Protocol Title:

Placebo-Controlled, Single-Dose Challenge Study of Gaboxadol in Adult Males with Fragile X Syndrome (FXS)

Protocol Number:

3.0

Study Name:

Single dose gaboxadol study in Fragile X Syndrome

Compounds: Gaboxadol

Study Phase: Phase II

Sponsor-Investigator Name and Legal Registered Address:

Cincinnati Children's Hospital Medical Center, 3333 Burnet Ave, Cincinnati, OH 45229

Version and Approval Date:

Version 3.0 Dated 23Jan2025

Confidentiality Statement

The information in this document is provided to you for review by you and your staff and the appropriate Institutional Review Board or Ethics Committee. By accepting this document, you agree that the information contained herein will not be disclosed to others without written authorization from the Sponsor, except to the extent necessary by law.

Table of Contents:

1. **Protocol Summary**
2. **Schedule of Activities (SoA)**
3. **Introduction**
4. **Additional Review of Study Elements**
5. **Screen Failures**
6. **Treatments**
7. **Discontinuation Criteria**
8. **Study Assessments and Procedures**
9. **Statistical Considerations**
10. **References**
11. **Potential Abbreviations**
12. **Summary of Versions and Changes**

1 Protocol Summary

1.1 Synopsis

Protocol Title

An investigator initiated, placebo-controlled, single-dose challenge study of the target engagement of gaboxadol using high density EEG recording in adult males with Fragile X syndrome (FXS)

Rationale

Fragile X Syndrome is the most common single gene disorder that causes intellectual disability and autism spectrum disorder (ASD). As a single gene disorder with increasingly well understood neurobiology, FXS is a model disorder for targeted treatment development. A large number of biochemical pathways and potential targets have been linked to the physiopathology of FXS. FXS is a result of mutations in the Fragile X messenger ribonucleoprotein 1 (FMR1) gene that block the expression of the Fragile X messenger ribonucleoprotein (FMRP). FMRP is a ubiquitously expressed messenger RNA (mRNA) binding protein required for transport and translation of 4%–8% of synaptic proteins, and thus regulates a variety of synaptic functions being expressed in various tissues, most prominently in the central nervous system (CNS), and it is a key promoter of synaptic plasticity.

Gaboxadol is a delta (δ) subunit-selective extrasynaptic ɣ-aminobutyric acid type A ($GABA_A$) receptor agonist. Gaboxadol acts with high potency and efficacy at extrasynaptic δ-containing $GABA_A$ receptors (Budimirovic et al, 2020; Budimirovic et al, 2021). In the Fmr1 KO mouse, the absence of FMRP leads to excessive protein synthesis downstream of signaling pathways coupled to group I metabotropic glutamate receptors (mGluRs), in particular mGluR5. Inhibition of mGluR5 and downstream signaling has been shown to correct a wide array of disease phenotypes in animal models of FXS (Bhakar et al, 2012). Glutamatergic transmission is under tight regulation by GABAergic inhibition and deficiencies in GABA-mediated inhibitory neurotransmission have been identified in the hippocampus, striatum, somatosensory cortex and amygdala of Fmr1 KO mice (D'Hulst et al, 2007). The use of GABA agonists has been suggested as a therapeutic strategy for FXS (Olmos-Serrano et al, 2010). As such, the most consistent neurochemical change in the Fmr1 KO mouse is impaired GABAergic neurotransmission, and this dysfunction has been implicated in many of the symptoms of FXS, including anxiety, autism-like behavior, risk for epilepsy, and cognitive impairment.

Dysfunctional GABAergic tonic inhibition is believed to be the pathophysiologic process mediating many of the symptoms in both ASD and FXS, and through selective stimulation of $GABA_A$ receptors, gaboxadol targets the molecular modulators of this impaired process central to FXS. In a Phase 2 safety, tolerability, and efficacy study (OV101-17-001; ROCKET [NCT03697161]), 23 subjects (males aged 13 to 22 years) with FXS were randomized across 3 active-arm dose cohorts: 5 mg once daily (QD) gaboxadol (n = 4), 5 mg twice daily (BID) gaboxadol (n = 5), and 5 mg three times daily (TID) gaboxadol (n = 6). The primary objective of the study was safety and tolerability, and the secondary objective was to evaluate changes in behavior after 12 weeks of treatment.

This was the first interventional clinical study of gaboxadol in FXS, and it demonstrated an adequate safety profile and positive efficacy signals across multiple behavior domains from a small, active-arm study.

The general area of extrasynaptic GABAergic neurotransmission and tonic inhibition has been the focus of several recent reviews, and the consensus appears to be that a reduction in tonic inhibition is central to certain neurodevelopmental disorders, which speaks to the potential of gaboxadol in individuals with FXS (Olmos-Serrano et al, 2010; Martin et al, 2014; Olmos-Serrano et al, 2011) and (Brickley & Mody, 2012; Ramamoorthi & Lin, 2011; Egawa & Fukuda, 2013; Deidda et al, 2014).

We recently published findings demonstrating that the $GABA_B$ selective agonist baclofen acutely (single dose) decreases gamma power in FXS (n=17) compared to placebo, consistent with our findings in the Fmr1 KO mouse (Jonak et al., 2022). Notably, the degree of gamma reduction was highly correlated to FMRP expression, such that greater post-dose gamma reduction was observed in individuals with the lowest FMRP levels (unpublished data, Schmitt et al., in prep). Thus, further exploration of the utility of GABA agonists, especially in males with FXS, is warranted given that males expressing zero or trace levels of FMRP drove our overall patient gamma band rescue finding with baclofen treatment.

We now seek to scientifically evaluate whether GABA subtype receptor specificity exists regarding neurophysiologic target engagement (EEG signal rescue) in FXS. We will first explore this in males given that males with FXS showed a prominent rescue of aberrant EEG findings with single dose baclofen (GABAB). This protocol will generate scientific data enabling the field to compare GABAA receptor engagement with gaboxadol to our existing data generated with GABAB modulation with use of single-dose baclofen. These findings will significantly enable future planning for use of gaboxadol and other GABA modulators in future FXS-specific clinical treatment development efforts.

Objectives and Endpoints

Objectives	Endpoints
Primary	
• **To evaluate target engagement of gaboxadol treatment on high density EEG recordings**	• **Changes in EEG recordings with gaboxadol treatment (60-90 minutes post-dose compared with pre-dose on resting power values, peak alpha frequency, and chirp ITC)**
Secondary	
• **To investigate the feasibility of home research visits and procedures in adult males with FXS**	• **Quality and test-retest reliability of assessments performed at home compared with the hospital setting (as assessed by test-retest of FMRP levels, NIH Cognitive Toolbox measures, RBANS list learning, and exploratory eye tracking)**
• **To investigate the feasibility of high density EEG recording at home in adult males with FXS**	• **Quality and test-retest reliability of EEG recordings in the home setting compared with EEG recordings performed in the hospital setting (as assessed by 1) raw data quality ratings and 2) ICC values of resting power, peak alpha frequency, and chirp ITC)**
• **To potentially explore the pharmacokinetics of gaboxadol treatment in single-dose trial design**	• **PK profile (60 +/- 10 minutes post-dose only); plasma samples will be banked for potential future PK analysis**
• **To investigate the effect of gaboxadol treatment on neuropsychological assessments**	• **Changes in cognitive and neuropsychological assessment measures with gaboxadol treatment (post-dose compared with pre-dose on NIH Toolbox and RBANS)**
• **To investigate the effect of gaboxadol treatment on eye tracking assessments**	• **Change in eye tracking outcomes (60-90 minutes post-dose compared to pre-dose on percent viewing of social versus geometric scene**
• **To investigate the effect of gaboxadol treatment on clinician-rated measures**	• **Change in clinician-rates measures (post-dose compared to pre-dose on CGI-I**

Objectives	Endpoints
• **To potentially determine whether FMRP levels predict treatment response**	• **Relationship between FMRP level and degree of change (baseline/screen to post-dose) across measures**

Overall Design

This is a single dose, placebo-controlled study. The study includes a Screening Period of up to 2 weeks. Male subjects aged 18 to 40 years (inclusive) with a diagnosis of FXS, consistent with their clinical evaluation and confirmed by FMR1-DNA testing showing presence of full mutation, will be eligible to participate in the study. Eligible subjects may enroll in this study comprised of two in home and two in clinic visits each 14 days apart, for a total of four visits. Subjects will be given single dose gaboxadol (10 mg) or matched placebo at each of these visits to take orally. Thus, all enrolled subjects will receive placebo at home and in clinic and receive gaboxadol at home and in clinic in a blinded fashion. Following the treatment visits, a follow up safety phone call will occur 7 days after the final treatment visit.

Clinical outcomes will be assessed by:

- Neurophysiologic Biomarkers
    - EEG (resting and auditory evoked potential studies)
        - Resting: Relative power across 7 frequency bands: delta (2–3.5 Hz), theta (3.5–7.5 Hz), alpha1 (8–10 Hz), alpha2 (10–12.5 Hz), beta (13–30 Hz), and gamma1 (30–55 Hz), and gamma2 (65–90 Hz); individualized alpha peak frequency (IAPF)
        - Auditory chirp: theta/alpha ITPC to stimulus onset, ITPC to the chirp stimulus at 40 Hz, ITPC to the chirp stimulus centered at 80 Hz
- Cognitive and neuropsychological assessment measures

Measure	*Domain*	*Task Description*	*Estimated Time*
RBANS List Learning	Working memory	Brief neuropsychological assessment of verbal working memory	5 minutes
NIH Cognitive Toolbox 3.0	Oral Reading, Picture Vocabulary, Speed Matching, Pattern Comparison Processing Speed, Flanker, Dimensional Card Change Sort	Computerized tasks comprised of 6 subtests presented on iPAD to assess range of language and cognitive domains including processing speed, inhibitory control, cognitive flexibility.	45 minutes
Screen Based Eye-tracking (Optional)	Social gaze aversion	An examinee will observe a series of pictures and videos on a	10 minutes

		computer screen where a video will capture gaze preference.	

- Overall Clinical Picture
    - Clinical Global Impressions of Improvement (CGI-I) and Severity (CGI-S)

Blood will be drawn for safety assessments (hematology, chemistry and urinalysis) at Screening and at the second clinic visit. The optional post-dose blood draw for fragile X messenger ribonucleoprotein levels (FMRP) and exploratory potential future PK assessments will be drawn at drug dosing visits prior to post-dose EEG evaluations if the participant opts in..

Safety will be assessed by reported AEs (evaluated at every visit), physical examination (evaluated at screening and as needed), vital signs (evaluated at every visit), and laboratory values (complete blood count with differential, comprehensive metabolic panel, and urinalysis; evaluated at screening).

