# Clinical Trial Protocol

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| **Title:** | An Exploratory Single Blind Study of Ergoloid Mesylates, 5-Hydroxytryptophan, and the Combination in Adult Males with Fragile X Syndrome |
| **Substance Identifier:** | Ergoloid mesylates, 5-hydroxytryptophan |
| **IND Number:** | Exempt |
| **Indication:** | Behavior, functional skills, cognition |
| **Phase:** | Phase 2 |
| **Sponsor:** | Elizabeth Berry-Kravis, MD, PhD – Study Sponsor<br>Purposeful IKE (Funding Study Drug)<br>FRAXA Research Foundation (Funding Study only) |
| **Principal Investigator:** | Elizabeth Berry-Kravis MD PhD<br>1725 West Harrison, Suite 718<br>Chicago, IL 60612 |
| **Protocol Version:** | ERG/5-HTP in FXS<br>Date: 23 May 2022<br>**V4.0** |

SYNOPSIS

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| **Name of Sponsor/Company:** | Elizabeth Berry-Kravis, MD, PhD – Study Sponsor<br>Purposeful IKE (Funding Study Drug)<br>FRAXA Research Foundation (Funding Study only) |
| **Name of Investigational Product:** | Ergoloid mesylates, 5-Hydroxytryptophan |
| **Study Title:** | An Exploratory Single Blind Study of Ergoloid Mesylates, 5-Hydroxytryptophan, and the Combination in Adult Males with Fragile X Syndrome |
| **Study Phase:** | Phase 2 |

**Study Objectives:**

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

- To obtain preliminary assessments of the efficacy of Ergoloid Mesylates, 5-Hydroxytryptophan, and the Combination
- To evaluate the safety and tolerability of Ergoloid Mesylates, 5-Hydroxytryptophan, and the Combination
- To obtain biomarker (ERP and eye tracking) data on Ergoloid Mesylates, 5-Hydroxytryptophan, and the Combination

**Exploratory Efficacy Outcome Measures**

- NIH Toolbox Cognitive Battery for Intellectual Disabilities (NIH-TCB)
- KiTAP executive battery – alertness, distractibility, go-nogo and flexibility subtests
- Clinical Global Impression Severity – Investigator rated (CGI-S)
- Clinical Global Impression Improvement – Investigator rated (CGI-I)
- Visual Analog Scale (VAS) rating for function, language and behavior using participant-specific anchors
- Aberrant Behavior Checklist (ABC)
- Anxiety, Depression, and Mood Scale (ADAMS)
- Vineland-3 Adaptive Behavior Scale
- Event-Related Potentials (ERP)
- Eye Tracking

**Safety and Tolerability Endpoints**

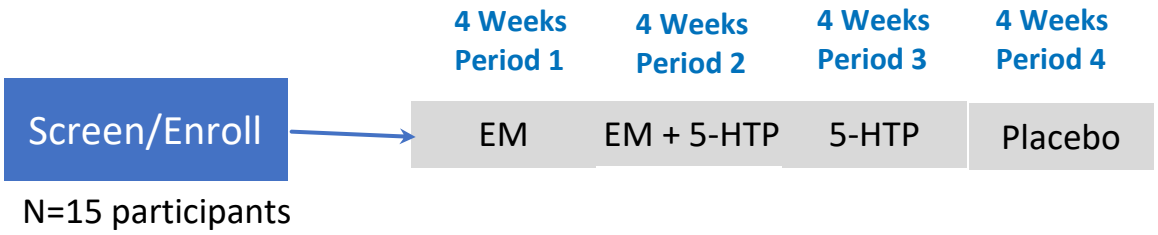
- Treatment-emergent Adverse events
- Changes in vital signs
- Clinical laboratory values for hematology and chemistry

**Study Design**

This is a single-center, Phase 2, single-blind, 4-period sequential study will obtain a preliminary assessment of the effects of ergoloid mesylates (EM) 1 mg TID and 5-hydroxytryptophan (5-HTP) 100 mg TID and the combination compared to a placebo period in participants 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 four 4 week single-blind treatment periods. The screening and baseline visits may occur at the same time, provided the results of safety labs can be obtained. A final follow-up visit or phone contact for safety is planned one week after the conclusion of Period 4.

Eligible participants will be started on EM for 4 weeks in Period 1, then will take EM and 5-HTP for 4 weeks in Period 2, then, 5-HTP for 4 weeks in Period 3, then placebo for 4 weeks in Period 4. Study visits will occur at baseline and every 4 weeks at transition times between periods, then at the end of the study.

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




EM=ergoloid mesylates 1 mg TID, 5-HTP = 5-hydroxytryptophan 100 mg TID

**Planned Numbers of Participants**

Enrollment of a total of 15 eligible participants is planned.

**Study Duration**

The total duration of the study for each participant will be up to 21 weeks, including a maximum of 4-week screening period, four 4-week Single-Blind treatment Periods, and a follow-up telephone call approximately 1 week after the last treatment visit.

**Study Procedures:**

The Screening Visit will occur up to 28 days prior to the first study drug administration on Day 1. During screening, participants 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, participants 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. Participants will undergo a full physical exam, and have vital signs measured. Height, weight, and BMI will also be collected. Fasting blood samples will be collected for chemistry and hematology. Urine will be collected for urinalysis. The Stanford-Binet version 5 will also be administered at screening, as will an assessment of suicidality risk.

At the Baseline visit (Period 1/Day 1), participants 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, behavioral, ERP and eye tracking assessments 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 all treatment Periods.

During all four single-blind periods, participants will receive three times a day treatment with single-blinded study medication, two caps three times a day, each dose at least 4 hours apart. In the first period they will take an EM capsules and a placebo capsule at each dose, the second period an EM and 5-HTP caps at each dose, the third period a 5-HTP and placebo capsule each dose, and the fourth period 2 placebo capsules each dose. EM and 5-HTP will be over-encapsulated to maintain the single-blind.

Participants will return to the clinic at the end of each Period at weeks 4, 8, 12, and 16. Cognitive and behavioral evaluations will be repeated at Weeks 4, 8, 12, and 16 (end of Period 1, 2, 3, 4). Additionally, participants 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 4 or following early discontinuation. During clinic visits, adverse effects will be assessed, and laboratory measures and vital signs will be repeated according to the Schedule of Assessments. Suicidality risk will also be evaluated during the treatment periods per the Schedule of Assessments; if a concern is detected, the participant will be referred for further evaluation and treatment.

**Participant 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 participant eligibility will be verified on Baseline Day 1.

**Participant Inclusion Criteria**

1. Male aged 18 to 45 years, inclusive.
2. Participant has Fragile X Syndrome with a molecular genetic confirmation of the full Fragile X Mental Retardation (FMR1) mutation (≥200 CGG repeats).
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. Participants 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. Participant 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. Participant has a parent, legal authorized guardian or consistent caregiver.
10. Participant and caregiver are able to attend the clinic regularly and reliably.
11. Participant is able to swallow capsules.
12. For participants who are not their own legal guardian, participant's parent/legal authorized guardian is able to understand and sign an informed consent form to participate in the study.
13. If participant is his own legal guardian, he can understand and sign informed consent to participate in the study.
14. If participant is not their own legal guardian, the participant provides assent for participation in the study, if the participant has the cognitive ability to provide assent.

