FULL PROTOCOL TITLE:
Effects of AFQ056 on Language Learning in Young Children with Fragile X Syndrome (FXS)

Protocol Version: v15.0
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SHORT PROTOCOL TITLE:
AFQ056 for Language Learning in Children with FXS
(FX-LEARN)

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INVESTIGATOR AGREEMENT

I have read the foregoing protocol Version 15.0 dated 02/03/2021 and agree to conduct the study as described herein.

By signing the protocol, the Investigator agrees to keep all information provided by the NeuroNEXT Network in strict confidence and to request the same from his/her staff and the Institutional Review Board. Study documents provided by the NeuroNEXT Network will be stored appropriately to ensure their confidentiality. The Investigator should not disclose such information to others without authorization, except to the extent necessary to conduct the study.

_____ _____

Investigator Signature **Date**

_____

Print Investigator's Name

SIGNATURE PAGE

Study Number:

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Principal Investigator Approval:

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NeuroNEXT Clinical Coordinating Center Approval:

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LIST OF ACRONYMS, ABBREVIATIONS, AND DEFINITIONS OF TERMS

ABC-FX	Aberrant Behavior Checklist – Community Edition – Factored for FXS
AE	Adverse Event
AKT	serine/threonine-specific protein kinase (also PKB – protein kinase B)
APP	Amyloid Precursor Protein
CBC	Complete blood count
CCC	Clinical Coordination Center
CDE	Common Data Elements
CFR	Code of Federal Regulations
CGI-I	Clinical Global Impression – Improvement
CGI-S	Clinical Global Impression - Severity
CIRB	Central Institutional Review Board
CMP	Comprehensive metabolic profile
CRF	Case report form
CS	Clinically Significant
CSS PI	Clinical Study Site PI
DCC	Data Coordination Center
DM	Data Management
DSMB	Data Safety Monitoring Board
DQ	Developmental quotient
eCRF	Electronic Case Report Form
EDC	Electronic data capture
EKG	Electrocardiogram
ELC	Early Learning Composite
ERK	Extracellular signal-related kinase
ERP	Event related potentials
FDA	Food and Drug Administration
FMR1	Fragile X Mental Retardation-1 gene
FMRP	Fragile X Mental Retardation Protein
FXS	Fragile X syndrome
GCP	Good Clinical Practice
HBsAg	Hepatitis B surface antigen
HIV	Human Immunodeficiency Virus
HIPAA	Health Insurance Portability and Accountability Act
ICH	International Conference on Harmonization
IMM	Independent Medical Monitor
mGluR5	Metabotropic Glutamate Receptor – Type 5
MedDRA	Medical Dictionary for Regulatory Activities
MMP9	Matrix Metalloprotein 9
MSEL	Mullen Scales of Early Learning
MTD	Maximum tolerated dose
NINDS	National Institute of Neurological Disorders and Stroke
PK	Pharmacokinetics
PLS-5	Preschool Language Scale Version 5
PPI	Protocol Principal Investigator
PSC	Protocol Steering Committee
SAE	Serious adverse event
SOA	Schedule of Activities
TSH	Thyroid stimulating hormone
VAS	Visual analog scale
VABS	Vineland Adaptive Behavior Scale

SYNOPSIS

Study Title**: Effects of AFQ056 on Language Learning in Young Children with Fragile X Syndrome (FXS)**

Short Title: **AFQ056 for Language Learning in Children with FXS**

Objectives

The overall goals are to change the paradigm for development of mechanism targeted pharmacotherapy in neurodevelopmental disorders and provide a definitive test of the mGluR theory in humans by determining whether AFQ056, an mGluR5 negative modulator, can enhance neural plasticity in the form of language learning during an intensive language intervention in very young children with fragile X syndrome. This trial therefore will use an innovative but exploratory new trial design to develop a different way to examine efficacy of an agent with substantial support as a drug targeting CNS plasticity in preclinical models of a developmental disorder. If the design is successful, this trial can serve as a model for future trials of mechanistically-targeted treatments operating on neural plasticity in other neurodevelopmental disorders.

Primary objective: To demonstrate greater improvement in language in young children with FXS treated with AFQ056, using an overall weighted communication score, in combination with an intensive standardized parent-implemented language learning intervention, relative to those treated with the language learning intervention and placebo, after 6 months of intervention, as a marker of drug effect on neural plasticity, the core problem in the disorder.

Key Secondary Objective: To show greater improvement in specific language, cognitive, and adaptive measures in the combination AFQ056/language intervention group relative to the language intervention/placebo group.

Other Secondary and Exploratory Objectives: (1) To show improvement in specific measures from ERP and eye tracking biomarkers in the combination AFQ056/language intervention group relative to the language intervention/placebo group. (2) Examine long-term safety and tolerability of AFQ056 in young children with FXS; (3) To explore whether greater improvement occurs in additional language, cognitive, adaptive and behavioral measures in the combination AFQ056/language intervention group relative to the language intervention/placebo group; (4) To explore improvement in multiple additional measures from the ERP and eye tracking in the combination AFQ056/language intervention group relative to the language intervention/placebo group; (5) To explore whether the normalization of eye tracking/pupillometry biomarkers is correlated with improvement in clinical outcome markers including language; (6) To explore whether normalization of blood biomarkers of translational pathways regulated by FMRP and mGluR5 receptors is associated with language, functional and/or biomarker improvements from AFQ056 treatment; (7) To examine if blood levels of AFQ056 correlate with improvement in language, functional or biomarker measures, (8) To examine if longitudinal analysis shows an improvement in the rate of change of language, functional or biomarker responses with the language intervention alone, and further improvement in rate of change when AFQ056 Is added to the language intervention

Design and Outcomes

Trial Design: The trial will be a phase II double blind placebo-controlled parallel-group flexible-dose forced-titration study that will use a novel design to address the impact of AFQ056 on language learning in 3-6 year old children with FXS. The flexible dose design will mimic practice,

take into account differential responsiveness and the known inter-child variability in drug levels with AFQ056, and allow use of the maximum tolerated dose (MTD) where MTD is defined as the maximum tolerated dose of the available dose levels in the protocol, which is felt likely to be most effective. There will be a 4-month single-blind placebo lead in after screening assessments during which subjects will have therapy treatments as usual to control for the effects of the language intervention. The placebo lead-in will also control for placebo effects which are prominent in clinical trials in FXS. This will be followed by an 8-month placebo-controlled phase in which 100 subjects with FXS will have AFQ056 baseline assessments and be randomized 1:1 to AFQ056 or placebo. Dose titration to MTD starting at 25 mg AFQ056/matching placebo will occur over 2 months and then subjects will have language intervention baseline assessments and initiate the language intervention, remaining on a stable AFQ056/placebo dose for the next 6 months. After 8 months of treatment in the placebo-controlled phase (6 months of language intervention), all subjects will have final assessments and will enter the open label extension and be treated with active drug according to the same schedule as in the placebo-controlled phase with 2 months of flexible dose titration to the MTD followed by a period of stable treatment. The duration of the stable treatment will depend on when the subject was enrolled into the study; those enrolled prior to June 15-30, 2019 will receive a 6-month period of stable treatment, and the open label extension may be shortened on a sliding scale for those enrolled after June 15, 2019, such that their treatment including weaning, if necessary, will end before August 31, 2021, with the exact length of the stable treatment period in the extension being determined by how long after June 15 they enroll and what dose of drug they are receiving in the open label treatment period (higher doses will have a longer weaning period of maximally 2 weeks for those on 75-100 mg BID, but 1 week for 50 mg BID, and no weaning period for lower doses). The total duration of the stable treatment for those enrolled after June 15, 2019 will range from 6 months to no open label extension, depending on the number of months after June 15, 2019 the subject is enrolled into the study. As such, some subjects will have an acute dose decrease if they were on a dose of 50-100 mg BID of active drug in the initial phase but experience from the Pediatric PK study conducted previously suggests that children can stop AFQ056 without withdrawal symptoms and the dose decrease and titration in the open label extension are unavoidable to maintain the blind of the study through the completion of the placebo-controlled period by the final participant. The open-label extension will allow all children a period of active drug exposure to make recruitment feasible, despite the long placebo period, and will allow additional collection of long-term safety data. Subjects will continue the language intervention through the extension phase to gain additional data on long-term effects of the combination of AFQ056 and the language intervention on developmental trajectory and the ERP biomarker.

Interventions and Duration

AFQ056: AFQ056 is a highly selective mGluR5 negative allosteric modulator shown to be effective in reversing dendritic spine and behavioral phenotypes in the mouse model of FXS. It is administered orally as a suspension at a concentration of mg/ml. Dosing range is expected to be 25-100 mg BID.

Language Intervention: The language intervention, which was developed by Dr. Leonard Abbeduto and colleagues, showed benefit for language development over 6 months (the period assessed in the placebo-controlled phase of this trial) in a pilot study of young children with FXS, can be taught to families with standardized delivery across sites, and implemented in the home through video-conferencing with families.

Time of Study Participation: Subjects will be in the study a total of 21 months (Screening and placebo run-in 4 months prior to randomization, 8 months placebo-controlled treatment, 8 months open-label extension, 1 month follow up after final assessment visit/visit 14).

**Note: Due to study drug expiration, the duration of the open label extension may be shortened on a sliding scale for those enrolled after June 15, 2019, such that their treatment including weaning, if necessary, will end before August 31, 2021, with the exact length of the stable treatment period in the extension being determined by how long after June 15 they enroll and what dose of drug they are receiving in the open label treatment period. The total duration of the stable treatment for those enrolled after June 15, 2019 will range from 6 months to no open label extension, depending on the number of months after June 15, 2019 the subject is enrolled into the study.

Duration of Enrollment: It is expected enrollment will occur over 18 months. Enrollment must end at the end of February 2020 due to study drug expiration at the end of August 2021.

Schedule and Type of Evaluations: The Language primary outcome assessment (weighted communication scale, WCS) will be obtained at screening, randomization (AFQ baseline), 2 (language intervention baseline), 4, 6, 8, 10, 12, 16 months and post-intervention follow up at 17 months. Cognition, language and behavioral assessments including the, MacArthur Bates Communicative Development Inventory, Preschool Language Scale – 5th Edition (PLS-5), Visual Analog Scale (VAS) of language, Clinician Global Impression-Improvement and Severity (CGI-I, CGI-S) for language and overall function, Mullen Scales of Early Learning (MSEL) Composite and Subscales, Vineland Adaptive Behavior Scale-Version 3 (Vineland-3); Aberrant Behavior Checklist-fragile X validated version (ABC–FX), and a VAS of behavior will be performed regularly throughout the protocol according to the Schedule of Assessments (below, Section 6.1, Table 7) ; Biomarkers including ERP, eye tracking/pupillometry, and blood biomarkers (ERK, AKT, S6 kinase, APP, MMP9, RNA, FMRP, DNA) will also be obtained throughout the protocol according to the Schedule of Assessments. Safety measures will include CBC, CMP, TSH, EKG, adverse events, suicidality.

Endpoints Derived from Evaluations:
Primary endpoints: Language - Weighted child intentional communication score coded from a 22-minute structured task which is an examiner/child dyadic play session, including a structured play session and an unstructured play session.

Key secondary endpoints:
- Functional (all raw scores)
  - Mullen Scales of Early Learning (MSEL)
    - Developmental Quotient (DQ)
    - Expressive Language Subscore
  - Vineland Adaptive Behavior Scale – Version 3
    - Composite Score
    - Communication Subscore
  - Preschool Language Scale – 5th Edition (PLS-5)
    - Expressive Communication Score
  - McArthur Communicative Development Inventory
    - Number of spoken words
  - Clinical Global Impression-Improvement (CGI-I)
    - Overall Function

Other Secondary endpoints:
Safety measures including clinically significant laboratory values and adverse events will be tabulated for descriptive comparison between the placebo and active drug groups

- Functional

- Weighted Communication
  - Structured play score
  - Nonstructured play score
- Preschool Language Scale – 5th Edition (PLS-5)
  - Auditory Comprehension Subscore
  - Total Language Score
- Mullen Scales of Early Learning (MSEL)
  - Receptive Language Subscore
  - Visual Reception Subscore
  - Gross Motor Subscore
  - Fine Motor Subscore
- Vineland Adaptive Behavior Scale – Version 3
  - Communication
  - Daily Living Skills
  - Socialization
  - Motor
- Aberrant Behavior Checklist – Fragile X Validated Version (ABC-FX)
  - Irritability Subscore
  - Stereotyped Behavior Subscore
  - Abnormal Speech Subscore
  - Hyperactivity Subscore
  - Social Avoidance Subscore
  - Lethargy Subscore
- Visual Analog Scale (VAS) of Target Behavior (1, 2, & 3 sum)
- Visual Analog Scale (VAS) of Language
- Clinical Global Impression-Improvement (CGI-I)
  - Language
- Clinical Global Impression-Severity (CGI-S)
  - Overall Function
  - Language

A number of additional exploratory endpoints will be considered as well.

Sample Size and Population

100 subjects with FXS age 32 months to 6 years will be studied. In order to prevent age imbalance, randomization will be stratified by age with two strata, 3-4 years and 5-6 years.

Inclusion Criteria

1. Age 32 months to 6 years inclusive at Screening (visit 1).
2. Has a documented *FMR1* full mutation.
Note Presence of mosaicism is allowed.
3. DQ<75 calculated from the Mullen Scales of Early Learning at time of screening.
4. Parent or legal guardian is available and able to communicate well with the investigator, comply with study requirements and provide written informed consent.
NoteThe parent or legal guardian who will be signing consent form, should be the individual administering the language intervention.
5. English is the primary language spoken in the home and the subject's first language is English.
6. Meet criteria indicating evidence of intentional communication based on parent interview via a communication eligibility screening tool.
Note On the Eligibility Screening Tool – Communication, the child must have at time of screening:

a) Section 1: Answer of YES; the child uses at least 5 spoken words to label items on a daily basis.

OR

b) Section 2: At least 3 YES answers to items 1-10 if child does not have at least 5 spoken words.

7. Produces 3 or more intentional acts of communication on the structured portion of the Weighted Communication play sample at time of screening**.**
   Note Subjects are permitted to use augmentative communication devices throughout the study if the device is the subject's primary form of communication and the device has been prescribed for the subject by an SLP.
8. Stable behavioral and other therapy regimen for 30 days prior to screening.
   Note Patients will be allowed to continue their standard-of–care therapies throughout the trial but these will not be changed during the placebo lead-in or placebo-controlled portion of the trial, outside of standard changes occurring from school schedules.
9. Stable dosing of all concurrent psychotropic medications except stimulants for at least 60 days prior to screening. Due to the very short half-life of stimulants (specifically methylphenidate and amphetamine variants), a stable regimen of these medications is required for 2 weeks only.
   Note Medications impacting GABA, glutamate and/or mGluR5 pathway receptors are exclusionary and not permitted during study participation. Additionally, stimulant regimens may include combinations of short- and long-acting forms and may be taken with different timing or dosing on different days of the week (e.g. Doses may be skipped on weekends or days off school and extra doses may be given some days for therapy sessions later in the day). The intent is to keep the doses and regimen being used at the time of screening consistent during the trial even if there is some variation in how the medication is taken on different days. Use of CBD oil or hemp based substances legal for sale over the internet are allowed provided that the dosing regimen has been consistent for at least 60 days prior to screening and will remain the same throughout the trial.

Exclusion Criteria

1. Use of medications impacting GABA, glutamate and/or mGluR5 pathway receptors or transmission.
   Note Treatment with acamprosate, amantadine, budipine, carbetocin, cycloserine, dextromethorphan, felbamate, ketamine, lithium*, minocycline, memantine, oxytocin, remacemide, racemic baclofen, riluzole, fycampa, investigational mGluR5 medications, and/or statins are exclusionary.
   Note Lithium taken as a dietary supplement is permitted if the dose is less than 5mg/day. A 5mg/day dose is the recommended dietary intake level, and is therefore not considered to be therapeutic. Lithium dosage must remain the same throughout the duration of the trial, and documented in the concomitant medication log.
2. Unstable seizure disorder as defined by any seizure in the 6 months prior to the screening visit, and/or any change in anti-convulsant drug dosing in the 60 days prior to screening.
   Note Use of levetiracetam and oxcarbazepine are among permitted anticonvulsants.
3. Use of any other investigational drug at the time of enrollment or within 30 days or 5 half-lives (whichever is longer) of the investigational drug prior to screening until end of study visits (or longer if required by local regulations).
4. History of hypersensitivity to AFQ056 or any mGluR antagonist.
5. History or presence of any clinically significant disease of any major system organ class, within the past 2 years prior to screening including but not limited to neurological, cardiovascular, endocrine, metabolic, renal, or gastrointestinal disorders. This does not include typical features of FXS such as psychological symptoms or history of epileptic seizures.

6. Significant acute illness that did not completely resolve at least four weeks prior to the Screening visit.
7. Abnormal laboratory values at screening that are in the opinion of the investigator are clinically significant and may jeopardize the safety of the study subject.
8. Use of (or use within at least 5 half-lives before dosing) concomitant medications that are strong/moderate inhibitors or inducers of CYP1A1/2, CYP2C9/19 or CYP3A4 (see Appendix B).
9. Subjects who are, in the opinion of the investigator, unable to comply with the requirements of the study.
10. Presence of immunodeficiency diseases at the time of screening, based on medical history, including a positive HIV test result.
11. History of a positive Hepatitis B surface antigen (HBsAg) or Hepatitis C result at time of screening.
12. History or presence of suicidal thoughts and/or suicide attempts.

1 STUDY OBJECTIVES

The overall goals are to change the paradigm for development of mechanism targeted pharmacotherapy in neurodevelopmental disorders and provide a definitive test of the mGluR theory in humans by determining whether AFQ056, an mGluR5 negative modulator, can enhance neural plasticity in the form of language learning during an intensive language intervention in very young children with fragile X syndrome. This trial therefore will use an innovative but exploratory new trial design to develop a different way to examine efficacy of an agent with substantial support as a drug targeting CNS plasticity in preclinical models of a developmental disorder. If the design is successful, this trial can serve as a model for future trials of mechanistically-targeted treatments operating on neural plasticity in other neurodevelopmental disorders.

1.1 Primary Objective

Efficacy
(1) To demonstrate greater improvement in language in young children with FXS treated with AFQ056, using an overall weighted communication score, in combination with an intensive standardized parent-implemented language intervention, relative to those treated with the language intervention and placebo, after 6 months of intervention, as a marker of drug effect on neural plasticity, the core problem in the disorder.

The *Hypothesis* is that the children treated with AFQ056 will show greater improvement in language and its nonverbal precursors as reflected in the change in a weighted communication score designed to assess both the proclivity to engage in communication and the sophistication of the means employed for communication from baseline (visit 3 assessment) to 8 months (visit 9 assessment) than those treated with placebo.

1.2. Key Secondary Objectives

1.2.1 Functional
To show greater improvement in specific standardized language, cognitive, and adaptive measures (see Endpoints Derived From Evaluations Section) from the Mullen Scales of Early Learning (MSEL), Preschool Language Scale – Version 5 (PLS-5), The MacArthur-Bates Communicative Development Inventory, the Vineland Adaptive Behavior Scale- 3RD Edition

(Vineland-3), and Clinician Global Impression for improvement (CGI-I) in the combination AFQ056/language intervention group relative to the language intervention/placebo group;

The ***Hypothesis*** is that the children treated with AFQ056 will show greater normalization of specific measures of language and one or more other domains (including cognitive, adaptive behavior, global functioning based on change from baseline (visit 3 assessment) to 8 months (visit 9 assessment) than those treated with placebo.

1.3 Other Secondary Objectives

1.3.1 Safety

To demonstrate long-term safety and tolerability of AFQ056 in young children with FXS.

The ***Hypothesis*** is that AFQ056 will be safe and well tolerated for periods up to 16 months and that children treated with AFQ056 will have a similar side effect profile relative to placebo-treated children as that seen in previous studies with AFQ056 in FXS.

The following Other Secondary Objectives will be explored using the data from this study.

1.3.2 Functional

To show greater improvement in additional cognitive, language, adaptive and behavioral (see Endpoints Derived from Evaluations Section) standardized measures including the MSEL, PLS-5, MacArthur-Bates Communicative Development Inventory, Vineland-3, Aberrant Behavior Checklist – fragile X validated version (ABC-FX), CGI-I and Visual Analog Scales (VAS) for language and behavior, in the combination AFQ056/language intervention group relative to the language intervention/placebo group

1.4 Exploratory Objectives

The following Exploratory Objectives will be evaluated using the data from the study.

1.4.1 Blood Biomarkers

To explore whether normalization of blood biomarkers of translational pathways regulated by FMRP and mGluR5 receptors, including ERK, AKT, S6 kinase, APP, and MMP9, is associated with language and/or developmental improvements from AFQ056 treatment.