Study Population

Inclusion Criteria

1.	Subject consents to participate, or if they are not their own legal guardian, offers assent supported by legally authorized representative consent
2.	Subject is willing and able to comply with the study procedures as specified in the protocol and to comply with the study drug administration. Caregiver also commits to the study requirements prior to any study-related procedures
3.	Subject and caregiver are both able to understand the spoken national language clearly and caregiver can read and write to complete study assessments
4.	Males age 18 to 40 years (inclusive)
5.	Has FXS with molecular genetic confirmation of the full FMR1 mutation (>200 cysteine-guanine-guanine [CGG] repeats). May have been confirmed historically or at Screening
6.	Is in general good health as deemed by the Investigator, determined by physical examination, medical history, and laboratory tests
7.	If receiving serotonin-selective reuptake inhibitor (SSRI), serotonin-norepinephrine reuptake inhibitor (SNRI), or serotonin antagonist and reuptake inhibitor (SARI), is on a stable, well-tolerated dose for the previous 3 months with no further changes anticipated
8.	Is not sexually active or can confirm at least one form of contraceptive

Exclusion Criteria

1.	Any chronic major medical comorbid condition deemed by the Investigator as presenting added risk to the subject, including but not limited to, refractory hypertension, kidney disease, or liver disease
2.	Diagnosed with diabetes (Type 1 or II) or receiving any anti-diabetic medication
3.	Unstable seizure disorder, defined by any seizure within 6 months prior to baseline visit and/or a change in any anti-convulsant drug dosing in the 60 days prior to study consent
4.	Changes in psychotropic or anti-convulsant (where taken for reasons other than seizure control) drug treatment within 30 days prior to Screening[1]
5.	Significant changes in any educational, behavioral, and/or dietary interventions the month prior to Screening[1]
6.	Planned initiation of new, or modification of ongoing, interventions during the study
7.	Unable or unwilling to take oral medication (whole capsule, despite assistance with a spoonful of applesauce, yoghurt, or equivalent liquid food)
8.	Consumption of liver enzyme inducers or inhibitors including and not limited to foods, medicines, herbal remedies and supplements three days prior to any Visit. Foods or beverages containing CYP3A4/5 inhibitors (e.g., grapefruit, pomegranate, pomelo, and star fruit) should be avoided before taking study medication and for up to 1 hour post dose throughout the study
9.	Has abnormal baseline laboratory assessments including, but not limited to, alanine aminotransferase (ALT) or aspartate aminotransferase (AST) or total bilirubin >1.5 × the upper limit of normal (ULN), serum creatinine >1.5 x ULN or other clinically relevant laboratory abnormality
10.	Has a clinically significant heart rate or blood pressure (BP) at Screening as judged by the Investigator
11.	Has received an investigational drug in any prior clinical study within 30 days or 5 half-lives (whichever is longer) prior to Screening

[1] Significant changes exclude changes at the end or beginning of the school year and school vacations since these are normal/constant/expected.

Number of Subjects

Ten subjects will be enrolled. This sample size is based on clinical considerations and considered large enough to demonstrate target engagement as well as the feasibility of the home assessments. As this is a target engagement study, the aim is to look for possible signals or trends, and all statistical analyses are considered exploratory. Subjects included in the study will be replaced if they do not complete at least one home and one in-clinic treatment visit.

Recruitment

We will recruit participants from our CCHMC clinic. We will also recruit through the National Fragile X foundation. We will provide the foundation with an Ad to be posed on their social media (Twitter/X, Facebook and LinkedIn). The Ad will link to the foundations MyFXResearch portal (https://fragilex.org/professional-resources/myfxresearch-portal/) where anyone can scroll through study opportunities and look for those that match their eligibility criteria and direct them to the contact info for the study.

Duration

Each subject will be in the study for a minimum of 9 weeks (4 visits, each 2 weeks (+/- 2 days) apart) or up to 12 weeks if screening and first dosing visit cannot occur on same day.

Safety Analyses

Safety variables will be analyzed according to their scale using descriptive methods as described above, and results will be presented by time point where appropriate.

2 Schedule of Activities (SoA)

Activity	Visit 1 Screening/First Dosing Day (Day 0)	Study period			Follow up phone call (Day 49+/-5)
		Visit 2 Second dosing day (at home; Day 14+/-3)	Visit 3 Third dosing day (in clinic; Day 28+/-3)	Visit 4 Fourth dosing day (at home; Day 42+/-3)	
Obtain informed consent	x				
Verify eligibility	x	x	x	x	x
Administer Stanford-Binet Test, SB5	x				
Record demographics	x				
Safety Evaluations					
Record health history	x				
Perform physical examination (including height and weight)	x				
Record vital signs (blood pressure, heart rate, temperature)	x	x	x	x	
Record AEs, unscheduled visits and medical resource utilization		Continuously through follow up phone call			
Record concomitant medications	x	x	x	x	x
Suicidality Checklist	x	x	x	x	x
Study drug					
Dispense and administer study drug	x	x	x	x	
Laboratory Assessments					
Collect samples for safety labs (hematology (CBC), comprehensive metabolic panel, urinalysis)	x		x		
Collect blood for FMR1 (if prior testing unavailable)	x				
Collect blood for FMRP and banked plasma for future potential PK analysis (optional)	x	x	x	x	
Pharmacokinetic gaboxadol sampling (optional)	x	x	x	x	
Clinical Outcome Measures (COM)					
Electrophysiology (EEG; pre- and post-dose at each treatment visit	x	x	x	x	

Activity	Visit 1 Screening/First Dosing Day (Day 0)	Study period			Follow up phone call (Day 49+/-5)
		Visit 2 Second dosing day (at home; Day 14+/-3)	Visit 3 Third dosing day (in clinic; Day 28+/-3)	Visit 4 Fourth dosing day (at home; Day 42+/-3)	
Administer NIH Cognitive Toolbox 3.0	x	x	x	x	
RBANS: List Learning	X	X	X	x	
Clinical Global Impressions of Improvement (CGI-I)		x	x	x	
Screen-Based Eye Tracking (emotional faces and social preference) [Optional]	x	x	x	x	
Baseline Characterization Measures					
Complete Aberrant Behavior Checklist (ABC-C_{FXS})	x				
Administer ADAMS	x				
Stanford Binet, 5th Edition (SB-5) IQ testing	x				
Clinical Global Impressions-Severity (CGI-S)	x				

Abbreviations: ABC-C_{FXS} = Aberrant Behavior Checklist; ADAMS = Anxiety, Depression and Mood Scale; AE = adverse event; EEG = electroencephalography; EOT = End of Treatment; FMR1 = Fragile X messenger ribonucleoprotein 1; FMRP = Fragile X messenger ribonucleoprotein; FXS = Fragile X Syndrome; NIH = National Institutes of Health; PK = pharmacokinetic(s);

Note: SB5 test results taken less than 1 year prior to Screening are acceptable as long as raw totals for each subset are available. Repeat unscheduled AEs arising after treatment administration may be recorded, all other ongoing medical issues at the time up to study drug administration should be recorded as medical

3 Introduction

3.1 Background

FXS is the most common inherited form of intellectual disability. Approximately 1 in 4,000 people worldwide are diagnosed with FXS. FXS is the result of a cysteine-guanine-guanine (CGG) trinucleotide repeat expansion (>200 repeats) within the Fragile X messenger ribonucleoprotein 1 (FMR1) gene located near the long arm of the X chromosome. Translation of the FMR1 gene leads to the synthesis of FMRP. Mutations in FMR1 lead to a lack of FMRP, resulting in FXS.

FXS is associated with a substantially increased risk, particularly in males, for a particular neurobehavioral phenotype marked by severe interfering behavioral symptoms in addition to cognitive delay. Approximately 2 in 3 males with FXS are thought to exhibit behavior consistent with an additional diagnosis of autism spectrum disorder (ASD; Clifford et al, 2007).

Additionally, FXS is associated with significant behavioral disturbance which contributes not only to the disability of the affected individual, but also has a significant negative impact on parents and caregivers. In one report, 89.8% of 49 boys with FXS exhibited significant interfering externalizing behaviors, most often driven by anxiety, which contributed to more familial stress compared to families of children with other chronic illness and controls (von Gontard et al, 2002). Fifty-eight percent of boys with FXS exhibited self-injurious behavior (SIB) in a survey report that included 55 families (Symons et al, 2003). Behaviors such as aggression and SIB in FXS are most often driven by treatment-refractory anxiety (Tranfaglia et al, 2012). Problematic associated behaviors, including aggression to self and others, and property destruction, can significantly limit the ability of an individual with FXS to participate in critical early intervention strategies such as speech and language therapy and formal educational programs. Later in life, behaviors commonly seen in FXS contribute to lifelong disability as they can limit vocational opportunities and curtail the individual's ability to contribute to more typical societal efforts and norms. The costs necessary to care for these individuals throughout their lives are therefore escalating and ever-increasing.

As a single gene disorder with increasingly well understood neurobiology, FXS is a model disorder for targeted treatment development. Commercially repurposed drugs, whose selection are based on recommendations initiated from gene expression analysis and then investigated with success in FXS preclinical models, may be effective in reducing impairments commonly seen in FXS (Tranfaglia et al, 2019). Combination drug therapy may be required to effectively improve behavior in FXS given the wide-ranging impact of deficient FMRP on brain function. This approach is consistent with the many failed clinical studies in FXS in the last decade (Berry-Kravis et al, 2018).

The primary objective of the study will be to assess the effect of single dose gaboxadol on EEG in context of a single dose treatment trial.

Secondary objectives are to assess the feasibility of home assessments as well as the effect of single dose gaboxadol on clinical assessment, both at home and in the clinic.

3.2 Study Treatments

3.2.1 Gaboxadol

3.2.1.1 Non-clinical pharmacology

Gaboxadol, a selective and potent $GABA_A$ agonist for δ-subunit-containing extrasynaptic GABAA receptors, was evaluated as a therapeutic agent for FXS by assessing its ability to normalize aberrant behaviors in a relatively uncharacterized mouse model of FXS (Fmr1 KO2 mice). Four behavioral domains (hyperactivity, anxiety, aggression, and repetitive behaviors) were probed using a battery of behavioral assays (Cogram et al, 2019).

Clinical symptoms of FXS can include hyperactivity, anxiety, memory and learning deficiencies, social abnormalities, aggression, and repetitive behaviors. In all cases, treatment with gaboxadol 0.5 mg/kg restored the behavior of the Fmr1 KO2 mice to wild-type levels.

Locomotor hyperactivity is a feature of human FXS. In the current study, Fmr1 KO2 mice showed increased locomotor activity that was normalized by gaboxadol. Previous work in the Dutch-Belgian Fmr1 KO mice has also shown a consistent hyperactivity phenotype that was reversed with gaboxadol treatment, however, at a higher dose.

The results from three anxiety tests, center distance in the Open Field Test (OFT), light/dark box, and successive alleys test (SAT), demonstrated a robust phenotype in the Fmr1 KO2 mouse that is reversed by treatment with gaboxadol. It should be noted that hyperactivity could confound the OFT and light/dark box results. However, anxiety-related behavior observed in the SAT occurs independent of locomotor activity changes, and while the anxiety effect in the KO is in the opposite direction of what is observed in patients with FXS, the results are consistent with previous reports describing the FXS mouse phenotype. Notably, the data demonstrated that gaboxadol normalized the aberrant anxiety behavior observed in the FXS mouse model as well as in all the other behaviors assessed in our analyses.

Irritability and aggressive behaviors, modeled by measuring social isolation with a novel cage mate, were also increased in the Fmr1 KO2 mice and reversed by gaboxadol. A recent study reported that nearly all (>90%) surveyed males and females with FXS engaged in some aggression in the previous 12 months. Among them, 33% of males and 20% of females showed severe aggression, i.e., enough to cause injury to care givers. Self-injury and impulsive behavior were also more prevalent in individuals with FXS.

Repetitive and stereotyped behaviors were consistently increased in Fmr1 KO2 mice and alleviated by gaboxadol treatment. Repetitive behaviors can cause serious problems for daily functioning and can become a barrier to learning and social isolation. Paradigms that assess stereotypic behaviors and socioemotional deficits might thus be useful to assess novel drugs for their effectiveness to ameliorate the autistic phenotypes in FXS.

Gaboxadol normalized all the tested behavioral deficits of Fmr1 KO2 mice at a dose of 0.5 mg/kg. While higher doses also normalized irritability and aggressive behaviors, this was not observed for other behavioral domains evaluated. One explanation for the somewhat narrow efficacy window observed here may come from previous work showing compromised information processing by either insufficient or excess tonic inhibition, which is the physiological process that gaboxadol

potentiates. Under this model, the behavioral benefit of drug at high doses would be offset by pharmacologically introduced FXS-independent deficits.

