (NIH-TCB)
    - Test of Attentional Performance (KiTAP)
    - Clinical Global Impression Severity – Investigator rated (CGI-S)
    - Visual Analog Scale (VAS) assessment of patient-specific behavioral anchors
    - Aberrant Behavior Checklist (ABC)
    - Anxiety, Depression, and Mood Scale (ADAMS)
    - Vineland-3 Rating Scale
        - If the site has performed a Vineland-3 assessment on the subject in the 6 months prior to screening, the results of that assessment may be used for baseline (Day 1) in this study.
    - Event-Related Potentials (ERP)
    - Eye Tracking

If subject remains eligible for the study:

- Randomize subject;
- Dispense study medication.

10.3.2 Week 1 (Periods 1 and 2)

The following assessments will be completed at the Week 1 Visit during each Period:

- Telephone call to:
    - Assess suicidality;
    - Record any updates to concomitant medication use , and
    - Assess and record any adverse events.

10.3.3 Week 2 (Periods 1 and 2)

The following assessments will be completed at the Week 2 Visit during each Period:

- Collect vital signs;
- Conduct ECG (single);
- Draw fasting blood samples for Chemistry and Hematology (following a minimum 8-hour fast);

- Collect study medication and record pill count remaining in bottle to assess dosing compliance;
- Assess suicidality;
- Record any updates to concomitant medication use; and
- Assess and record any adverse events.

10.3.4 Week 6 (Periods 1 and 2)

The following assessments will be completed at the Week 4 Visit during Periods 1 and 2:

- Collect vital signs;
- Assess suicidality;
- Conduct the following cognitive tests:
    - NIH Toolbox Cognitive Battery for Intellectual Disabilities (NIH-TCB)
    - Test of Attentional Performance (KiTAP)
    - Clinical Global Impression Severity – Investigator rated (CGI-S)
    - Clinical Global Impression Improvement – Investigator rated (CGI-I)
    - Visual Analog Scale (VAS) assessment of patient-specific behavioral anchors
- Collect study medication and record pill count remaining in bottle to assess dosing compliance;
- Dispense study medication;
- Record any updates to concomitant medication use; and
- Assess and record any adverse events.

10.3.5 Week 12 (Periods 1 and 2)

The following assessments will be completed at the Week 12 Visit during Periods 1 and 2:

- Collect weight;
- Collect vital signs;
- Conduct ECG (single);
- Draw fasting blood samples for Chemistry and Hematology (following a minimum 8-hour fast);
- Collect urine for safety urinalysis;
- Assess suicidality;
- Draw blood sample for pharmacokinetic analyses;
- Conduct the following cognitive tests:
    - NIH Toolbox Cognitive Battery for Intellectual Disabilities (NIH-TCB)
    - Test of Attentional Performance (KiTAP)
    - Clinical Global Impression Severity – Investigator rated (CGI-S)
    - Clinical Global Impression Improvement – Investigator rated (CGI-I)
    - Visual Analog Scale (VAS) assessment of patient-specific behavioral anchors
    - Aberrant Behavior Checklist (ABC)
    - Anxiety, Depression, and Mood Scale (ADAMS)
    - Vineland-3 Rating Scale

- Event-Related Potentials (ERP)
- Eye Tracking

- Collect study medication and record pill count remaining in bottle to assess dosing compliance;
- Record any updates to concomitant medication use; and
- Assess and record any adverse events.

Period 1/Week 12 and Period 2 /Day 1 activities are conducted at the same clinic visit. It is not necessary to repeat assessments of vital signs and ECG.

10.3.6 Week 13 (Period 2, Final Contact at End of Study)

- Telephone call to:
  - Record any updates to concomitant medication use ,
  - Assess and record any adverse events, and
  - Record final subject disposition.