1.4.2. Other Biomarkers

To show improvement in specific measures from ERP (auditory habituation first 15% versus last 15% of pediatric p50/N1 (P1) peak occurring at approximately 100 ms post-stimulus and amplitude of auditory response – pediatric p50/N1, N2, P2), and eye tracking (looking time and fixations to the eyes) biomarkers in the combination AFQ056/language intervention group relative to the language intervention/placebo group.

The ***Hypothesis*** is that the children treated with AFQ056 will show greater improvement of these specific ERP and eye tracking measures based on change from baseline (visit 3 assessment) to 8 months (Visit 9 assessment) than those treated with placebo.

Additionally, to show improvement in multiple additional measures from the ERP and eye tracking (see Endpoints Derived from Evaluations Section) in the combination AFQ056/language intervention group relative to the language intervention/placebo group.

An additional exploratory hypothesis will examine whether the improvement in biomarkers listed as key secondary outcomes will be correlated with improvement in the weighted communication score, and potentially with scores on the key and other secondary measures throughout the study.

1.4.3 Pharmacokinetics

To determine if blood levels of AFQ056 correlate with improvement in language, functional or biomarker measures.

1.4.4 Longitudinal Analysis of Effects of Language Intervention and AFQ056

To determine if longitudinal analysis shows an improvement in the rate of change of language, functional or biomarker responses with the language intervention alone, and further improvement in rate of change when AFQ056 Is added to the language intervention.

2 BACKGROUND

2.1 Rationale

This trial is being proposed as a model trial for a paradigm shift to change the process for development of drugs targeted to correct specific dysfunctional pathways contributing to neurobiological and synaptic dysfunction in genetic neurodevelopmental disorders, based on animal model work. Fragile X syndrome (FXS) is the ideal disorder in which to conduct such a model trial, given the tremendous progress in understanding the basic neuroscience of synaptic dysfunction and the extensive background of preclinical studies in FXS models. Treatment of FXS animal models (both Drosophila and mouse) with drugs targeted to synaptic dysfunction has been massively successful. The body of translational pre-clinical research in FXS has been more consistent and convincing than any other model of disease correction for a single gene developmental disorder in neuroscience, with over 60 different research publications showing either genetic or pharmacological reversal of phenotypes associated with absence of FMRP in cellular models and animal models from two different species.

Yet over the past decade of work, it has been challenging to translate this extremely promising large body of work to benefit humans with FXS. Positive behavioral results in early proof of concept trials have been difficult to reproduce in larger phase 2b and phase 3 trials, leading to questions about the best way to design the trials and conduct the translational work itself. Sub-optimal outcome measures (placebo effects, floor effects, variability in parent ratings), lack of good biomarkers, population variability, and variability in the nature of responses have been problems. But the largest issue with work done to date is the concern that what is being measured in trials in humans with FXS is simply not the same as what is being demonstrated in the animal models. Human trials to date have been largely focused on behavior in adolescent or adult populations with FXS, and after years of development and shaping of behavior by the environment and exposures, it is unclear that adult behavior can be viewed as a surrogate marker for effects on neural plasticity seen in the FXS animal models. Given the prolonged prior development time, it is also unclear that changes of sufficient effect size (especially given the variability in the population) can be seen at all in adolescents and adults with FXS treated with mechanistically targeted agents. At this juncture, it is important for the translational effort in the FXS field to undergo a paradigm shift and begin to design trials to look for the cognitive and learning outcomes that might be predicted from the preclinical work. It will be important for this shift to focus on

moving these studies to very young children, to maximize the possible plasticity and avoid contamination of outcomes from "wiring" present in the mature brain that reflects accumulation of decades of environmental exposure. It stands to reason that treatments for a developmental disorder would best be studied in young children when the minimum amount of aberrant development has had time to take place.

As such this protocol proposes a trial in which the drug to be studied will target the best studied and most successfully reversed synaptic pathway dysfunction in preclinical work in FXS models, but, unlike other trials, the group of patients to be studied will be very young children (age 3-6 years), use of the drug will be coupled with an intensive language learning intervention, and the primary outcome to be measured will be progress in language skills and their nonverbal precursors resulting from the intervention. It is expected that the learning outcome in this trial will be a much better surrogate for the plasticity and synaptic effects in the FXS models than non-specific behavioral effects studied previously in older patients.

The following pages present the "story" of FXS and translation of progress in basic science research to humans, which has brought the field to the point of needing a paradigm shift to move toward targeted treatment trials aimed at studying "plasticity" as outlined in this protocol. FXS is the most common known single gene cause of intellectual disability and ASD, with an estimated frequency of about 1:4000-5000. The disorder affects all ethnic groups worldwide. FXS is one of the fragile X-associated disorders (FXDs), all of which arise from a trinucleotide repeat (CGG) expansion mutation in the promoter region of *FMR1* (*Fragile X mental retardation 1* gene). The CGG sequence is transcribed into the 5' untranslated region of the *FMR1* mRNA and thus length of the repeat sequence does not affect the sequence of the protein product of *FMR1* (fragile X mental retardation protein, or FMRP) [1]. Large expansions in *FMR1* (>200 CGG repeats), termed the "full mutation," cause FXS, which results from methylation and transcriptional silencing of *FMR1* with consequent loss or significant reduction in expression of FMRP.[1] Males with FXS typically present with developmental delay, particularly language delay, and ultimately display intellectual disability that can range from mild to severe.[2] Hypotonia is often seen early in life and evolves into coordination and praxis problems in older children and adults. Physical features include macroorchidism in most adult males, and variable presence of craniofacial characteristics including prominent ears, macrocephaly, long face, prominent jaw and forehead, midfacial hypoplasia, and high arched palate. Loose connective tissue leads to hyperextensible joints, flat feet, and soft redundant skin on the palms.[2] Females with a full mutation are more variably and usually more mildly affected than males because of production of FMRP in cells that express the normal X chromosome with the non-mutated *FMR1* allele. Severity of cognitive impairment in females with a full mutation is inversely related to the activation ratio for the normal *FMR1* allele and levels of expressed FMRP.[3] Males with size (full and premutation) or methylation (partially unmethylated full mutation) mosaicism may be more mildly affected, with severity related to the percent of unmethylated *FMR1* alleles and FMRP levels. A number of medical problems appear to be more prevalent in FXS than in the general population, including seizures, strabismus, frequent otitis media, gastroesophageal reflux, and sleep apnea and other sleep disorders [4].

The majority of males with FXS will meet criteria for mild to severe intellectual disability.[5] Average IQ in adult males with FXS is 40-50. Females with FXS are often less affected than males with about 25% having cognitive impairment and others frequently being diagnosed with learning disabilities[6]. Average IQ in females is about 80, with a range from severe impairment to normal or even superior ability. Multiple studies have shown a decrease in full-scale IQ scores and adaptive behavior with age as children with FXS become older.[7-10]. Decline in standard scores for intelligence and adaptive function is not the results of loss of skills or regression but rather failure to keep pace with the normal rate of intellectual development. Behavioral dysfunction is very commonly seen in FXS and includes attention deficits/hyperactivity, hyperarousal, anxiety,

perseverative behavior and aggression. About a 50-67% of males and about 20% of females with FXS meet criteria for ASD.[11] Although psychopharmacologic treatment for behavioral symptoms (with stimulants, SSRIs, antipsychotics depending on most problematic symptoms) is often employed in FXS [12], these medications are incompletely effective[13] likely due to the environmental contingencies maintaining the behaviors, and there are no treatments for the underlying cognitive disorder. ***Thus, treatment of both behavior and cognition/development in FXS represent areas of high need.***

Language development is globally delayed for most individuals with FXS. It is not uncommon for individuals with FXS to remain prelinguistic communicators until much later in life than is seen in typical development [14]. The average age of a first word in FXS is about 25 months and many boys with FXS do not develop phrase speech until after age 4. However, the majority of males and females with FXS will obtain spoken language at some point, and they will continue to gain language skills throughout the lifespan, albeit at a slower pace than typically developing individuals [15]; [16]. In general, individuals with FXS have stronger receptive than expressive language skills. Pragmatic language (communication in social interactions) is delayed very early in development. Young children with FXS have been shown to have difficulty with joint attention, reciprocating positive facial expressions, eye gaze, and turn-taking [16]; [17]. Even when verbally fluent, males with FXS demonstrate high rates of tangential (off-topic) language, overly literal interpretation of language, as well as decreased topic initiation and maintenance [18]; [19]. Perseverative language is a hallmark of FXS and causes significant social difficulty. ***Thus, treatment of language deficits in FXS as a subsection of development is an area of high need, especially in young children with FXS.***

Recent dramatic progress in understanding the neurobiology and synaptic mechanisms resulting from absence of FMRP in FXS has become an important window to future targeted treatments for FXS, ASD and related neurodevelopmental disorders (NDDs).[11,20] The *Fmr1* knockout mouse model of FXS, which makes no functional FMRP, has been a critical resource to understand the role of FMRP in neurons, identify cellular targets for treatment and explore effects of potential disease-modifying agents. FMRP is an mRNA binding protein involved in the transport and translational regulation of a subset of dendritic mRNAs.[20] FMRP regulates (inhibits) dendritic protein translation in response to synaptic activation by Group 1 metabotropic glutamate receptors (mGluR1 and mGluR5),[21] muscarinic (M1) acetylcholine receptors and probably multiple Gq-linked receptors. Activation of these receptors results in signaling through ERK- and mTOR-dependent signaling pathways, ultimately resulting in loss of FMRP repressor function at the ribosome, and a pulse of new protein synthesis. Translation of multiple synaptic proteins is regulated by FMRP, for example STEP and Arc, which are linked to AMPA receptor internalization.[22] FMRP also regulates activity of some pre- and postsynaptic ion channels such as BK and SLACK channels through direct protein-protein interactions.[23] These regulatory functions of FMRP appear to be critical for synaptic maturation and strength, as in the absence of FMRP (Figure 4), there is elevation of levels of synaptic proteins usually controlled by FMRP, immature elongated dendritic spines,[24] abnormal spine density, abnormal synaptic plasticity including enhanced mGluR-activated hippocampal and cerebellar LTD, and impaired LTP in hippocampus, cortex and amygdala, and proneness to abnormal epileptiform discharges.[20,23] The morphological abnormalities and synaptic plasticity deficits found in the *fmr1* knockout mouse and the *Drosophila* model of FXS, in which there is loss of *dfmr1* (homolog of the *FMR1* gene in the *Drosophila* genome), are associated with numerous cognitive, behavioral and electrophysiological phenotypes [20,23].

The abnormalities observed in the absence of FMRP in the mouse model of FXS has led to identification of treatment targets for potential clinical development and directed at (1) reduction of excess activity in signal transduction pathways connecting group 1 mGluRs or other Gq-linked

receptors to the dendritic translational machinery, either through (1A) receptor modulation at the cell surface or (1B) through modulation of the intracellular signaling pathway (2) reduction of excessive activity of proteins normally regulated by FMRP, (3) increasing expression and activation of surface AMPA receptors, (4) modification of activity of GABA and other receptors/proteins that regulate glutamate signaling or translational signaling pathways, (5) using miRNAs to block excessive translation of mRNAs normally regulated by FMRP, and (6) correction of abnormal channel activities normally directly regulated by FMRP Treatments aimed at all of these types of targets have shown success in reversing phenotypes in the *Fmr1* knockout mouse [25-27] and *dfxr* fly[28] models, however the most extensive body of research surrounds the use of mGluR5 negative allosteric modulators (NAMs) and genetic receptor reduction to reduce excessive translational pathway signaling through mGluR5 receptors.

Reduction of mGluR5 receptor activity with mGluR5 NAMs has resulted over 30 publications documenting correction of abnormalities or deficits in dendritic spine morphology, ocular dominance plasticity, olfactory learning, synaptic plasticity (LTP and LTD), motor learning, open-field hyperactivity, marble burying, social behaviors, prepulse inhibition (PPI), epileptiform bursts, neuronal network activity states, audiogenic seizures, growth patterns, cellular signaling and protein synthesis in the *fmr1* knockout mouse (see Table 1 for a summary of studies in the FXS mouse), and synaptic branching, courtship behavior, and learning phenotypes (ref) in the *Drosophila* model of FXS. Although not every phenotype studied has been corrected, it is clear that more phenotypes have been corrected than not corrected. The majority of the studies in the FXS mouse have been done with examiners measuring outcome blinded to treatment status, although formal randomization in animal studies has not been done. Phenotypes have been corrected in adulthood [29], however several studies in which age groups were compared suggest better effectiveness in young animals.[30,31] Numerous mGluR5 NAMs have been used to produce these effects.

This solid foundation of pre-clinical work has led to attempts to translate findings to humans with FXS. A phase 1 open label proof-of-concept (POC) trial of fenobam, a very short-acting mGluR5 NAM, showed reversal of abnormal sensorimotor gating in humans with FXS, despite high interpersonal variability in PK curves. Subsequently, development of highly selective longer acting mGluR5 NAMs with improved PK profiles for treatment of FXS was initiated at both Roche (RO4917523) and Novartis (AFQ056). At Novartis, AFQ056 was originally developed for levo-Dopa induced dyskinesia in patients with Parkinson's disease (PD-LID). PET scan data were generated at Novartis, demonstrating the AFQ056 binds to and is highly selective for mGluR5 receptors in brain the brain (PET).

Table 1. Studies Documenting Reversal of Phenotypes in FXS Mouse Model

Specific Target	Rescue Type	Rescued/Improved Phenotypes	Phenotypes tested but not rescued
mGlu5	Genetic (mGluR5 hetorozygote – 50% receptor reduction)	**Molecular & Cellular:** protein synthesis, dendritic spine density, body weight [32] **Plasticity & Hyperexcitability:** ocular dominance, **AGS***, LTD [32] **Behavior:** social interactions [33]; partial rescue of social dominance [34] **Cognition:** inhibitory avoidance memory [32]	macroorchidism [32,33]; locomotor activity, **AGS**, **PPI*,** startle response, OFA, MB [33] ***The protocols used for AGS induction were slightly different, but still do not fully explain these contradictory results, method for PPI was very different from 210, 211 and human studies**

	Pharmacological (mGluR5 negative modulators)	**Molecular & Cellular:** mTOR [29,35] and ERK1/2 signaling [29]; GSK3β phosphorylation [27,36]; synaptic protein composition [37]; PSD-95 [38], mRNA granule levels [39], protein synthesis [29,40,41]; AMPA receptor internalization [42]; dendritic spine length and density (*in vitro:* [31,43,44], *in vivo*: [29,31,45]; partial rescue of macroorchidism [29] **Plasticity & Hyperexcitability:** LTD (*after in vivo administration*) [29]; mEPSC frequency (amygdala) [46]; spontaneous EPSC amplitude and charge (hippocampus)[30]; UP states [47] neuronal network activity [48]; synaptic and network potentiation [49], OFA [35,50,51]; AGS [149, 152, 214], prolonged spike discharges [52], limbic epileptogenesis [53], **startle response*** [29], PPI [43,44] **Behavior:** social dominance [34]; sociability [54], open field hyperactivity **Cognition:** object recognition (*chronic, not acute*) [35]; fear conditioning [29]; associative motor learning (*acute*)[55], inhibitory avoidance (*chronic and acute*) [50,55] extinction memory (*chronic*) [50]; maze learning [38]	LTP deficits in the amygdala and hippocampus (*bath application*) [56,57]; LTD (*bath application*) [46] **startle response***, rotarod performance [58]; object recognition (*acute*) [56], coordinate and categorical tasks (*acute*) [56], analgesic response [35] **mGlu5 NAMs used:** ***MPEP***:[27,30,31,38-43,48,49,51,52,56-58] ***MTEP***: [35] ***Fenobam:*** [37,43,55] ***AFQ056:*** [34,44,45,54] ***CTEP:*** [29,50] ***Two different mGlu5 NAMs were used for these studies.**

AGS: audiogenic seizure, EPSC: excitatory post-synaptic current, LTD: long-term depression, LTP: long-term potentiation, MB: marble burying, OFA: open field activity, PPI: prepulse inhibition data in Appendix A).

Based on the extensive and promising pre-clinical and early clinical work with mGluR5 NAMs in FXS, studies in FXS were initiated with AFQ056 at Novartis. A small (N=30) phase 2a POC placebo-controlled crossover study suggested benefit of AFQ056 treatment of behavior on the Aberrant Behavior Checklist – Community Edition (ABC-C) in fully methylated adult males with FXS (N=7). A small phase 2a placebo controlled PK and safety study was conducted by Roche with RO4917523 demonstrating a favorable safety profile. These phase 2a studies prompted two larger (N=160 each) phase 2b placebo-controlled, methylation stratified trials examining effects of 3 months of treatment with AFQ056 on behavior in adolescents (AFQ056B2214 or "2214") and adults (AFQ056A2212 or "2212") with FXS (Novartis, clinicaltrials.gov), and a large (N=200) placebo-controlled trial of RO4917523 for anxiety in FXS (Roche, clinicaltrials.gov), none of which ultimately showed efficacy of drug for behavioral outcomes. Despite the early results in the Novartis POC trial suggesting behavior might be a surrogate endpoint for disease modification that could be used for drug registration in adult/adolescent studies, at the time of design of phase 2b studies, uncertainty remained surrounding whether 1) behavior is an adequate surrogate for disease state in FXS, 2) deficits in a developmental disorder such as FXS can be expected to be sensitive to any form of reversal over short time frames in adults or adolescents, and 3) disease modification in the form of enhanced learning capability can be detected at any age in a time frame assessable by traditional short-term placebo-controlled study, and (4) trials might require a learning intervention to see the proposed effects on synaptic plasticity observed in the animal models.

Thus, early in the discussion of the AFQ056 and RO4917523 development strategy (before any trials were conducted), it was proposed that: 1) measurement of developmental skills, cognition or function would be needed to demonstrate disease modification, 2) disease reversal might be detectable only in young children and thus trials in adults could not predict response in children given the developmental nature of FXS and higher level of plasticity at younger ages, and 3) implementation of a learning intervention within the trial design to capture putative drug effects on neural plasticity and accelerate progress would likely be necessary to show a functional or cognitive response in the time frame of even the longest possible placebo-controlled trial. Plans to address these issues were proposed by a team of academic researchers specializing in FXS and Novartis program scientists, and included a 6-month placebo-controlled study evaluating AFQ056 effects on intensive reading training in children and adolescents with FXS, and a separate placebo-controlled study evaluating AFQ056 effects on behavior, cognition and development in children potentially as young as age 3 years. To meet FDA requirements to implement these two studies and determine appropriate drug doses for children, a phase 2a PK study was conducted in children with FXS, age 3-11 (clinicaltrials.gov). Concomitantly open label extension studies ("2278" and "2279") were conducted, in which participants from 2212 and 2214 were treated with AFQ056 for over 2 years, to evaluate long-term safety (clinicaltrials.gov). It was not expected that conduct of either of the trials in children would depend on results of the adult/adolescent studies, given the above concerns. However, when recent analysis of results from 2212 and 2214 showed no 3-month behavioral efficacy on the ABC total score or subscores for AFQ056, and the Roche adult study showed no benefit of RO4917523 for anxiety and behavior, the additional Novartis studies were cancelled. Roche also discontinued their FXS studies based on their "negative" adult study. Although Roche ran a Pediatric PK study that was not powered for efficacy, they decided not to pursue the effects of RO4917523 in children with an efficacy trial.

The combined Novartis studies completed to date have generated a large amount of safety data suggesting no major concerns. ***None of the "negative" studies, however, for the reasons noted above, have really answered the question of whether mGluR5 NAMs can modify the core features and course of FXS because they have not studied the core deficit: cognition and learning in sufficiently young populations with this developmental disorder.*** Although all the pieces are now in place to run the trial(s) that would actually begin to address the question of whether the extensive basic science and animal model work predicts disease modification in humans by mGluR5 negative modulators, these trials will not be done though the Novartis program or through industry in general due to lack of precedent for the type of trial that needs to be done. Novartis will however provide AFQ056 and perform PK studies should the FXS scientific community obtain funding to explore this question by conducting a trial utilizing a learning intervention in young children. Thus, this NeuroNEXT protocol represents the next step for the FXS research community to be able to study the effects of mGluR5 negative modulation on plasticity in the youngest (age 3-6 years) children possible. At the same time, the protocol will result in generation of more information on language learning through intensive language intervention without pharmaceutical intervention; and will seek to develop a badly needed brain-based outcome measure of treatment effects in FXS.