**Participant Exclusion Criteria**

1. History of, or current cardiovascular, renal, hepatic, respiratory, gastrointestinal, psychiatric, neurologic, cerebrovascular, or other systemic disease that would place the participant 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. Clinically significant abnormalities, in the investigator's judgment, in safety laboratory tests, vital signs, as measured during Screening.
3. History of substance abuse within the past year, according to investigator assessment.
4. Use of CYP3A4 inhibitors, beta-blockers, MAO inhibitors or triptans at any time during participation in the study.
5. Significant hearing or visual impairment that may affect the participant's ability to complete the test procedures.
6. Concurrent major psychiatric condition (e.g., Major Depressive Disorder, Schizophrenia or Bipolar Disorder) as diagnosed by the investigator. Participants with additional diagnosis of Autism Spectrum Disorder or Anxiety Disorder will be allowed as these are characteristics of FXS.

7. Participant has active diseases that would interfere with participation, such as acquired immunodeficiency disorder, hepatitis C, hepatitis B, or tuberculosis.
8. Participant 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.
9. Participant has participated in another clinical trial within the 30 days preceding Screening.

**Investigational Product, Dosage, and Mode of Administration:**
In this single-blind, 4-period study, 15 participants will be enrolled and will be treated with the following during the 4 periods of the study.

- Ergoloid mesylates 1 mg TID (Medisca)* and matching placebo for 5-Hydroxytrptophan 100mg TID***
- Ergoloid mesylates 1 mg TID (Medisca) and 5-Hydroxytryptophan 100 mg TID (5-HTP, Basic Vitamins)**
- 5-Hydroxytryptophan 100 mg TID (5-HTP, Basic Vitamins) and matching placebo for Ergoloid mesylates 1mg TID***
- Matching placebo for Ergoloid mesylates 1mg TID and Matching placebo for 5-Hydroxytryptophan 100mg TID***
-

* 1 mg will be mixed with methyl cellulose and placed in a size 00 capsule
** will be over-encapsulated in identical size 00 capsules
***will be ascorbic acid powder in identical size 00 capsules

All study medications will be prepared at the Rush Oak Park Compounding Pharmacy and provided to the RUMC Investigational Pharmacy for dispensing as identical-appearing capsules in bottles. Two bottles of capsules will be provided at each visit and one capsule from each bottle will be taken 3 times a day. Doses should be taken at least 4 hours apart.

**Overview of Endpoints**

**Exploratory Efficacy Outcome Measures**

- NIH-TCB: Cognitive battery assessing different domains of 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 participant-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 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); physical examinations; and vital signs (including blood pressure, heart rate, and respiratory rate).

**Statistical Considerations**

Efficacy Analyses

The primary efficacy population will be the intent to treat (ITT) efficacy population, which will include all participants 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 participants who complete all 4 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.

Safety Analyses

The Safety population will include all participants who received at least one dose of study treatment. Adverse Events (AEs), including clinically meaningful laboratory abnormalities and significant behavioral changes will be tabulated. AE severity and relatedness to treatment will be assessed. AEs will be tabulated for placebo, EM, EM + 5-HTP and 5-HTP 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.

# 1 TABLE OF CONTENTS

# 2 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 |
| bid | Twice daily |
| BMI | Body mass index |
| BP | Blood pressure |
| BPM | Beats per minute |
| BUN | Blood urea nitrogen |
| °C | Degrees centigrade |
| CBT | Cognitive Behavioral Therapy |
| 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 |
| CNS | Central nervous system |
| CP | Completers population |
| CRF | Case report form |
| DCCS | Dimensional change card sort (test) |
| EDTA | Ethylenediaminetetraacetic acid |
| EEG | Electroencephalogram |
| 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 |
| HgbA1C | Hemoglobin A1C |
| ICF | Informed consent form |
| ICH | International Conference on Harmonization |
| IRB | Institutional Review Board |
| ITI | Inter-trial interval |
| ITT | Intent to treat (population) |
| kg | kilogram |
| KiTAP | Test of Attentional Performance |
| mg | Milligram |
| mL | Milliliter |
| msec | Millisecond |
| µ | Micro |
| ng | Nanogram |
| NIH-TCB | NIH Toolbox Cognitive Battery for Intellectual Disabilities |
| PO | By mouth (per os) |
| RBC | Red blood cell |
| ROI | Region of interest |
| SAE | Serious adverse event |
| SD | Standard deviation |
| SOP | Standard operating procedure |
| VAS | Visual analog scale |

# 3 INTRODUCTION

## 3.1 Background

Mutational inactivation of the *FMR1* gene encoding the Fragile X Mental Retardation protein (FMRP) causes a spectrum of symptoms including seizures, sleep disorders, anxiety, irritability, autism, mild to severe cognitive impairment and intellectual disability. The constellation of symptoms is known as Fragile-X syndrome (FXS).

FXS in humans is caused by a CGG triplet expansion mutation (> 200 repeats) in the 5′ untranslated region of the *FMR1* gene located on the X chromosome, which leads to gene methylation, inactivation, and resultant loss of fragile X mental retardation protein (FMRP) expression. FMRP functions as a translational regulator, affecting synthesis of many proteins including those involved in synaptic pruning during development.[1] Meta-analysis estimates the frequencies of individuals with the full mutation FXS allele to be approximately 1 in 7000 males and 1 in 11,000 females.[2] FXS is severely debilitating in males. Females generally are less affected than males due to mosaicism resulting from X-chromosome inactivation which occurs randomly early in embryogenesis.[3]

FXS presents with a variable clinical phenotype. In affected individuals, the disease presents during childhood with delayed developmental milestones. Intellectual deficit can be of variable severity and may include problems with working and short-term memory, executive function, language, mathematics and visuospatial abilities. Behavioral anomalies can be mild (e.g. anxiety, mood instability) to severe (e.g. aggressive behavior, autism). Autistic-like behavior can include hand flapping, poor eye contact, hand biting, gaze avoidance, social phobia, social and communication deficits and tactile defensiveness. In females, intellectual and behavioral disorders are typically milder than in males and usually consist of shyness, social and other forms of anxiety, and variable learning problems, with IQ ranging from normal to intellectually impaired. About 25% of girls have an IQ less than 70. Attention deficit hyperactivity disorder (ADHD) is present in over 89% of males and 30% of females and behavioral disinhibition is very common. Recurrent otitis media (50%) and seizures (12%) can also be observed. Individuals with FXS display a range of neuropsychiatric symptoms including intellectual disability, delayed language acquisition, poor social interaction, features of autism spectrum disorder, anxiety, hyperarousal, hypersensitivity, repetitive behaviors, disrupted sleep, attention deficit hyperactivity disorder and irritability/aggression.[3] These behavioral changes are modelled in adult male *Fmr1* knockout (KO) mice which display a spectrum of behavioral phenotypes due to the *Fmr1* gene deletion. The mutant mice show hyperarousal in the open field test, have impaired social interaction, are less likely to build nests when provided cotton batting and are less likely to bury marbles in the cage bedding. Adult male mice have been used for most studies with animal models, as male FXS patients typically suffer more severe symptoms than do female patients due to the single X chromosome. In both humans with FXS `and the *Fmr1* KO mice, there are alterations in the density, size, shape and maturity of dendritic spines, the principle recipients of excitatory inputs from other neurons.[1]

Efforts to treat FXS have included numerous investigations have not been widely successful, which has led to revision of trial designs and exploration for additional and new therapies.[3] Management of FXS is currently symptom-based and requires a multidisciplinary approach. Speech, physical and sensory integration therapy as well as individualized educational plans and behavioral interventions may be combined with medication, such as stimulants for attention deficit-hyperactivity disorder; selective serotonin reuptake inhibitors (SSRIs) for anxiety, depression, obsessive-compulsive

disorder; and atypical antipsychotic agents for self-injury and aggressive behaviors. New targeted treatments for FXS are being studied. Despite this slow progress, treatment of FXS remains still an unmet medical need.