10.3.7 Early Termination

It is hoped that all subjects can be followed through to the conclusion of the study at Period 2/Week 13. However, if an early termination occurs, the Week 12 procedures should be completed at the time of the subject's withdrawal (if possible) and an explanation provided as to why the subject is withdrawing or being withdrawn from the study. Procedures to be completed early termination:

- Collect weight;
- Collect vital signs;
- Conduct ECG (single);
- Draw fasting blood samples for Chemistry and Hematology (following a minimum 8-hour fast);
- Collect urine for safety urinalysis;
- Assess suicidality;
- Draw blood sample for pharmacokinetic analyses;
- Conduct the following cognitive tests:
  - NIH Toolbox Cognitive Battery for Intellectual Disabilities (NIH-TCB)
  - Test of Attentional Performance (KiTAP)
  - Clinical Global Impression Severity – Investigator rated (CGI-S)
  - Clinical Global Impression Improvement – Investigator rated (CGI-I)
  - Visual Analog Scale (VAS) assessment of patient-specific behavioral anchors
  - Aberrant Behavior Checklist (ABC)
  - Anxiety, Depression, and Mood Scale (ADAMS)
  - Vineland-3 Rating Scale
  - Event-Related Potentials (ERP)
  - Eye Tracking

- Collect study medication and record pill count remaining in bottle to assess dosing compliance;
- Record any updates to concomitant medication use;
- Assess and record and adverse events; and
- Record final subject disposition.

11 PLANNED STATISTICAL METHODS

11.1 Sample Size

Given the exploratory nature of this study, a definitive sample size calculation is not possible. However, for the sake of providing an evaluation of the potential power to detect a meaningful difference between treatments, a crossover study having no carryover effects with 80% power, a 5% alpha level, and a common standard deviation of 3 units, a sample size of 30 evaluable patients (15 per sequence group) will allow detection of an effect size of approximately 2.25 units on the ABC Total Score outcome measure. If carryover effects or excessive dropouts allow only for a first Period analysis, a sample size of 15 per group during the first period would allow for detection of an effect size of 3.19 units.

11.2 Demographics

Summary statistics [number (n), mean, standard deviation (SD), minimum, median, and maximum] will be tabulated for the observed values for all continuous demographic parameters. Frequencies and percentages will be tabulated for categorical data.

11.3 Analysis Populations

The primary efficacy population will be the intent to treat (ITT) efficacy population, which will include all randomized subjects who received at least one dose of treatment and returned for at least one follow-up visit. The completers population (CP), defined as all randomized subjects who completed both treatment periods with no significant protocol violations, will be used to evaluate the robustness of the ITT results.

The Safety population will include all randomized subjects who received at least one dose of study treatment.

11.4 Efficacy: Cognitive Testing Analysis

All cognitive parameters will be summarized at each time point collected and standard descriptive statistics provided. Baseline measurements are defined as those obtained on Day 1/Period 1 prior to receipt of any study medication. For cognitive parameters measured at baseline, change from baseline will also be calculated and standard descriptive statistics provided for each time point. Linear mixed models will be used to explore potential treatment effects.

11.5 Safety Analysis

Safety analysis will be based on all subjects receiving at least one dose of study medication. Treatment emergent AEs will be summarized based on the frequency of AEs and their severity for all dosed subjects. Adverse Events (AEs), including clinically meaningful laboratory abnormalities and significant behavioral changes, will collected. AE severity and relatedness to treatment will be assessed. AEs will be tabulated for placebo and BPN14770 treatments using preferred terms and system organ classes. Summaries will also be provided by severity and relationship to treatment. Serious Adverse Events (SAEs) will be summarized separately, in a manner similar to that used for Adverse Events.

Overall safety and tolerability will be assessed by clinical and/or statistical review of all safety parameters including adverse experiences, laboratory values, vital signs, physical exams, and ECG data. General clinical and laboratory adverse events will be presented in frequency tables. Summary statistics (n, mean, SD, median, and range (minimum, maximum) will be tabulated for the observed values and the change (or percent change) from baseline for all continuous parameters for vital signs, ECG, and laboratory tests.

11.6 Pharmacokinetic Analysis

BPN14770 plasma levels will be presented in listing form by Period, Treatment and subject.

11.7 Biomarker Analysis

Biomarker analysis will be exploratory and will follow reporting of the clinical study, as the biomarker analyses to be conducted may be partially determined by clinical results observed in the study.