Thus, far all mGluR5 NAMs appear to rescue phenotypes in the FXS mouse and in pre-clinical studies in which two different drugs were compared, results were similar (refs). AFQ056 (Mavoglurant, Novartis) has been chosen for this study because: 1) it is the only mGluR5 NAM that has been studied in children down to age 3; 2) PK data exists for children age 3-11; 3) there were no significant safety issues and the drug was well-tolerated in children age 3-11; 4) there is PET data at Novartis that shows the drug crosses the blood-brain barrier and is hitting the desired target (mGluR5 receptor) in brain; 5) Novartis is willing to provide the drug, help with obtaining an IND and perform PK studies for the study. Specifically, four pre-clinical studies have shown

reversal of phenotypes in the FXS mouse with AFQ056, including dendritic spine length and density *in vitro* and *in vivo*, motor learning, PPI, and several social behaviors. AFQ056 will be dosed orally as a suspension that was used in the prior Novartis PK study for children age 3-11 with FXS (clinicaltrials.gov). In this study, the investigational drug (AFQ056) was prepared by Novartis and provided to the Investigator as open label medication in a form of a powder for an oral suspension, resulting after reconstitution with water in a 10 mg/mL oral suspension. This suspension was well-tolerated and PK data is available documenting the oral bioavailability of the drug. Use of the suspension will eliminate the need for young children to swallow pills or capsules. The dosage range to achieve blood levels expected to produce over 50% mGluR5 receptor occupancy in brain will be 25 to 100 mg which is a higher oral dose relative to weight than that used in the adult and adolescent studies (up to 100 mg), however in the pediatric PK study metabolism of the drug in children appeared to be enhanced relative to the adult population and thus similar doses were needed for equivalent blood levels, despite smaller body weight. Because PK showed interperson variation in blood levels for a given dose of AFQ056 in children, the flexible dose forced-titration to the maximum tolerated dose has been employed to maximize the chances that a stable dose that will be tolerated will be employed during the 6 months of the language intervention.

The language learning intervention to be coupled with the AFQ056 targeted pharmaceutical treatment and employed in the treated and placebo groups was chosen because to be appropriate for very young children with FXS the intervention must support child participation and engagement and must target a skill still emerging in the age group to be studied. Both males and females will be studied and although females on average are higher functioning, those enrolled in the study will have a DQ<75 and thus will represent the lower end of functioning for females with FXS and will be more comparable to the males and will have sufficient developmental impairment to evaluate improvement from the interventions. On average children with FXS say their first word at just over 2 years of age [59] and gradually work toward speech in multiword phrases by around age 4-6 years. Thus, at age 3-6 years, children with FXS are often in a period of transition in language skills suggesting language should be an area of development particularly sensitive to intervention at this age, and that if the mGluR5 NAM can make it easier to learn, the potential rate of change at this age should make both the effects of the language intervention alone and in combination with the drug effects easier to observe. If the drug works on synaptic plasticity the effects of the drug should be best seen during the period of maximum plasticity for the function being studied and age 3-6 should be the period of maximum plasticity for language learning for the majority of children with FXS. Given that language is the best domain of learning to study at this age range, the particular language intervention to be employed was chosen because: 1) it has been studied in FXS and has been shown to accelerate language progress in young children with FXS on a background of standard therapy [60]; 2) it relies on training of parents or caregivers to maximize the total amount of time during which the intervention can be delivered in the home; 3) it has been successfully delivered in a standardized fashion by a combination of in person visits at the clinic or research center and in-home sessions conducted via video-conferencing thereby reducing the number of physical study visits needed to implement and monitor parental mastery and delivery of the intervention, reducing family burden and making compliance more likely.

The language intervention within the placebo-controlled period for this study will be 6 months. Even though the study population will be young, in general it is expected that effects on learning resulting from either the language intervention or AFQ056 will only be measurable over time. The language intervention has shown effects after 6 months of treatment and due to the problematic nature of maintaining placebo treatment for more extended time periods, 6 months was chosen as the period of time over which to examine the effects of AFQ056 on language learning in conjunction with the language intervention. Although virtually all children with FXS receive some speech and other therapies as well as special education, the specific amounts and intensity of

therapy vary quite widely and thus the language intervention in this study will be more intensely focused on very specific areas (promoting more frequent expressive communication, as well as building skills in expressive vocabulary and grammar) relative to background therapy allowing us to look at amplification of progress in this specific area during the trial.

Because it will be critical to have the AFQ056 dose titrated to the maximum dose tolerated before initiating the language intervention, there will be a two-month flexible dose titration period before initiating the language intervention to make sure the dose is optimized before the language intervention starts. Thus, the total placebo-controlled period will be 8 months. After the end of the placebo-controlled period, all subjects will initiate titration of AFQ056 as per the titration protocol in the placebo-controlled period, starting with 25 mg BID to the maximum tolerated dose (up to 100 mg BID) over 2 months and then will be treated with AFQ056 through an open label extension. All subjects, families and investigators will remain blinded to treatment status during the placebo-controlled period during the extension period until the final subject has completed the placebo-controlled period and all data from this period is entered in the DCC database and locked. As such, some subjects will have an acute dose decrease if they were on a dose of 50-100 mg BID of active drug in the initial phase but experience from the Pediatric PK study conducted previously suggests that children can stop AFQ056 without withdrawal symptoms and the dose decrease and titration in the open label extension are unavoidable to maintain the blind of the study through the completion of the placebo-controlled period by the final subject.

The purpose of the extension period will be the following:

1. To systematically establish safety of AFQ056 in a larger cohort of subjects (which will include all those treated with placebo in the first period of the trial) and over a longer period of time (up to 16 months in those treated with drug during the first period) in the 3-6 year age group. If AFQ056 were ever to be used for treatment of FXS based on this and other subsequent studies, patients would be treated for life or at least for many years until something better is developed. Although there is safety data over long periods in older patients, there is no extended safety data for young children. Even though we are not predicting specific safety issues, this extended treatment of up to 16 months will help build an important chronic safety profile for future use in very young children.
2. To examine the trajectory of effects of AFQ056 on language development with the language intervention over a longer period of time (14 months) relative to the 6 months in the placebo-controlled period.
3. To examine whether a change in trajectory of language development can be seen after the placebo group goes on AFQ056in the open label extension period.
4. In the absence of drug effect, to examine the trajectory of the effects of the language intervention over a longer time period (14 months).
5. To examine the trajectory of the ERP biomarker performance across a longer time period of development, and sensitivity to both the drug and language intervention.

Due to study drug expiration in August 2021, the length of the open label extension may be shortened depending upon the date the subject is consented. For subjects consented prior to June 2019, the open label extension period will be 8 months allowing the entire 21-month protocol to be completed. For any subject that is consented in or after June 2019 the open label extension period may be shortened on a sliding scale for those enrolled after June 15, 2019, such that their treatment including weaning, if necessary, will end before August 31, 2021, with the exact length of the stable treatment period in the extension being determined by how long after June 15 they enroll and what dose of drug they are receiving in the open label treatment period. The total duration of the stable treatment for those enrolled after June 15, 2019 will range from 6 months to no open label extension, depending on the number of months after June 15, 2019 the subject is enrolled into the study.

Auditory event-related potentials (ERP), is a CNS measure that is significantly abnormal (both amplitude and habituation) in FXS, has shown improvement in habituation in an oddball paradigm in a prior pilot trial of minocycline in FXS, and would be expected to relate to language processing and learning. The ERP biomarker will be utilized in this protocol as an assay of direct effects of AFQ056 and/or language intervention on CNS activity. The prolonged time course of the study should allow the biomarker to be linked to developmental parameters over time so that its value as a predictive marker for long-term clinical effects of interventions in FXS can be determined. A marker of CNS activity that is linked to clinical outcomes is badly needed in the field and would be a tremendous resource for future interventions research.

In order to be able to relate the primary outcomes of improved language and communication as measured in a structured and coded play-based assay and normalization of auditory ERP to general development and behavior in FXS over the relatively long time course of the study, secondary outcomes will track domains of 1) cognitive development (Mullen Scales of Early Learning), adaptive development (Vineland Adaptive Behavior Scale), behavioral development (Aberrant Behavior Checklist – FX Version) eye tracking (biomarker of social engagement), language development (MacArthur Bates Communicative Development Inventory, Expressive Vocabulary Test, Peabody Picture Vocabulary Test, Visual Analog Scale for Language), and global function (CGI-I, CGI-S). The study will explore molecular measures of the translational pathway regulated by FMRP, including ERK[61], AKT[62], S6 kinase[63], APP[64], and MMP9[65] activities and phosphorylation (when applicable) state, to see if a molecular biomarker linked to clinical improvement can be identified.

This trial will **not only** address the impact of AFQ056 on language learning in young children with FXS, hence providing the first true test of the mGluR theory of intellectual disability in FXS by using a learning/plasticity model, but it will **also** test formally the effects of the language intervention itself in the largest group than has been studied to date, and will test whether abnormal auditory ERP can be a readout for improvement in language and speech sound processing and development related to either the language intervention itself, or the combination of the language intervention and AFQ056. Thus, important information will be generated even in the absence of a drug effect.

This trial protocol will serve as a model for a new generation of similar trials that need to incorporate a learning paradigm to translate targeted treatments from animal models to other genetic neurodevelopmental disorders. Hence, even in the absence of a drug effect for AFQ056 valuable knowledge will still be gained about how to conduct this type of novel trial. If successful, this trial is expected to create a template for a paradigm shift that will change clinical practice and change the process of translation of mechanistically targeted, animal model-based treatments for FXS and for other neurodevelopmental disorders, so as to move the focus of initial efficacy studies from behavior in adults and adolescents to learning and cognition in young children. Without such a paradigm shift, it is entirely possible the benefits of the advances in basic neuroscience and the promise of reversal of disease in animal models will never be realized in humans due to a flawed process of human testing and inability to detect change in adult populations with developmental disorders. This study is the highest priority in translational medicine in the FXS field right now, as it will allow an understanding of whether the animal model data (even when very extensive and reproducible) does not translate directly to humans or whether we have been doing the wrong clinical trials in humans. It is imperative we do this study soon so as not to continue to waste millions of dollars in the FXS and other highly similar genetic neurodevelopmental disability fields doing clinical trials using translational models that are doomed to failure. Whatever the result of this study, it will illuminate the relevance of animal

model data to human translation, and guide the steps in the process of moving from bench to bedside.

2.2 Supporting Data

Pharmacokinetics of AFQ056 in Pre-Clinical Studies
AFQ056 is a structurally novel, subtype-selective, non-competitive inhibitor of the metabotropic glutamate receptor 5 (mGluR5). The details of the pharmacokinetic profiles in the preclinical species can be found in the current Investigator's Brochure of AFQ056.

In vitro blood distribution of AFQ056 was different across species with fractions in plasma of 0.56 (rat), 0.76 (dog), and 0.92 (human). The unbound fractions in plasma were 6.5% (rat), 0.3% (dog), and 2.8% (human). Blood distribution as well as protein binding were independent of the AFQ056 concentration within the range of 10 to 10000 ng/mL. In human hepatocytes, liver microsomes, liver S9 fraction, and recombinant CYP enzymes, the *in vitro* hepatic metabolism of AFQ056 was *via* CYP3A4, CYP2C8, CYP2C9, and CYP2C19. Results from recombinant CYP1A1 suggested a possible contribution of extrahepatic metabolism. Inhibitors and/or inducers of CYP3A4, CYP2Cs and/or CYP1A1 will likely influence the hepatic clearance of AFQ056 in humans. AFQ056 could not be identified as a substrate of active hepatic or intestinal uptake and efflux transport processes in primary cultures of rat and human hepatocytes and Caco-2 cells. In human hepatocytes, AFQ056 did not induce expression or activity of CYP enzymes at the therapeutically relevant concentration of 2 µM. AFQ056 showed weak inhibition of CYP2C19 and CYP3A4. Weak time-dependent, irreversible inhibition of CYP3A4-mediated midazolam 1'-hydroxylation by AFQ056 was also observed. AFQ056 was found to be a weak inhibitor of MDR1 (multidrug-resistant protein 1), MXR (mitoxantrone resistant protein), OAT3 (organic anion transporter 3), OCT1, OCT2 (organic cation transporter 1 and 2), OATP1B1 and OATP1B3 (organic anion transporting polypeptide 1B1 and 1B3). AFQ056 does not inhibit MRP2 (multidrug resistant-associated protein 2) and OAT1 (organic anion transporter 1). The weak inhibition potential of AFQ056 towards these enzymes and transporters is unlikely to be of clinical significance, as the IC 50 values were much higher than the mean Cmax values of AFQ056 in subjects treated with twice daily oral doses of 150 mg (in CSF form) was approximately 2.0 µM (concentration in the therapeutic relevant range, CAFQ056A2102).

Pharmacodynamics of AFQ056 in Pre-Clinical Studies
In-vitro data
AFQ056 is a highly selective inhibitor of mGluR5 indicated by its lack of in-vitro inhibition of human recombinant mGluR1/2/4/7 and GABAB1/2 (gamma-Aminobutyric acid) receptors (up to 10 µM).

In-vivo data
Animal studies assessing the pharmacodynamic effects of AFQ056 have been conducted with respect to treatment of the following indications: anxiety, drug abuse, gastro-esophageal reflux disease (GERD), dyskinesia/Parkinson's disease.

In several animal models, anxiolytic-like effects of AFQ056 were determined in a dose range of 0.03-30 mg/kg. AFQ056 decreased the extent of intravenous nicotine self-administration in a dose range of 0.03-10 mg/kg. In terms of GERD, AFQ056 was generally active at doses ≥1mg/kg (and comparable with the active comparator baclofen). In a monkey model of parkinsonism, AFQ056 potentiated the anti-parkinsonian locomotor activity of low-dose L-Dopa and decreased the severity of L-Dopa induced dyskinesia.

Safety, Pharmacology and Toxicology in Pre-Clinical Studies
Cardiovascular safety pharmacology investigations showed a weak inhibition of the human ether-a-go-go related gene (hERG) channel that did, however, not translate into effects on the QT-interval in dog telemetry studies. Dose-related increases in heart rate and respiratory rate seen in dog studies (at a dose range of 12.5 – 625 mg/kg) were not reproduced in human clinical studies.

Single dose toxicity studies with AFQ056 revealed non-lethal intravenous doses of 50/80 mg/kg (female/male) in mice and 40/60 mg/kg (female/male) in rats. Repeated oral dose toxicity studies with AFQ056 were conducted for up to 13 weeks in mice, 26 weeks inrats and 52 weeks in dogs. Target organs of toxicity were the CNS (all species as evidenced by adverse clinical signs), liver (rat and dog), thyroid and pituitary gland (rats), ovaries and the mammary gland (female rats). **In mice**, the No-observed adverse effect level (NOAEL) was 400 mg/kg/day. **In rats**, target organs of toxicity at the maximum dose level included the thyroid and pituitary gland suggesting a stimulation of hypothalamic- pituitary-thyroid axis. These observations are considered to be secondary to a liver weight increase and enhanced clearance of thyroxin that is commonly observed in rat toxicity studies. **In dogs**, a NOAEL of 60 mg/kg was determined in a 52-week chronic toxicity study. In the same study, no significant neuromotor effects were seen at the top dose of 180 mg/kg. A common finding in all repeated-dose dog studies was increased excretion of protoporphyrin into the bile duct canaliculi. However, this finding was not considered adverse as it was not associated with any functional or histopathological findings in studies up to 26 weeks in duration. However, the data for AFQ056 indicates that this compound has a low likelihood to cause drug-induced porphyria in humans by inhibition of ferrochelatase. At the no-effect-level for increased protoporphyrin excretion after 52 weeks of dosing (60 mg/kg), the AUC-based safety margin relative to the exposure at a human dose of 150 mg b.i.d. is 7.4 and 9.45 for females and males, respectively. AFQ056 does not appear to have a genotoxic potential and is not phototoxic.

Teratogenicity and Reproductive Toxicity Data in Pre-Clinical Studies
No effects on male or female fertility were observed in rats up to the top dose level of 500 mg/kg. A rat embryo-fetal development study indicated that AFQ056 has a potential to induce fetal toxicity. No overt toxicity was observed in a pre- and postnatal development study in rats. In conclusion, AFQ056 is not considered to have a teratogenic potential. A juvenile toxicity study was conducted in the rat since this species is well accepted for such studies and showed pharmacodynamic responses to AFQ056. In this study AFQ056 was administered at 0, 10, 30, 200 mg/kg/day (from day 7-70 post-partum) followed by 6-weeks recovery period. No mortality was observed. The top dose induced transient adverse clinical signs (reduced body weight gain; lower body weights). Other findings included non-adverse hepatic hypertrophy and thyroid follicular cellular hypertrophy which are considered an adaptive response to high drug load. Hence, the NOAEL was set at 30 mg/kg/day corresponding to the following exposure data: Cmax of 2.6-3.0 µg/mL and AUC0-24hr: 20-23.5 µg*hr/mL) for males and females, respectively. In conclusion, juvenile animals were not more sensitive compared to adult rats and no new target organs of toxicity were determined. The therapeutic index at the NOAEL relative to the maximum recommended human therapeutic dose of 150 mg b.i.d. (CSF formulation) is 6.1 vs. 7.0 (Cmax) and 3.4 vs. 4.0 (AUC0-24hr) for males and females, respectively.

Pharmacokinetics of AFQ056 in Human Studies
Absorption, Distribution, Metabolism and Elimination
After a single oral dose of 100 mg (MF) in adult healthy volunteers the absolute bioavailability of AFQ056 was 40%, and the time (Tmax) to reach maximum plasma concentrations (Cmax) was about 2 hours (median). AFQ056 demonstrated to be highly bound to proteins in plasma with an unbound fraction of 2.8%. AFQ056 demonstrates dose proportional PK properties up to the

maximum therapeutic dose range of 150 mg with CSF. After repeat dosing, the steady state was achieved in 48-96 hours with accumulation ratio of 1.78 to 1.94; there was no evidence of time-dependent change in the PK. After an intravenous infusion of AFQ056 (25 mg over 10 min), total clearance (CL) was 33.2 L/h and the mean volume of distribution (Vd) was 281 L. The mean apparent elimination half-life (T1/2) was approximately 8 to 9 hours.

The elimination of AFQ056 occurred primarily via CYP-enzyme mediated metabolism by CYP1A1, CYP2Cs and CYP3A4. The excretion of AFQ056 and its metabolites was shown to occur mainly via the fecal route (58.6% of dose) and to a smaller extent by the renal route (31.4% of dose). In feces, 41.8% of the dose was eliminated as unchanged AFQ056 and 15.0% of the dose in the form of metabolites. No parent drug was detected in urine. Co-administration of AFQ056 with ketoconazole (400 mg qd) increased Cmax and AUCinf of AFQ056 by approximately 2.5-fold and 3-fold respectively. No PK interaction was observed when AFQ056 was co-administered with Sinemet® (levodopa/carbidopa). Cigarette smoking appears to lower the exposure of AFQ056 as cessation of cigarette smoking resulted in 2.7-fold increase in trough concentrations of AFQ056. The development of the CYP450 enzymes (CYP2Cs and CYP3A4) involved in the AFQ056 metabolism in the pediatric group aged 3 years upwards is expected to be similar to adults (Benedetti, Whomsley and Baltes 2005) whereas the activity of the CYP1A1 enzyme in the intestine is low (<50 % compared to adult) during early life but attains the same activity level as the adults by the age of 5 years (Kearns and Winter 2003). Similar exposure levels observed in the adolescent study (CAFQ056B2131) compared to the adults also imply that the CYP450 enzyme activity were similar in adolescents (12 to 18 years) compared to the adults. Little or no effect on the exposure levels due to the difference in CYP450 maturation compared to the adults is also expected in the age group (3 to 11 years) involved in this study. Additionally, in previous studies, the pharmacokinetic properties of AFQ056 were not significantly altered as a function of age or body-weight.

The extent of increase of AFQ056 exposure (represented by higher Cmax and AUCinf values) upon intake of a high fat meal before oral administration of AFQ056 appears to be formulation dependent. In studies CAFQ056A2102 (CSF), CAFQ056A2113 (MF), and CAFQ056A2115 (FMI1 and FMI2) respectively, the increase in Cmax under fed condition was 79%, 4% and 52% while the increase in AUCinf was 22%, 21% and 13%. In contrast, both oral suspension formulations suited for pediatric studies termed POS-1 and POS-2 showed a 23 – 33% lower Cmax and a delayed Tmax under fed compared to fasted conditions, while comparable AUC data was obtained.

PK of AFQ056 in Adolescents with FXS

In the adolescent PK study (CAFQ056B2131), a terminal elimination half-life (T1/2) of approximately 7 hr and slightly lower dose-normalized exposure levels were observed in male patients with FXS patients after single oral AFQ056 doses of 25 mg, 50 mg or 100 mg (MF) compared to historical adult PK data. Hence, the same absolute doses of AFQ056 were recommended in adolescent patients with FXS as in adult patients with FXS. There was no maximum tolerated dose (MTD) determined due to the excellent tolerability of AFQ056 in this study (single subject with two abnormal laboratory values). The PK profile of AFQ056 after single-dose administration was also assessed in Japanese subjects. Based on comparison with previous studies in Caucasian subjects, exposure levels of AFQ056 appear to be comparable between the Japanese and Caucasian populations in the relevant dose range (up to 100 mg) (CAFQ056A1103), indicating that there was no influence of race on the PK of AFQ056.