These results provide robust evidence of the potential benefit of gaboxadol in reversing ASD-related behaviors, aggression and sociability. Taken together, these results support the hypothesis that potentiation of extrasynaptic $GABA_A$ receptors by gaboxadol may be of benefit in individuals with FXS.

3.2.1.2 Effect in humans

Gaboxadol is a novel δ-selective $GABA_A$ agonist. In the signal-finding, randomized, double-blind, parallel-group study (ROCKET) to evaluate the safety, tolerability and efficacy in males aged 13 to 22 years with a confirmed diagnosis of FXS, 23 subjects were randomized across three active-arm dose cohorts: gaboxadol 5 mg once daily (QD), gaboxadol 5 mg twice daily (BID), and gaboxadol 5 mg three times daily (TID). The study met its primary objective and appeared to be well tolerated over 12 weeks of treatment with no serious adverse events (SAEs) or deaths reported across the three dose cohorts. The lower and middle dosing regimens appeared to be superior to the higher, TID dose, which is consistent with the emerging profile of gaboxadol (Ovid Therapeutics, 2020).

3.3 Benefit/Risk Assessment

The single dose of gaboxadol given two times throughout study is not expected to provide clinical benefit. However, it may provide information that can be used in future studies, including evidence of target engagement on EEG measures (our primary aim). A single dose design allows us to evaluate the effect on neurophysiology in a minimally invasive manner. We have previously demonstrated target engagement using another GABAergic drug, baclofen, specifically demonstrating the reduction of gamma power following single dose administration (Jonak et al., 2022). The risks associated with the single dose of gaboxadol are assessed as minimal. However, somnolence is a potential treatment-related adverse event and will be monitored for as an adverse event of special interest. More detailed information about the known and expected benefits and risks and reasonably expected AEs of gaboxadol may be found in the cross references gaboxadol safety data. Each subject will receive one gaboxadol dose in clinic, one gaboxadol dose in the home, one placebo dose in clinic, and one placebo dose in the home.

The information gathered via high density EEG recordings may be of considerable benefit to future FXS studies as it has the potential to make “go no-go” decisions. Given our previous demonstration of target engagement with baclofen, a $GABA_B$ selective agonist, and the known deficient GABA neurotransmission in FXS, it is reasonable to predict that gaboxadol, a $GABA_A$ agonist, may have a similar impact on neurophysiology. The proposed design allows us in a small patient sample and with a single dose to determine whether gaboxadol has any impact on neurophysiology. If we show neurophysiological target engagement, this would allow us to move to a larger scaled trial to determine longer-term treatment impact. In addition, given we are obtaining FMRP levels and additional clinical measures on participants, we also may determine whether specific baseline characteristics predict gaboxadol response so that future studies may selectively enroll those individuals who are most likely to respond. We have demonstrated in our single dose baclofen study that individuals with the lowest FMRP levels demonstrated the most target engagement, or reduction of gamma power.

Furthermore, this study will add important information to the scientific data on EEG changes in people with FXS, allowing a better understanding of how this objective assessment varies within and between individuals. All of this is important for the field in terms of better understanding FXS and, in turn, helping potential treatments to be developed.

Last, the information provided by the home assessments may be of considerable benefits to patients participating in future FXS studies since, if these assessments can be performed reliably at home, this will greatly reduce the burden of study participation on patients and families.

4 Additional Review of Study Elements

4.1 EEG rationale

Electroencephalogram (EEG) has become an established methodology for the assessment of brain activity (Herrmann et al, 2010; Buzsáki and Schomburg, 2015; Stoyell et al, 2021). Electrodes in contact with the scalp can, non-invasively, detect changes in electrical currents near the surface of the brain (Clancy, 1988). These changes are recorded as an EEG signal and can be analyzed in a multitude of ways (Sanei and Chambers, 2013; Morales and Bowers, 2022). Event-related potentials (ERPs) assess signal changes with features like N1 and P2 characterizing stimuli response. Time frequency plots allow different frequencies of brain activity to be visualized and analyzed with the signal segmented into delta (1–4 Hz), theta (4–8 Hz), lower alpha (8–13 Hz), beta (13–30 Hz), and low gamma (30–55 Hz) and high gamma (65-80 Hz) frequency bands, (Jonak et al, 2022). A variety of analysis methodologies can then be employed to analyze the individual frequency power, coupling behavior between (Pedapati et al, 2022) and within (Wang et al, 2017; Schmitt et al, 2022) different frequency bands, and synchronization of frequency bands with external stimuli (Ethridge et al, 2019).

Measurements are taken when the Subjects are either at rest or during exposure to external (auditory) stimuli. Resting EEG measures the behavior of the brain without auditory stimuli, but with silent standardized videos to ensure compliance while paradigms such as chirp measure the behavior of the brain when exposed to external stimuli. The chirp paradigm (Dau et al, 2000) presents a repeating oscillating sound wave. Such experiments measure the synchronization of brain activity to external stimuli.

While there is limited sensitivity with regards to depth of signal detection and identification of the signal's region of origin, EEG has been shown to be a valuable assessment tool in the field of epilepsy (Boonyakitanont et al, 2020), neurodevelopmental conditions (Ewen et al, 2019), and is rapidly becoming regarded as a valuable biomarker in Fragile X (Kenny et al, 2022).

Fmr1 KO mouse models of Fragile X have been observed to demonstrate neocortical hyperactivity (Gibson et al, 2008; Hays et al, 2011, Goswami et al, 2019). Additionally, increased resting EEG power and reduced synchronization with the chirp paradigm was recorded (Jonak et al, 2020).

Such EEG biomarkers are translatable across to the Fragile X human populations with both auditory and resting paradigms demonstrating hyperactivity, changes in frequency power levels compared to controls and altered synchronization response in Fragile X individuals. Auditory stimuli were found to invoke enhanced N1, P2 responses on ERPs (Ethridge et al, 2019). Increased gamma power is seen in Fragile X cohorts, both at rest (Wang et al, 2017) and when subjected to auditory (Ethridge et al, 2019) stimuli, with males elevated gamma power more pronounced**.** Increases in theta power are coupled to this gamma increase, with alpha power seen to reduce. Additionally, phase locking, or synchronization of gamma with external stimuli, such as that in the auditory chirp paradigm is reduced in Fragile X patients (Ethridge et al, 2019).

There have been preliminary studies investigating the usability of these biomarkers to identify target engagement in drug development (Jonak et al, 2022). Single dose racemic baclofen (RBAC) was found to increase low frequency power and suppress elevated background gamma power at rest, in both Fragile X humans and Fmr1 KO mouse models. Increased synchronization with the chirp stimulus was seen in the mouse cohort however not replicated in the human cohort.

This was however attributed to the analysis being underpowered due to data loss. Increase in theta and reduced gamma power was seen across both cohorts post dosing as measured by chirp. Moreover, there was evidence of gamma changes being localized instead of global.

These findings have drawn to light the issue of signal localization in EEG. These limitations can be somewhat overcome by employing source imaging (Michel and Brunet, 2019). Source imaging, where EEG signals are projected through an electronic field model based on MRI, allows increased localization of the EEG signals leading to separation of overlapping signal regions (Pedapati et al, 2022). In resting state, increases in gamma power were found to be localized to the bilateral temporal lobes with low frequency changes found to be global. Cross frequency correlations, such as theta- gamma, have also been identified as potential future biomarkers with power coupling and regional coupling of interest. Moreover, EEG measures correlate with clinical outcome measures, including hyperactivity, irritability, social withdrawal, and adverse speech scales (Ethridge et al, 2017; Pedapati et al, 2022).

Employing resting and chirp paradigms in conjunction with ERP, time frequency and advanced sourcing imaging analysis we aim to leverage these gamma EEG biomarkers to identify target engagement and explore further the potential of theta and cross frequency couple biomarkers and possible clinical correlations.

4.1 Justification for Dose

4.1.1 Gaboxadol

The gaboxadol dosage selected for this study is 10 mg or Placebo as a single dose on each study visit (two at home and two in clinic). The justification for the selected gaboxadol dosage is based on previous studies in FXS (Budimirovic, 2021).

Preclinical margins of safety over a 5 mg BID dose have been established across a wide range of species (mice, rats, dogs and baboons) and study lengths (13, 26 and 52 weeks). Systemic exposure cover (C_{max} and AUC) ranges from 6-47-fold for rodents and dogs to 2 to 12-fold in baboons based on the NOAEL or no observed effect level (NOEL) in terms of baboons.

A total daily dose ranging from 5 mg to 15 mg per day is within the dose range previously found to be well tolerated in adult subjects. Results from Study gaboxadol-16-001 indicate that the PK profile of gaboxadol following administration of a single 5 mg oral dose to adolescent (13- to 17-year-old) male and female subjects with FXS or AS is comparable to the PK profile previously observed in healthy adult subjects. Therefore, gaboxadol exposure in the present study population of 18-to 40-year-old subjects with FXS is expected to be comparable to the exposure range previously studied across the Phase 1, 2, and 3 studies that evaluated gaboxadol in adult subjects with insomnia.

After the last dose of study drug there will be no tapering of gaboxadol dosing, as no withdrawal effects have been observed in previous clinical studies.

5 Screen Failures

Screen failures are defined as subjects who consent to participate in the clinical study but are not subsequently enrolled. A minimal set of screen failure information is required to ensure transparent reporting of screen failure subjects to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any SAE.

Individuals who do not meet the criteria for participation in this study (screen failures) may be rescreened once if the reason for failing Screening is anticipated to resolve in a short period of time. Individual lab parameters or Screening tests may be repeated within the 2-week screening period if the result is considered aberrant or not clinically consistent with the subject's health status. Decisions to retest should be made in conjunction with the study medical monitor.

6 Treatments

6.1 Study Treatments Administered

Study drug is the active Gaboxadol or matching placebo.

Treatments administered are summarized in Table below.

Treatments Administered

Study Drug Name:	**Gaboxadol**	**Placebo to match Gaboxadol**
Dose Formulation:	**Gelatin Capsule (Size 2)**	**Gelatin Capsule (Size 2)**
Unit Dose Strength(s)/Dose Level(s):	**5 mg**	**Placebo**
Route of Administration	**oral**	**oral**
Dosing Instructions:	**10 mg as a single dose (two 5mg capsules)**	**match active dosing (2 placebo capsules)**
Packaging:	**White HDPE 40 cc bottles containing 30 capsules**	**White HDPE 40 cc bottles containing 30 capsules**
Responsible Manufacturer:	**Mayne Pharma (US)**	**Mayne Pharma (US)**
Assembly of Clinical Supplies:	**Sharp Clinical (US)**	**Sharp Clinical (US)**
Supplied by:	**Healx Limited**	**Healx Limited**

HDPE=high-density polypropylene

6.2 Method of Treatment Assignment

This is a double-blind, placebo-controlled single dose challenge study. Each subject will receive a single dose of drug or placebo in two settings at home and in clinic. Randomization will ensure that in each setting- home (visits 2 and 4) and in clinic (visits 1 and 3) - that each subject receives alternating gaboxadol or placebo.

6.3 Blinding

The placebo drug product matches the Gaboxadol 5 mg caps (two placebo or two gaboxadol capsules given on each dosing day). Dr. Paul Horn, biostatistician, will provide the Cincinnati Children's Hospital Medical Investigational Drug Pharmacy with the subject randomization schema. All study investigators involved with all aspects of executing the human research work will remain blinded to treatment assigned. The blind will be broken in the event of a study SAE

deemed possibly related to study drug treatment. All unblinding will be done via consultation of the protocol PI Dr. Craig Erickson and the study medical monitor Dr. Sam Vaughn.