12 DATA MANAGEMENT

12.1 Data Handling

Data will be recorded at the site on CRFs and reviewed by the CRA during monitoring visits. The CRA will verify data recorded in the EDC system with source documents. All corrections or changes made to any study data must be appropriately tracked in an audit trail in the EDC system. A CRF will be considered complete when all missing, incorrect, and/or inconsistent data has been accounted for.

12.2 Computer Systems

Data will be collected and processed using a validated EDC system. The system and procedures are designed in compliance with Title 21 of the Code of Federal Regulations (21 CFR Part 11).

12.3 Data Entry

Data must be recorded using the EDC system as the study is in progress. All site personnel must log into the system using their secure user name and password in order to enter, review, or

correct study data. These procedures must comply with 21 CFR Part 11 and other appropriate international regulations. All passwords will be strictly confidential.

The Investigator has ultimate responsibility for the accuracy, authenticity, and timely collection and reporting of all clinical, safety, and laboratory data entered on the CRFs and any other data collection forms.

12.4 Data Validation

Validation checks programmed within the EDC system, as well as supplemental validation performed via review of the downloaded data, will be applied to the data in order to ensure accurate, consistent, and reliable data. Data identified as erroneous, or data that are missing, will be referred to the investigative site for resolution through data queries.

The CRFs must be reviewed and signed by the Investigator.

13 INVESTIGATOR REQUIREMENTS AND QUALITY CONTROL

13.1 Ethical Conduct of the Study

The Investigator agrees, when signing the protocol, to adhere to the instructions and procedures that are described in the protocol and to conduct the study in accordance with the CFRs (21 CFR § 11, 50, 54, 56, and 312 Subpart D), which originate from the ethical principles laid down in the current revision of the Declaration of Helsinki, Good Clinical Practice (GCP), and the policies and procedures as outlined by the ethical requirements for IRB review and informed consent form.

The Investigator agrees to allow monitoring and auditing of all essential clinical study documents by the Sponsor or its authorized representatives and inspection by the FDA or other appropriate regulatory authorities. Monitoring and auditing visits by the Sponsor will be scheduled with the appropriate staff at mutually agreeable times periodically throughout the study.

The Investigator will ensure proper implementation and conduct of the trial, including those study-related duties that are delegated to other appropriately qualified individuals. The Investigator will ensure that study staff cooperates with monitoring and audits and will demonstrate due diligence in recruiting and screening study subjects.

For this study and all studies conducted under an IND, the Investigator must sign and return a completed Form FDA 1572, “Statement of Investigator,” to the Sponsor.

13.2 Institutional Review Board (IRB)

Before initiation of the study, the Investigator must obtain approval or favorable opinion of the research protocol, informed consent form, and any advertisement for subject recruitment from an IRB complying with the provisions specified in 21 CFR §56 and applicable pertinent state and federal requirements of each participating location including International Conference on Harmonization (ICH) and GCP guidelines.

Institutional review boards must be constituted according to the applicable laws. It is the responsibility of each investigational site to submit the protocol, Investigators' Brochure, subject informed consent, subject recruitment materials (if applicable), and other documentation as required by the IRB to their IRB for review and approval. A copy of the written approval must be provided to the Sponsor.

Sites must adhere to all requirements stipulated by their respective IRB. This includes notification to the IRB regarding: protocol amendments, updates to the subject informed consent, recruitment materials intended for viewing by subjects, IND Safety Reports, serious and unexpected AEs, reports and updates regarding the ongoing review of the trial at intervals specified by the respective IRB, and submission of final summaries to IRB.

The Investigator must promptly inform his/her IRB of all unexpected SAEs considered possibly related to the treatment or other critical safety information reported by the clinical site.

13.3 Informed Consent

The informed consent form (ICF) and any changes to the ICF made during the course of the study must be agreed to by the Sponsor or designee and the IRB prior to its use and must be in compliance with all ICH GCP, provisions specified in 21 CFR §50, and applicable pertinent state and federal requirements.