## 3.2 Rationale

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, *FMR1* gene.

Two previously untested products in FXS were predicted *in silico* to have beneficial activity in FXS and proposed for *in vivo* follow-up in *Fmr1* KO mice, namely ergoloid mesylates (EM) and 5-hydroxytryptophan (5-HTP). Both agents showed an interesting polypharmacological predicted bioactivity spectrum, which is a necessity for complex disorders, such as FXS. The most strongly associated targets after the statistical enrichment were HTR1A, HTR2A, DRD2 and OPRM1 for EM, and ESR1, HTR1A, GRIA1, and GRIK1 for 5-HTP. Both products worked in the majority of tests *in vivo* in the *Fmr1* KO mouse model (see below Section 3.3.1 Preclinical Pharmacology), which led to the hypothesis that their serotonergic action would be additive, whilst the diversity in target and pathway modulation could indeed produce some synergistic effect, which was also confirmed *in vivo*, leading to the alleviation of all FXS phenotypes in the *Fmr1* KO mouse.

## 3.3 Risk/Benefit

### 3.3.1 Preclinical Pharmacology

Preclinical *in vivo* data are available for the suggested formulations and their combination. Blinded experiments were conducted to assess the effect of the two treatments and their combination in *Fmr1* KO mice (*Fmr1* gene deletion). Adult male mice were used for all experiments. *Fmr1* knockout mice recapitulate the human phenotype and represent a valuable preclinical model for assessment of putative drug treatments. The *Fmr1* KO carries an insertion in exon 5.[4] It is a protein null, although *Fmr1* mRNA is still present.[5] These mice have been backcrossed to the C57/Bl6 or the FVB strains. The *Fmr1* KO2 is a null allele at *Fmr1* generated by deletion of the promoter and first exon of *Fmr1*.[6] It is both protein and mRNA null. This mutation is the same as is produced by Cre-mediated excision of the loxP sites present in the *Fmr1* cKO described below (we house these and other mice models of FXS). According to literature, *Fmr1* KO mice show alterations in the density, size, shape and maturity of dendritic spines, the principle recipients of excitatory inputs from other neurons.[1] Toxicological information on the acute toxicity of 5-HTP in animals are available[7], as is the corresponding information for EM[8]. The treatment of the mice with 5-HTP, EM was conducted according to the matrix shown below in Table 1.

**Table 1**. Treatment matrix for EM, 5-HTP and the combination in *Fmr1* KO mice.

| Group Number | Route | Concentration | Regimen | No. animals |
|---|---|---|---|---|
| 1. WT | - | - | QD | 10 |
| 2. KO | - | - | QD | 10 |
| 3. KO 5-HTP | IP | 80 mg/kg | QD | 10 |
| 4. KO EM | PO | 4 mg/kg | QD | 10 |
| 5. KO EM + 5-HTP | PO + IP | 2 mg/kg + 40 mg/kg | QD | 10 |

The behavioural tests performed were for hyperactivity (open field), stereotypy (self-grooming), sociability (three chamber partition test), memory and learning (novel object recognition), anxiety (hyponeophagia), daily living (nest building). Concise definitions follow below.
One-way Analysis of Variance and Dunnett's multiple comparisons against the KO group were performed suggesting that both treatments as well as their combination were significantly different from the KO group (adjusted p-value <0.0001) whilst the actual scoring across tests for both the treatments and their combination was similar to the WT group. Specifically, for all six tests, the treatment combination scoring measurements were found to be very similar to those provided for the WT group. Additional *in vivo* experiments were conducted to assess the effect of SSRIs on 5-HTP + EM treatment, specifically the standard of care SSRI Fluvoxamine was administered (40mg/kg) in another 10 diseased mice. Results confirmed that Fluvoxamine did not alter the effectiveness of 5-HTP + EM treatment (adjusted p-value<0.0001).