PK of AFQ056 in Children with FXS

The child PK study (CAFQ056B2154) was a sequential, two-period, open-label study to assess the PK and S&T of AFQ056 in children with FXS. The study population was patients with FXS

aged 3-11 yrs (Cohort 1: 5-11 yrs (n=12) was enrolled first and then Cohort 2**:** 3-4 yrs (n=9). Dosing was individualized for the multiple dosing during Period 2 based on pre-defined PK criteria from Period 1 during which sunjects recieved a single dose of 15mg. Based on PK measurements following the single dose, and indiviualized dose was calculated for Period 2 which consisted of 1.25mg/kg, 50mg or 100mg bid for 1 week.

In cohort 1, period 2, three subjects received 50 mg b.i.d., six subjects received 100 mg b.i.d. and the other three subjects received individualized doses of 1.25 mg/kg which was equivalent to 20 mg b.i.d., 25 mg b.i.d. and 60 mg b.i.d. respectively. When administered in Cohort 1 FXS subjects, AFQ056 was rapidly absorbed. Median Tmax was 0.542 h with the range of 0.467-2.15 h for the dosing group 100 mg b.i.d. on Day 7. Geometric mean of Cmax,ss of AFQ056 was 196, 113, 229, 234 and 286 ng/mL for the patients dosed at 20, 25, 50, 60 and 100 mg of AFQ056 (b.i.d. regimen), respectively on Day 7. Geometric mean exposure of AFQ056 was 510, 419, 843, 1210, and 1050 ng.h/mL for AUClast in the patients dosed at 20, 25, 50, 60 and 100 mg of AFQ056 (b.i.d. regimen), respectively on Day 7, The summary statistics of PK parameters such as Cmax, AUC0-12h, AUClast and Tmax of AFQ056 are shown in Figure 1 and Table 2 (source: Novartis CSR for study CAFQ056B2154).

In cohort 2, period 2, four subjects received 50 mg b.i.d. and one subject received 100 mg b.i.d. and other four subjects received individualized doses of 1.25 mg/kg which was equivalent to 15 (n=1), 20 (n=1) and 25 mg (n=2) b.i.d. respectively. When administered in FXS patients aged between 3-4 years, AFQ056 was rapidly absorbed. Median Tmax was 1.310 h for the dosing group 50 mg b.i.d. on Day 8. Geometric mean of Cmax,ss of AFQ056 was 177, 75.4, 144, 161 and 96 ng/mL for the patients dosed at 15, 20, 25, 50 and 100 mg of AFQ056 (b.i.d. regimen), respectively on Day 8. Geometric mean exposure of AFQ056 (AUClast) was 457, 293, 493, 711 and 267 ng.h/mL for AUClast in the patients dosed at 15, 20, 25, 50 and 100 mg of AFQ056 (b.i.d. regimen), respectively on Day 8, The summary statistics of PK parameters such as Cmax, AUC0-12h, AUClast and Tmax of AFQ056 are shown in Figure 1 and Table 3 (source: Novartis CSR for study CAFQ056B2154).

Figure 1. Cmax and AUC Plots from Pediatric PK Study with AFQ056 (Source: Novartis CSR for study CAFQ056B2154)

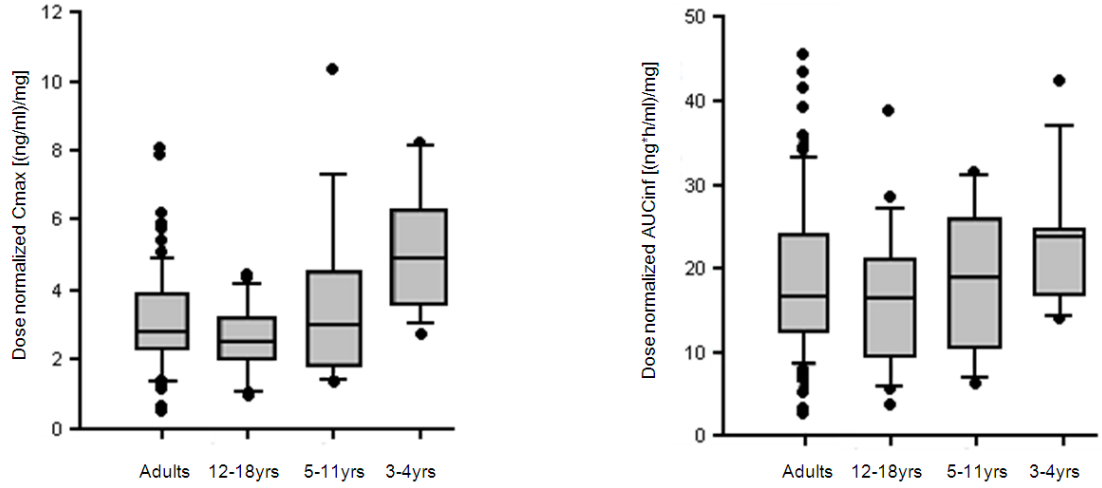


Table 2. Summary statistics for PK parameters after multiple oral doses of AFQ056 on Day 7 in patients with FXS aged 5-11 years

Treatment	Statistic	Cmax (ng/mL)	AUC0-12h (hr*ng/mL)	AUClast (hr*ng/mL)	Tmax (hr)
AFQ056 20mg b.i.d.	N	1	-	1	1
	Mean (SD)	196 (-)	-	510 (-)	2.00 (-)
	CV% mean	-	-	-	-
	Geo-mean	196	-	510	2.00
	CV% geo-mean	-	-	-	-
	Median	196	-	510	2.00
	[Min; Max]	[196;196]	-	[510;510]	[2.00;2.00]
AFQ056 25mg b.i.d.	N	1	1	1	1
	Mean (SD)	113 (-)	461 (-)	419 (-)	0.500 (-)
	CV% mean	-	-	-	-
	Geo-mean	113	461	419	0.500
	CV% geo-mean	-	-	-	-
	Median	113	461	419	0.500
	[Min; Max]	[113;113]	[461;461]	[419;419]	[0.500;0.500]
AFQ056 50mg b.i.d.	N	3	1	3	3
	Mean (SD)	229 (101)	585 (-)	913 (451)	1.57 (0.823)
	CV% mean	43.8	-	49.4	52.6
	Geo-mean	215	585	843	1.37
	CV% geo-mean	44.9	-	51.8	78.2
	Median	201	585	790	2.00
	[Min; Max]	[146;341]	[585;585]	[536;1410]	[0.617;2.08]
AFQO56 60mg b.i.d.	N	1	-	1	1
	Mean (SD)	234 (-)	-	1210 (-)	2.00 (-)
	CV% mean	-	-	-	-
	Geo-mean	234	-	1210	2.00
	CV% geo-mean	-	-	-	-
	Median	234	-	1210	2.00
	[Min; Max]	[234;234]	-	[1210;1210]	[2.00;2.00]
AFQ056 100 mg b.i.d.	N	6	3	6	6
	Mean (SD)	286 (107)	1210 (114)	1110 (375)	1.03 (0.809)
	CV% mean	37.5	9.4	33.9	78.3
	Geo-mean	272	1210	1050	0.815
	CV% geo-mean	35.0	9.4	36.7	83.5
	Median	271	1200	1060	0.542
	[Min; Max]	[189;484]	[1100;1330]	[608;1660]	[0.467;2.15]

Source: Table 14.2-2.2, Novartis CSR for study CAFQ056B2154

Pharmacodynamics of AFQ056 in Human Studies

The degree of mGlu5 receptor occupancy has been investigated in six healthy volunteers following oral administration of radio-labeled AFQ056 using [11C]-ABP688 radioligand – positron emission tomography (PET) (CAFQ056A2104). After single, oral doses of the CSF

ranging from 25 mg to 400 mg the degree of mGlu5 receptor occupancy dose-dependently increased. The degree of receptor occupancy amounted to approximately 55% at the therapeutic dose of 100 mg and 85% at the maximum tolerated single dose of 400 mg. PET data is shown in Appendix A.

Table 3. Summary statistics for PK parameters after multiple oral doses administration of AFQ056 on Day 8 in patients with FXS aged between 3-4 years

Treatment	Statistic	Cmax (ng/mL)	AUC0-12h (hr*ng/mL)	AUClast (hr*ng/mL)	Tmax (hr)
AFQ056 15mg b.i.d.	N	1	-	1	1
	Mean (SD)	177	-	457	0.500
	CV% mean	-	-	-	-
	Geo-mean	177	-	457	0.500
	CV% geo-mean	-	-	-	-
	Median	177	-	457	0.500
	[Min; Max]	[177;177]	-	[457;457]	[0.500;0.500]
AFQ056 20mg b.i.d.	N	1	-	1	1
	Mean (SD)	75.4	-	293	2.15
	CV% mean	-	-	-	-
	Geo-mean	75.4	-	293	2.15
	CV% geo-mean	-	-	-	-
	Median	75.4	-	293	2.15
	[Min; Max]	[75.4;75.4]	-	[293;293]	[2.15;2.15]
AFQ056 25mg b.i.d.	N	2	2	2	2
	Mean (SD)	149 (50.2)	689 (435)	541 (315)	0.684 (0.260)
	CV% mean	33.8	63.1	58.2	38.0
	Geo-mean	144	617	493	0.658
	CV% geo-mean	35.5	76.5	68.2	40.4
	Median	149	689	541	0.684
	[Min; Max]	[113;184]	[381;997]	[318;763]	[0.500;0.867]
AFQO56 50mg b.i.d.	N	4	2	4	4
	Mean (SD)	179 (102)	768 (14.2)	726 (176)	1.32 (0.879)
	CV% mean	57.0	1.9	24.3	66.8
	Geo-mean	161	768	711	1.07
	CV% geo-mean	54.4	1.9	23.4	89.3
	Median	144	768	685	1.31
	[Min; Max]	[99.4;328]	[758;778]	[559;975]	[0.500;2.15]
AFQ056 100 mg b.i.d.	N	1	-	1	1
	Mean (SD)	96.0	-	267	2.00

	CV% mean	-	-	-	-
	Geo-mean	96.0	-	267	2.00
	CV% geo-mean	-	-	-	-
	Median	96.0	-	267	2.00
	[Min; Max]	[96.0;96.0]	-	[267;267]	[2.00;2.00]

Source: Table 14.2-2.2, Novartis CSR for study CAFQ056B2154

Human Safety and Tolerability Data

Over 1100 study subjects have been enrolled in a total of 25 completed human studies, of which 17 were phase I studies (single-dose: n=11; multiple-dose: n=6), six were phase II studies and two were open-label extensions. In these studies, a total of approximately 800 study subjects were exposed to AFQ056 while the remaining subjects received either placebo or an active comparator [AFQ056 Investigator Brochure]. Seven of these studies were in patients with FXS, resulting in exposure to AFQ056 in approximately 300 patients with FXS.

Overall, AFQ056 was safe and well tolerated and the AEs observed throughout the clinical development program of AFQ056 were mostly of mild or moderate intensity and resolved spontaneously. There was only a single serious adverse event (SAE) of psychosis reported in healthy volunteer studies that was suspected to be study drug-related. An additional 13 patients with PD-LID were reported with a drug-related SAE, while none of the patients with FXS developed any SAE (for details see below under SAEs). In two single-ascending CAFQ056A1103, CAFQ056A2101) and two multiple ascending dose studies (CAFQ056A2102, CAFQ056A2120) in healthy volunteers, AFQ056 has been administered up to a maximum single dose of 800 mg (clinical service formulation (CSF)) and multiple dose of 450 mg b.i.d. (CSF) for 10 days preceded by 4 days of up-titration. Based on these four safety and tolerability studies a single maximum tolerated dose (MTD) of 400 mg and multiple MTD of 150 mg b.i.d. has been determined. Single doses of >400 mg or multiple doses of >150 mg b.i.d. resulted in drug-related adverse events (AEs) that were considered to be dose-limiting (e.g. hallucinations, psychosis-like symptoms, dyskinesia). Visual hallucinations or illusions were also reported at the multiple MTD of 150 mg b.i.d. (CSF) in chronic smokers (CAFQ056A2109), but not in non-smoking subjects at this dose level (CAFQ056A2102) suggesting that smokers exhibit reduced tolerability to AFQ056 compared to non-smokers. In patients with FXS, AFQ056 has been administered up to 100 mg b.i.d. over duration of over 2 years.

In the 6 studies conducted in adolescents and adults with FXS, CAFQ056A2204 (phase 2 POC), CAFQ056A2131 (adolescent PK), CAFQ056A2212 (phase 2b placebo-controlled adults), CAFQ056B2214 (phase 2b placebo-controlled adolescents), CAFQ056B2278 (open label extension adolescents), CAFQ056B2279 (open label extension adults) safety and tolerability profiles were in general benign. A summary of adverse events seen in the treated and placebo groups from the combined placebo-controlled studies in adults and adolescents is given in Table 4. There was an increased rate of dizziness, insomnia, headache, decreased appetite and vomiting, although all side effects were mild-moderate in degree. There were no clinically significant changes in laboratory parameters, EKGs, or vital signs during these studies. Side effects led to discontinuation in 5 subjects in the adult study (all in the 100 mg BID group and all due to insomnia) and in 2 subjects in the adolescent study (also at 100 mg BID and due to psychomotor agitation). In the open label extension studies 2278 and 2279, side effect patterns were similar and over the course of 2 or more years of treatment with AFQ056, only 9 of 103 and 12 of 148 subjects discontinued from the extension studies, largely due to behavioral side effects consistent with activation (irritability, aggression, hyperactivity, anxiety, and impulsive behavior).

Table 4. Adverse events from baseline to Week 12, by treatment (safety population)

Event	Placebo N=86 n (%)	Mavoglurant 25 mg bid N=75 n (%)	Mavoglurant 50 mg bid N=69 n (%)	Mavoglurant 100 mg bid N=84 n (%)
Patients with any adverse event	53 (61.6)	50 (66.7)	43 (62.3)	71 (84.5)
Dizziness	1 (1.1)	1 (1.3)	0	10 (11.9)
Insomnia	0	1 (1.3)	4 (5.8)	14 (16.7)
Nasopharyngitis	8 (9.3)	11 (14.7)	9 (13.4)	11 (13.1)
Decreased appetite	1 (1.1)	0	2 (2.9)	8 (9.5)
Headache	6 (7.0)	5 (6.7)	4 (5.8)	14 (16.7)
Nausea	0	0	1 (1.4)	4 (4.8)
Upper respiratory tract infection	6 (7.0)	5 (6.7)	3 (4.3)	9 (10.7)
Vomiting	2 (2.3)	5 (6.7)	6 (8.7)	10 (11.9)
Agitation	0	1 (1.3)	2 (2.9)	3 (3.6)
Diarrhea	3 (3.5)	5 (6.7)	1 (1.4)	5 (6.0)
Irritability	0	2 (2.7)	2 (2.9)	3 (3.6)
Aggression	1 (1.1)	1 (1.3)	3 (4.3)	2 (2.4)
Pyrexia	1 (1.1)	2 (2.7)	1 (1.4)	3 (3.6)
Abdominal pain upper	0	2 (2.7)	0	2 (2.4)
Anxiety	1(1.1)	0	0	2 (2.4)
Oral herpes	0	2 (2.7)	0	2 (2.4)
Self-injurious behavior	0	0	0	2 (2.4)
Cough	1 (1.1)	3 (4.0)	1 (1.4)	1 (1.1)
Nasal congestion	0	3 (4.0)	0	1 (1.1)
Oropharyngeal pain	1 (1.1)	2 (2.7)	2 (2.9)	1 (1.1)
Rhinitis	0	2 (2.7)	1 (1.4)	1 (1.1)
Rash	0	2 (2.7)	0	0
Influenza	2 (2.3)	2 (2.7)	0	2 (2.4)
Fatigue	0	0	1 (1.4)	2 (2.4)

Source: Compiled AE data from papers in press for CAFQ056A2212 and CAFQ056B2214

In the Pediatric PK study (CAFQ056B2154) in cohort 1 (age 5-11), nine (75%) patients reported with at least a single AE. The most commonly affected system organ classes (SOC) were gastrointestinal disorders (n=8; 66.7%), followed by nervous system (n=5; 41.7%) and psychiatric disorders (n=4; 33.3%). AEs belonging to other SOCs occurred in 2 subjects only. AEs observed in cohort 1 (age 5-11) are provided in Table 5 (source: Novartis CSR for study CAFQ056B2154). The most commonly reported individual AE was vomiting (mild, without stomach contents, brief duration) with an overall incidence of 50.0 % (n=6) followed by diarrhea, dizziness, initial insomnia and psychomotor activity with an overall incidence of 16.7% (n=2), while all other AEs had an incidence of <10%. All AEs were of mild intensity. Most of these had an early onset and spontaneously resolved within <2 hours after last dosing. The AEs typically occurred on the first day of the multiple dosing period 2 and resolved despite continuous dosing at the target dose.

In Cohort 2 (age 3-4 years), six (66.7%) patients reported at least a single AE. The most commonly affected system organ classes (SOC) were psychiatric disorders (n=3; 33.3%),

followed by infections and infestations (n=2; 22.2%). AEs belonging to other SOCs occurred in single subjects only. AEs observed in cohort 2 (age 3-4) are provided in Table 6 (source: Novartis CSR for study CAFQ056B2154). The most commonly reported individual AE was insomnia (n=2) or initial insomnia (n=1) reported in three of nine subjects. All other AEs occurred in single subjects only. All AEs were of mild intensity. These were not suspected to study-drug related except for four AEs of insomnia (n=2), initial insomnia (n=1) and somnolence (n=1). These AEs occurred on treatment days 2, 3 or 4 and spontaneously resolved despite continuous dosing at the target dose except for a single subject for which sleep disturbances were still present at the EOS visit.

There were no SAEs or any AE-related study discontinuations in CAFQ056B2154. All AEs were of mild intensity. Moreover, there were no clinically relevant alterations of laboratory, EKG or vital sign data. Hence, each of the 21 children with FXS exposed to AFQ056 completed the study per protocol at the assigned target dose.

Based on the PK and safety data presented above, the dose range of 15-100 mg has been chosen for this study as a dose range likely to give blood levels consistent with >50% receptor occupancy and which have generally shown a favorable safety profile in prior studies. Some behavioral activation and insomnia may be expected as potential relatively common side effects, but these are actually common side effects of standard psychopharmacology with stimulants and SSRIs often used in FXS, and these side effects may be managed by dose reduction in the 2 months of flexible dose titration and the beginning of dosing, The flexible dose design of this will allow for this kind of side effect to be minimized by allowing dose adjustment up or down to find the maximum tolerated dose before implementing the language intervention so that stable dosing can be maintained during the language intervention. Based on the allowance for flexible dose titration to manage the common side effects seen with AFQ056 and the discontinuation rates of less than 10% over up to 2 years of participation in the adult and adolescent open label extension studies CAFQ056B2278 and B2279, it is expected that there will be less than 10% attrition from this study over the course of the 14 months of participation.