6.4 Preparation/Handling/Storage/Accountability

The Investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study drug received and any discrepancies are reported and resolved before use of the study drug (see drug handling instructions in the Pharmacy Manual). Only subjects enrolled in the study may receive study drug and only authorized site staff may supply or administer study drug.

Study drug must be stored in a secure, environmentally controlled and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the Investigator and authorized site staff while at the study site. The Investigator, institution or the head of the medical institution (where applicable) is responsible for study drug accountability, reconciliation and record maintenance (i.e., receipt, reconciliation and final disposition records). For home visits, study drug will be ordered by a study physician and taken to the home visit by CCHMC study staff and administered to the subject in the home in accordance with CCHMC standard operating procedure for research study drug transport.

Further guidance and information for the final disposition of unused study drug are provided in the Pharmacy Manual.

6.5 Concomitant Therapy

Any medication or vaccine (including over the counter or prescription medicines, vitamins and/or herbal supplements) that the subject is receiving at the time of enrollment or receives during their participation in the study must be recorded along with:

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency

6.6 Treatment After the End of the Study

Since this is a feasibility study to assess home visits, no study-related treatment will be offered after the end of the study.

7 Discontinuation Criteria

7.1 Discontinuation of Study Drug

7.1.1 Temporary Discontinuation

Study drug dosing may be suspended in the event of:

1. Clinically important laboratory abnormalities
2. Other intercurrent illnesses or major surgery
3. Use of prohibited treatment
4. Any other protocol deviation that results in a significant risk to the subject's safety
5. Investigator decision

7.1.2 Rechallenge

Resumption of study drug after temporary discontinuation is the decision of the Investigator.

7.1.3 Dose Adjustment

No dose adjustment is available in this trial design.

7.2 Discontinuation from the Study

- A subject may withdraw from the study at any time at their own request, or the request of the legal guardian or may be withdrawn at any time at the discretion of the Investigator for safety, behavioral or administrative reason.
- If the subject consents, the End of Treatment visit procedures should be completed as soon as possible after the decision to discontinue the study.
- If the subject withdraws consent for disclosure of future information, the Investigator may retain and continue to use any data collected before such a withdrawal of consent.
- If a subject withdraws from the study, they may request destruction of any samples taken and not tested, and the Investigator must document this in the site study records.
- Refer to the SoA for data to be collected at the time of study discontinuation (End of Treatment) and follow-up and for any further evaluations that need to be completed.
- If the subject wishes to withdraw from treatment but not the study the End of Treatment visit should be scheduled at 14 ± 3 days from the date of study drug discontinuation.

7.3 Lost to Follow-up

A subject will be considered lost to follow-up if they repeatedly fail to return for scheduled visits and is unable to be contacted by the study site.

The following actions must be taken if a subject fails to return to the clinic for a required study visit:

- The site must attempt to contact the subject and reschedule the missed visit as soon as possible and counsel the subject on the importance of maintaining the assigned visit schedule and ascertain whether or not the subject wishes to and/or should continue in the study.
- Before a subject is deemed lost to follow-up, the Investigator or designee must make every effort to regain contact with the subject (where possible, 3 telephone calls and, if necessary, a certified letter to the subject's last known mailing address or local equivalent methods).
- Should the subject continue to be unreachable, they will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

Early Stopping

The study can be stopped early if warranted for safety, logistical, or strategic reasons. No statistical stopping rules will be applied.

8 Study Assessments and Procedures

Informed Consent Process

Screening and consent procedures for the study will be completed prior to enrollment. Participants (if they have decisional capacity) or legally authorized representatives together with participants initially will receive written materials and be given an oral explanation of the study. Informed consent procedures will be conducted by research staff or investigators. In addition, members of the protocol team will be available to address any questions for participants or families.

Each participant must give informed consent to participate when possible. Given the developmental disability associated with FXS, particularly in males, some subjects themselves will not be able to provide consent. Consent also will be provided by the legally authorized representatives of adult subjects who are not their own legal guardian.

Participants (and if they have one, their legally authorized representative) will be informed during their initial consent, prior to the initiation of the pharmacologic study, and on several additional occasions throughout their experience in the study that they may elect to not to participate at any time.

Consent will be collected using the procedures outlined above. This will take place after initial screening but before any formal assessments and experimental procedures begin. Both written and oral information will be provided to participants and legally authorized representatives. Refusal to participate in the study will not limit their ability to seek services at Cincinnati Children's Hospital Medical Center. One signed and witnessed copy of the consent form will be stored in secure storage and the participant and legally authorized representative will each be given a copy of the signed forms, as is appropriate. Participants will receive payment for taking part in this study.

Some individuals may be interested in study participation but live far away from CCHMC. In these circumstances, the consent forms will be mailed to potential participants after they have initiated contact with our research program. Our research team will arrange for a conversation with the potential participant by phone after the documents have been received. During the phone conversation, our research team member will review the consent form (i.e., study purpose, voluntary participation, study procedures, risks and benefits, and confidentiality) and answer any questions. Then, the participant will sign the documents and mail the documents back to the researcher. Once the researcher at our Center receives them, he/she will sign the consent forms and return copies of the documents to the participant for their records and place the originals in a secure area and a screening visit will be set up with the participant at CCHMC.

The study team will attempt to obtain consent from all participants potentially eligible for the study. All participants who are cognitively unable due to limited cognitive functioning will require a legally authorized representative to provide consent.

Study procedures and their timing are summarized in the SoA.

- Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.
- The Investigator and responsible clinical study site staff will review all assessment documentation for each subject to confirm eligibility at the Screening/Day 1 visits, as well as throughout the subject's participation in the study to ensure continued eligibility. The Investigator should confirm study eligibility at Screening and Day 1 in the subject's source documentation.
- All Screening evaluations must be completed and reviewed to confirm that potential subjects meet all eligibility criteria. Screening may occur on the same day as initial drug visit. The Investigator will maintain a Screening log to record details of all subjects screened and to confirm eligibility or record reasons for Screening failure, as applicable.
- The maximum amount of blood collected from each subject over the duration of the study, including any extra assessments that may be required, will not exceed 250 mL.
- Time windows outlined in the SoA should be adhered to given single-dose challenge. If the duration of each visit is too long for the patient, eligibility may need to be reconsidered. If Visit 1 is too long for the patient, Screening Day may be separated from Drug Visit 1.
- If Subjects cannot swallow whole capsules, it should be clearly documented and considered as screen failures. This would fulfil exclusion criteria number 9 and the patient should be withdrawn from the study.

Data Protection

- Subjects will be assigned a unique identifier by the Sponsor. Any subject records or datasets that are transferred to the Sponsor will contain the identifier only; subject names or any information which would make the subject identifiable will not be transferred.
- The subject must be informed that his personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the subject.
- The subject must be informed that his medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

8.1 Screening/Baseline Characterization Only Assessments

8.1.1 Stanford-Binet Test

The Stanford-Binet Test version 5 (SB5) with deviation z-scores is a commonly utilized IQ test in persons with FXS. Specific scoring algorithms derived in persons with FXS using the SB5 that avoid floor effects have been developed (Sansone et al, 2014). The SB5 Abbreviated will be used to characterize the subject's baseline cognitive status. The SB5 equivalent mental age will be used to determine which NIH Toolbox Cognition Battery test version to use. SB5 test results taken less than 1 year prior to Screening are acceptable as long as raw totals for each subset are available.

8.1.2 Genetic Mutation Confirmation

For any subjects who do not present at Screening with available FXS confirmatory testing using Southern Blot and polymerase chain reaction (PCR), confirmatory testing will be completed locally, and results will be available prior to the Day 1 visit to ensure a diagnosis marked by an over 200 CGG repeat expansion of the FMR1 gene with at least partial gene methylation.

8.1.3 Physical Examinations

Subjects will undergo a full physical examination at baseline. The physical examination will consist of an examination of the following: General Appearance, Skin, Head, Ears, Eyes, Nose and Throat, Cardiovascular, Pulmonary, Abdomen/GI, Neurological, Lymph Nodes, Spine, Extremities, Musculoskeletal systems, and any clinical signs of aseptic meningitis.

Investigators should pay special attention to clinical signs related to previous serious illnesses.

Height and weight will also be measured and recorded.

8.1.4 Vital Signs

Vital signs will be measured as outlined in the SoA in a semi-supine or supine position after 5 minutes rest and will include tympanic or oral temperature (ºC), systolic and diastolic BP [mmHg] and heart rate. Clinically significant findings will be captured as AEs. Vital signs will be conducted by trained study staff members as confirmed on the study delegation of authority log.

8.2 Study Assessments

8.2.1 Electrophysiology (via EEG) Assessments

An EEG will be administered according to the time points outlined on the SoA. Research-based EEG acquisition will be obtained using 128 lead channels. This work will include data outputs for resting state and auditory chirp. Research EEG testing post-dose will occur immediately following optional FMRP and pK sampling in the 60-90 minute post-dose time frame.

EEG gamma wave features will form the primary endpoint for this study. Additionally, changes in lower frequency bands, cross frequency coupling (CFC) and correlation of significant EEG features with clinical outcomes will be analyzed as exploratory outcome measures.

EEG primary endpoints will be measured using the resting state and chirp paradigms with auditory steady state included as an exploratory measure. The chirp paradigm will be broadband auditory chirp, with 2 second repetitions, designed to measure synchronization of all EEG frequency waves with external auditory stimuli. Resting state EEG will evaluate intrinsic brain activity unrelated to a specific task. EEG features will be analyzed with both electrode potential analysis (EPA) and source imaging models. EPA will be centered on the frontal lobe, due to signals originating from the auditory system being projected to access the frontal lobe from the temporal lobes. For the signal from the temporal and frontal lobes to be isolated and analyzed separately a source imaging model will be employed. Source imaging allows improved localization of the EEG signal by combining high density EEG with an MRI atlas.

At baseline patients with FXS are expected to present with elevated gamma power in the frontal regions where EPA is used and in the temporal lobes where source imaging is employed for both the resting state and chirp paradigms. Additionally, a reduction in what is termed synchronized gamma, where gamma waves synchronize with the external auditory stimuli in chirp, is expected for the FXS cohort prior to any treatment.

The primary and exploratory endpoints for each EEG paradigm are:

8.2.1.1 Resting state

Primary endpoint: Intraclass correlation coefficient (ICC) values for resting power (delta, theta, alpha, beta, gamma) and peak alpha frequency ≥ 0.6 and within 90% confidence interval of previously published ICC values (Liu et al., 2023). However, we expect the majority of ICC values to be 0.7 – 0.8. ICCs will compare pre-dose values only (i.e., pre-dose home vs. pre-dose lab). In addition, data quality ratings (1-5 scale, 1=unusable data, 5=perfect data) between corresponding home and lab visits that are equal or +/- 1.

Exploratory endpoint: Reduction in resting state gamma power 1 (low gamma) and gamma power 2 (high gamma) from baseline to end of treatment.

Treatment could potentially reduce gamma power in the FXS treatment group. This will be measured firstly, by electrode topography, to assess electrode level power changes and secondly, the source imaging model will be used to localize the gamma power to specific brain regions, with focus on power in the bilateral lobes of the brain.

Global and regional CFC as well as individualized alpha peak frequency will be analyzed as exploratory findings to understand the cross-functioning behavior. This will include but not be limited to coupling of gamma and theta signals as well as local and global gamma coupling.

8.2.1.2 Chirp

Primary endpoint: Intraclass correlation coefficient (ICC) values for gamma synchronization ≥ 0.6. ICCs will compare pre-dose values only (i.e., pre-dose home vs. pre-dose lab). In addition, data quality ratings (1-5 scale, 1=unusable data, 5=perfect data) between corresponding home and lab visits that are equal or +/- 1.