**Figure 1.** Open field WT-V, KO-V, 5-HTP, EM and combination (EM + 5-HTP)

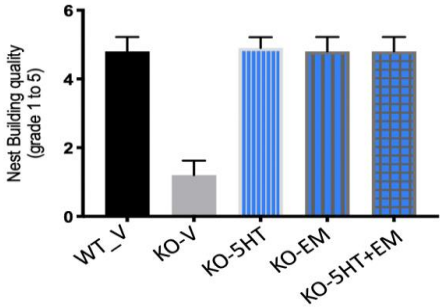



**Figure 2**. Stereotypy WT-V, KO-V, 5-HTP, EM and combination (EM + 5-HTP)

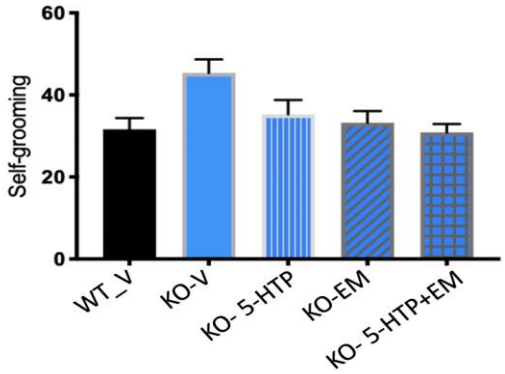



**Figure 3**. Sociability WT-V, KO-V, 5-HTP, EM and combination (EM + 5-HTP)

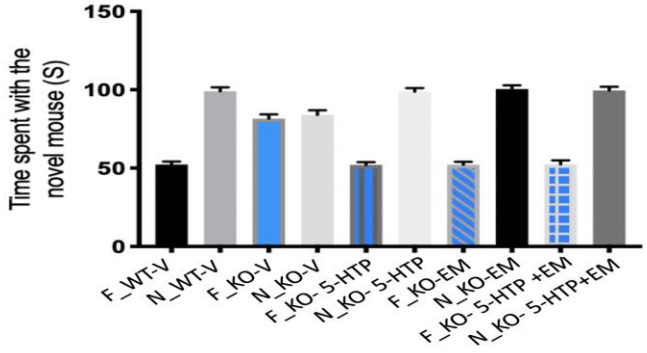


**Figure 4.** Memory and learning for WT-V, KO-V, 5-HTP, EM and combination (EM + 5-HTP)

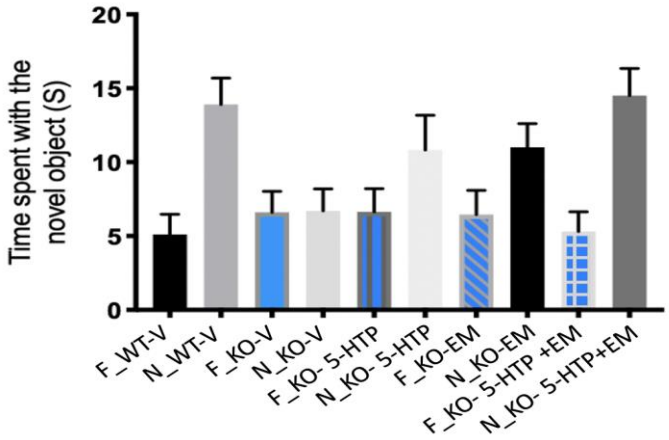



**Figure 5.** Hyponeophagia for WT-V, KO-V, 5-HTP, EM and combination (EM + 5-HTP)

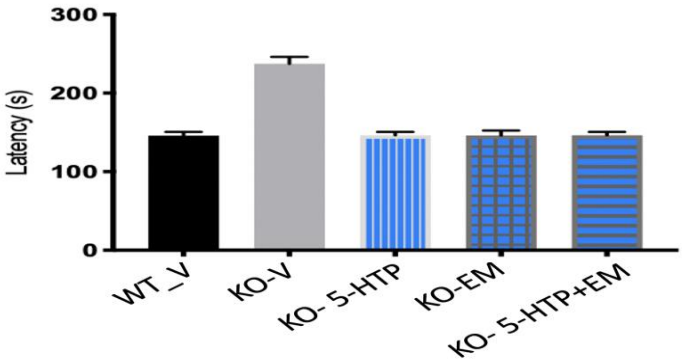
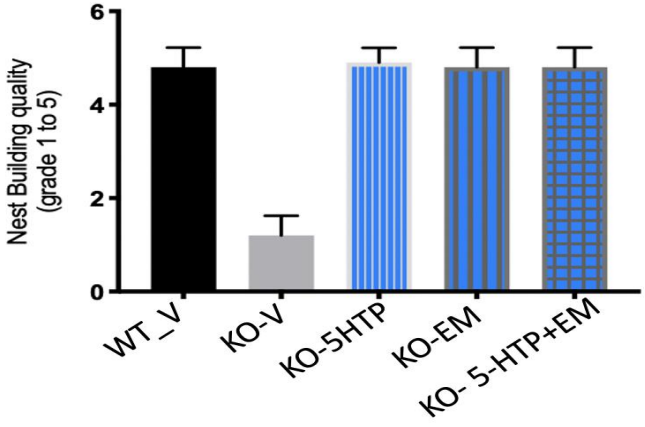



**Figure 6.** Test of daily living in WT-V, KO-V, 5-HTP, EM and combination (EM + 5-HTP)




### 3.3.2 Clinical Experience

**Ergoloid Mesylates**, trade name **Hydergine**, is a mixture of the methanesulfonate salts of three dihydrogenated ergot alkaloids, dihydroergocristine, dihydroergocornine, and alpha- and beta-dihydroergocryptine. Ergot alkaloids are dopamine agonists which activate dopamine receptors (in the basal ganglia and other parts of the brain involved in motor function) and a prolactin inhibitor. Ergot is a strong vasoconstrictor and thus helps to reduce bleeding by narrowing of the blood vessels.

Ergot was perhaps first used in medicine as an oxytocic drug, to promote uterine contraction during childbirth. They have been used for treating migraine headaches since 1883 and also in treating Parkinson's disease, restless leg syndrome, Alzheimer's disease, dementia, hyperprolactinemia and other purposes.[9]

There have been 26 clinical drug trials which investigated the effectiveness of EM in geriatric psychopharmacology, with significant improvement in at least 50% of the studies in the areas of cognitive dysfunctions, mood depression, and the composite scores as measured by subjective clinical behavioral rating scales.[10] Assessments of studies on the effect of EM in dementia have shown strong effects in Vascular Dementia but only a modest efficacy in Alzheimer's Dementia, with a stronger potential on higher dosage,[11] as well as a statistically significant improvement on all ten components of the Sandoz Clinical Assessment-Geriatric scale.[12] Olin *et al.*[13] assessed its overall effects on patients with possible dementia, and showed significant treatment effects. EM was found to be well tolerated in controlled studies of elderly patients with age-related cognitive decline.[14]

5-hydroxytryptophan, also known as 5-HTP has a nutraceutical status and has never been approved as a drug for any indication, but as a dietary supplement has been used extensively for several disorders for many years such as in the therapy of depression, fibromyalgia, obesity, insomnia and chronic headache. The starting dose is generally 50 mg, three times a day, which can be doubled after a couple of weeks, if necessary.[15]

Despite its relevance to L-tryptophan for which there are safety concerns, 5-HTP has demonstrated a very good safety profile. In humans, 5-HTP has been administered in combination with SSRIs and tricyclic antidepressants,[16,17] Monoamine oxidase inhibitors (MAOIs)[18,19,20] and tryptophan.[21] According to Turner and Blackwell,[22] serotonin syndrome has not been reported in humans in association with 5-HTP, either as monotherapy or in combination with other medications. In clinicaltrials.gov (*accessed on 5 Feb 2021*) there are 8 registered clinical studies in phases 2 and 3 involving 5-HTP in one of the arms as a treatment for various indications such as major depressive disorder [NCT04395183], spinal cord injuries [NCT04520178] Crohn's syndrome [NCT03574948] and others.

## 3.4 Study objectives and endpoints

### 3.4.1 Study Objectives

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

- To obtain a preliminary assessment of the efficacy of ergoloid mesylates (EM) 1 mg TID, 5-hydroxytryptophan (5-HTP) 100 mg TID, and the combination
- To evaluate the safety and tolerability of EM, 5-HTP, and EM + 5-HTP in fragile X syndrome
- To obtain pharmacodynamic (ERP, eye tracking) data on EM, 5-HTP, and EM + 5-HTP

### 3.4.2 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 participant-specific behavioral anchors
- Aberrant Behavior Checklist (ABC)
- Anxiety, Depression, and Mood Scale (ADAMS)

- Vineland-3 Rating Scale
- Event-Related Potentials (ERP)
- Eye Tracking

### 3.4.3 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)

# 4 INVESTIGATIONAL PLAN

## 4.1 Overall Study Design

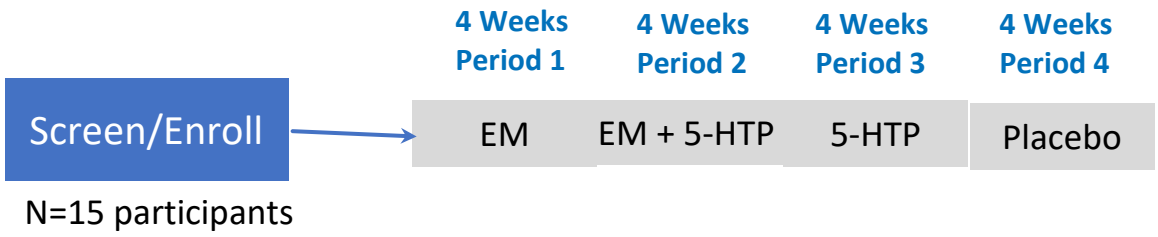
This is a single center, Phase 2, single-blind, 4 period study to obtain preliminary assessment of the effects of ergoloid mesylates (EM), 5-hydroxytryptophan (5-HTP), and the combination in participants with Fragile X Syndrome. As schematic display of the study design is shown in Figure 7.

The study will consist of a Screening period of up to 28 days prior to initial study drug administration, followed by four 4-week single-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 treatment periods. A final follow-up phone contact for safety is planned one week after the conclusion of Period 4.

Eligible participants will be started on over-encapsulated EM 1 mg TID during Period 1, then will take EM 1 mg TID and 5-HTP 100 mg TID in Period 2, then 5-HTP 100 mg TID in Period 3, and placebo in Period 4. Throughout all 4 periods, participants will take two identical capsules three times a day. If only taking one over-encapsulated drug, they will take one over-encapsulated placebo pill with the drug at each dose, and when in period 4 they will take two over-encapsulated placebo pills at each dose.

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

**Figure 7 Study Design Schematic**




### 4.1.1 Dose Selection

The doses of EM and 5-HTP were chosen as these are standard doses used in clinical management.

EM label for elderly demented patients suggests 1 mg tablets, 3 times a day, according to the product label[23]. 5-HTP labels commonly suggest 1-4 tablets totaling 200mg per day, however for depression, most commonly, 150-800 mg daily is taken for 2-6 weeks.[24] Given this range, as well as the duration of the study, 100mg TID is suggested for 5-HTP.