Table 5. Incidence of AEs by preferred term - n(percent) of patients (Safety analysis set) – Cohort 1 (age 5-11 years)

Preferred term	-Period1- 15mg AFQ056 N=12 n (%)	-Period 2- 20mg AFQ056 N=1 n (%)	25mg AFQ056 N=1 n (%)	50mg AFQ056 N=3 n (%)	60mg AFQ056 N=1 n (%)	100mg AFQ056 N=6 n (%)	Total N=12 n (%)
Patients with AE(s)	4(33.3)	1(100)		3(100)	1(100)	4(66.7)	9(75.0)
Preferred term							
Vomiting		1(100)		2(66.7)		3(50.0)	6(50.0)
Diarrhea	1(8.3)			1(33.3)			2(16.7)
Dizziness						2(33.3)	2(16.7)
Initial insomnia		1(100)				1(16.7)	2(16.7)
Psychomotor hyperactivity		1(100)		1(33.3)			2(16.7)
Anticipatory anxiety	1(8.3)					1(16.7)	1(8.3)
Anxiety					1(100)		1(8.3)
Coordination abnormal						1(16.7)	1(8.3)

Dyskinesia			1(33.3)			1(8.3)
Eye pain					1(16.7)	1(8.3)
Feeling hot					1(16.7)	1(8.3)
Food poisoning	1(8.3)					1(8.3)
Headache					1(16.7)	1(8.3)
Hordeolum	1(8.3)					1(8.3)
Hot flush					1(16.7)	1(8.3)
Hyperhidrosis					1(16.7)	1(8.3)
Hypersomnia					1(16.7)	1(8.3)
Mydriasis					1(16.7)	1(8.3)
Nasopharyngitis			1(33.3)			1(8.3)
Non-cardiac chest pain					1(16.7)	1(8.3)
Oropharyngeal pain					1(16.7)	1(8.3)
Pollakiuria			1(33.3)			1(8.3)
Swelling face					1(16.7)	1(8.3)

Arranged in descending order of frequency by total column.
Source: Table 14.3.1-1.1, Novartis CSR for study CAFQ056B2154

Table 6. Incidence of AEs by preferred term - n(percent) of patients (Safety analysis set) – Cohort 2 (age 3-4 years)

Preferred term	-Period1- 15mg AFQ056 N=9 n (%)	-Period 2- 15mg AFQ056 N=1 n (%)	20mg AFQ056 N=1 n (%)	25mg AFQ056 N=2 n (%)	50mg AFQ056 N=4 n (%)	100mg AFQ056 N=1 n (%)	Total N=9 n (%)
Patients with AE(s)	2(22.2)	1(100)	1(100)		3(75.0)	1(100)	6(66.7)
Preferred term	-	-	-				
Insomnia	-	-	-		2(50.0)		2(22.2)
Gastrointestinal viral infection	-	1(100)	-				1(11.1)
Infected bites	1(11.1)	-	-				1(11.1)
Initial insomnia	-	-	1(100)				1(11.1)
Nasal congestion	-	-	-			1(100)	1(11.1)
Pharyngitis	1(11.1)	-	-				1(11.1)
Rash	-	-	1(100)				1(11.1)
Somnolence	-	-	-		1(25.0)		1(11.1)
Urticaria	-	-	1(100)				1(11.1)

Arranged in descending order of frequency by total column.
Source: Table 14.3.1-1.1, Novartis CSR for study CAFQ056B2154

3 STUDY DESIGN

The trial will use a double-blind placebo-controlled parallel-group flexible-dose forced titration design in which 100 subjects with FXS, age 32 months to 6 years of age will enter a single-blind 4-month placebo lead-in, followed by an 8-month placebo-controlled phase in which they are randomized 1:1 to AFQ056 or placebo followed by an 8 month open label extension phase in which all participants enrolled by June 15, 2019 will be treated with active drug. For participants enrolled after June 15, 2019, the duration of the open label extension will be shorter and will depend upon when the subject is enrolled and what dose of AFQ056 the participant is taking for stable treatment in the open label extension. The total duration of the stable treatment for those

enrolled after June 15, 2019 will range from 6 months to no open label extension, depending on the number of months after June 15, 2019 the subject is enrolled into the study. The flexible dose design will mimic practice, take into account differential responsiveness and the known inter-child variability in drug levels with AFQ056, and allow use of maximum tolerated dose (MTD) which is likely to be most effective.

Participants will be seen for a screening visit during which they will have assessments (to include language, communication, cognitive, adaptive, behavioral measures, CGI, blood for safety labs and biomarkers, U/A, EKG, ERP and eye tracking) and if they meet entry criteria will start a single-blind 4-month placebo lead-in to provide a control group with treatment as usual for the language intervention placebo group. Subjects will be seen for a study visit 2 months into the placebo lead in (-2 months). It should be noted that although 32 month old children can enter the placebo lead-in, these children will be age 3 years before they are randomized and are potentially exposed to drug, thus the age of treatment will remain within the age range that has been studied in the prior pharmacokinetic study with AFQ056.

At the end of 4 months in the placebo lead-in all subjects will have a repeat battery of assessments and be randomized to drug or placebo at an AFQ baseline visit. The initial dose will be 25 mg BID and dose titration will be done every week based on safety report and side effects alone (efficacy will not be a factor in dose titration due to known strong early placebo effects in prior FXS studies). If the subject has no side effects at each call, the dose will be force titrated (mandatory titration if no side effects) to the next level, 50 mg BID, 75 mg BID and 100 mg BID in order. If there is concern for side effects the dose may be left the same for an additional week or reduced. If there are side effects after the first week of treatment, the 25 mg dose will be allowed to be reduced to 12.5 mg BID. Patients who cannot tolerate 12.5 mg BID will be withdrawn from the study. Subjects will be seen for a study visit for a safety check at 1 month from baseline, during the titration period. The dose can be adjusted weekly through week 7 after baseline. This will give extra weeks after the full titration schedule to ensure the final dose chosen is the optimal one for the given patient. After 7 weeks, the dose will be fixed and at the 2 month visit (language intervention baseline visit) all subjects will have the battery of assessments again and will initiate the language intervention, remaining on a stable AFQ056/placebo dose for the next 6 months.

Families will have training for the language intervention at the 2 month visit, and subsequent instruction and monitoring of delivery of the intervention will occur by weekly video conferencing (per institutional policy) with the language therapist for the first 4 months of the intervention (6 months from randomization), and then monthly for the duration of the study.

Study visits will occur at the 3, 4, 6 and 8 month visits (v6 – 9) after implementation of the language intervention (occurs at 2 months).

- At 3 months: only VAS and CGI assessments will be done
- All baseline assessments <u>except</u> the Mullen, PLS-5, and Children's Sleep Habits Questionnaire will be repeated at 4 and 6 months (visits 7 and 8). Auditory ERP and Eye Tracking will only be repeated at 4 months (visit 7) and not at 6 months (visit 8).
- All baseline assessments will be repeated at the 8 month visit (visit 9), in addition to the EKG and blood/urine tests.

At all visits, the following will be done: an interim history, vital signs, weight, height, a physical and neurological exam, adverse event review, suicidality review, dosing diary/compliance review, concomitant medication/therapy review, language intervention monitoring/review, and inclusion/exclusion criteria review will be done.

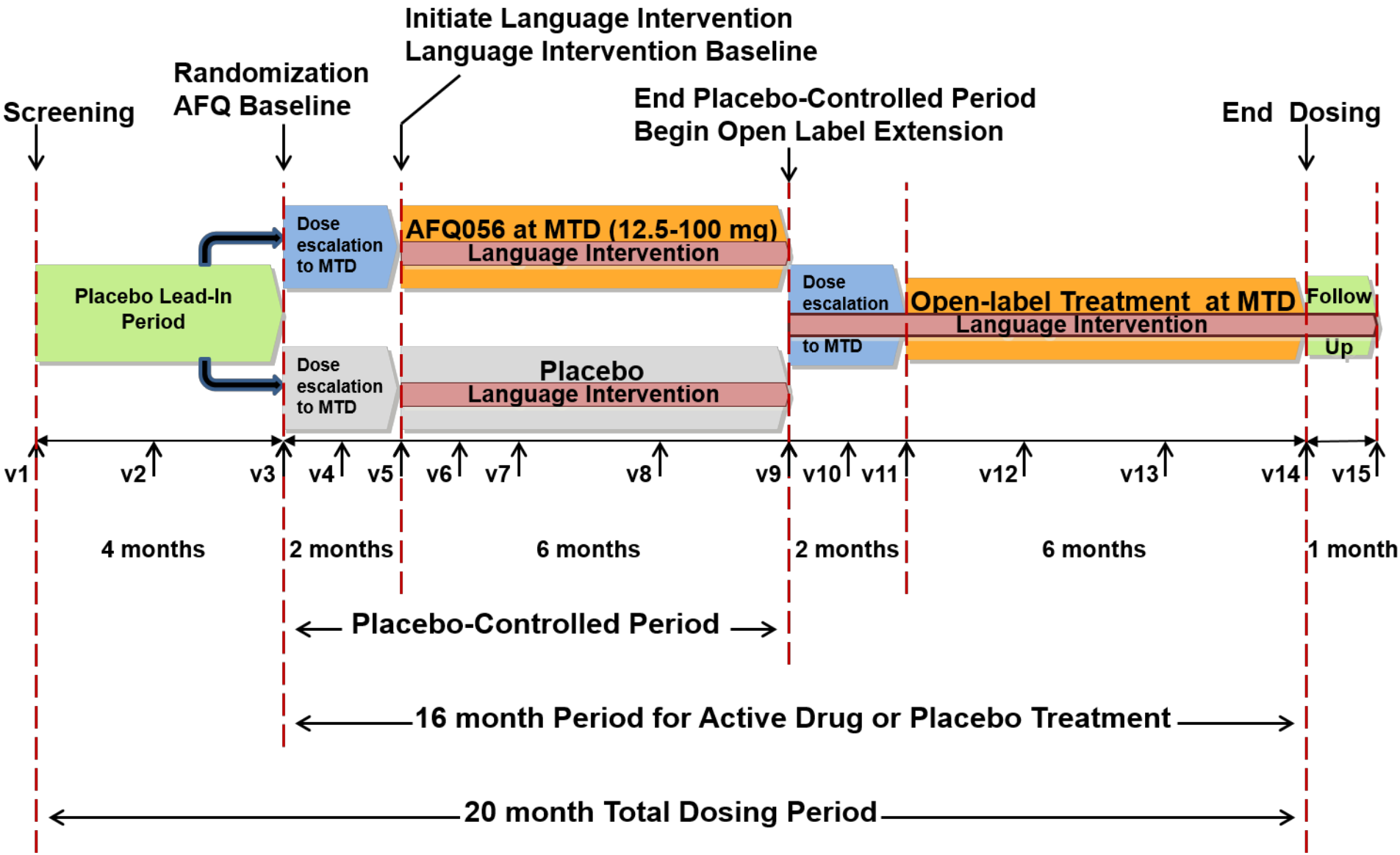
- The 8 month (visit 9) assessments will serve to evaluate whether AFQ056 was able to amplify language and communication progress over 6 months in a language intervention.
- The 4 and 6 month (visits 7 and 8) assessments will allow an evaluation of the trajectory over time of effects of the language intervention and any potential additional drug effect and whether it occurs early or there is a progressive building of the effect(s) over time.
- The 4 month assessment will also allow some data to be collected in the case of families who are not able to sustain the language intervention with the same intensity in the later part of the 6 months as opposed to the early months. It is not expected this will be a frequent problem, however.

The 4 month placebo lead-in will allow for placebo effects to stabilize, and for the group ultimately randomized to placebo, there will be 6 months (4 month placebo lead-in + 2 months of placebo dose titration after randomization) of placebo treatment without the language intervention, which will allow this group to serve as a treatment-as usual/medication placebo control group for the 6 month language intervention placebo group, thus allowing the effects of the language intervention alone in FXS to be quantified.

After 8 month assessments in the placebo-controlled phase (including 6 months of language intervention), all subjects will enter the extension and be treated with active drug according to the same schedule as in the placebo-controlled phase. All subjects will be dosed with 25 mg BID of unblinded AFQ056 upon completion of the 8 month visit, followed by 2 months of flexible dose forced-titration to the MTD of the dose levels available in the protocol (dose adjustments to be done as described above for the placebo-controlled period for the flexible dose forced-titration). This will be followed by a period of stable treatment. The length of stable treatment will be dependent upon how long after June 15, 2019 the participant enrolls and what dose of drug they are receiving in the open label treatment period. The total duration of the stable treatment for those enrolled after June 15, 2019 will range from 6 months to no open label extension, depending on the number of months after June 15, 2019 the subject is enrolled into the study.

Some subjects will have an acute dose decrease if they were on 50-100 mg BID of active drug in the initial phase but experience from the PK study suggests that children can come off AFQ without withdrawal symptoms and the dose decrease and titration in the open label extension are unavoidable to maintain the blind of the study through the completion of the placebo-controlled period by the final participant. Subjects will continue the language intervention through the extension phase. The open-label extension will allow all children a period of active drug exposure to make recruitment feasible, despite the long placebo period. It will also allow an assessment of more long term-effects of the language intervention and its impact on the ERP and eye tracking biomarker outcomes and other developmental and behavioral outcomes, will allow examination of the trajectory of the language intervention going from placebo to drug treatment in a blinded setting, and will furthermore allow additional long-term safety data to be collected on a larger cohort of children exposed to AFQ056. A diagram of the study design is provided below in Figure 2.

Figure 2. Effects of AFQ056 on Language Learning in Young Children with Fragile X Syndrome (FXS) – Protocol Design



4 SELECTION AND ENROLLMENT OF SUBJECTS

4.1 Inclusion Criteria

1. Age 32 months to 6 years inclusive at Screening (visit 1).
2. Has a documented *FMR1* full mutation.
   Note Presence of mosaicism is allowed.
3. DQ<75 calculated from the Mullen Scales of Early Learning at time of screening.
4. Parent or legal guardian is available and able to communicate well with the investigator, comply with study requirements and provide written informed consent.
   NoteThe Parent or legal guardian who will be signing consent form, should be the individual administering the language intervention
5. English is the primary language spoken in the home and the subject's first language is English.
6. Meet criteria indicating evidence of intentional communication based on parent interview via a communication eligibility screening tool at time of screening.
   Note On the Eligibility Screening Tool – Communication, the child must have:
   a. Section 1: Answer of YES; the child uses at least 5 spoken words to label items on a daily basis.
      OR
   b. Section 2: At least 3 YES answers to items 1-10 if child does not have at least 5 spoken words.
7. Produces 3 or more intentional acts of communication on the structured portion of the Weighted Communication play sample at time of screening.
   Note Subjects are permitted to use augmentative communication devices throughout the duration of the study if the device is the subject's primary form of communication and the device has been prescribed for the subject by an SLP.
8. Stable behavioral and other therapy regimen for 30 days prior to screening.
   Note Patients will be allowed to continue their standard-of–care therapies throughout the trial but these will not be changed during the placebo lead-in or placebo-controlled portion of the trial, outside of standard changes occurring from school schedules.
9. Stable dosing of all concurrent psychotropic medications except stimulants for at least 60 days prior to screening. Due to the very short half-life of stimulants (specifically methylphenidate and amphetamine variants), a stable regimen of these medications is required for 2 weeks only.
   Note Medications impacting GABA, glutamate and/or mGluR5 pathway receptors are exclusionary and not permitted during study participation. Additionally, stimulant regimens may include combinations of short- and long-acting forms and may be taken with different timing or dosing on different days of the week (e.g. Doses may be skipped on weekends or days off school and extra doses may be given some days for therapy sessions later in the day). The intent is to keep the doses and regimen being used at the time of screening consistent during the trial even if there is some variation in how the medication is taken on different days. Use of CBD oil or hemp based substances legal for sale over the internet are allowed provided that the dosing regimen has been consistent for at least 60 days prior to screening and will remain the same throughout the trial.

4.2 Exclusion Criteria

1. Use of medications impacting GABA, glutamate and/or mGluR5 pathway receptors or transmission.
   Note Treatment with acamprosate, amantadine, budipine, carbetocin, cycloserine, dextromethorphan, felbamate, ketamine, lithium*, minocycline, memantine, oxytocin,

remacemide, racemic baclofen, riluzole, fycampa, investigational mGluR5 medications, and/or statins are exclusionary.
Note Lithium taken as a dietary supplement is permitted if the dose is less than 5mg/day. A 5mg/day dose is the recommended dietary intake level, and is therefore not considered to be therapeutic. Lithium dosage must remain the same throughout the duration of the trial, and documented in the concomitant medication log.

2. Unstable seizure disorder as defined by any seizure in the 6 months prior to the screening visit, and/or any change in anti-convulsant drug dosing in the 60 days prior to screening.
Note Use of levetiracetam and oxcarbazepine are among permitted anticonvulsants.
3. Use of any other investigational drug at the time of enrollment or within 30 days or 5 half-lives (whichever is longer) of the investigational drug prior to screening until end of study visits (or longer if required by local regulations).
4. History of hypersensitivity to AFQ056 or any mGluR antagonist.
5. History or presence of any clinically significant disease of any major system organ class, within the past 2 years prior to screening including but not limited to neurological, cardiovascular, endocrine, metabolic, renal, or gastrointestinal disorders. This does not include typical features of FXS such as psychological symptoms or history of epileptic seizures.
6. Significant acute illness that did not completely resolve at least four weeks prior to the Screening visit.
7. Abnormal laboratory values at screening that are in the opinion of the investigator are clinically significant and may jeopardize the safety of the study subject.
8. Use of (or use within at least 5 half-lives before dosing) concomitant medications that are strong/moderate inhibitors or inducers of CYP1A1/2, CYP2C9/19 or CYP3A4 (see Appendix B).
9. Subjects who are, in the opinion of the investigator, unable to comply with the requirements of the study.
10. Presence of immunodeficiency diseases at the time of screening, based on medical history, including a positive HIV test result.
11. History of a positive Hepatitis B surface antigen (HBsAg) or Hepatitis C result at time of screening.
12. History or presence of suicidal thoughts and/or suicide attempts.

4.3 Subject Withdrawal Criteria

Any study subject/legal guardian has the right to voluntarily withdraw informed consent and discontinue study participation at any time without providing specific reasons. Subjects may be considered withdrawn if they state an intention to withdraw, fail to return for visits, or become lost to follow-up for any other reason. If premature withdrawal occurs for any reason, the investigator must make every effort to determine the primary reason for a subject's premature withdrawal from the study and record this information on the Study Completion/Termination CRF.

If a subject is withdrawn from the study during the placebo-lead in period or prior to randomization, they will be brought back in for an abbreviated follow-up/termination visit. At this visit, the following will be done:

- Drug accountability (family will be asked to return all drug kits and supplies)
- Adverse Event Review

The above information will need to be entered into the EDC. If this takes place prior to Baseline, please contact the Study Team, and they will provide instructions on how to deem them ineligible to proceed to randomization in the system.

A physical exam, psychiatric exam, vital signs, and any other assessment per PI discretion may be conducted, but is not required to be done or entered into the EDC.

The investigator should discontinue study treatment for a given subject or withdraw the subject from study if he/she believes that continuation would be detrimental to the subject's well-being. If a subject has intolerable side effects on the lowest dose of AFQ056 allowed in the study, 12.5 mg BID, that subject will be discontinued from the study.

Study treatment *must* be discontinued [and the subject withdrawn from the trial] under the following circumstances and according to Investigator discretion:

- Legal guardian withdraws informed consent.
- Serious AE occurs that is in the opinion of the investigator suspected to be related to the study medication.
- Severe CNS-related AE occurs that is in the opinion of the investigator suspected to be related to the study medication.

The subject may be withdrawn from the study, if:

- Clinically significant changes of laboratory parameters and/or vital signs occur that are in the opinion of the investigator suspected to be related to the study medication. The clinical significance of laboratory abnormalities and other abnormal assessments will be assessed and interpreted by the investigator in the context of the overall clinical presentation.
- Subject is lost to follow-up.
- ≥ 7 consecutive days of study medication are missed or a total of ≥ 14 days of study medication are omitted once the subject has begun dosing at any time during the study
- Protocol deviation occurs that in the opinion of the Site PI or Study Team may alter the study results (e.g. administration of concomitant medication).

If a subject withdraws from the study after randomization, a Termination visit should be conducted as soon as possible. The assessments and procedures completed during the termination visit will depend upon whether the subject discontinued study drug prior to the termination visit. Assessments and procedures to be conducted as possible based on agreement of the caregiver at the termination visit(s) as follows:

- The subject is on study drug at the time of the visit
  - The termination visit will include all the assessments and procedures for the 16 month visit (visit 14).
  - If possible, the patient will return for a follow up visit after weaning drug one month after the termination visit and do all the assessments for the 17 month visit (visit 15)
- The subject discontinued study drug prior to the visit
  - The termination visit will include the following safety assessments and procedures as possible
    - AE review
    - Blood draw
    - Urine dipstick
    - EKG
    - Vitals
    - Physical, neurological and psychological exams
    - Suicidality assessment
    - Fundoscopic exam
    - Study drug accountability
- The subject discontinued study drug but had a full battery of tests within the past two months after being on study drug for at least two months

- The termination visit will include all the assessments and procedures for the month 17 visit (visit 15)

- A follow up visit via phone or email after the Termination Visit with the subject off study drug should be conducted approximately 30 days after the termination visit.

Subjects that are withdrawn after randomization will not specifically be replaced as it is expected that there will be less than a 10% withdrawal rate. If the rate exceeds 10% an amendment will be considered to replace subjects.

4.4 Study Enrollment Procedures

Potentially eligible subjects making contact with the study site or recruited from a registry by phone will be scheduled for a screening visit. The Central IRB-approved consent document will be sent to families who wish to read the consent and have a chance to ask questions ahead of time at their leisure (during time when they are not directly managing their child with FXS), either by phone or email. The consent will not be signed until the family arrives at the site and meets with the study coordinator and the site PI. At the screening visit, the family will meet with the study coordinator and site PI, who will review the consent and answer any remaining questions. Only after the consent has been signed at the site at the beginning of the screening visit by the parent or legal guardian and individuals obtaining consent, will any study-specific procedures for the screening visit as outlined in the protocol be done. The process of obtaining informed consent including those present and their relationship to the subject and persons explaining the consent will be documented in the subject source documents for the screening visit.

Each subject/patient entering the study for screening will be assigned a unique study number. The study number will be a combination of the NeuroNEXT center identifier and a four-digit number representing the subject number at the site. (e.g., If the site is 101, the first patient would be assigned 1001 and the subject study number would be 101-1001). Numbers will be used sequentially study-wide. If a subject screen fails (or withdraws during the placebo lead-in period), the study number will not be used again and that number will simply not advance into the list of randomized subjects. If a subject is deemed eligible for enrollment into the study and will commence dosing, that subject will be randomized through an interactive website, and will be stratified based on age (Ages 3-4, and 5-6). The DCC will generate a randomization table for each of the strata using a permuted block design with random block sizes. At the time of randomization, the study coordinator logs into the study website and enters and confirms the potential subject's eligibility criteria. The subject will then be randomized to one of the two treatments. The study ID must be added to all subsequent CRF's and on the dispensed study medication.