The Investigator, or a person delegated the responsibility by the Investigator, must ensure that each study subject (or legally acceptable representative) is fully informed about the nature and objectives of the study and possible risks associated with participation and must ensure that the subject has been informed of his/her rights to privacy. The Investigator or delegate will allow the subject adequate opportunity to read the written informed consent and ask any questions. The Investigator will obtain written informed consent from each subject before any study-specific activity is performed and should document in the source documentation that consent was obtained prior to any study-specific activity. The original signed copy of the ICF must be maintained by the Investigator and is subject to inspection by a representative of the Sponsor, their representatives, auditors, the IRB/EC and/or regulatory agencies. A copy of the signed ICF will be given to the subject.

13.4 Study Monitoring

The role of the study monitor is to verify the rights and well-being of the subjects are protected, the data is accurate, complete, and verifiable from source documents, and the conduct of the study is in compliance with the protocol, Declaration of Helsinki, ICH GCP, and applicable regulatory requirements.

To achieve this objective, the monitor's duties are to aid the Investigator and, at the same time, the Sponsor in the maintenance of complete, legible, well organized and easily retrievable data. Before the enrollment of any subject in this study, the Sponsor or their designee will review with the Investigator and site personnel the following documents: Clinical Protocol, Investigator's Brochure, CRFs and procedures for their completion, informed consent process, management of investigational product, and the procedure for reporting adverse events such as SAEs.

The Investigator will permit the Sponsor or their designee to monitor the study as frequently as deemed necessary to determine that data recording and protocol adherence are satisfactory. During the monitoring visits, information recorded on the CRFs will be verified against source documents and requests for clarification or correction may be made. After the CRF data is entered by the site, the CRA will review the data for safety information, completeness, accuracy, and logical consistency. Computer programs that identify data inconsistencies may be used to help monitor the clinical study. If necessary, requests for clarification or correction will be sent to investigators. The Investigator and his/her staff will be expected to cooperate with the monitor and provide any missing information, whenever possible.

All monitoring activities will be reported and archived. In addition, monitoring visits will be documented at the investigational site by signature and date on the study-specific monitoring log and findings documented in a follow-up letter.

It is important that the Investigator and their relevant personnel are available during the monitoring visits and that sufficient time is devoted to the process.

13.5 Disclosure of Information

It is understood by the Investigator that the information and data included in this protocol is confidential and proprietary to the Sponsor, Tetra Discovery Partners, Inc. This information may be disclosed to study personnel under the Investigator's supervision and the Institutional Review Board for the purpose of evaluating or conducting the study under the condition that they maintain confidentiality. The contents of this document may not be used in any other clinical trial, disclosed to any other person or entity, and/or published without the prior written permission of the Sponsor. The foregoing shall not apply to disclosure required by any regulations; however, the Investigator will give prompt notice to the Sponsor of any such disclosure.

The medical information and completed original CRFs are the sole property of the Sponsor and should not be made available in any form to third parties, except for authorized representatives of the Sponsor or appropriate regulatory authorities, without written permission from the Sponsor. Information for a specific subject may be provided to the subject, caregiver, and/or guardian if deemed by the investigator to be helpful in the clinical management of the subject.

13.6 Record Storage and Retention

Records of subjects, source documents, monitoring visit logs, CRFs, inventory of study product, regulatory documents, and other Sponsor correspondence pertaining to the study must be kept in the appropriate study files at the site. Source data is defined as all information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical study necessary for the evaluation and reconstruction of the clinical study. Source data are contained in source documents (original records or certified copies). These records will be retained in a secure file for the period as set forth in the Clinical Study Agreement. Prior to transfer or destruction of these records, the Sponsor must be notified in writing and be given the opportunity to further store such records.

To enable evaluations and/or audits from regulatory authorities or the Sponsor, the Investigator will keep records, including the identity of all participating subjects (sufficient information to link records, e.g., CRFs and hospital records), all original signed ICFs, copies of all CRFs, SAE forms, source documents, and detailed records of treatment disposition. The records should be retained by the Investigator according to specifications in the ICH guidelines, local regulations, or as specified in the Clinical Study Agreement, whichever is longer. **The Investigator must obtain written permission from the Sponsor before disposing of any records, even if retention requirements have been met.**

If the Investigator relocates, retires, or for any reason withdraws from the study, the Sponsor should be prospectively notified. The study records must be transferred to an acceptable designee, such as another Investigator, another institution, or to the Sponsor.