Exploratory endpoint: Reduction in gamma power 1 (low gamma) and gamma power 2 (high gamma) and increase gamma synchronization from baseline to end of treatment.

Treatment could potentially reduce gamma power in the FXS treatment group and increase gamma synchronization with the external auditory stimuli. This will be measured firstly by electrode potential analysis looking at gamma power and synchronization in the frontal region of the brain and secondly, the source imaging model will be used to localize the gamma power synchronization to specific brain regions, with focus on power in the bilateral lobes of the brain.

The ratio of gamma power to gamma synchronization and the correlation of this ratio with clinical outcome assessments will be analyzed. Synchronization onset, which is heightened in FXS, will also be evaluated. Other global and regional frequency power and synchronization, with a focus on theta, will be analyzed as exploratory findings.

8.2.2 NIH Cognitive Toolbox - Cognition Battery

The NIH Toolbox will be administered according to the timepoints outlined on the SoA. For post-dose Toolbox assessments, this measure will be administered immediately following completion of EEG assessments.

The NIH Toolbox assesses 4 different domains: cognition, emotion, motor and sensation. In this clinical study only the cognition battery of tests will be used to assess attention and executive functioning, language and processing speed.

The subject's developmental age recorded from the SB5 at Screening will be used to determine the tests administered for the study. Only subjects provide responses for these assessments (Hessl et al, 2016; Gershon et al, 2013; Casaletto et al, 2015).

The selection of subtests (Oral Reading, Picture Vocabulary, Speed Matching, Pattern Comparison Processing Speed, Flanker, Dimensional Card Change Sort) were chosen based on feasibility within the FXS population (Hessl et al., 2016), demonstration of sensitivity to change (Shields et al., 2023; Berry-Kravis et al., 2021), and relevance to the cognitive phenotype (Schmitt et al., 2019).

Exploratory endpoint: Intraclass correlation coefficient (ICC) values for NIH Cognitive Toolbox measures ≥ 0.7 and within 90% confidence interval of previously published ICC values (Hessl et al., 2016). ICCs will compare pre-dose values only (i.e., pre-dose home vs. pre-dose lab).

8.2.3 Clinical Assessment of Suicidal Ideation and Behavior

The clinical assessment of suicide ideation and behavior will be administered according to the time points outlined on the SoA.

This assessment is a series of "yes" or "no" questions. The clinician will ask if the subject understands the concept of suicidal ideation. If the answer is "No", the remaining questions about the subject's behavior will be asked to the caregiver. If the answer is "Yes", the remaining questions will be directed to the subject.

8.2.4 Clinical Global Impressions-Severity (CGI-S) and Clinical Global Impressions-Improvement (CGI-I)

The CGI-S will be administered at Baseline per the SoA. The CGI-S will be used for clinical characterization only. The CGI-s requires the clinician to assess how severe the subject's current severity of illness based on clinician's total clinical experience with the relevant population from 1(not ill at all) to 7 (among the most severely ill).

The CGI-I will be administered according to the time points outlined on the SoA (treatment/dosing visits only). The CGI-I will be completed after the subject receives study medication. The CGI-I requires the clinician to assess how much the patient's illness has changed relative to pre-dose, from 1 (very much improved) to 7 (very much worse).

8.2.5 Aberrant Behavior Checklist Score

The ABC-C_{FXS} will be administered at screen/baseline as a characterization measure per the SoA. The ABC-C_{FXS} will be used for clinical characterization only.

The ABC-C_{FXS} is a measure of interfering behavior commonly seen in individuals with FXS (Wheeler et al, 2014; Sansone et al, 2012). The ABC-C_{FXS} is a caregiver-rated questionnaire with demonstrated reliability and validity that has been used in multiple studies of individuals with developmental disabilities (Aman et al, 1985). Items on the ABC-C_{FXS} are rated on a scale of 0 to 3 ("behavior not a problem" to "behavior is a severe problem"). The ABC-C_{FXS} is factored specifically for persons with FXS has 6 subscales including irritability, social withdrawal, stereotypy, hyperactivity, inappropriate speech and social avoidance that the individual questions map to (Sansone et al, 2012). Higher scores indicate more severe behavior problems.

8.2.6 Anxiety, Depression and Mood Scale

The ADAMS will be administered at screen/baseline as a characterization measure in accordance with the SoA (Esbensen et al, 2003). The ADAMS will be used for clinical characterization only.

The ADAMS is a caregiver-rated questionnaire that assesses anxiety and depression in people with intellectual disabilities. This questionnaire has 5 domains: Manic/active Behavior, Depressed Mood, Social Avoidance, General Anxiety and Compulsive Behavior. Each domain is rated on a scale of 0 (not a problem) to 3 (severe problem) with higher scores indicating greater anxiety and depression.

8.2.7 Repeatable Battery for the Assessment Neuropsychological Status Update (RBANS), List Learning Subtest

The RBANS List Learning subtest will be administered according to the timepoints outlined on the SoA. For post-dose RBANS assessments, this test will be administered following the NIH Toolbox Assessment.

The RBANS List Learning subtest is a performance-based measure of verbal working memory. The subject is given a list of 10 words and asked to repeat them back over the course of four trials. The total score reflects the total number of words remembered from the list across the four trials. Four different forms (versions) are available, which will be randomized across visits and pre- and post-dosing timepoints.

8.2.8 Clinical Safety Laboratory Assessments

Clinical laboratory tests, including hematology, chemistry and urinalysis will be performed as specified in Appendix 2 and in the SoA (Section 2). Safety labs including a comprehensive metabolic panel, complete blood count, urinalysis and lipid profile will be obtained locally with results reviewed prior to enrolment.

The Investigator must review the laboratory report, document this review and record any clinically relevant changes occurring in the AE section of the eCRF. The laboratory reports must be filed with the source documents. Clinically significant abnormal laboratory findings are those which are not associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the subject's condition.

All laboratory tests with values considered clinically significantly abnormal during participation in the study or within 30 days after the last dose of study intervention should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the Investigator or medical monitor. If such values do not return to normal/baseline within a period of time judged reasonable by the Investigator, the etiology should be identified, and the Sponsor notified. If laboratory values from non-protocol specified laboratory assessments performed at the institution’s local laboratory require a change in subject management or are considered clinically significant by the Investigator (e.g., SAE or AE or dose modification), then the results must be recorded in the eCRF. Safety laboratory tests are to be performed and reviewed by the Investigator or qualified designee (e.g., physician’s assistant, nurse practitioner).

8.2.9 Optional Post-Dose Blood Draw:

On drug dosing days participants will have the option to opt into an additional blood draw collected 60 minutes (+/- 10) following the drug dose. The blood collected from that sample will be used for Pharmacokinetic and FMRP level testing. If participants opt into the blood draw both tests will be completed on the sample. If participants do not opt into the blood draw then no sample will be collected and neither test will be completed. The time of blood sample collection and the time prior to study drug dose will be recorded.

8.2.10 Screen Based Eye Tracking (Optional)

Videos, static faces and inanimate images/marks will be shown on a digital screen to all subjects throughout the study as noted in the SoA. The eye tracking task will be optional for Subjects.

In people with FXS, eye tracking findings show gaze abnormalities, including reduced eye gaze to eye regions (Farzin 2009; Farzin 2011). Additionally, aberrant pupil response to viewing emotional faces has been noted in FXS, thus pointing to aberrant autonomic reactions in this disorder (Farzin 2009; Farzin 2011). Eye tracking and pupillometry evaluations have been used in a number of FXS specific targeted treatment studies including studies of minocycline (Leigh 2013; Schneider et al, 2013) and mavoglurant (Berry-Kravis et al, 2016) where early reports indicated potential normalization of eye tracking parameters with treatment.

8.3 Home Visits

Home visits will be staffed by a minimum of two CCHMC study personnel. There will be a primary lead coordinator whose responsibilities will include but not be limited to: study recruitment and scheduling, coordinating the study visits, running the study visit, and data entry following the visit. An additional coordinator will be present at each home visit to provide additional behavioral support and complete any testing the lead coordinator is not trained in. At least one of the CCHMC study personnel will be a trained phlebotomist who will be responsible for the blood draws. At least one of the CCHMC study personnel will be trained to complete vital signs. At least one of the CCHMC study personnel will be trained to administer psychological and cognitive measures (e.g., RBANS List Learning, NIH Toolbox). At least one of the CCHMC study personnel will be trained to lead EEG (and eye tracking, if applicable) data collection. The CCHMC study personnel will be crossed trained as needed to perform multiple roles. A tele-health call with a CCHMC physician will be scheduled during home visits to complete: Suicidality checklist and CGI-I.

Home visit procedures can occur anywhere in the home that the participant and their family is comfortable with. A gauss meter will be used to place EEG equipment in areas of least electrical noise.

8.4 Additional exploratory outcome measures

For any significant findings, including those under the primary gamma outcome measures correlations with clinical outcome measures, including but not limited to the hyperactivity, irritability, social withdrawal and adverse speech scales will be analyzed. EEG and cognitive outcome measures also will be examined in relation to FMRP levels. Additionally, the findings from the endpoints will be used to investigate connectivity in FXS patients and any changes that may occur due to treatment. Adverse Events and Serious Adverse Events

All AEs will be reported by the subject (or, when appropriate, by a caregiver, surrogate or the subject's legally authorized representative).

The Investigator and any qualified designees are responsible for detecting, documenting and recording events that meet the definition of an AE or SAE and remain responsible for following

up AEs that are serious, considered related to the study intervention or study procedures, or that caused the subject to discontinue the study.

8.5 AE and SAEs

8.5.1 Time Period and Frequency for Collecting AE and SAE Information

All medical occurrences that meet the definition of an AE will be collected from the signing of the informed consent form (ICF) until the follow-up visit at the time points specified in the SoA.

Medical occurrences that begin before the start of study drug but after obtaining informed consent will be recorded on the Medical History section of the eCRF not the AE eCRF. All events that occur after the first dose of study drug will be recorded on the AE eCRF.

All SAEs will be recorded and reported to the Sponsor or designee immediately and under no circumstance should this exceed 24 hours. The Investigator will submit any updated SAE data to the Sponsor within 24 hours of it being available.

Investigators are not obligated to actively seek AE or SAE after conclusion of the study participation, however, if the Investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study, and he considers the event to be possibly or probably related to the study intervention or study participation, the Investigator must promptly notify the Sponsor.

8.5.2 Method of Detecting AEs and SAEs

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the subject is the preferred method to inquire about AE occurrences.

8.5.3 Follow-up of AEs and SAEs

After the initial AE/SAE report, the Investigator is required to proactively follow each subject at subsequent visits/contacts. All SAEs will be followed until resolution, stabilization, the event is otherwise explained, or the subject is lost to follow-up.

The Investigator must record follow-up information. Follow-up information is reported by eCRF or paper SAE form as with initial AE/SAEs.

Adverse Event Reporting

Adverse events that do not meet the definition of a SAE will be reported to the IRB and FDA at the time of annual review and will be provided for review to the Medical Monitor at the time of regularly scheduled quarterly meetings.

Serious Adverse Event Reporting

All serious adverse events (SAEs) will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the Subject is stable. Other supporting documentation of the event may be requested and should be provided as soon as possible.

The study sponsor will be responsible for notifying the Food and Drug Administration (FDA) of any unexpected fatal or life-threatening adverse event that is at least possibly related to the study intervention as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the information for events that are fatal or life-threatening and no later than 15 calendar days for other SAEs (21 CFR 312.32(c)(2).

In addition, the sponsor must notify FDA and all participating investigators in an Investigational New Drug (IND) safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting.