4.4.1 Subject Recruitment and Retention

Participants will be recruited through participating FXS Clinics which exist at NeuroNEXT Network sites. All current FXS Clinics in the USA belong to the Fragile X Clinical and Research Consortium (FXCRC). These Clinics also participate in FORWARD which is a registry and database that captures the natural history of FXS through longitudinal follow up of patients at all the FXCRC FXS Clinics. All FXCRC Clinics can recruit patients through the FORWARD registry, both from their own clinic and other FXCRC Clinics not participating in the trial. The study will also be announced through information disseminated at FXCRC meetings, through the National Fragile X Foundation (NFXF) and through the FRAXA Research Foundation. Individual FXS Clinics will do mailings to their clinic population, and email blasts to families about the study will be sent out through local parent support groups in the NFXF Community Support Network (CSN).

Postings will be placed on www.fragileX.org, https://fragilex.org/our-research/fragile-x-clinics/and www.fraxa.org websites. Flyers about the study will be sent to community neurologists at NeuroNEXT clinical sites. Webinars will be conducted for participant recruitment as needed. Interested participants will be contacted by the investigators or their staff and invited to participate. These recruitment strategies will include a mechanism by which the patients can provide their contact information. We will use the NeuroNEXT Recruitment and Retention Committee to identify recruitment strategies. Flyers can also be sent to community Geneticists, Genetic Counselors and Developmental Pediatricians if needed.

Given the extensive information network in the FXS field, the pre-existing presence of a network of clinics in the FXCRC with opportunities to enlist up to 14 NeuroNEXT sites with FXCRC clinics, the fact that no other clinical trials of targeted treatments will be available to this age group, and the high level of interest in new treatments and research participation in newly diagnosed families with young children with FXS, it is not expected the recruitment will be a problem. Retention can be problematic in a 20-month trial such as this, however high retention rates were seen in the prior extension studies of AFQ056 for up to 36 months. Also, the close relationships between families with FXS and FXCRC clinics and staff, the frequent video-conferencing interactions with staff to monitor drug reconstitution and to monitor the language intervention, and the opportunity for a free ongoing intensive language intervention will help with retention, as will the promise of the open label extension at the end of the study when all participants will know they are going to receive the active drug AFQ056. Based on the above considerations it is estimated that retention will be 80-90% through the full 20 months of the study. Subjects withdrawing during the placebo lead-in before randomization will be replaced such that the full 100 subjects will be randomized.

4.4.2 Screening Logs

All clinical sites will keep these screening logs documenting demographic information including age, gender, race and ethnicity, how all subjects scheduled for screening learned of the trial (e.g., national foundation website or information blast, information from CSN groups, mailing from participating FXCRC/NeuroNEXT Clinic site, recruitment through FORWARD registry, study described at clinic visit, found trial at clinicaltrials.gov, heard about it from someone else, etc.), how subjects were referred to contact site about the trial, reasons for ineligibility at the screening visit, and reasons for non-participation if eligible.

Screening logs to document demographic information and reasons for ineligibility and non-participation will be stored centrally at the NeuroNEXT Clinical Coordination Center. Information about screening failures and successes will be compiled and reviewed on a periodic basis so that feedback can be provided to sites.

Screening log data will be provided to the study team, de-identified and analyzed to determine the most successful routes for information dissemination and recruitment strategies for ongoing recruitment in this study and for future studies. The study team will work to identify any common reasons for screen failure that might be identified before a patient comes in for screening in order to prevent unnecessary burden for FXS families, and to consider amendments to the study to adjust inclusion/exclusion criteria if a large number of patients are screen failing for specific reasons that would not ultimately affect the study results if allowed. Likewise, reasons for non-enrollment will be tracked centrally. If there are recurring themes adjustments will be attempted to target this problem, as long as the adjustments do not affect the study conduct or data quality.

4.4.3 Informed Consent

Written informed consent will be obtained from the parent(s) or legal guardian of each study participant before any study-specific procedures or assessments are done and after the aims, methods, anticipated benefits, and potential hazards are explained. Both parents should be consented, if they are present at the Screening visit. The willingness of the parent(s) or legal guardian of the participant to participate in the study will be documented in writing in a consent form approved by the NeuroNEXT Central Institutional Review Board (CIRB), which will be signed by the parent(s) or legal guardian of the participant with the date of that signature indicated. The investigator will keep the original consent forms and a copy will be given to the parent or legal guardian of the participant. It will also be explained to the parent(s) or legal guardian of the participant that they are free to refuse entry into the study and free to withdraw from the study at any time without prejudice to future treatment. Written and/or oral information about the study in a language understandable by the parent(s) or legal guardian of the participant will be given to all participant families.

Informed consent will be obtained by licensed physician investigators only, who are trained on the study and listed on delegation logs as being authorized to obtain consent. The site PI will typically be involved in explaining the study and will play a role during the consent process and answering any questions in conjunction with the coordinator, as well as overseeing that the process is carried out correctly. The consent in this study will always be signed by the parent or legal guardian due to the age and cognitive ability of the participants. Basic parts of the study the child is capable of understanding will be explained to the child as possible in very simple language. The consenting process will be documented on a screening visit source document that will list the steps of the consenting process and document those present at the consenting process and their relationship to the study participant or role as study staff. The signed consent form will be kept in the study subject files with the other source documents from the screening visit.

Note: The parent or legal guardian signing the informed consent form must be the same individual who will administer the language intervention throughout the trial. If both parents sign the informed consent, the parent who is the primary care provider for the child must be the one who administers the language intervention throughout the trial.

4.4.4 Assent

Assent will not be done as subject with FXS age 32 months to 6 years of age with a DQ less than 75 (study entry criteria) are by definition functioning at less than the 5 year old level and are not capable of meaningful assent.

4.4.5 Global Unique Identifier (GUID) and Data Sharing

Data from this study may be submitted to the National Database for Autism Research (NDAR). NDAR is a computer system run by the National Institutes of Health that allows researchers studying autism to collect and share information with each other. With an easier way to share, researchers hope to learn new and important things about autism more quickly than before. During and after the study, the researchers will send health and behavioral information about the subject, in some cases, the subject's genetic information, to NDAR. However, before they send it to NDAR, names, addresses, phone numbers, and other identifiable information will be removed, and replaced that information with a code number called a Global Unique Identifier (GUID). Other researchers nationwide can then file an application with the National Institutes of Health to obtain access to this study data for research purposes.

To obtain this number, information will need to be obtained from the subject's parent/caregiver regarding the subject. Site staff must collect the child's last name at birth, first name at birth, gender at birth, day of birth, month of birth, year of birth, city of birth, country of birth, and mother's maiden name. The information will be entered into a secure website to create a 9-digit unique identification number called the Global Unique Identifier (GUID) that will be used for data sharing purposes. Some subjects may already have a GUID assigned to them. Details regarding this process can be found in the Manual of Operations. Information used to make the GUID will not be stored. More details regarding generating the GUID can be found in the Manual of Operations.

4.4.6 Randomization/Treatment Assignment

If a subject is deemed eligible for enrollment into the study at the screening visit, that subject will be entered into the placebo lead-in. When he/she returns for the baseline visit 4 months later, the subject will complete the baseline visit assessments, will be randomized if eligible and will commence dosing, with the first dose to be taken in the morning the day after all the Baseline visit (visit 3) procedures are completed. The subject must be at least 3 years old at the time of randomization.

Randomization will be performed through an interactive website. In order to prevent age imbalance, randomization will be stratified by age with two strata, 3-4 years and 5-6 years. Block randomization will be used with random block sizes. Subjects will be assigned a study ID at the time of enrollment. The study ID includes the identification of the center and a unique subject ID. The DCC will generate a randomization table for each of the strata using a permuted block design with random block sizes. Kit numbers assigned at each local site pharmacy will be tracked at the NeuroNEXT Central Pharmacy through the interactive IWRS system. For sites using a research pharmacy, a prescription for the study drug (AFQ056 or placebo) with subject number on the prescription may be written by a licensed physician investigator on the study and exchanged at the site research pharmacy for the packet to which the subject was randomized.

5 STUDY INTERVENTIONS/STUDY MEDICATION/STUDY DRUG OR DEVICE

5.1 Study Medications/Interventions, Administration, and Duration

AFQ056

Novartis will provide AFQ056 and matching placebo labeled for investigational use. The NeuroNEXT Central Pharmacy, the Clinical Materials Services Unit (CMSU) at the University of Rochester Medical Center (URMC) will create active and placebo study drug kits with unique identifiers not associated with the randomization ID. These kits, which will be used after Screening (placebo bottles) and after randomization will be provided to the local pharmacies from the CCC Central Pharmacy.

An immediate-release oral suspension dosage form will be used ("powder for oral suspension" with flavoring to be re-constituted in water at 10 mg/ml and dosed in measured aliquots). The AFQ056/placebo powder in bottles, each bottle containing 500 mg of AFQ056 or matching placebo will be provided by Central Pharmacy. Central Pharmacy Kits will include 60 cc syringes for use for reconstitution of the powder as well as the study medication and all necessary items for the dosing process. During the blinded placebo–controlled period bottles will be assigned by the CCC Central Pharmacy and will be dispensed by the local pharmacy or other designated staff as applicable at study visits, with numbers of bottles dispensed based on the number of bottles needed depending on the subject's dose.

At screening, all subjects will be started on placebo suspension dosing at the same volume as the starting dose for the dose titration after randomization (2.5 cc BID) during the 4 months of placebo lead-in. The parent/caregiver will receive bottles containing placebo powder and the 60 cc syringes, for use in reconstitution of the powder with bottled water. Because the active drug powder is only stable for approximately 10 days in suspension, the placebo powder will be resuspended in a new bottle every 10 days or less during the placebo lead in, so as not to unblind the parent/caregiver as to when they may be starting active drug.

Parent/caregivers will be trained at the site in the procedure for reconstituting the drug each week, with the study coordinator demonstrating the reconstitution procedure and the families practicing the process with the coordinator at Screening. Please note that dosing will not begin until a few days after Screening, once subject eligibility is confirmed. The parent/caregiver will be given an Instruction Sheet for Preparation of the AFQ056/Placebo Study Medication to take home with them to reinforce the instructions for reconstitution. This will be made available in the Manual of Operations (Section I). The reconstitution procedure will involve the parent/caregiver pouring water from the bottle into a glass, withdrawing over 50 cc into the syringe by immersing the syringe in the water in the cup and pulling up to fill to past a 50 cc fill-line premarked on the syringe by the study coordinator at the site, inverting the syringe and pushing out air bubbles and extra water to bring the water level to the 50 cc fill line, and then adding the water in the syringe to a bottle containing the 500 mg drug or placebo powder and then mixing thoroughly by repeated inversion.

Beginning at the start of the placebo run-in and occurring the first two weeks of dosing, video-conferencing calls will be done each week with the parent/caregiver to watch them reconstitute the bottle(s) of powder to be used for the following week with bottled water, to make sure this is being done correctly at home. All efforts will be made to schedule the conference call on the same day and time every week. If this is not possible on a given week, the recommended window for the drug dilution calls is -1/+3 days. Bottles will be counted when the subject and parent/caregiver return for study visits to ensure the correct amount of drug has been removed to give to the subject, in order to carry out drug accountability. This process will allow identification of any problems parent/caregivers may be having with the reconstitution process and/or compliance and will allow correction before randomization with the reconstitution process. If after two weeks of video-conferencing calls for drug reconstitution, the coordinator believes the family is having trouble with the reconstitution process or is still requiring a lot of coaching from the coordinator, an additional two weeks of calls for bottle reconstitution can be done. More calls can be added after that if needed during the placebo lead-in period. After the family is no longer doing conference calls they can reconstitute bottles as needed as long as no bottle is used for over 10 days.

Once a subject is randomized and a kit assigned, the parent/caregiver will continue to receive at every 2-month in-person study visit bottles containing 500 mg of active drug or matching placebo powder, as well as bottled water and premarked 60 cc syringes with 50 cc fill line to use for reconstitution of the AFQ056 powder. Subjects will start on 25 mg BID (2.5 cc BID) or matching placebo after randomization and the dose will be titrated weekly depending on tolerability. If the subject has no side effects at each call, the dose will be force titrated to the next level, 50 mg BID, 75 mg BID and 100 mg BID in order. If there is concern for side effects the dose may be left the same for an additional week or reduced. If there are side effects after the first week of treatment, the 25 mg dose will be allowed to be reduced to 12.5 mg BID. See section 9.1 also for dose adjustment considerations. Patients who cannot tolerate 12.5 mg BID will be withdrawn from the study. Subjects will be seen for a study visit for a safety check at 1 month from baseline, during the titration period. The dose can be adjusted weekly through week 7 after baseline. After randomization, monitoring procedures to ensure correct reconstitution

with parent/caregivers will be identical to those described above for the placebo lead-in. Drug accountability/compliance will be assessed at every 2-month in-person visit after Baseline (i.e. when drug is dispensed). Parents/legal guardians should be reminded to bring all study drug bottles with them to each 2-month visit when drug accountability is to be done. For more details, please refer to the Study Medications/Central Pharmacy section of the Manual of Operations.

The following table provides recommendations and guidelines regarding dose titration. Please note the Site Investigators will have the opportunity to contact the Independent Medical Monitor (IMM) should they be unclear as to how to proceed, and need additional input and guidance. Details about how to contact the IMM can be found in the Manual of Operations.

AE Grade/Severity	**Relationship**	**Most Likely Drug Action**	**Possible Other Drug Actions**
Grade 1 (Mild)			
	Unrelated	Increase	
	Unlikely	Increase	
	Possibly	Increase	
	Probably	Increase	Hold at physician discretion if AE non tolerable for patient/family and/or concern for worsening AE with increase
	Definitely	Increase	Hold at physician discretion if AE non tolerable for patient/family and/or concern for worsening AE with increase
Grade 2 (Moderate)			
	Unrelated	Increase	
	Unlikely	Increase	
	Possibly	Increase	Hold at physician discretion if AE non tolerable for patient/family and/or concern for worsening AE with increase
	Probably	Hold	Increase at physician discretion if AE considered tolerable, Decrease if AE considered intolerable by family
	Definitely	Hold	Increase at physician discretion if AE considered tolerable, Decrease if AE considered intolerable by family
Grade 3 or higher (severe)			
	Unrelated	Increase	Increase at physician discretion, if AE not overly interfering with dose finding
	Unlikely	Hold	Increase at physician discretion, if AE not overly interfering with dose finding
	Possibly	Hold	
	Probably	Decrease	
	Definitely	Decrease	

Please note, adverse events will be graded based on CTCAE version 4.0, with the exception of behavioral adverse events, including but not limited to the following behaviors commonly seen in FXS:

- Hyperactivity
- Attention
- Anxiety
- Perseverative behavior
- Irritability/tantrumming/aggressive behavior/self-injury

- Hypersensitivity

The above behaviors will be noted as absent (none), mild, moderate or severe and captured on the Psychiatric/Behavioral exam CRF. If worsening in any one of the above categories is determined to be a clinically significant change from baseline by the treating physician, it would then also be captured as an adverse event and recorded on the AE CRF accordingly. A "clinically significant" change from baseline is defined as behavior that is not just related to a single event or lack of cooperation in certain settings such as the study visit. Rather, the change should be seen consistently. Minor fluctuations in behavior typical to the individual should not be captured as clinically significant.

For the purposes of the behavioral events being followed in this study, the following definitions of mild, moderate and severe will be utilized:

- **Mild**: the behavior may be present in just one domain of functioning and may be confined to one setting, but results in a marginal worsening in quality of life.
- **Moderate**: Behavior has been noted in multiple domains of functioning and is not confined to one setting. The behavior impacts quality of life in several areas and the child may be requiring implementation of more supports in at least one setting.
- **Severe**: The behavior is seen in all settings and has a pervasive impact on quality of life and the child is requiring support across multiple settings.

After the initial dosing calls in the placebo lead-in, the parent/caregiver will dilute bottles as needed, diluting a new bottle when they can no longer get drug suspension out of the prior bottle. A partial dose may be drawn up into the dosing syringe from one bottle and then filled the rest of the way from the next bottle diluted. The parent/caregiver will be encouraged to use as much of the suspension in each bottle as possible without going beyond 10 days of use for any bottle. The parent/caregiver will write the date on the bottle when diluted to ensure they know when the bottle is no longer good. Each bottle will typically allow dosing of about 40 ml of the diluted drug as the bottle adapter does not allow the syringe to pull out the last 10 ml of suspension. The following chart shows the volume of reconstituted AFQ056 to be used for each possible dose in the study and the resulting number of doses that can be obtained from a bottle and the number of days before a new bottle will need to be reconstituted. No other doses will be allowed.

12.5 mg BID	1.25 ml/dose	32 doses/bottle	10 days/bottle (bottle will not be empty when need to reconstitute based on time)
25 mg BID	2.5 ml/dose	16 doses/bottle	8 days/bottle
50 mg BID	5 ml/dose	8 doses/bottle	4 days/bottle
75 mg BID	7.5 ml/dose	5 doses/bottle	2.5 days/bottles
100 mg BID	10 ml/dose	4 doses/bottle	2 days/bottle

The suspension will be drawn up into a syringe fitted to the cap on the bottle in which the powder has been reconstituted and administered directly into the subject's mouth at home and in the outpatient setting by the parent/caregiver. The subject should swallow after the suspension is administered, and the subject's mouth should be checked to ensure that the suspension was swallowed. Approximately 8 oz. of fluid (e.g. water, juice, milk) will be administered with the drug administration. If the child does not tolerate having the suspension administered directly with the syringe, the suspension may be mixed with fluid (e.g. water, juice, milk) and then given to the child. The parent/caregiver will fill out a medication diary to indicate every dose administered to the child. All dose changes during the study will be recorded on a Dose Titration CRF and captured in the EDC.

Doses in the range of 1.25 ml (12.5 mg) to 10 ml (100mg) twice daily will be administered orally during the study. The dose will be given in the morning and early evening. The two doses should not be taken less than 4 hours apart. If insomnia is a side effect the second daily dose may be moved to early afternoon.

Expected side effects would be those observed in the prior adult/adolescent studies in FXS and the pediatric PK study. These include dizziness, insomnia, headache, decreased appetite, vomiting, diarrhea, and behavioral “activation” with irritability, aggression, hyperactivity, anxiety, and impulsive behavior. These side effects are expected to be relatively infrequent, mild-moderate in degree, and insomnia and behavioral side effects will in most cases be manageable by dose adjustments in the flexible dosing period. Insomnia may be managed by moving the dose earlier in the day as noted above.

Medications required to treat acute intercurrent medical problems (e.g. antibiotics for ear infections) should be employed as per standard of care, as long as prohibited medications expected to interact with AFQ056 are avoided. The study site should be notified of any acute problems and medications to be used so that guidance can be given as to any drug interactions and prohibited specific medications can be avoided. Melatonin may be used to manage sleep problems. If surgery is required for a minor problem (e.g. myringotomy tube placement, tonsillectomy/adenoidectomy, dental work, strabismus surgery) the patient may continue in the study but the study site should be contacted to discuss use of anesthesia compatible with the study drug. Major surgery may require study discontinuation.

Language Intervention

The language intervention will be administered by a trained language specialist (training to be completed with videotapes and practice activities to reach fidelity at initiation of the study through at-home synchronous video conferencing sessions). Initial administration of the parent education session of the intervention will be done around the time of the 2 month visit using a didactic education session and delivery of the intervention will be started at that visit. The intervention will subsequently be delivered to the parent by a speech-language clinician through weekly clinician coaching, homework, and feedback sessions.

The entire intervention will be delivered to the parent in the home or at scheduled study visits through the use of a laptop computer equipped with distance video-teleconferencing software. Coaching, homework, and feedback sessions will occur weekly for 4 months, then monthly for the remainder of the study through the final two months of the placebo-controlled portion and the subsequent open-label extension.
The parent will be trained in stepwise fashion to administer the language intervention to the child over the entire subsequent course of participation in the study.

SLPs will set up 1-2 distance technology training sessions with the subject family between month 1 and month 2 (visits 4 and 5). During the call, SLPs will review the technology procedures, work with the parent/primary caregiver to set up the intervention environment and work on setting up the equipment as necessary for the language intervention. These practice calls will need to be completed prior to visit 5 (month 2), after which the language intervention will begin. Please refer to the SLP Training Manual for more details.