13.7 Protocol Amendments and Deviations

Any amendments to the study protocol will be communicated to the Investigator by the Sponsor or authorized representative. All protocol amendments will undergo the same review and approval process as the original protocol. A protocol amendment may be implemented only after it has been approved by the IRB, unless immediate implementation of the change is necessary for subject safety. In this case, the situation must be documented and reported to the Sponsor immediately and to the IRB within 5 working days.

13.8 Access to Source Documentation

The Sponsor (or the Sponsor's authorized representative) must have access to inspect all documents and records that are to be maintained by the Investigator, including, but not limited to, medical records (office, clinic, or hospital) for the subjects in this trial. These regulations also allow the Sponsor's records to be inspected by authorized representatives of the FDA or other regulatory authorities. The names and identities of all research subjects will be kept in strict confidence and will not appear on CRFs or other records that are provided to or retained by the Sponsor (or the Sponsor's authorized representative).

13.9 Financial Disclosure

Clinical Investigators are required to provide financial disclosure information to the Sponsor to permit the Sponsor to fulfill its obligations under 21 CFR §54. In addition, investigators must commit to promptly updating this information if any relevant changes occur during the study and for a period of 1 year after the completion of the study.

For this study, any personnel listed on the FDA 1572 Form will be required to provide financial disclosure information.

13.10 Publication Policy

Manuscript(s) and abstract(s) may only be prepared through cooperation between the Sponsor (or designee) and the study Investigator(s). The Investigator agrees not to publish or publicly present any results of the study without prior written consent of the Sponsor.

14 ADMINISTRATIVE INFORMATION

14.1 Sponsor

Tetra Discovery Partners, Inc.
38 Fulton Street West, Suite 303
Grand Rapids, MI 4953-2684
Phone: 616.635.0937
[redacted]

14.2 Biological Specimens

CMIC, Inc.
2860 Forbs Avenue
Hoffman Estates, Illinois 60192-3702
Phone: 847.645.0407
Phone: 224.293.6800
Email: www.cmic-inc.com

15 REFERENCES

1. Declaration of Helsinki (Fortaleza, October 2013) Seventh revision, 64th World Medical Association General Assembly Meeting.
2. International Conference on Harmonisation (ICH) E6 (R1) Good Clinical Practice Guideline.
3. Burgin AB, Magnusson OT, Singh J, et al. Design of Phosphodiesterase Type 4D (PDE4D) Allosteric Modulators for Cognition with Improved Safety. Nature Biotechnology 2010; 28:63-70.
4. Fox D, 3rd, Burgin AB, Gurney ME. Structural basis for the design of selective phosphodiesterase 4B inhibitors. Cellular signaling 2014; 26:657-63.
5. Gurney M, Cogram P, Deacon RM, Rex C, Tranfaglia M. Multiple Behavior Phenotypes of the Fragile-X Syndrome Mouse Model Respond to Chronic Inhibition of Phosphodiesterase-4D (PDE4D). Scientific Reports 7, Article number: 14653 (2017); doi:10.1038/s41598-017-15028-x
6. Sansone et al. Improving IQ measurement in intellectual disabilities using true deviation from population norms. Journal of Neurodevelopmental Disorders 2014; 6:16.
7. NIH Toolbox, web address: http://www.healthmeasures.net/explore-measurement-systems/nih-toolbox .
8. Weintraub S, Dikmen SS, Heaton RK, Tulsky DS, Zelazo PD, Bauer PJ, et al. Cognition assessment using the NIH Toolbox. Neurology. 2013;80(11 Suppl3):S54–64.
9. Gershon RC, Cella D, Fox NA, Havlik RJ, Hendrie HC, Wagster MV. Assessment of neurological and behavioural function: the NIH Toolbox. Lancet Neurol. 2010;9(2):138–9.
10. Hessl et al. The NIH toolbox Cognitive Battery for intellectual disabilities: three preliminary studies and future direction. Journal of Neurodevelopmental Disorders (2016) 8:35; DOI 10.1186/s11689-016-9167-4.
11. Knox et al. Feasibility, reliability, and clinical validity of the Test of Attentional Performance for Children (KiTAP) in Fragile X syndrome (FXS). Journal of Neurodevelopmental Disorders. 2012; 4:2.
12. Berry-Kravis E, Sansone S, Crestodina C, McLennan Y, Chin J, Joshi R, Schneider A, Leigh MJ, Hagerman R, Hessl D. Sensitivity of the KiTAP executive function battery and an eye tracking paradigm to effects of AFQ056 in fragile X syndrome. Ann Neurol 2016;80:S413.
13. Berry-Kravis E, Rubin J, Harary E, Daniely Y. A 6-week, randomized, multicenter, double-blind, parallel, flexed- and fixed-dose study of MDX (Metadoxine extended-release; MG01CI) compared with placebo in adolescents and adults with fragile X syndrome. American Academy of Child and Adolescent Psychiatry meeting, San Antonio, October 2015.
14. McCracken JT, McGough J, Shah B, et al. Risperidone in children with autism and serious behavioral problems. N Engl J Med. 2002; 347:314–321. [PubMed: 12151468].
15. Berry-Kravis E, Sumis A, Hervey C, et al. Open-label treatment trial of lithium to target the underlying defect in fragile X syndrome. J Dev Behav Pediatr. 2008; 29:293–302. [PubMed:18698192].
16. Guy, William (1976). "Clinical Global Impressions". *ECDEU Assessment Manual for Psychopharmacology—Revised*. Rockville, MD: U.S. Department of Health, Education, and Welfare; Public Health Service, Alcohol; Drug Abuse, and Mental Health