The sponsor is responsible for ensuring that the single IRB overseeing the conduct of the trial and the Medical Monitor are notified in conjunction with reporting to the FDA or per established policies and procedures.

Follow-up information must be reported according to the following:

- **SAEs:** All SAEs must be followed until the outcome of the events is “recovered/resolved”, “recovered/resolved with sequelae”, or “fatal”, and until all queries have been resolved. Cases of chronic conditions, cancer or AEs ongoing at time of death (where death is due to another AE) may be closed with the outcome “recovering/resolving” or “not recovered/not resolved”. Cases can be closed with the outcome of “recovering/resolving” when the subject has completed the Follow-up Period and is expected by the Investigator to recover.

The SAE follow-up information should only include new (e.g., corrections or additional) information and must be reported **within 24 hours** of the Investigator’s first knowledge of the information. This is also the case for previously non-serious AEs which subsequently become SAEs.

- **Non-serious AEs**: Non-serious AEs must be followed until the outcome of the event is “recovering/resolving”, “recovered/resolved”, or “recovered/resolved with sequelae” or until the end of the Follow-up Period stated in the protocol, whichever comes first, and until all queries related to these AEs have been resolved. Cases of chronic conditions, cancer or AEs ongoing at time of death (where death is due to another AE) may be closed with the outcome of “recovering/resolving” or “not recovered/not resolved”. Cases can be closed with the outcome of “recovering/resolving” when subject has completed the Follow-up Period and is expected by the Investigator to recover.

If a potential hypersensitivity reaction is observed, additional blood samples may be required to further characterize the potential hypersensitivity reaction. If an anaphylactic shock is suspected, samples may be taken for the measurement of tryptase. In this case, a blood sample should be taken 3 to 4 hours after the event and again approximately 1 to 2 weeks later to determine tryptase baseline levels. In addition, assessments for elevated histamine levels may be considered.

The Investigator must ensure that the worst-case severity and seriousness of an event is kept throughout the study, i.e., if the severity of an AE changes over time, then it should be reported as a single AE with the most severity. A worsening of an unresolved AE must be reported as follow-up with re-assessment of severity and/or seriousness of the event.

If an AE is resolved and re-appears later then it should be reported as a new AE.

Queries or follow-up requests for non-serious AE must be responded to within 7 calendar days from the date of receipt of the request, unless otherwise specified in the follow-up request.

8.5.4 Adverse Events of Special Interest

Somnolence is an expected AE of special interest defined following review of the available safety data.

8.5.5 Regulatory Reporting Requirements for SAEs

Prompt notification by the Investigator to the Sponsor of an SAE is essential so that legal obligations and ethical responsibilities toward the safety of subjects and the study intervention under clinical investigation are met.

The Sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study intervention under clinical investigation. The Sponsor will comply with regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRB)/Independent Ethics Committees (IEC) and Investigators.

Safety reports must be prepared for suspected unexpected serious adverse reactions (SUSAR) according to local regulatory requirements and Sponsor policy and forwarded to Investigators as necessary.

8.5.6 Periodic Safety Data Review

The Sponsor (Dr. Craig Erickson), Medical Monitor (Dr. Sam Vaughn) will conduct every other month Safety Review Meetings to review safety data and protocol deviations during the study. Data will remain blinded during this activity.

The main focus of the Safety Review Meeting will be the evaluation of safety data including AEs/SAEs, labs, vitals, physical examination, concomitant medications and protocol deviations.

9 Statistical Considerations

9.1 Statistical Analysis

General Statistical Methods

Descriptive analyses will include summary statistics (n, mean, standard deviation, median, and minimum and maximum) for quantitative variables or number and percentage of subjects in each category of interest for qualitative data.

Our primary endpoint analysis for test-retest reliability will use interclass correlation coefficients (ICCs). The ICC model is a 2-way simple random effects model, with one factor a randdom sample of Subjects from the FXS population, and the other factor a random representation of visits from a large pool of same-condition EEG collections. As we are interested in the absolute agreement of single measurement, the suitable model is ICC(A,1) (McGraw & Wong, 1996). The corresponding sample ICC estimation applies $ICC(A,1) = \frac{BMS - EMS}{BMS + (k-1)EMS + \frac{k}{n}(RMS - EMS)}$, where k is number of visits, n is number of Subjects. BMS denotes the between-subject mean square, RMS denotes the within-subject between-visit mean square, and EMS denotes the residual mean square including small-valued subject-visit interaction. ICC quantifies the ratio between between-subject variability and total variability with a range in [0, 1], where 0 indicates no reliability and 1 indicates perfect reliability. ICC calculation was conducted using the icc function of irr package v0.84.1(Gamer et al., 2019) in R v4.1.0 (*R: A Language and Environment for Statistical Computing*, 2021).

Where applicable, data will be presented by time point. Any statistical analysis applied will be considered purely exploratory. The following exploratory analyses will be conducted:

The exploratory statistical analysis will be a linear mixed model analysis of repeated measures. A separate mixed model analysis will be conducted on each outcome. If deemed necessary, the outcomes will be appropriately transformed to obtain a more normal distribution. The fixed effects of drug, drug sequence, and drug period will be examined. Subject will be accounted for as a random effect (G-Side) in the model. The period within subject will be included as a random effect (R-side). Various covariance structures within subject will be examined, e.g., first-order antedependence, and that with the lowest corrected Akaike Information Criterion will be used. Age, IQ, and mosaic status will be considered as covariates in the mixed models, as will baseline clinical and cognitive data for analyses of those parameters. Restricted maximum likelihood estimation, Type 3 tests of fixed effects, and generalized least-squares will be used, with the Kenward-Roger correction (Kenward & Roger, 1997) applied to the appropriate and best fitting covariance structure. The robust covariance matrix estimator will also be considered. If there is no sequence effect then the model will be re-fit without that term and the six pairwise differences of least squares means among the drug effects will be estimated (Jones & Kenward, 2015). Hedges' ĝ will be calculated and interpreted as the effect size estimator for the pairwise contrasts between the drug effects. Findings will be examined in relation to Fmr1 gene expansion and methylation. Baseline and changes in neurophysiologic measures will be examined for relationships to cognitive and clinical outcomes as an exploratory evaluation of these variables as potential biomarkers of drug effects in FXS.

The mixed model procedure of PROC GLIMMIX in SAS® version 9.4 software (SAS Institute, Inc., Cary, NC) will be used to conduct the mixed model analysis making use of all available data from all Subjects to provide a robust mechanism for handling data that are assumed missing at

random (Little, 1995) (Mallinckrodt et al., 2003) (Rubin, 1976) (Wolfinger & Chang, 1995). The level of significance for all tests will be set at $\alpha = .05$ (two-tailed) and, to address multiple testing, p-values will be adjusted using the False Discovery Rate (Benjamini & Hochberg, 1995).

9.1 Populations for Analyses

For purposes of analysis, the following populations are defined:

Population	Description
Enrolled	**All subjects who sign the ICF.**
Safety	**All subjects who are administered at least 1 dose of study drug. Analyses will be 'as treated'.**
Modified Intention-to-Treat (mITT)	**All subjects who receive at least 1 dose of study drug and who have at least 1 post-dose measurement of the primary endpoint.**

9.2 Safety Analyses

Adverse Events

AEs will be coded using the most recent version of the Medical Dictionary for Regulatory Activities (MedDRA) available at the time of study start.

Treatment emergent adverse effects (TEAEs) are defined as any AE occurring or worsening on or after the first dose of study drug.

If a subject experiences the same preferred term under the same system organ class multiple times, then these events will be counted as multiple events unless deemed recurrent under one event by the Investigator or Sponsor.

The frequency and incidence of TEAEs will be presented by system organ class and preferred term (number and percentage of subjects experiencing at least 1 AE per preferred term as well as the number of observed events), in order of decreasing frequency.

Separate tables will be presented by severity and by relationship.

All AEs will be presented in full in a comprehensive listing including subject number, severity, seriousness, action taken, outcome, relationship to treatment, onset/stop and duration. Details of SAEs and AEs leading to withdrawal will be listed separately.

Vital Signs

Vital signs will be summarized descriptively as actual values and change from baseline by visit.

Physical Examination

Physical examination results will be listed by subject and body system.

Clinical Laboratory

Descriptive statistics will be presented for quantitative laboratory parameters for each time point. Similarly, changes from baseline will be summarized.

Values outside the normal range will be categorized as H (above the normal range) or L (below the normal range) based on the laboratory's reference range and these will be flagged in the listings of individual subject data, including information on clinical significance.

10 References

Aman MG, Singh NN, Stewart AW, Field CJ. Psychometric characteristics of the Aberrant Behavior Checklist. *Am J Ment Defic.* 1985 Mar;89(5):492-502.

Berry-Kravis E, Hicar M, Ciurlionis R. Reduced cyclic AMP production in Fragile X syndrome: cytogenetic and molecular correlations. Pediatr Res. 1995 Nov;38(5):638-643.

Berry-Kravis E, Hessl D, Abbeduto L, Reiss AL, Beckel-Mitchener A, Urv TK; Outcome Measures Working Groups. Outcome measures for clinical trials in Fragile X syndrome. J Dev Behav Pediatr. 2013 Sep;34(7):508-522.

Berry-Kravis E, Des Portes V, Hagerman R, Jacquemont S, Charles P, Visootsak J, Brinkman M, Rerat K, Koumaras B, Zhu L, Barth GM, Jaecklin T, Apostol G, von Raison F. Mavoglurant in Fragile X syndrome: Results of two randomized, double-blind, placebo-controlled trials. Sci Transl Med. 2016 Jan;8(321):321ra5.

Berry-Kravis EM, Lindemann L, Jønch AE, Apostol G, Bear MF, Carpenter RL, et al. Drug development for neurodevelopmental disorders: lessons learned from Fragile X syndrome. Nat Rev Drug Discov. 2018 Apr;17(4):280-299.

Berry-Kravis E, Horrigan JP, Tartaglia N, Hagerman R, Kolevzon A, Erickson CA, et al. A Double-Blind, Randomized, Placebo-Controlled Clinical Study of Trofinetide in the Treatment of Fragile X Syndrome. Pediatr Neurol. 2020 Sep;110:30-41.

Bhakar AL, Dölen G, Bear MF. The Pathophysiology of Fragile X (and What It Teaches Us about Synapses). Annu Rev Neurosci. 2012;35(1):417–43

Boonyakitanont P, Lek-uthai A, Chomtho K, Songsiri J.A review of feature extraction and performance evaluation in epileptic seizure detection using EEG. Biomedical Signal Processing and Control. 2020;57:101702. doi:10.1016/j.bspc.2019.101702.

Brickley SG, Mody I. Extrasynaptic GABAA Receptors: Their Function in the CNS and Implications for Disease. Neuron. 2012;73(1):23–34.

Budimirovic D, Lee A, Dominick K, Tartaglia N, Berry-Kravis E. The Phase 2a ROCKET trial investigating gaboxadol in adolescents and young adults with Fragile X Syndrome: Study design (1344). Neurology Apr 2020;94(15 supplement):1344

Budimirovic DB, Dominick KC, Gabis LV, Adams M, Adera M, Huang L, et al. Gaboxadol in Fragile X Syndrome: A 12-Week Randomized, Double-Blind, Parallel-Group, Phase 2a Study. Front Pharmacol. 2021;12:757825.

Butler MG, Rames LJ, Wadlington WB. Acrodysostosis: report of a 13-year-old boy with review of literature and metacarpophalangeal pattern profile analysis. Am J Med Genet. 1988 Aug;30(4):971-980.

Buzsáki, G. and Schomburg, E.W. What does gamma coherence tell us about inter-regional neural communication? Nature Neuroscience. 2015;18(4):484–489. doi:10.1038/nn.3952.