Furthermore, when compared to the mouse doses tested in vivo (2 mg/kg for EM and 40 mg/kg for 5-HTP), if these are extrapolated to humans by allometric scaling, i.e. assuming that metabolic rate scales proportionally to body weight raised to the ¾ power, the proposed doses of 1 and 100 mg TID for EM and 5-HTP, respectively, are safely lower. Namely the 2 mg/kg mouse dose scales to about 20 mg daily dose for a typical 70 kg male, while for 5-HTP. a 40 mg/kg mouse dose scales to about 400 mg daily dose.[25]

## 4.2 Study Duration

The total duration of the study for each participant will be up to 21 weeks, including a maximum of a 4-week screening period, four 4-week single-blind Treatment Periods, and a follow-up call approximately 1 week after last treatment.

# 5 SELECTION AND WITHDRAWAL OF PARTICIPANTS

## 5.1 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.

## 5.2 Participant Inclusion Criteria

1. Participant is male aged 18 to 45 years, inclusive.
2. Participant has Fragile X Syndrome with a molecular genetic confirmation of the full Fragile X Mental Retardation 1 (FMR1) mutation (≥200 CGG repeats).
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. Participants 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. Participant 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. Participant has a parent, legal authorized guardian or consistent caregiver.
10. Participant and caregiver are able to attend the clinic regularly and reliably.
11. Participant is able to swallow tablets and capsules.
12. For participants who are not their own legal guardian, participant's parent/legal authorized guardian is able to understand and sign an informed consent form to participate in the study.
13. If participant is his/her own legal guardian, he/she can understand and sign informed consent to participate in the study.
14. If participant is not their own legal guardian, the participant provides assent for participation in the study, if the participant has the cognitive ability to provide assent.

## 5.3 Participant 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 participant 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. Clinically significant abnormalities, in the investigator's judgment, in safety laboratory tests, vital signs, or ECG, as measured during Screening.
3. History of substance abuse within the past year, according to investigator assessment.
4. Use of CYP3A4 inhibitors, beta-blockers, MAO inhibitors or triptans at any time during participation in the study.
5. Significant hearing or visual impairment that may affect the participant's ability to complete the test procedures.
6. Concurrent major psychiatric condition (e.g., Major Depressive Disorder, Schizophrenia or Bipolar Disorder) as diagnosed by the investigator. Participants with additional diagnosis of Autism Spectrum Disorder or Anxiety Disorder will be allowed.
7. Participant has known or suspected human immune deficiency virus-positive status or has diseases such as acquired immunodeficiency disorder, hepatitis C, hepatitis B, or tuberculosis.
8. Participant 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.
9. Participant has participated in another clinical trial within the 30 days preceding Screening.

## 5.4 Participant Withdrawal

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

- Participant withdraws consent or requests discontinuation from the study for any reason;
- Occurrence of a medical condition or circumstance that exposes the participant to substantial risk and/or does not allow the participant to adhere to the requirements of the protocol as determined by the Investigator;
- Any serious adverse event (SAE), clinically 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 participant;
- Requirement for prohibited concomitant medication;
- Termination of the study by the Sponsor or the regulatory authority.

Should a participant withdraw after administration of EM in Period 1, or should the Investigator decide to withdraw the participant, all efforts will be made to complete and report the protocol-stipulated observations up to the time of withdrawal. Week 16 procedures (see Section 10.3.5 and Section 14.1 Schedule of Study Assessments) will be completed at the time of the participant's withdrawal and an explanation provided as to why the participant is withdrawing or being withdrawn from the study.

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

The Investigator must document the primary reason for discontinuation of a study participant 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.

## 5.5 Replacement of Participants

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

# 6 TREATMENT OF PARTICIPANTS

## 6.1 Treatment Arms

In this single-blind, 4-period study, 15 participants will be enrolled and will be treated with the following during the 4 periods of the study.

- Ergoloid mesylates 1 mg TID (Medisca)* and matching placebo for 5-Hydroxytryptophan 100mg TID***
- Ergoloid mesylates 1 mg TID (Medisca) and 5-Hydroxytryptophan 100 mg TID (5-HTP, Basic Vitamins)**
- 5-Hydroxytryptophan 100 mg TID (5-HTP, Basic Vitamins) and matching placebo for Ergoloid mesylates 1mg TID***
- Matching placebo for Ergoloid mesylates 1mg TID and Matching placebo for 5-Hydroxytryptophan 100mg TID ***

* 1 mg will be mixed with methyl cellulose and placed in a size 00 capsule
** will be over-encapsulated in identical size 00 capsules
***will be ascorbic acid powder in identical size 00 capsules

All study medications will be prepared at the RUMC research pharmacy and provided as identical-appearing capsules in bottles. Two bottles of capsules will be provided at each visit and one capsule from each bottle will be taken 3 times a day. Doses should be taken at least 4 hours apart.

## 6.2 Study Medications

The Ergoloid Mesylate is provided as powder manufactured by Medisca and 5-HTP 100 mg capsules available to the RUMC pharmacy are manufactured by Basic Vitamins. All study medications and their matching placebos will be compounded and over-encapsulated at the Rush Oak Park Compounding Pharmacy.

## 6.3 Drug Accountability

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

All drug returned will be disposed of by the study team after medication reconciliation to determine compliance. Amount of drug returned and disposed will be recorded in the participant study binders. In accordance with Good Pharmacy Practices, gloves will always be worn by study personnel when handling study drug.

## 6.4 Blinding

This is a single-blind study, meaning the participant and his family/caregivers will not know what drug or combination the participant is receiving. The investigator and study coordinator will know what drug the participant is taking. The remainder of the study team performing assessments such as the Vineland, eye tracking, ERP, Toolbox, and the KiTAP will not know the type of treatment the patient is on when testing is done.

The PI or designee will be responsible for maintaining the single blind, consistent with protocol design, throughout the study, except in the case of an emergency need to unblind the participant for medical reasons. Blinding is not to be broken during the study unless considered necessary by the Investigator for emergency situations for reasons of participant safety. When the blind is broken, the reason must be fully documented.

## 6.5 Study Drug Administration

Two capsules will be taken orally in the morning, afternoon and evening. Doses should be taken at least 4 hours apart.

## 6.6 Prior and Concomitant Medications

A prior medication is defined as any psychotropic or anti-epileptic medication taken by the participant 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 14 Follow-up Visit. Concomitant medications 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.

## 6.7 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.

## 6.8 Dietary Guidelines

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

## 6.9 Lifestyle Guidelines

There no lifestyle restrictions or guidelines associated with this study.

# 7 STUDY PROCEDURES AND ASSESSMENTS

The study will be conducted 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),[26,27] and the policies and procedures as outlined by the ethical requirements for IRB review and informed consent form.

## 7.1 Informed Consent

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

## 7.2 Medical /Surgical History

Medical/Surgical history will be recorded at the Screening Visit as specified in Section 10.2. Participant eligibility will be evaluated to determine all inclusion and none of the exclusion criteria are met. The Investigator will inquire with the participant on Period 1/Day 1 (Baseline, prior to

randomization) to determine if there have been any changes in the participants' s health affecting eligibility or requiring an update to their medical and surgical history.

## 7.3 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.

## 7.4 Stanford Binet Intelligence Scale

The Stanford Binet Intelligence Scale – Version 5 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.[28]

## 7.5 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 the visit at the end of each Period 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.

## 7.6 Height, Weight, Body Mass Index

Body weight and height are to be measured at Screening. Body weight will be repeated at the visit at the end of each Period as specified in the Schedule of Assessments. Participants will wear indoor

clothing and remove their shoes prior to the measurements. Body Mass Index (BMI) will be calculated and recorded.