The didactic sessions will involve power point presentations with video examples of implementation. The coaching sessions will involve real-time instruction and feedback to the parent (via MacBook computers and Bluetooth “bug in the ear” technology both provided to the parent at the 1 month visit) while the parent interacts with the child in a play-based format. The

parent will independently record and submit homework practice sessions assigned by the language therapist. These will be submitted to the clinical team via a file sharing system. The language clinician will then provide a feedback and joint problem-solving session related to implementation of targeted strategies and management of child challenging behaviors to ensure optimal levels of participation in the intervention.

Parents will also report on how often and how much time they have used the language intervention between contacts with the SLP using the Language Intervention Diary, and will be graded on their ability to deliver the intervention during videotaped structured homework sessions.

The intervention is designed to maximize the extent to which parents engage in the types of verbally responsive interactions that have been well documented as facilitating language learning and use in children with typical and atypical development. These interactions are characterized by frequent parent talk about the child's focus of attention, contingent parental responses to child actions and communication, parental language to the child slightly in advance of child language levels, and affectively positive parental talk. These strategies are often difficult to arrive at naturally for parents of children with FXS because of the children's developmental delays and comorbid challenging behaviors.

The language intervention is designed to help parents learn and use verbally responsive interactional strategies more frequently and effectively throughout the course of their daily interactions with their children. Nevertheless, it is expected that there will be considerable variability in parental rate of mastery and frequency of use of the targeted strategies, which means that there will be variability in the effective dose received by the children enrolled. By examining clinician-rated parental fidelity of implementation during coaching and homework sessions and parent-reported mastery and frequency of use during feedback sessions in relation to child outcomes, we will be able to examine the effects of the language intervention separately from, and in combination with, the drug.

During months 2 through 5 there will be four parent education sessions as well as weekly coaching, homework, and feedback sessions. During months 6 through 17 there will be once per month coaching, homework, and feedback sessions.

The parent must complete:

- All four parent education sessions;
- 41 of 48 (85%) combined coaching, homework, feedback sessions during months 2 through 5
- 25 of 33 (76%) combined coaching, homework, feedback sessions during months 6 through 17

Sites must contact the Study Team if the parent is approaching the threshold for missed sessions.

It is not expected there will be any side effects of the language intervention. Supportive standard of care speech, developmental, occupational, and physical therapy will be continued throughout the study as per standard treatment. Attendance at preschool, kindergarten or first grade school programs with special education programs per standard of care management will be continued throughout the study. New intensive therapy programs in addition to the language intervention should not be started during study participation. If the participant is receiving ABA at the time of study entry this may be continued as per the baseline therapy program but should not be

intensified. Minor adjustments in the number of hours or time distribution of a therapy that a child is already receiving are allowed.

5.2 Handling of Study Medications/Interventions

Study drug consisting of AFQ056 or matching placebo with identical flavoring will be shipped as powder in bottles (500 mg AFQ056 per bottle) from Novartis to the University of Rochester NeuroNEXT Central Pharmacy under the name of the PPI. Placebo powder in bottles will be dispensed to parent/caregiver during the placebo lead-in, AFQ056 (500 mg) or placebo powder in bottles will be dispensed to the parent/caregiver during the randomized placebo-controlled period, and AFQ056 (500 mg) in bottles will be dispensed to the parent/caregiver during the open label extension and will be dissolved in 50 cc of bottled water measured with a syringe (as described in Section 5.1) to give a 10mg/ml suspension. New bottles of AFQ056 powder will be dissolved every 10 days or as needed by the parent or caregiver at home (stability of the suspension is estimated at 10 days). Appropriate documentation of the reconstitution both at study visits and at home will be maintained at the study sites. Also, documentation of the subject specific dispensing process will be maintained by the site pharmacy or designated study staff.

Labels for the study medication on the bottles dispensed during the placebo lead-in and placebo-controlled period, up to the 8 month visit (visit 9), will be provided by the University of Rochester Central Pharmacy on bottles in kits sent to the site pharmacy, will indicate AFQ056/placebo and will include the following information:

- Study Name/Acronym
- Unique Kit Identifier (sites will interact with the DCC's IWRS to determine the kit number that corresponds to the appropriate treatment arm at the time of dispensing).
- Unique bottle identifiers within each kit.
- Description of Contents
- Storage conditions
- Dosing instructions
- Caution Statement (i.e. investigational use only and keep out of reach of children)
- Space to record date dispensed, date reconstituted, subject initials
- Name of manufacturer and name of distributor

The study number for the subject and study visit number will be added to the label by the coordinator at each study visit when the study drug is given to the subject. Study drug will be dispensed to subjects at all study visits after screening, until the Follow Up visit, after which no more study drug will be provided. From the month 8 visit on, study drug will no longer be blinded and all subjects will receive active drug. Drug dispensed at the month 8 visit and after will be labeled as AFQ056 and will contain the same information as the AFQ056/placebo labels for bottles dispensed up to month 8. Amount of study drug dispensed during flexible dosing periods will be calculated to accommodate any possible dose increases that would occur prior to the next study visit. All bottles of study medication including empty, partially used and unused bottles will be returned to the study site at each visit, and a new set of bottles will be dispensed.

Study drug will be received at the study site by a designated person, handled and stored safely and properly at the site pharmacy or other designated location, and kept in a secured location to which only the Study pharmacist and staff, site Investigator and designated staff have access. Upon receipt, the study drugs will be stored according to the instructions specified on the drug labels. Storage conditions will be adequately monitored and temperature in the area in which the study drug is stored will be controlled, monitored and recorded, at a minimum on a daily

basis. Appropriate temperature logs will be maintained. For more details, please refer to the Study Medications/Central Pharmacy section of the Manual of Operations.

In accordance with local regulatory requirements, the Investigator, designated site staff, or head of the medical institution (where applicable) at each site must document the amount of investigational product dispensed and/or administered to study subjects, the amount received from the Central Pharmacy, and the amount destroyed upon completion of the study. An investigator is responsible for ensuring product accountability records are maintained throughout the course of the study. The research pharmacist or designated study staff will be responsible for maintaining an accurate record of the shipment and dispensing of study drug in a drug accountability log. The inventory in the ledger will include details of AFQ056 or placebo bottles received and dispensed to subjects, batch, and ID numbers. All unused bottles and labels must be kept until reconciliation of delivery records with accountability logs by the monitor.

After the monitor has performed accountability, the site will be instructed by the CCC or designee to destroy the used and unused bottles of study medication. At the end of the study the CCC Central Pharmacy will communicate with Novartis and then destroy all unused bottles still on site. An accounting will be made of any drug deliberately or accidentally destroyed. Discrepancies between the amount of AFQ056 received and dispensed drug will be reconciled. A Drug Summary Log (Appendix C) will be used to track drug substance at all site pharmacies and the CCC Central Pharmacy and this will be provided to Novartis along with drug destruction certificates from all sites and from the CCC Central Pharmacy at the end of the study.

All drug supplies will be used only for this protocol and not for any other purpose. Unless specifically instructed by the CCC, the Investigator or pharmacist must not destroy any drug labels, or any partly used or unused drug supply. At the conclusion of the study, the site pharmacies will provide a copy of the drug accountability logs and Drug Summary Log to the study monitor, the PPI and to the CCC and Central Pharmacy. Only after receiving a written authorization by the CCC, will the unused and partly used drug supplies as well as the empty containers be destroyed by the site’s pharmacist according to pharmacy SOPs at the study sites, subsequently providing a drug destruction certificate.

Emergency unblinding will only be undertaken when it is essential to treat the patient safely and efficaciously. In most cases, study drug discontinuation and knowledge of the possible treatment assignments will be sufficient to treat a study patient who presents with an emergency condition. However, if unblinding is necessary, the DCC's IWRS system that will be used to track/dispense study drug, will also be used by authorized personnel (usually the site PI) to disclose treatment assignment in the event of an emergency situation. The system will automatically inform the monitor for the site and the PPI that the code has been broken. The site investigator will have a procedure in place to allow access to the emergency code break process at any time in case of an emergency. Parents/caregivers of all subjects will be informed how to contact the site investigator or his/her backup in cases of emergency when the site investigator is unavailable. An assessment will be done by the appropriate site personnel and the PPI after an unblinding to assess whether or not study drug should be discontinued for a given patient and if the patient may enter the open-label extension.

5.3 Concomitant Interventions

All concomitant diseases and conditions will be treated in accordance with prevailing medical practice. Medications/significant non-drug therapies used within the 6 months prior to Screening (visit 1) and at any time during the study will be recorded on the Prior/Concomitant Medications

and Significant Non-Drug Therapies source documents and CRF. The Investigator should instruct the patient/caregiver to notify the study site about any new medications the patient takes after the start of the study. All medications and significant non-drug therapies (including intensive developmental therapies) administered after the patient starts the study must be listed on the Prior/Concomitant Medications and Significant Non-Drug Therapies source document and CRF.

Virtually all children with FXS are expected to receive at least some speech and other therapies as well as special education, the specific amounts and intensity of therapy vary quite widely and thus the language intervention in this study will be more intensely focused on very specific areas, increased expressive communication and enhanced expressive vocabulary and grammar, relative to background therapy. This will allow examination of amplification of progress in this specific area during the trial, in a setting where this specific language intervention is used to equalize differences between co-therapies children are receiving at baseline in the community.

Despite inter-patient variability, amount of baseline therapy being used is expected to be roughly similar in the placebo and AFQ056 groups based on random group assignment, however if this is found to be different between groups, it will be added to the model for analysis of results as a co-variate.

5.3.1 Required Concomitant Medications/Interventions

There are no required concomitant medications or interventions outside of the language intervention which is an integral part of the study which must be carried out with all subjects.

5.3.2 Prohibited Medications/Interventions

Medications with GABA, glutamate or mGluR pathway activity are not allowed.
Stimulants, antidepressants, antipsychotics, alpha-adrenergic agonists are permitted for management of behavioral symptoms. Alpha-agonists, trazodone, and melatonin are permitted for the treatment of sleep problems. If any of these medications are used, a comorbid diagnosis for which the drug is FDA approved must be documented (e.g. ADHD for stimulants or alpha-agonists, anxiety disorder for SSRIs). If any of these medications are used, the dose must be stable for 60 days or 2 weeks for stimulants (see inclusion criteria #9) prior to screening and must remain stable during the placebo run-in and double-blind placebo-controlled treatment period. If the investigator feels that it is essential to add medications to manage behavior during the placebo-controlled treatment period, the patient will need to be withdrawn from the study. The reasons for the withdrawal should be carefully documented.

Doses of the above medications may be adjusted if needed during the open label extension period, and medications for behavior can be added during this time if absolutely needed. Reasons why it was critical to add the behavioral medication and co-morbid diagnosis will be clearly documented. Data from subjects for whom behavioral medication was used will be used to evaluate safety of concomitant psychoactive treatments when used with AFQ056 in the age group being studied.

Use of the following treatments is NOT allowed after randomization during either the placebo-controlled period or the open-label extension period:

Glutamatergic and GABAergic drugs due to potential mechanistic interactions with AFQ056 include the following list. This list is by no means exhaustive and medical judgment should always prevail. The site Investigators must refer to the product information for any concomitant medication to ascertain potential drug interactions and contact the PPI when there are questions about whether a medication is allowed.

Prohibited Agents:

- Treatment with acamprosate, amantadine, budipine, carbetocin, cycloserine, dextromethorphan, felbamate, ketamine, memantine, remacemide, racemic baclofen, riluzole, fycampa or investigational medications interacting with mGluR5 receptors
- Moderate or strong inhibitors or inducers of CYP1A1/2, CYP2C9/19 or CYP3A4 due to potential for increased or decreased metabolism of AFQ056. A list of these agents is given in Appendix B. This list is by no means exhaustive and medical judgment should always prevail. The site investigators must refer to the product information for any concomitant medication to ascertain potential drug interactions and contact the PPI when there are questions about whether a medication is allowed.
- Lithium, warfarin and digoxin – narrow therapeutic windows and not enough data regarding potential interactions between AFQ056 and these compounds
  Note Lithium taken as a dietary supplement is permitted if the dose is less than 5mg/day. A 5mg/day dose is the recommended dietary intake level, and is therefore not considered to be therapeutic. Lithium dosage must remain the same throughout the duration of the trial, and documented in the concomitant medication log. Isoflurane – potential interaction with AFQ056
- Any investigational drugs – potential for confounding the safety and/or efficacy of AFQ056. In this study oxytocin, minocycline and lovastatin are considered to potentially confound efficacy due to potential effects in FMRP-regulated pathways and so they are prohibited.

Parents/caregivers will be instructed to notify the study site about any new medications the subject takes after the start of the study. If a subject accidently uses a prohibited medication it must be stopped immediately as soon as this is discovered and will be listed as a protocol deviation with a statement about any potential effect on subject safety. Based on the assessment of the site investigator together with the PPI about how much the use of the medication may have confounded efficacy assessments, a decision will be made about whether the subject can stay in the trial.

Any intensive behavioral, cognitive, or therapeutic intervention not utilized at the beginning of the study may not be added to the subject's therapy regimen at any time during the study in either the placebo-controlled or the open-label extension periods (except the language intervention provided through the study). Adjustments of routine standard-of-care once to several times weekly therapies and those administered in preschool or school programs are allowed. Minor adjustments in the number of hours or time distribution of a therapy that a child is already receiving are allowed. The parent/caregiver should discuss with the study staff any new therapies to be added to the subject's baseline regimen so that it can be determined whether these are sufficiently intensive to compromise the efficacy assessments for the language intervention in this trial. If a subject uses a prohibited therapy it must be stopped immediately as soon as this is discovered and will be listed as a protocol deviation. Based on the assessment of the site Investigator together with the PPI about how much the use of the therapy may have confounded efficacy assessments, a decision will be made about whether the subject can stay in the trial.

5.3.3 Precautionary Medications/Interventions

There are no precautionary medications or interventions.

5.4 Subject compliance

To assess compliance with study medication, a dosing diary will be provided to all parents/caregivers of subjects at each visit from screening and the beginning of the placebo

lead-in to the 16 month visit. The parent/caregivers will record all doses given and the time given in the dosing diary. To assess compliance the diary will be reviewed with the study coordinator at each visit and missed doses noted. Empty bottles will be counted and volume remaining in partially used and unused bottles approximated. Bottle counts and expected/actual bottle use will be recorded in source documents. Correspondence between study drug used and logs will be determined and discrepancies reconciled with the parents/caregivers at each study visit. If 7 or more consecutive days (14 consecutive doses) or 14 or more total days (28 total doses) are missed between any two study visits, the subject may be withdrawn from the study. If any doses are missed compliance will be reinforced with the family at the visit.

Parental compliance with the language intervention will be assessed in three ways:

- First, clinicians will rate parent engagement and competence with the intervention during the coaching sessions. We will use a rating system adapted from that used successfully by Kasari et al.[66] in their research on parent-implemented interventions for children with autism.
- Second, clinicians will rate parent engagement and competence with the intervention during the parental homework sessions; again, using the adaptation of the Kasari et al.[66] coding system.
- Finally, clinicians will query the parent about mastery and ease of use of the taught strategies and the frequency of use during the past week. These queries will occur during feedback sessions.

These three quantitative measures will be analyzed separately and in combination to determine parental mastery and use of the targeted strategies and thus the effective “dose” of the language intervention actually received by the subject.

Parents will have a worksheet (Language Intervention Diary) they can use as a tool to keep a chart of their use of the intervention strategies to include the number of times and the length of time each day the intervention was used. The time chart will be formatted like a daily planner with each day divided into blocks of time in columns on the chart and the family will color in the time period when the intervention was used. One week of time will be represented on each page of the time chart. Based on times filled in by the family, the number of times the intervention is used can be tracked by the family to help them report this information systematically to the SLP during ratings of caregiver fidelity. This Language Intervention Diary will be reviewed at study visits and kept in the source documents but not entered into CRFs. The CRFs will contain only the bulleted information above. The Language Intervention Diary may serve as a guide for site staff to review intervention compliance with the family and encourage additional use when needed.

Clinician fidelity in implementing the didactic, coaching, and homework sessions will also be rated following a rubric developed previously. [60] It is expected that scores of 80-90% correct will be achieved. Clinician fidelity will be assessed for a subset of up to 10% of a clinician’s sessions.

Months 2 through 5 will involve four parent education sessions as well as weekly coaching, homework, and feedback sessions. Months 6 through 17 will involve once per month coaching, homework and feedback sessions.

We recommend that the parent must complete:

- All four parent education sessions;
- 41 of 48 (85%) combined coaching, homework, feedback sessions during months 2 through 5

- 25 of 33 (76%) combined coaching, homework, feedback sessions during months 6 through 17

Sites must contact the Study Team if the parent is approaching the threshold for missed sessions.

6 CLINICAL AND LABORATORY EVALUATIONS/STUDY PROCEDURES

6.1 Schedule of Activities - Table 7

Evaluation	Screening (Month -4, v1)	Month[3]	Random-ization/ Baseline (Month 0, v3)	Months (visit #)[3]											Follow up (Month 17, v15)[16]
		-2 (v2)		1 (v4)[20]	2 (v5)	3 (v6)[20]	4 (v7)	6 (v8)	8 (v9)	9 (v10)[20]	10 (v11)	12 (v12)	14 (v13)	16[16] (v14)	
Written Informed Consent	X														
Inclusion/Exclusion Review	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Documentation of Disease/Disorder	X														
Medical History/Demographics	X														
Autism Diagnostic Observation Scale[21]		X[11]													
Vital Signs: HR, BP (sitting)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical & Psychiatric Examination	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Neurological Examination	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Funduscopic Examination[8]	X	X	X		X		X		X		X	X		X	X
EKG	X[15]				X				X		X			X	
Concomitant Medication/Therapy Review	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Safety Labs[1]	X[17]				X				X		X			X	
Research Labs for Biomarkers[2]	X[13]				X				X		X			X	
Blood PK Sampling	X[13]				X				X		X			X	
Urine dipstick	X[14]				X				X		X			X	
Randomization[12]			X												

Evaluation	Screening (Month -4, v1)	Month[3]	Random-ization/ Baseline (Month 0, v3)	Months (visit #)[3]											Follow up (Month 17, v15)[17]
		-2 (v2)		1 (v4)[20]	2 (v5)	3 (v6)[20]	4 (v7)	6 (v8)	8 (v9)	9 (v10)[20]	10 (v11)	12 (v12)	14 (v13)	16[16, 17] (v14)	
Dispense Study Drug	X	X	X		X		X	X	X		X	X	X	X	
Dose Review/Titration[4]			X	X					X	X				X[5]	
Drug Accountability/Compliance[18]		X	X		X		X	X	X		X	X	X	X	X
Adverse Event Review		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Suicidality Screen	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Implement/Review Language Intervention/Compliance[6]				X[10]	X	X	X	X	X	X	X	X	X	X	X
Weighted child intentional communication score	X		X		X		X	X	X		X	X		X	X
Auditory ERP[7]	X[15]		X		X		X		X					X	X
Mullen Scales of Early Learning	X		X		X				X					X	
Vineland Adaptive Behavior Scale-3	X		X		X		X	X	X			X		X	X
Preschool Language Scale - 5	X		X		X				X					X	
MacArthur Vocabulary Scale	X		X		X		X	X	X			X		X	X
VAS-Language	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CGI-I[9]		X	X	X	X	X	X	X	X	X	X	X	X	X	X
CGI-S[9]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
VAS-Behavior	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ABC-FX	X		X		X		X	X	X		X	X		X	X
Eye Tracking[7]	X[15]		X		X		X		X					X	X
Children's Sleep Habits Questionnaire	X		X	X	X				X	X	X			X	X

1. CBC, Comprehensive Metabolic Profile (CMP), TSH at screening, 2, 8, 10, 16 months.

2. Cellular biomarkers: ERK, AKT, S6 kinase, APP, and MMP9 at screening, 2, 8, 10, 16 months, DNA banking at screening, RNA, protein, serum banking at screening, 2, 8, 10, 16 months.

3. Visit windows are ±5 days for v2-v3 based on the date of Visit 1, and ±5 days for v4-v15 based on date of visit 3.

4. Dose titration done weekly from the baseline visit (v3) to the 2 month visit (v5) and from the 8 month visit (v9) to the 10 month visit (v1). Please refer to the Manual of Operations for more details regarding how the dose titration reviews should be done.

5. Wean over 1-2 weeks for dose >25 mg.

6. The 2 month visit is the language intervention baseline assessment visit and in addition to monitoring of the language intervention at study visits, video-conferencing calls will be done with the language therapist to monitor and for ongoing training with the language intervention weekly for 4 months with a ±2 day window between v5 –v8, then monthly with a ±7 day window for the duration of the trial.

7. As possible (expected >50% of cohort).

8. The PI (a fragile X specialist) will perform the funduscopic examination. Many of the study assessments depend on the child's visual function and constitute functional vision assessments. All clinicians and assessors will be trained to observe for evidence of visual compromise during other routine visits and assessments. If any evidence of visual compromise is noted during any of these assessments, the patient will be sent for an ophthalmology exam for further evaluation.