Casaletto KB, Umlauf A, Beaumont J, Gershon R, Slotkin J, Akshoomoff N, Heaton RK. Demographically corrected normative standards for the English version of the NIH toolbox cognition battery. J Int Neuropsychol Soc. 2015 May;21(5):378–391.

Choi CH, Schoenfeld BP, Weisz ED, Bell AJ, Chambers DB, Hinchey J, et al. PDE-4 inhibition rescues aberrant synaptic plasticity in Drosophila and mouse models of Fragile X syndrome. J Neurosci. 2015 Jan;35(1):396-408.

Ciaccio C, Fontana L, Milani D, Tabano S, Miozzo M, Esposito S. Fragile X syndrome: a review of clinical and molecular diagnoses. Ital J Pediatr. 2017 Apr;43(1):39.

Clancy, R. Electroencephalography: Basic principles, clinical applications and related fields, ed 2. Edited by Ernst Niedermeyer Fernando Lopes da Silva Baltimore, Urban and Schwarzenberg, Annals of Neurology, 1988;24(6):799–799. doi:10.1002/ana.410240620.

Clifford S, Dissanayake C, Bui QM, Huggins R, Taylor AK, Loesch DZ. Autism spectrum phenotype in males and females with Fragile X full mutation and premutation. J Autism Dev Disord. 2007 Apr;37(4):738-747.

Cogram P, Deacon RMJ, Warner-Schmidt JL, Schimmelmann MJ von, Abrahams BS, During MJ. Gaboxadol Normalizes Behavioral Abnormalities in a Mouse Model of Fragile X Syndrome. Front Behav Neurosci. 2019;13:141.

Deidda G, Bozarth IF, Cancedda L. Modulation of GABAergic transmission in development and neurodevelopmental disorders: investigating physiology and pathology to gain therapeutic perspectives. Front Cell Neurosci. 2014;8:119.

Dau, T. et al. Auditory brainstem responses with optimized chirp signals compensating basilar-membrane dispersion. The Journal of the Acoustical Society of America. 2000;107(3):1530–1540. doi:10.1121/1.428438.

D'Hulst C, Kooy RF. The GABAA receptor: a novel target for treatment of Fragile X? Trends Neurosci. 2007;30(8):425–31.

Dockendorff TC, Su HS, McBride SM, Yang Z, Choi CH, Siwicki KK, et al. Drosophila lacking dfmr1 activity show defects in circadian output and fail to maintain courtship interest. Neuron. 2002 Jun;34(6):973-984.

Egawa K, Fukuda A. Pathophysiological power of improper tonic GABAA conductances in mature and immature models. Front Neural Circuit. 2013;7:170.

Esbensen AJ, Rojahn J, Aman MG, et al. Reliability and validity of an assessment instrument for anxiety, depression, and mood among individuals with mental retardation. J Autism Dev Disord. 2003 Dec;33(6):617-629.

Ethridge, L.E. et al. Neural synchronization deficits linked to cortical hyper-excitability and auditory hypersensitivity in Fragile X syndrome. Molecular Autism. 2017;8(1):22. Doi:10.1186/s13229-017-0140-1.

Ethridge, L.E. et al. Auditory EEG Biomarkers in Fragile X Syndrome: Clinical Relevance. Frontiers in Integrative Neuroscience, 2019;13:60. Doi:10.3389/fnint.2019.00060.

Ewen, J.B., Sweeney, J.A. and Potter, W.Z. Conceptual, Regulatory and Strategic Imperatives in the Early Days of EEG-Based Biomarker Validation for Neurodevelopmental Disabilities. Frontiers in Integrative Neuroscience, 2019;13:45. doi:10.3389/fnint.2019.00045.

Farzin F, Rivera SM, Hessl D. Brief report: Visual processing of faces in individuals with Fragile X syndrome: an eye tracking study. J Autism Dev Disord. 2009 Jun;39(6):946-952.

Farzin F, Scaggs C, Hervey C, Berry-Kravis E, Hessl D. Reliability of eye tracking and pupillometry measures in individuals with Fragile X syndrome. J Autism Dev Disord. 2011 Nov;41(11);1515-1522.

Gershon RC, Wagster MV, Hendrie HC, Fox NA, Cook KF, Nowinski CJ. NIH toolbox for assessment of neurological and behavioral function. Neurology. 2013 Mar;80(11 Suppl 3):S2–S6.

Gervasi N, Tchénio P, Preat T. PKA dynamics in a Drosophila learning center: coincidence detection by rutabaga adenylyl cyclase and spatial regulation by dunce phosphodiesterase. Neuron. 2010 Feb;65(4):516-529.

Gibson, J.R. et al. Imbalance of Neocortical Excitation and Inhibition and Altered UP States Reflect Network Hyperexcitability in the Mouse Model of Fragile X Syndrome. Journal of Neurophysiology, 2008;100(5):2615–2626. doi:10.1152/jn.90752.2008.

Goswami S, Cavalier S, Sridhar V, Huber KM, Gibson JR. Local cortical circuit correlates of altered EEG in the mouse model of Fragile X syndrome. Neurobiol Dis. 2019 Apr;124:563-572. doi: 10.1016/j.nbd.2019.01.002. Epub 2019 Jan 9. PMID: 30639292; PMCID: PMC6371815.

Gurney ME, Cogram P, Deacon RM, Rex C, Tranfaglia M. Multiple Behavior Phenotypes of the Fragile-X Syndrome Mouse Model Respond to Chronic Inhibition of Phosphodiesterase-4D (PDE4D). Sci Rep. 2017 Nov;7(1):14653.

Hagerman RJ, Hagerman PJ. Fragile X Syndrome: Diagnosis, Treatment and Research. 3rd ed. Baltimore, Maryland: JHU Press; 2002.

Hays, S.A., Huber, K.M. and Gibson, J.R. Altered Neocortical Rhythmic Activity States in Fmr1 KO Mice Are Due to Enhanced mGluR5 Signaling and Involve Changes in Excitatory Circuitry. Journal of Neuroscience. 2011;31(40):14223–14234. doi:10.1523/jneurosci.3157-11.2011.

Herrmann, C.S., Fründ, I. and Lenz, D. Human gamma-band activity: A review on cognitive and behavioral correlates and network models. Neuroscience & Biobehavioral Reviews. 2010;34(7):981–992. doi:10.1016/j.neubiorev.2009.09.001.

Hessl D, Sansone SM, Berry-Kravis E, Riley K, Widaman KF, Abbeduto L, Schneider A, Coleman J, Oaklander D, Rhodes KC, Gershon RC. The NIH toolbox cognitive battery for intellectual disabilities: three preliminary studies and future directions. J Neurodev Disord. 2016 Sep;8(1):35.

Inácio AR, Bucala R, Deierborg T. Lack of macrophage migration inhibitory factor in mice does not affect hallmarks of the inflammatory/immune response during the first week after stroke. J Neuroinflammation. 2011 Jun;8:75.

Jonak, C.R. et al. Multielectrode array analysis of EEG biomarkers in a mouse model of Fragile X Syndrome. Neurobiology of Disease. 2020;138:104794. Doi:10.1016/j.nbd.2020.104794.

Jonak, C.R. et al. Baclofen-associated neurophysiologic target engagement across species in Fragile X syndrome. Journal of Neurodevelopmental Disorders. 2022;14(1):52. doi:10.1186/s11689-022-09455-9.

Kanellopoulos AK, Semelidou O, Kotini AG, Anezaki M, Skoulakis EM. Learning and memory deficits consequent to reduction of the Fragile X mental retardation protein result from metabotropic glutamate receptor-mediated inhibition of cAMP signaling in Drosophila. J Neurosci. 2012 Sep;32(38):13111-13124.

Kenny, A., Wright, D. and Stanfield, A.C. EEG as a translational biomarker and outcome measure in Fragile X syndrome. Translational Psychiatry. 2022;12(1):34. doi:10.1038/s41398-022-01796-2.

Ketas pharmaceutical interview form [package insert]. Tokyo, Japan: Kyorin Pharmaceutical Co., Ltd.; September 2022 revision (14th edition). https://www.pmda.go.jp/PmdaSearch/iyakuDetail/GeneralList/4490010. September 2022. Available Accessed at: 26 September 2022

Kidd SA, Lachiewicz A, Barbouth D, Blitz RK, Delahunty C, McBrien D, Visootsak J, Berry-Kravis E. Fragile X syndrome: a review of associated medical problems. Pediatrics. 2014 Nov;134(5):995-1005.

Kithcart AP, Cox GM, Sielecki T, Short A, Pruitt, J, Papenfuss T, Shawler, T, Gienapp I, Satoskar AR, Whitacre CC. A small-molecule inhibitor of macrophage migration inhibitory factor for the treatment of inflammatory disease. FASEB J. 2010 Nov;24(11):4459-4466.

Knott EP, Assi M, Rao SN, Ghosh M, Pearse DD. Phosphodiesterase Inhibitors as a Therapeutic Approach to Neuroprotection and Repair. Int J Mol Sci. 2017 Mar;18(4):696.

Ledeboer A, Liu T, Shumilla JA, Mahoney JH, Vijay S, Gross MI, Vargas JA, Sultzbaugh L, Claypool MD, Sanftner LM, Watkins LR, Johnson KW. The glial modulatory drug av411 attenuates mechanical allodynia in rat models of neuropathic pain. Neuron Glia Biol. 2006 Nov:2(4):279-291.

Lee H, Graham JM Jr, Rimoin DL, Lachman RS, Krejci P, Tompson SW, Nelson SF, Krakow D, Cohn DH. Exome sequencing identifies PDE4D mutations in acrodysostosis. Am J Hum Genet. 2012 Apr;90(4):746-751.

Leigh M, Nguyen D, Mu Y, Winarni TI, Schneider A, Chechi T, et al. A randomized double-blind, placebo-controlled trial of minocycline in children and adolescents with Fragile X syndrome. J Dev Behav Pediatr. 2013 Apr;34(3):147-155.

Linglart A, Fryssira H, Hiort O, Holterhus PM, Perez de Nanclares G, Argente J, et al. PRKAR1A and PDE4D mutations cause acrodysostosis but two distinct syndromes with or without GPCR-signaling hormone resistance. The Journal of Clinical Endocrinology & Metabolism, 1 December 2012;97(12):E2328–E2338, https://doi.org/10.1210/jc.2012-2326

Martin BS, Corbin JG, Huntsman MM. Deficient tonic GABAergic conductance and synaptic balance in the Fragile X syndrome amygdala. J Neurophysiol. 2014;112(4):890–902

McKechanie AG, Barnicoat A, Trender-Gerhard I, Allison M, Stanfield AC. Fragile X-associated conditions: implications for the whole family. Br J Gen Pract. 2019 Aug;69(686):460-461.

Michel, C.M. and Brunet, D. EEG Source Imaging: A Practical Review of the Analysis Steps. Frontiers in Neurology. 2019;10:325. doi:10.3389/fneur.2019.00325.

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);549-555.

Morales, S. and Bowers, M.E. Time-frequency analysis methods and their application in developmental EEG data. Developmental Cognitive Neuroscience, 2022;54:101067. doi:10.1016/j.dcn.2022.101067.

Nishio Y, Koda M, Hashimoto M, Kamada T, Koshizuka S, Yoshinaga K, et al. Deletion of macrophage migration inhibitory factor attenuates neuronal death and promotes functional recovery after compression-induced spinal cord injury in mice. Acta Neuropathol. 2009 Mar;117(3):321-328.

Olmos-Serrano JL, Paluszkiewicz SM, Martin BS, Kaufmann WE, Corbin JG, Huntsman MM. Defective GABAergic Neurotransmission and Pharmacological Rescue of Neuronal Hyperexcitability in the Amygdala in a Mouse Model of Fragile X Syndrome. Journal of Neuroscience. 2010;30(29):9929–38.