## 7.7 Vital Signs

Vital signs (pulse rate, blood pressure, respiration rate, and temperature) will be measured at Screening, during the Treatment Visits, and Follow-Up 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 participant, 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 participant in the sitting position, after the participant is at rest for 5 minutes.

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

## 7.8 Laboratory Assessments

Appendix 14.2 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 this is a single-site study, an accredited local laboratory will be used to analyze the clinical laboratory samples.

## 7.9 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 participant. 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.

## 7.10 Additional Safety Measures

Participants 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 participant, 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.

## 7.11 Exploratory Efficacy Assessments

### 7.11.1 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 composites where age-equivalent scores will also be collected.

#### 7.11.1.1 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.[29,30,31] 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.[32] Further validation of the adjusted measure was published subsequently.[33]

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).

#### 7.11.1.2 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.[34] 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.[35,36]

#### 7.11.1.3 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.[37,38]

##### 7.11.1.3.1 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 participant's overall condition based on a trained clinician's assessment of cognition, behavior and activities of daily living.[39] 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.

##### 7.11.1.3.2 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 participant'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.[39]

Completion of this scale requires the clinician to rate how much the participant'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 participant at all applicable visits throughout the trial.

#### 7.11.1.4 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 participant's behavior with respect to three domains: daily functioning, anxiety/irritability and language; with guidance form the investigator, the caregiver will select a behavior that is a problem affecting function within each domain that will be assessed throughout the study. The horizontal marks are measured in centimeter distance where they call from the "good behavior" side 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.[40] The caregiver completing the assessment should remain the same at all applicable visits throughout the trial.

#### 7.11.1.5 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.[41] 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.

#### 7.11.1.6 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.[42] 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 participant should have intimate knowledge of the participant'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.

#### 7.11.1.7 Vineland-3 Rating Scale

The Vineland Adaptive Behavior Scales, Third Edition (Vineland-3)[43] assesses adaptive behavior in five domains, each with subdomains:

1. Communication
    - Subdomains: Receptive, Expressive, Written
2. Daily Living Skills
    - Subdomains: Personal, Domestic, Community
3. Socialization
    - Subdomains: Interpersonal Relationships, Play and Leisure, Coping Skills
4. Motor Skills
    - Subdomains: Fine Motor, Gross Motor
5. Maladaptive Behavior
    - Internalizing, Externalizing, Critical Items

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 participant 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.

#### 7.11.1.8 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.[44,45] Auditory stimuli are presented and EEG events assessed in relation to timing of the stimuli. For this study, an ERP protocol developed to measure resting state delta and alpha power, alpha responses to eye closure, CHIRP responses,[46] and processing of oddball stimuli relative to standards[47] will be used. This protocol has shown feasibility in prior studies. The EEGs will be read by Dr. Lauren Ethridge who has developed multiple ERP protocols and has extensive experience in ERP evaluation.

#### 7.11.1.9 Eye Tracking

Eye tracking has been successfully used to assess social gaze in FXS.[48] Testing will be conducted in a quiet room with the lights turned off. The eye tracker (Tobii) will be calibrated for each participant at the beginning of each session. Following calibration, participants 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.

### 7.11.2 Timing of Efficacy Assessments

#### 7.11.2.1 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 participant-specific behavioral anchors
- Aberrant Behavior Checklist (ABC)
- Anxiety, Depression, and Mood Scale (ADAMS)
- Vineland-3 Rating Scale
- Event-Related Potentials (ERP)
- Eye Tracking

#### 7.11.2.2 Week 4, 8, 12, 16 (end Periods 1 through 4)

The full battery of cognitive, behavioral and pharmacodynamic tests will be performed at Week 4 of 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 participant-specific behavioral anchors
- Aberrant Behavior Checklist (ABC)
- Anxiety, Depression, and Mood Scale (ADAMS)
- Event-Related Potentials (ERP)
- Eye Tracking
- Vineland-3 Rating Scale

# 8 EVALUATION AND REPORTING OF ADVERSE EVENTS

## 8.1 Adverse Events

An adverse event is defined as any untoward medical occurrence in a clinical investigation participant 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 Informed Consent until study participation is

complete (the Follow-up Visit). Participants should be instructed to report any adverse event that they experience to the Investigator. Beginning with the signing of the informed consent until the time of the first dose of study drug, investigators should make updates to medical history and record any pre-existing medical condition or signs or symptoms that changes in severity, frequency, or seriousness in the medical history. Serious adverse events that occur prior to the first dose of study drug should be reported as an update to medical history as well as be reported on the appropriate adverse event CRF.

Beginning with the first dose of study drug, investigators should make an assessment for adverse events at each visit and record all adverse events, non-serious and serious, on the appropriate adverse event CRF.

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.

Any medical condition already present prior to the participant taking the first dose of study drug should be reported in the medical history. Any SAEs occurring prior to the first dose of study drug should be reported as an update to medical history as well as an adverse event. Any pre-existing medical condition or signs or symptoms that changes in severity, frequency, or seriousness after the participant takes the first dose of study drug and through the Follow-up Visit should be reported as an adverse event.

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.

## 8.2 Definitions

### 8.2.1 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.

### 8.2.2 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.

### 8.2.3 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 participant 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 inparticipant 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 inparticipant 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 participant 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 in participant hospitalizations, or the development of drug dependency or drug abuse.

## 8.3 Assessment of Adverse Events by the Investigator

### 8.3.1 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 participant may have.
- Concomitant drug - The other drugs the participant is taking or the treatment the participant 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.

### 8.3.2 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.

### 8.3.3 Adverse Event Monitoring and Follow-up

Participants 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. Any actions taken and follow-up results must be recorded either on the appropriate page of the CRF, as well as in the participant's source documentation. Follow-up laboratory results should be filed with the participant's source documentation.

For all AEs that require the participant 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).

Participants who have non-serious AEs that are ongoing at study completion or study withdrawal must be followed until resolution of the AEs or for 30 days after the last dose of study drug, whichever comes first. Participants who have an ongoing SAE at study completion or study withdrawal must be followed until the event resolves or the event or sequela stabilizes.

SAE documentations will include:
Investigator details (name, phone, fax, e-mail)
Participant number
Participant 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 participant until the SAE has subsided or until the condition becomes chronic in nature, stabilizes (in the case of persistent impairment), or the participant dies. Within 24 hours of receipt of follow-up information, the Investigator must update the Sponsor and submit any supporting documentation (e.g., participant 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. The clinical research site will be responsible for reporting SAEs to the Institutional Review Board (IRB) per FDA regulations.

# 9 STUDY PROCEDURES AND ASSESSMENTS BY VISIT

## 9.1 Schedule of Assessments

The study timetable in Section 14.1 shows the schedule of planned study procedures. Every effort should be made to adhere to this procedure schedule.

## 9.2 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;
- Draw blood samples for Chemistry and Hematology;
- Collect urine for safety urinalysis;
- Complete Stanford-Binet assessment;
- Evaluate inclusion and exclusion criteria.

## 9.3 Baseline (Period 1/Day 1)

Participants 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.

### 9.3.1 Prior to Drug Dispensing

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, behavioral and pharmocodynamic 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 participant-specific behavioral anchors
    - Aberrant Behavior Checklist (ABC)
    - Anxiety, Depression, and Mood Scale (ADAMS)
    - Vineland-3 Rating Scale
    - Event-Related Potentials (ERP)
    - Eye Tracking

If participant remains eligible for the study:

- Dispense study medication.