Administration; National Institute of Mental Health; Psychopharmacology Research Branch; Division of Extramural Research Programs. pp. 218–222. OCLC 2344751. DHEW Publ No ADM 76–338 – via Internet Archive.

17. Berry-Kravis E, Hessl D, Rathmell B, Zarovecs P, Cherubini M, Walton-Bowen K, Gonzales-Heydrich J, Wang P, Carpenter R, Bear M, Hagerman R. Effects of STX209 (arbaclofen) on neurobehavioral function in children and adults with fragile X syndrome: a randomized, controlled, phase 2 trial. Sci Transl Med 2012 Sep 19;4(152):152ra127.
18. Sansone SM, Widaman KF, Hall SS, et al. Psychometric Study of the Aberrant Behavior Checklist in Fragile X Syndrome and Implications for Targeted Treatment. Journal of Autism and Developmental Disorders. 2012;42(7):1377-1392. doi:10.1007/s10803-011-1370-2.
19. Cordeiro, L, Ballinger E, Hagerman R and Hessl D. Clinical assessment of DSM-IV anxiety disorders in fragile X syndrome: prevalence and characterization. J Neurodevelop Disord. 2010; **3**:9067. https://doi.org/10.1007/s11689-010-9067-y.
20. Vineland Adaptive Behavior Scales, Third Edition (Vineland-3). Sara S. Sparrow, PhD, Domenic V. Cicchetti, PhD, Celine A. Saulnier, PhD. Web link: https://www.pearsonclinical.com/psychology/products/100001622/vineland-adaptive-behavior-scales-third-edition--vineland-3.html#tab-resources.
21. Luck S. J. An Introduction to the Event-Related Potential Technique. 2005. Cambridge, MA: MIT Press.
22. Knoth IS, Lippé S. Event-related potential alterations in fragile X syndrome. Frontiers in Human Neuroscience. 2012;6:264. doi:10.3389/fnhum.2012.00264.
23. Ethridge et al. Neural synchronization deficits linked to cortical hyper-excitability and auditory hypersensitivity in fragile X syndrome. Molecular Autism (2017) 8:22 DOI 10.1186/s13229-017-0140-1.
24. Farzin, F., Scaggs, F., Hervey, C. et al. Reliability of eye tracking and pupillometry measures in individuals with Fragile X Syndrome. J Autism Dev Disord. 2011; 41:1515. https://doi.org/10.1007/s10803-011-1176-2.
25. Method No. HP074P, Validation of an Analytical Method for the Determination of BPN14770 in Human Plasma by High-Performance Liquid Chromatography/Tandem Mass Spectrometry (15April2016).