Olmos-Serrano JL, Corbin JG, Burns MP. The GABAA Receptor Agonist THIP Ameliorates Specific Behavioral Deficits in the Mouse Model of Fragile X Syndrome. Dev Neurosci-Basel. 2011;33(5):395–403.

Ovid Therapeutics Announces Positive Topline Results from the Phase 2 ROCKET Trial of OV101 for the Treatment of Fragile X Syndrome [Internet]. 2020 [cited 2023 Jan 17]. Available from: https://investors.ovidrx.com/news/news-details/2020/Ovid-Therapeutics-Announces-Positive-Topline-Results-from-the-Phase-2-ROCKET-Trial-of-OV101-for-the-Treatment-of-Fragile-X-Syndrome-05-07-2020/default.aspx

Pedapati, E.V. et al. Neocortical localization and thalamocortical modulation of neuronal hyperexcitability contribute to Fragile X Syndrome. Communications Biology. 2022;5(1):442. doi:10.1038/s42003-022-03395-9.

Powell ND, Papenfuss TL, McClain MA, Gienapp IE, Shawler TM, Satoskar AR, Whitacre CC. Cutting edge: macrophage migration inhibitory factor is necessary for progression of experimental autoimmune encephalomyelitis. J Immunol. 2005 Nov;175(9):5611-5614.

Ramamoorthi K, Lin Y. The contribution of GABAergic dysfunction to neurodevelopmental disorders. Trends in Molecular Medicine. 2011;17(8):452–62.

Sanei, S. and Chambers, J.A. (2013) EEG signal processing. John Wiley & Sons.

Sansone SM, Widaman KF, Hall SS, Reiss AL, Lightbody A, Kaufmann WE, et al. Psychometric study of the Aberrant Behavior Checklist in Fragile X Syndrome and implications for targeted treatment. J Autism Dev Disord. 2012 Jul;42(7):1377-1392.

Sansone SM, Schneider A, Bickel E, Berry-Kravis E, Prescott C, Hessl D. Improving IQ measurement in intellectual disabilities using true deviation from population norms. J Neurodev Disord. 2014;6(1):16.

Schmitt LM, Li J, Liu R, Horn PS, Sweeney JA, Erickson CA, Pedapati EV. Altered frontal connectivity as a mechanism for executive function deficits in Fragile X syndrome. Mol Autism. 2022 Dec 9;13(1):47. doi: 10.1186/s13229-022-00527-0. PMID: 36494861; PMCID: PMC9733336.

Schneider A, Leigh MJ, Adams P, Nanakul R, Chechi T, Olichney J, Hagerman R, Hessl D. Electrocortical changes associated with minocycline treatment in Fragile X syndrome. J Psychopharmacol. 2013 Oct;27(10):956-963.

Schepers M, Tiane A, Paes D, Sanchez S, Rombaut B, Piccart E, Rutten BPF, Brône B, Hellings N, Prickaerts J, Vanmierlo T. Targeting Phosphodiesterases-Towards a Tailor-Made Approach in Multiple Sclerosis Treatment. Front Immunol. 2019 Jul;10:1727.

Snape M, Horrigan J, Glass L, et al. Improving outcome measures for Fragile X syndrome clinical trials: development of Fragile X syndrome specific rating scales. In: Society for the Study of Behavioral Phenotypes New York City. 2014.

Stoyell, S.M. et al. High-Density EEG in Current Clinical Practice and Opportunities for the Future. Journal of Clinical Neurophysiology. 2021;38(2):112–123. doi:10.1097/wnp.0000000000000807.

Symons FJ, Clark RD, Hatton DD, Skinner M, Bailey DB Jr. Self-injurious behavior in young boys with Fragile X syndrome. Am J Med Genet A. 2003 Apr;118A(2):115-121.

Tranfaglia MR. Fragile X syndrome: a psychiatric perspective. Results Probl Cell Differ. 2012;54:281-295.

Tranfaglia MR, Thibodeaux C, Mason DJ, Brown D, Roberts I, Smith R, Guilliams T, Cogram P. Repurposing available drugs for neurodevelopmental disorders: The Fragile X experience. Neuropharmacology. 2019 Mar;147:74-86.

Von Gontard A, Backes M, Laufersweller-Plass C, Wendland C, Lehmkuhl G, Zerres K, Rudnik-Schöneborn S. Psychopathology and familial stress-comparison of boys with Fragile X syndrome and Spinal Muscular Atrophy. J Child Psychol Psychiatry. 2002 Oct;43(7):949-957.

Wang, J. et al. A resting EEG study of neocortical hyperexcitability and altered functional connectivity in Fragile X syndrome. Journal of Neurodevelopmental Disorders. 2017;9(1):11. doi:10.1186/s11689-017-9191-z.

Wheeler A, Raspa M, Bann C, Bishop E, Hessl D, Sacco H, Bailey DB Jr. Anxiety, attention problems, hyperactivity, and the Aberrant Behavior Checklist in Fragile X syndrome. Am J Med Genet A. 2014 Jan;164A(1):141-155.

Yoshioka A, Yamaya Y, Saiki S, Kanemoto M, Hirose G, Pleasure D. Cyclic GMP/cyclic GMP-dependent protein kinase system prevents excitotoxicity in an immortalized oligodendroglial cell line. J Neurochem. 2000 Feb;74(2):633-640.

Zhang YQ, Bailey AM, Matthies HJ, Renden RB, Smith MA, Speese SD, et al. Drosophila Fragile X-related gene regulates the MAP1B homolog Futsch to control synaptic structure and function. Cell. 2001 Nov;107(5):591-603.

Zhu J, Mix E, Winblad B. The antidepressant and antiinflammatory effects of rolipram in the central nervous system. CNS Drug Rev. 2001 Winter;7(4):387-398.

11. Potential Abbreviations

1MCP-1	**Monocyte chemoattractant protein-1**
ABC-C_{FXS}	**Aberrant Behavior Checklist**
ACRDYS2	**Acrodysostosis without hormone resistance**
ADAMS	**Anxiety, Depression and Mood Scale**
ADHD	**Attention-deficit hyperactivity disorder**
ADL	**Activities of Daily Living**
AE	**Adverse event**
AI	**Artificial Intelligence**
ALT	**Alanine aminotransferase**
ASD	**Autism spectrum disorder**
AST	**Aspartate aminotransferase**
AUC	**Area under the concentration-time curve (exposure)**
BID	**Twice daily**
BP	**Blood pressure**
BSA	**Body surface area**
cAMP	**Cyclic adenosine monophosphate**
CFC	**Cross frequency coupling**
CFR	**Code of Federal Regulations**
CGG	**Cysteine-guanine-guanine**
CGI-I	**Clinical Global Impressions of Improvement**
CGI-S	**Clinical Global Impressions of Severity**
CIOMS	**Council for International Organizations of Medical Sciences**
C_{max}	**Peak concentration**
CNS	**Central Nervous System**
COA	**Clinical Outcome Assessments**
CONSORT	**Consolidated Standards of Reporting Trials**
CRA	**Clinical research associate**
CRO	**Contract research organization**
CYP450	**Cytochrome P450**
Dfmr1	**Drosophila Fragile X messenger ribonucleoprotein 1**
Dnc	**Dunce gene (Drosophila)**
eCRF	**Electronic case report form**
EDC	**Electronic data capture**
EEG	**Electroencephalography**
eGFR	**Estimated glomerular filtration rate**

EOT	**End of Treatment**
EPA	**Electrode potential analysis**
ePRO	**Electronic patient reported outcome**
ERP	**Evoked response potential**
EU	**European Union**
FDA	**Food and Drug Administration**
FMR1	**Fragile X messenger ribonucleoprotein 1**
FMRP	**Click here to enter text.Fragile X messenger ribonucleoprotein**
FXS	**Click here to enter text.Fragile X Syndrome**
GABA	**ɣ-aminobutyric acid**
GCP	**Good Clinical Practice**
GGT	**Gamma-glutamyl transferase**
GI	**Gastrointestinal**
HDPE	**High-density polyethylene**
HED	**Human equivalent dose**
HGC	**Hard gelatin capsule**
HIPAA	**Health Insurance Portability and Accountability Act**
HPMC	**Hydroxypropyl methyl cellulose**
IB	**Investigator's Brochure**
IC_{50}	**half maximal inhibitory concentration**
ICF	**Informed consent form**
ICH	**International Council for Harmonisation**
IEC	**Independent Ethics Committee**
IFN	**Interferon**
IND	**Investigational New Drug**
INR	**International normalized ratio**
IP	**Intraperitoneal**
IRB	**Institutional Review Board**
KO	**Knockout**
MDRI	**Multiple Domain Responder Index**
MedDRA	**Medical Dictionary for Regulatory Activities**
mGluRs	**Metabotropic glutamate receptors**
MIF	**Migration inhibitory factor**
mITT	**Modified intention-to-treat**
mRNA	**Messenger RNA**
MS	**Multiple sclerosis**
NCAS	**Non-Clinical Assessment of Safety**

NIH	**National Institutes of Health**
NOAEL	**No observed adverse effect level**
NOEL	**No observed effect level**
OFT	**Open Field Test**
PAD	**Pharmacological active dose**
PCR	**Polymerase chain reaction**
PD	**Pharmacodynamic(s)**
PDE	**Phosphodiesterase**
PDE4	**Phosphodiesterase-4**
PDE4D	**Phosphodiesterase-4D**
PDE4DNAM	**PDE4D negative allosteric modulators**
PK	**Pharmacokinetic(s)**
PKA	**cAMP-dependent kinase**
PO	**Orally**
PRO	**Patient-reported outcome**
QD	**Once daily**
QTcF	**Corrected QT interval using Fridericia's correction method**
RBC	**Red blood cell**
RTMS	**Randomization and Trial Supply Management System**
SAE	**Serious adverse event**
SAP	**Statistical Analysis Plan**
SARI	**Serotonin Receptor Antagonists and Reuptake Inhibitors**
SAT	**Successive alleys test**
SB5	**Stanford-Binet Test version 5**
SIB	**self-injurious behavior**
SNRI	**Serotonin and Norepinephrine Reuptake Inhibitors**
SoA	**Schedule of Activities**
SRM	**Study Reference Manual**
SS	**Steady state**
SSRI	**Selective Serotonin Reuptake Inhibitors**
SUSAR	**Suspected unexpected serious adverse reactions**
TEAE	**Treatment-emergent adverse event**
TID	**Three times daily**
T_{max}	**Time to maximum concentration**
TNF	**Tumor necrosis factor**
TTL	**Transistor-Transistor-Logic**
UCR1	**Upstream conserved region 1**

UCR2	**Upstream conserved region 2**
ULN	**Upper limit of normal**
US	**United States of America**
WBC	**White blood cell**
WT	**Wild-type**

12. Summary of Versions and Changes

Protocol	Version	Date	Summary of Change
Original	1.0	7th September 2023	Original Version
Updated	2.0	6th November 2023	Changes made due to FDA IND clinical hold: • Protocol title change to simplify • To highlight scientific rationale, increased focus and justification of single-dose design as well as exploration of GABA agonists throughout the protocol • Modification of 'Primary Objective' to evaluate target engagement of gaboxadol treatment on high density EEG recordings based on scientific rationale • Modification of age to exclude children and only include individuals aged 18 years and older • Additional blood draw for safety assessments at the second clinic visit (Visit 3) • Additional inclusion criteria of contraception use • AEs updated to include somnolence
Updated	3.0	23rd January 2025	• Adding recruitment information • Change post-dose blood draw to be optional