### 9.3.2 Weeks 1, 5, 9, 13 (Periods 1 through 4)

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

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

### 9.3.3 Week 4, 8, 12, 16 (end Periods 1 through 4)

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

- Collect weight;
- Collect vital signs;
- Draw blood samples for Chemistry and Hematology;
- Collect urine for safety urinalysis;
- Conduct the following cognitive, behavioral and pharmocodynamic 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 participant-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 capsule count remaining in bottle to assess dosing compliance;
- Record any updates to concomitant medication use; and
- Assess and record any adverse events.

### 9.3.4 Week 17 (Period 4, 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 participant disposition.

### 9.3.5 Early Termination

It is hoped that all participants can be followed through to the conclusion of the study at Period 4/Week 17. However, if an early termination occurs, the Week 16 procedures should be completed at the time of the participant's withdrawal (if possible) and an explanation provided as to why the participant is withdrawing or being withdrawn from the study. Procedures to be completed early termination:

- Collect weight;
- Collect vital signs;
- Draw blood samples for Chemistry and Hematology;
- Collect urine for safety urinalysis;
- 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 participant-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 capsule count remaining in bottle to assess dosing compliance;
- Record any updates to concomitant medication use;
- Assess and record and adverse events; and
- Record final participant disposition.

# 10 PLANNED STATISTICAL METHODS

## 10.1 Sample Size

Given the exploratory nature of this study, a definitive sample size calculation is not possible.

## 10.2 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.

## 10.3 Analysis Populations

The primary efficacy population will be the intent to treat (ITT) efficacy population, which will include all randomized participants 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 participants who completed all 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 participants who received at least one dose of study treatment.

## 10.4 Efficacy: Cognitive, Behavioral and Pharmocodynamic Measures Analysis

All cognitive and behavioral 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.

## 10.5 Safety Analysis

Safety analysis will be based on all participants 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 participants. 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 and laboratory tests.

# 11 DATA MANAGEMENT

## 11.1 Data Handling

Data will be recorded at the site on CRFs. All corrections or changes made to any study data will be noted on the CRFs and dated appropriately.

## 11.2 Data Entry into EDC

Data will be collected and processed in RedCap. Data will be recorded in RedCap 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. 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.

## 11.3 Data Validation

Validation checks programmed within RedCAP, 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 corrected.

The paper CRFs must be reviewed and signed by the Investigator.

# 12 INVESTIGATOR REQUIREMENTS AND QUALITY CONTROL

## 12.1 Ethical Conduct of the Study

The Investigator agrees 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),[26,27] and the policies and procedures as outlined by the ethical requirements for IRB review and informed consent form.
The Investigator agrees to allow inspection by the FDA or other appropriate regulatory authorities as needed.

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 participants.

## 12.2 Institutional Review Board (IRB)

Before initiation of the study, the Investigator will obtain approval or favorable opinion of the research protocol, informed consent form, and any advertisement for participant 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, participant

informed consent, participant recruitment materials (if applicable), and other documentation as required by the IRB to their IRB for review and approval.

The study site will adhere to all requirements stipulated by their respective IRB. This includes notification to the IRB regarding: protocol amendments, updates to the participant informed consent, recruitment materials intended for viewing by participants, 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 will 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.

## 12.3 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 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, will ensure that each study participant (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 participant has been informed of his/her rights to privacy. The Investigator or delegate will allow the participant adequate opportunity to read the written informed consent and ask any questions. The Investigator will obtain written informed consent from each participant 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 participant to inspection by the IRB/EC and/or regulatory agencies. A copy of the signed ICF will be given to the participant.

## 12.4 Record Storage and Retention

Records of participants, 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 10 years, prior to transfer or destruction of the records.

The Investigator will keep records, including the identity of all participating participants (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 or local regulations. If the Investigator relocates, retires, or for any reason withdraws from the study, the study records will be transferred to an acceptable designee, such as another Investigator.

## 12.5 Protocol Amendments and Deviations

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 participant safety. In this case, the situation must be documented and reported to the IRB within 5 working days.

## 13 REFERENCES

1. Gross C, Hoffmann A, Bassell GJ, Berry-Kravis EM. Therapeutic strategies in fragile X syndrome: from bench to bedside and back. Neurotherapeutics 2015;12:584-608..
2. Hunter, Jessica, et al. "Epidemiology of fragile X syndrome: A systematic review and meta-analysis." American Journal of Medical Genetics Part A 164.7 (2014): 1648-1658.
3. Berry-Kravis EM, Lindemann L, Jønch AE, Apostol G, Bear MF, Carpenter RL, Crawley JN, Curie A, Des Portes V, Hossain F, Gasparini F, Gomez-Mancilla B, Hessl D, Loth E, Scharf SH, Wang PP, Von Raison F, Hagerman R, Spooren W, Jacquemont S. Drug development for fragile X syndrome: Lessons learned and implications for treatment of neurodevelopmental disorders. Nat Rev Drug Discov. 2018 Apr;17(4):280-299. PMID: 29217836
4. Bakker et al., Fmr1 knockout mice: a model to study fragile X mental retardation. The Dutch-Belgian Fragile X Consortium. Cell. 1994 Jul 15;78(1):23-33. PMID: 8033209
5. Yan QJ et al., A phenotypic and molecular characterization of the fmr1-tm1Cgr Fragile X mouse, Genes, Brain and Behavior, Wiley Online Library 2004, https://doi.org/10.1111/j.1601-183X.2004.00087.x
6. Mientjes EJ, Nieuwenhuizen I, Kirkpatrick L, Zu T, Hoogeveen-Westerveld M, Severijnen L, Rifé M, Willemsen R, Nelson DL, Oostra BA. The generation of a conditional Fmr1 knock out mouse model to study Fmrp function in vivo. Neurobiol Dis. 2006 Mar;21(3):549-55. doi: 10.1016/j.nbd.2005.08.019. Epub 2005 Oct 26. PMID: 16257225.
7. 5-Hydroxytryptophan Toxicity data, Pubchem, Online (accessed 05 Feb 2021) at<https://pubchem.ncbi.nlm.nih.gov/compound/5-Hydroxytryptophan#section=Toxicity&fullscreen=true>
8. Hydergine Summary of data for chemical selection, Online (accessed 05 Feb 2021) at<https://ntp.niehs.nih.gov/ntp/htdocs/chem_background/exsumpdf/hydergine_508.pdf>
9. Sharma, N., Sharma, V. K., Kumar Manikyam, H., & Bal Krishna, A. (2016). Ergot Alkaloids: A Review on Therapeutic Applications. European Journal of Medicinal Plants, 14(3), 1-17. https://doi.org/10.9734/EJMP/2016/25975
10. McDonald, R. J. (1979). Hydergine: A Review of 26 Clinical Studies. Pharmacopsychiatry, 12(06), 407–422. https://doi.org/10.1055/s-0028-1094637
11. Schneider LS, Olin JT. Overview of Clinical Trials of Hydergine in Dementia. Arch Neurol. 1994;51(8):787–798. doi:10.1001/archneur.1994.00540200063018
12. Ammon, R., Sharma, R., Gambert, S.R. et al. Hydergine revisited: A statistical analysis of studies showing efficacy in the treatment of cognitively impaired elderly. AGE 18, 5–9 (1995). https://doi.org/10.1007/BF02434076
13. Olin J, Schneider L, Novit A, Luczak S. Hydergine for dementia. Cochrane Database Syst Rev. 2001;(2):CD000359. doi: 10.1002/14651858.CD000359. PMID: 11405961.
14. Wadworth, A.N., Chrisp, P. Co-Dergocrine Mesylate. Drugs & Aging 2, 153–173 (1992). https://doi.org/10.2165/00002512-199202030-00002
15. Das YT, Bagchi M, Bagchi D, Preuss HG. Safety of 5-hydroxy-L-tryptophan. Toxicol Lett. 2004 Apr 15;150(1):111-22. doi: 10.1016/j.toxlet.2003.12.070. PMID: 15068828.
16. Meltzer H, Bastani B, Jayathilake K, Maes M. Fluoxetine,but not tricyclic antidepressants, potentiates the 5-hydroxytryptophan-mediated increase in plasma cortisol and prolactin