16 Appendices

16.1 Schedule of Assessments

	Screening	PERIOD 1					Cross-over	PERIOD 2					Follow-Up/
	Days -28 thru 0	P1 Day 1	P 1 Wk 1	P 1 Wk 2	P 1 Wk 6	P1 Wk 12*		P2 Day 1*	P2 Wk 1	P2 Wk 2	P2 Wk 6	P2 Wk 12 (Early Term[a])	P2 Wk 13
Target Day within each Period	-28-0	1	7±2	14±2	42±3	84±3		1	7±2	14±2	42±3	84±3	91±2
Clinic Visit	X	X		X	X	X				X	X	X	
Telephone Call			X						X				X
Informed Consent	X												
Eligibility	X	X[b]											
Medical /Surgical History	X												
Demographic /Social History	X												
Physical Exam	X												
Abbreviated PE		X[b]										X	
Ht, Wt, BMI	X					X[c]						X[c]	
Vital Signs[d]	X	X[b]		X	X	X		X		X	X	X	
ECGs (single)	X			X		X		X		X		X	
Chemistry and Hematology	X			X		X				X		X	
Urinalysis	X					X						X	
Suicidality	X		X	X	X	X			X	X	X	X	
PK and Biomarker Sampling	X					X						X	
Randomization		X											
Dispense Test Medication		X			X			X			X		
1st Dose of Medication		X											
Stanford-Binet	X[e]												
NIH-TCB		X			X	X					X	X	
KiTAP		X			X	X					X	X	
CGI-S[f]		X			X	X					X	X	
CGI-I[f]					X	X					X	X	
VAS		X			X	X					X	X	
ABC		X				X						X	
ADAMS		X				X						X	
Vineland-3[f]		X[e]				X						X	

	Screening	PERIOD 1					Cross-over	PERIOD 2					Follow-Up/
	Days -28 thru 0	P1 Day 1	P 1 Wk 1	P 1 Wk 2	P 1 Wk 6	P1 Wk 12*		P2 Day 1*	P2 Wk 1	P2 Wk 2	P2 Wk 6	P2 Wk 12 (Early Term[a])	P2 Wk 13
Target Day within each Period	-28-0	1	7±2	14±2	42±3	84±3		1	7±2	14±2	42±3	84±3	91±2
Clinic Visit	X	X		X	X	X				X	X	X	
Telephone Call			X						X				X
ERP		X				X						X	
Eye Tracking		X				X						X	
IP accountability				X	X	X				X	X	X	
Con Meds	X	X	X	X	X	X			X	X	X	X	X
Adverse Events		X	X	X	X	X		X	X	X	X	X	X
Final Disposition													X

Note: Period 1 Wk 12 and Period 2 Day 1 visits may be combined

[a] If early termination occurs, the Week 12 procedures should be completed at the time of the subject's withdrawal (if possible).
[b] Do not need to repeat if screening and baseline done at same visit.
[c] Weight only
[d] Vital signs include pulse rate, blood pressure, respiration rate, and temperature.
[e] If the site has performed a Stanford-Binet test or Vineland-3 assessment on the subject in the 6 months prior to screening, the results of these assessments may be used for this study.
[f] The CGI-S, CGI-I and Vineland-3 assessments may be conducted over the telephone, if necessary.

16.2 Clinical Laboratory Analytes

Standard Chemistry

Alanine aminotransferase (ALT)	Albumin
Alkaline phosphatase	Aspartate aminotransferase (AST)
Bicarbonate	Blood urea nitrogen (BUN)
Calcium	Chloride
Creatinine	Glucose
Potassium	Sodium
Total bilirubin	Total protein

Hematology

Hematocrit	Hemoglobin
Platelet count	Red blood cell (RBC)
Mean corpuscular hemoglobin concentration (MCHC)	Mean corpuscular hemoglobin (MCH)
White blood cell count and differential (basophils, eosinophils, lymphocytes, monocytes, and neutrophils)	Mean corpuscular volume (MCV)

.

Urinalysis

Bilirubin	Blood
Glucose	Ketones
Nitrite	pH
Protein	Specific Gravity
Urobilinogen	Leukocyte esterase

If protein, urine occult blood, nitrite, or leukocyte esterase values are out of range, the microscopic examination will be reported.