secretion in subjects with major depression or with obsessive compulsive disorder. Neuropsychophar-macology 1997;17:1–11.
17. Nardini M, De Stefano R, Iannuccelli M, Borghesi R,Battistini N. Treatment of depression with L-5-hydroxytry-ptophan combined with chlorimipramine, a double-blindstudy. Int J Clin Pharmacol Res 1983;3:239–50.
18. Kline N, Sacks W. Treatment of depression with an MAOinhibitor followed by 5-HTP – an unfinished research project. Acta Psychiatr Scand Suppl 1980;280:233–41.
19. Alino JJ, Gutierrez JL, Iglesias ML. 5-Hydroxytryptophan (5-HTP) and a MAOI (nialamide) in the treatment of depression. A double-blind controlled study. Int Pharmacopsy-chiat 1976;11:8–15.
20. Nicolodi M, Sicuteri F. Fibromyalgia and migraine, two faces of the same mechanism. Serotonin as the commonclue for pathogenesis and therapy. Adv Exp Med Biol 1996;398:373–9.
21. Quadbeck H, Lehmann E, Tegeler J. Comparison of the antidepressant action of tryptophan, tryptophan/5-hydroxytryptophan combination and nomifensine. Neuro-psychobiology 1984;11:111–5.
22. Turner EH, Blackwell AD. 5-Hydroxytryptophan plus SSRIs for interferon-induced depression: synergistic mechanisms for normalizing synaptic serotonin. Med Hypotheses. 2005;65(1):138-44. doi: 10.1016/j.mehy.2005.01.026. PMID: 15893130.
23. Ergoloid Mesylates Dosing Information, Sunpharma, Online (accessed 05 Feb 2021) at<https://www.sunpharma.com/sites/default/files/Ergoloid%20Mesylates%20PI.pdf>
24. 5- Hydroxytryptophan Dosing Information, Web MD, Dosing Tab, Online (accessed 05 Feb 2021) at< https://www.webmd.com/vitamins/ai/ingredientmono-794/5-htp) >
25. Mahmood I, Martinez M, Hunter RP. Interspecies allometric scaling. Part I: prediction of clearance in large animals. J Vet Pharmacol Ther. 2006 Oct;29(5):415-23. doi: 10.1111/j.1365-2885.2006.00786.x
26. Declaration of Helsinki (Fortaleza, October 2013) Seventh revision, 64th World Medical Association General Assembly Meeting.
27. International Conference on Harmonisation (ICH) E6 (R1) Good Clinical Practice Guideline.
28. Sansone et al. Improving IQ measurement in intellectual disabilities using true deviation from population norms. Journal of Neurodevelopmental Disorders 2014; 6:16.
29. NIH Toolbox, web address: http://www.healthmeasures.net/explore-measurement-systems/nih-toolbox .
30. 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.
31. 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.
32. 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.
33. Shields RH, Kaat A, McKenzie FJ, Drayton A, Sansone SM, Coleman J, Michalak C, Riley K, Berry-Kravis E, Gershon RC, Widaman KF, Hessl D. Validation of the NIH Toolbox Cognitive Battery in intellectual disability. Neurology 2020;94:1-12. PMID: 32094241
34. 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.

35. 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.
36. 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.
37. 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].
38. 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].
39. 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.
40. 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.
41. 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.
42. Lisa Cordeiro, Elizabeth Ballinger, Randi Hagerman and David Hessl. 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.
43. 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.
44. Luck S. J. An Introduction to the Event-Related Potential Technique. 2005. Cambridge, MA: MIT Press.
45. 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.
46. 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.
47. Ethridge L, Thaliath A, Kraff J, Nijhawan K, Berry-Kravis E. Development of neural response to novel sounds in fragile X syndrome: potential biomarkers. Am J Intellect Dev Disabil. 2020;125:449-464.
48. 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.

# 14 Appendices

## 14.1 Schedule of Assessments

| | | PERIOD 1 | | | PERIOD 2 | | PERIOD 3 | | PERIOD 4 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Screening Days -28 thru 0** | **P1 Day 1** | **P 1 Wk1 (±1)** | **P 1 Wk4 (±5)** | **P2 Wk5 (±1)** | **P2 Wk8 (±5)** | **P3 Wk9 (±1)** | **P3 Wk12 (±5)** | **P4 Wk13 (±1)** | **P4 Wk16 (±5)** | **P4 Wk16 (ET[a]) (±5)** | **P4 Wk17 (±1)** |
| **Clinic Visit** | **X** | **X** | | **X** | | **X** | | **X** | | **X** | **X** | |
| **Telephone Call** | | | **X** | | **X** | | **X** | | **X** | | | **X** |
| Informed Consent | **X** | | | | | | | | | | | |
| Eligibility | **X** | **X[b]** | | | | | | | | | | |
| Medical /Surgical History | **X** | | | | | | | | | | | |
| Demographics | **X** | | | | | | | | | | | |
| Physical Exam | **X** | | | | | | | | | | | |
| Abbreviated PE | | **X[b]** | | **X** | | **X** | | **X** | | **X** | **X** | |
| Ht, Wt, BMI | **X** | | | **X[c]** | | **X[c]** | | **X[c]** | | **X[c]** | **X[c]** | |
| Vital Signs[d] | **X** | **X[b]** | | **X** | | **X** | | **X** | | **X** | **X** | |
| Chemistry and Hematology | **X** | | | **X** | | **X** | | **X** | | **X** | **X** | |
| Urinalysis | **X** | | | **X** | | **X** | | **X** | | **X** | **X** | |
| Suicidality | **X** | | | **X** | | **X** | | **X** | | **X** | | **X** |
| Dispense Study Medication | | **X** | | **X** | | **X** | | **X** | | | | |
| 1st Dose of Medication | | **X** | | | | | | | | | | |
| Stanford-Binet | **X** | | | | | | | | | | | |
| NIH-TCB | | **X** | | **X** | | **X** | | **X** | | **X** | **X** | |
| KiTAP | | **X** | | **X** | | **X** | | **X** | | **X** | **X** | |
| CGI-S | | **X** | | **X** | | **X** | | **X** | | **X** | **X** | |
| CGI-I | | | | **X** | | **X** | | **X** | | **X** | **X** | |
| VAS | | **X** | | **X** | | **X** | | **X** | | **X** | **X** | |
| ABC | | **X** | | **X** | | **X** | | **X** | | **X** | **X** | |
| ADAMS | | **X** | | **X** | | **X** | | **X** | | **X** | **X** | |
| Vineland-3 | | **X** | | **X** | | **X** | | **X** | | **X** | **X** | |
| ERP | | **X** | | **X** | | **X** | | **X** | | **X** | **X** | |
| Eye Tracking | | **X** | | **X** | | **X** | | **X** | | **X** | **X** | |
| IP accountability | | | | **X** | | **X** | | **X** | | **X** | **X** | |
| Con Meds | **X** | **X[b]** | **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: Screening and Period 1 Day 1 visits may be combined***

[a] If early termination occurs, the Week 16 procedures should be completed at the time of the participant'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

## 14.2 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) |

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**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.