9. For this study two sets of CGI-S/CGI-I will be used. The Language/Communication CGI-S/CGI-I will be anchored to language development. The Overall Function CGI-I/CGI-S will take into account all areas of function including cognition, adaptive behavior, and maladaptive behavior and will be anchored to the child's overall functioning in the home and environments outside the home relative to a typical child of the same age.

10. SLPs will set up 1-2 distance technology training sessions with the subject family during the first month of the trial (after visit 4 – Month 1). During the call, SLPs will review the technology procedures, work with the parent/primary caregiver to set up the intervention environment and work on setting up the equipment as necessary for the language intervention. These practice calls will need to be completed prior to visit 5 (Month 2), after which the language intervention will begin. Please refer to the SLP Training Manual for more details.

11. The ADOS can be administered at any point between Screening and Baseline (i.e. visits 1, 2 or 3), but must be done prior to randomization.

12. Subjects must be at least 3 years old at the time of randomization.

13. If the PK and biomarker blood samples cannot be collected at the screening visit, sites may collect them at the -2 month or baseline visits (v2 or v3) as long as it is prior to randomization. If the PK and biomarker samples cannot be obtained at a visit following randomization (v5, 9, 11 and 14), then the sample can be re-drawn at the next visit to the site.

14. Urine dipstick testing should be completed during the screening visit when possible. If the urine dipstick testing cannot be completed at the screening visit, sites may collect them at the -2 month or baseline visits (v2 or v3) as long as it is prior to randomization. If the urine dipstick testing cannot be obtained at a visit following randomization (v5, 9, 11 and 14), then the sample can be collected at the next visit to the site. For more details please refer to the Manual of Operations.

15. If the auditory ERP, eye tracking, and/or EKG cannot be administered at the screening visit, sites may administer them at the -2 month visit (v2).

16. For subjects with an open label extension that is less than 6 months, a termination visit will occur prior to drug expiration), the length of the open label extension, and the number of months between Visit 11 and Visit 14, will be determined based on when the subject is consented and their dose in the open label extension. .

For those for whom the open label extension is shortened, all study visits will occur at the usual times in the protocol, but an early termination visit will be conducted by August 31, 2021 and if possible visit 15 occurring approximately 1 month later.

17. If a subject is withdrawn from the study during the placebo-lead in period or prior to randomization, they will be brought back in for an abbreviated follow-up/termination visit. Please refer to Section 4.3. Subject Withdrawal Criteria for more details. Subjects withdrawing early (after randomization has occurred) will complete an end of study visit at which assessments and procedures for the 16 month visit (v14) will be conducted, and then if possible will complete a follow up visit at which the same procedures as listed for the 17 month follow up visit (v15) will be conducted. If subjects discontinue study drug prior to the early termination visit, only safety outcomes are assessed during the termination visit (Blood draw, urine dipstick, EKG, vitals, physical, neurological and psychological exams, suicidality assessment, fundoscopic exam, AE review, study drug accountability) and a follow-up email or phone call to document adverse events will be completed 30 days later.

18. If the safety sample obtained at screening visit cannot be analyzed, the sample can be re-drawn at visit 2. In this case dosing can begin, but the results from the repeat blood test must be obtained and acceptable before randomization can be done at Visit 3 If a lab result is abnormal and deemed not clinically significant by the Site Investigator, dosing may proceed. If the safety samples cannot be obtained at a visit following randomization (v5, 9, 11 and 14), then the sample can be re-drawn at the next visit to the site.

19. Depending upon the timing of the visit, the evening/p.m. dose may be administered after the completion of the visit. If the visit is done earlier in the day, the family will retain the current bottle being used in order to administer the evening dose on the day of visit 3. The bottle diluted at visit 3 will then be used staring with the morning dose the day after the visit. This bottle will be then be returned at visit 4 so that site study staff may complete accountability documentation. Please see the NN107 FX LEARN MOP for details about data entry related to accountability.

20. Visit 4 (Month 1), Visit 6 (Month 3) and Visit 10 (Month 9) can be completed remotely if travel to these visits is difficult for the family. All parent rated measures and questionnaires should be completed over the phone. Vitals are optional for these visits. Flexible assessments missed at a visit just prior to a remote visit can be completed after the remote visit, during the next visit to the site. See footnotes 13, 14, and 18 above.
21. The ADOS can be administered using the alternative instructions found in section 6.3.9 “Autism Diagnostic Observation Scale – 2 (ADOS-2)” if the participant is using personal protective equipment which would prevent full completion of the ADOS.

6.2 Timing of Study Activities

Visits are shown in the schedule of activities Table as:

- Screening (visit 1, when the subject will be evaluated to determine if he/she qualifies for the study, assessments will be done and subject will start placebo lead-in)
- -2 months (visit 2) when subject will follow up to track progress
- Baseline (visit 3, when pre-intervention assessments are done and the patient is randomized to AFQ056 or placebo)
- Monthly and Bi-monthly visits after Baseline as well as assigned visit number, in order, from the 1 month (visit 4) to 16 month (visit 14) visits
    - The 2 month visit (visit 5) is the language intervention baseline visit when assessments will be obtained on the optimized dose of AFQ056/placebo, and then the language intervention will be implemented.
- Follow Up (the final visit when the subject returns for a safety check after the AFQ056 has been discontinued at the end of the study).

Visit windows will be as follows:

- Month -2 (v2) and Baseline (v3) ±5 days based on the date of Screening (v1)
- Month 1 (v4) through Month 17 (v15) ±5 days based on the date of Baseline (v3)

Visits may be split over two days if necessary to lessen the burden of the assessments on the parent(s)/legal guardian and child. The Screening visit may be split over 2 days within a week apart. If the visit will be split over two days more than a week apart, the site must contact the Study Team prior to proceeding. For all subsequent visits that are split over two days, both days must fall within the protocol-specified visit window. If one day should fall outside the window, please contact the Study Team prior to proceeding.

6.2.1 Screening/Pre-Randomization Evaluations/procedures

At the beginning of the Screening visit, informed consent to enable participation in the study will be obtained from a legally authorized representative, a parent or legal guardian, by licensed physician investigator delegated to obtain consent. Screening assessments will be completed after consent, after which the subject will receive bottles for placebo lead-in at the end of the visit. The purpose of the screening period is three-fold: to confirm eligibility before randomization, to stabilize placebo effect, and to allow a control group for the language intervention/placebo group.

If the subject does not meet eligibility criteria he/she will be discontinued before the -2 month visits (visit 2) and neither visit 2 nor the baseline visit will be completed. If eligible, the patient will complete the -2 month visit for dispensing of placebo bottle and adverse event review. The subject will be randomized during the baseline visit but will not receive new study medication until all procedures at the baseline visit have been completed. In case a baseline visit needs to be split between multiple days, baseline visits may begin up to 48 hours before the first dosing day with the medication post-randomization. Typically, the Baseline visit will occur the day before the first day of post-randomization dosing.

If a patient fails to meet an eligibility criterion that the investigator deems may change over time, such that the patient may qualify if screened again at a later date, the patient may be re-screened for eligibility. The re-screening visit must occur at least 4 weeks after the initial screening visit occurred. If a patient is re-screened, all inclusion and exclusion criteria must be reassessed prior to final eligibility determination.

6.2.2 On-Study/On-Interventions Evaluations/procedures

Study visits after Baseline will occur at 1 (visit 4), 2 (visit 5), 3 (visit 6), 4 (visit 7), 6 (visit 8), 8 (visit 9), 9 (visit 10), 10 (visit 11), 12 (visit 12), 14 (visit 13), and 16 (visit 14) months while the subject is taking study medication/placebo. Visit windows for visits 4 through 15 will be ±5 days base on the date of Baseline (v3).

Dose titration reviews will be done weekly with a recommended ±1 day window to evaluate side effects and adjust study medication dose between Baseline (visit 3) and visit 5 in the placebo-controlled period, and between visit 9 and 11 in the open-label extension. Video conference calls will be done with the language therapist to monitor and for ongoing training with the language intervention weekly for 4 months, then monthly with a ±7 day window for the duration of the trial (through the open label portion).

The schedule of activities at each study visit will depend somewhat on subject cooperation and family perception of the best order of activities for the subject in question. In general, if the visit is being conducted on a single day, the play-based assessment and developmental and language testing will be done first, followed by the eye tracking and auditory ERP, as applicable, and formal medical exams. EKG and blood work, will typically be done last. If the visit is conducted over multiple days these events will be re-organized with the general principle of doing activities for which patient performance is important (play-based assessment, developmental and language testing, and ERP/eye tracking) will be given priority as initial assessments on each day, again with parent input as to the preferences and types of activities that will be difficult for the child. The parent completed measures, adverse event review, dosing diary review and dosing/compliance discussion and interim history can be done with the family while the subject is undergoing testing at visits that require subject testing. If the subject cannot complete testing without the parent present in the testing room, the parent will accompany the subject to testing and discussions with the parent will be done when the medical assessments are completed.

6.2.3 Study Medication/Intervention Discontinuation Evaluations/procedures

Study medication/intervention discontinuation will occur when subjects complete the study or withdraw from the study early (after randomization). At study completion, subjects will complete all the assessments and procedures for the 16 month visit (visit 14).

If a subject withdraws from the study after randomization, a Termination visit should be conducted as soon as possible. The assessments and procedures completed during the termination visit will depend upon whether the subject discontinued study drug prior to the termination visit. Assessments and procedures are to be completed as possible based on agreement of the caregiver at the termination visit(s) as follows:

- The subject is on study drug at the time of the visit
  - The termination visit will include all the assessments and procedures for the 16 month visit (visit 14).
- The subject discontinued study drug prior to the visit
  - The termination visit will include the following safety assessments and procedures
    - Blood draw
    - Urine dipstick
    - EKG
    - Vitals
    - Physical, neurological and psychological exams
    - Suicidality assessment
    - Fundoscopic exam

- AE review
- Study drug accountability

A follow up visit after the Termination Visit with the subject off study drug should be conducted 30 days after the termination visit.

Study medication will be weaned over 1-2 weeks for subjects on a dose of >25 mg BID AFQ056 or placebo, and stopped without weaning for subjects on a dose of 12.5 or 25 mg BID. For participants on a dose of 50 mg BID, the dose will be weaned to 25 mg BID for a week and then stopped. For participants on 75 mg BID or 100 mg BID, the dose will be weaned to 50 mg BID for a week and then to 25 mg for a week, and then stopped.

Discontinuation criteria for individual subjects are listed in section 4.3 of the protocol – please refer to that section for more details.

The entire trial will be stopped if 3 serious adverse events considered probably or definitely due to drug occur or upon recommendation by the DSMB based on unblinded review of adverse events.

Dosage reductions will be allowed down to 12.5 mg BID during dose titration periods. If a down titration is needed during the fixed dose period because of side effects, the subject will be withdrawn from the study. If a subject cannot tolerate even the 12.5 mg BID dose, that subject will be withdrawn from the study. Subjects may be withdrawn from the study if there is a temporary suspension of dosing for more than 7 consecutive days or for more than 14 days total.

6.2.4 On-Study/Off-Intervention Evaluations

If subjects come off of AFQ056/placebo study medication treatment they will be withdrawn from the study according to the procedures described above and will not be followed in the study after completing the follow up visit.

6.2.5 Final On-Study Evaluations

All subjects will complete the Follow Up visit (visit 15) as the final visit of the study. At that time, subjects will be off of study medication and will turn in all empty bottles of study medication. Parents/caregivers of subject will be instructed to call the site Investigator or study staff should there be any concerns after the follow up visit. Subjects may continue to receive the language intervention indefinitely as delivered by the parent, but this will no longer be monitored by the language therapist. Please refer to the SOA for the complete list of procedures and assessments done at this visit.

If a subject withdraws from the study after randomization and stops taking study drug prior to the termination visit (Month 16, Visit 14), the follow up visit (visit 15) will consist of a phone call or email to check for adverse events that will occur 30 days after the termination visit.

6.2.6 Off-Study Requirements

There will be no specific requirements for subjects after completion of the study protocol. The subject will go back to being treated per best medical practice and standard of care.

6.2.7 Pregnancy (Optional)

Pregnancy guidance as not relevant to this study as pregnancy will not occur in children 3-6 years of age.

6.3 Special Instructions and Definitions of Evaluations

6.3.1 Informed Consent

Informed consent will be obtained by licensed physician investigators who are specifically trained on the study and listed on delegation logs as being authorized to obtain consent. The site PI will typically be involved in explaining the study and will play a role during the consent process and answering any questions in conjunction with the coordinator, as well as overseeing that the process is carried out correctly. The consent in this study will always be signed by the parent or legal guardian due to the age and cognitive ability of the participants. Basic parts of the study the child is capable of understanding will be explained to the child as possible in very simple language.

Written informed consent will be obtained from the parent(s) or legal guardian of each participant before any study-specific procedures or assessments are performed and after the aims, methods, anticipated benefits, and potential hazards are explained. Both parents should be consented, if they are present at the Screening visit. The willingness of the parent(s) or legal guardian to have the subject participate in the study will be documented in writing in a consent form, approved by the NeuroNEXT CIRB, which will be signed by the parent(s) or legal guardian of the participant with the date of that signature indicated. The investigator will keep the original consent forms and copies will be given to the parent or legal guardian of the participant. It will also be explained to the parent or legal guardian of the participants that they are free to refuse entry into the study and free to withdraw from the study at any time without prejudice to future treatment. Written and/or oral information about the study in a language understandable by the parent(s) or legal guardian of the participant will be given to all parents/legal guardians of the participants. HIPAA guidelines for confidentiality and the principles of medical ethics will be adhered to during the study.

Note: The parent or legal guardian signing the informed consent form must be the same individual who will administer the language intervention throughout the trial. If both parents sign the informed consent, the parent who is the primary care provider for the child must be the one who administers the language intervention throughout the trial.

6.3.2 Protocol Violations

Deviations from the written protocol will be considered a protocol violation and reported to the Central IRB and the safety monitoring board. A minor protocol deviation will be considered a departure from the protocol of relatively minor degree (such as study visit a day or two out of window due to child being sick). A major deviation will be considered any significant deviation from the protocol that could affect significantly the conduct of the study, compromise efficacy assessments or create a safety risk for the subject. The reason for the deviation for protocol deviations will be documented by the site PI in the source documents in CRFs, along with steps taken to avoid the deviation and future similar deviations.

This study will be conducted as described in this protocol, except during an emergency situation in which the protection, safety and well-being of the subject requires immediate intervention, based on the judgment of the Investigator (or appropriately qualified designee). In the event of a significant deviation from the protocol (protocol violation), the Investigator or designee must contact the PPI and/or the Study Team at the earliest possible time by telephone. This will allow an early joint decision regarding the subject's continuation in the study. The medical monitor

may also be contacted for consultation regarding dosing deviations and input on how the subject should proceed. The Investigator will document the outcome, and the Study Team likewise will retain a documentary record of the decision. Protocol Deviations will be submitted to the Central IRB per NeuroNEXT policy.

6.3.3 Documentation of fragile X syndrome

Subjects will be evaluated for the diagnosis of Fragile X Syndrome and will have to have documentation of a full *FMR1* mutation (CGG repeat length over 200 repeats). If the subject has been genotyped at any time in the past and has a report documenting this, DNA testing for FXS will not need to be repeated at Screening. Mutation testing is an absolute requirement prior to the screening visit for enrollment. A copy of the *FMR1* DNA test result will be filed with the source documents and the mutation present will be entered on the Medical History CRF.

6.3.4 Demographics

Demographic information will be collected at the Screening visit including: date of birth, sex, ethnicity, and race.

6.3.5 Medical History

A medical history will be taken at the Screening visit and this will include developmental history with age milestones such as when walking and first word were achieved, as well as a general description of the child's current developmental and language function. History of behavioral difficulties in typical areas of dysfunction in FXS (hyperactivity, distractibility, anxiety, perseverative behavior, irritability/tantrumming/aggressive behavior) and current levels of severity will also be obtained.

History of any medical problems or surgeries will be obtained, with special attention to common medical problems in FXS including:

- Recurrent otitis media and need for myringotomy tube placement
- Strabismus and need for medical management (patching) or surgery
- Bowel dysregulation with constipation or diarrhea
- Gastroesophageal reflux
- Sleep dysregulation
- Insomnia or sleep apnea
- Need for tonsillectomy and/or adenoidectomy
- Seizures
- Orthopedic issues such as foot pronation
- Cardiac murmurs.

History of prior diagnosis of autism spectrum disorder (ASD) will be obtained along with information regarding what type of practitioner made this diagnosis. Previous medical records that are germane to the diagnosis of intellectual disabilities (e.g. IQ test scores and developmental assessments and dates of prior testing) will also be collected. All of the above medical history including specific prior medical diagnoses and surgeries and current medical conditions prior to initiating study treatment will be documented in the source documents and CRFs.

6.3.6 Treatment History

History of all prior treatment directed at the core features of FXS within the 6 months prior to screening will be obtained. This will include largely therapeutic interventions, such as when the child began therapy through early intervention, early childhood programs, or private therapies. A past history of all prior therapies (including speech, occupational, physical, developmental and

behavioral therapies) within the 6 months prior to screening will be obtained, when these were started and stopped, in addition to how many hours per week of therapy were done over what time frames. This information will be documented in the source documents and CRFs. All ongoing therapy interventions at the time of screening will be documented also, including type of therapy, and number of hours per week. An educational history will be obtained and documented in the source documents and CRFs, with type of school programs attended, current school program, hours per day of school attended, and hours per day of special education services.

The language intervention is being used to equalize difference between co-therapies.

All medications used for over a month within the past 6 months and all medications used for control of behavioral symptoms related to FXS will be documented, along with the reason for their use and start and stop dates.

6.3.7 Concomitant Medication/Therapy Review

All current (concomitant) medications and non-prescription supplements, doses, frequency taken, reason for taking and start and stop dates will be reviewed at every study visit and recorded in the source documents and CRFs. All non-drug therapy interventions such as speech, OT, PT, and behavioral therapies including frequency and time of therapy sessions, reasons for implementation or discontinuation and start and stop dates will be reviewed and recorded on the source documents and CRFs.

6.3.8 Protocol Amendments and Study Termination

All revisions and/or amendments to this protocol must be approved in writing by the Sponsor and the CIRB. The Investigator will not make any changes to the conduct of the study or the protocol without first obtaining written approval from the Sponsor and the CIRB, except where necessary to eliminate an apparent immediate hazard to a study subject.

The Sponsor and NeuroNEXT Network reserve the right to discontinue the study at a clinical study site(s) for safety or administrative reasons at any time. Should the study be terminated and/or the clinical study site closed for any reason, all documentation and study medication pertaining to the study must be returned to the Sponsor or its representative.

If an amendment requires a change in the consent form because the amendment affects information provided to the parents or legal guardians of the participants in the original consent, all parent/legal guardians will be presented the amended consent once approved, new information will be explained and the parent/legal guardian will sign the amended consent if they agree that the subject should continue to participate given the new information.

6.3.9 Clinical Assessments

Appropriate qualified site staff will be qualified to administer standardized measures and will be trained on all assessments at an investigator meeting held before the study is initiated, or at the study site or by web -based training if the assessor is not able to attend the investigator meeting.

Vital Signs - Height (cm), weight (kg) and vital signs, including blood pressure (SBP and DBP in mmHg), heart rate (bpm), and body temperature (°C) will be assessed at all study visits. These will be recorded in the source documents and CRFs.

Physical and Neurological Examination will be performed at all study visits. It will include the examination of general appearance, skin, neck (including thyroid), eyes, ears, nose, throat, lungs, heart, abdomen, back, lymph nodes, and extremities. The neurological exam will include examination of cranial nerves, strength, deep tendon reflexes, sensory exam, cerebellar functions, and gait. Results of all physical and neurological examinations will be documented in the source documents and the CRFs. Significant abnormalities of the physical and neurological exam (not expected based on the diagnosis of FXS itself) that are present prior to the start of the study drug will be listed as a prior diagnosis in the medical history as current medical conditions in the source documents and CRFs. Significant findings identified after the start of study drug which meet the definition of an adverse event must be recorded as an adverse event in the source documents and CRFs.

Funduscopic Examination will be performed at Screening, -2 month visits, AFQ Baseline and 2, 4, 8, 10, 12, and 16 month and follow up visits (visits 1, 2, 3, 5, 7, 9, 11, 12, 14 and 15) by the PI (fragile X specialist). This will be done to screen for retinal abnormalities based on retinal degeneration findings observed in a 2-year rat carcinogenicity study conducted by Novartis. If any abnormal findings not previously documented in the history or any significant changes in the exam occur at any time during the study, the patient will be referred for evaluation and care in Ophthalmology.

Many of the study assessments depend on the child's visual function and constitute functional vision assessments. All clinicians and assessors will be trained to observe for evidence of visual compromise during other routine visits and assessments. If any evidence of visual compromise is noted during any of these assessments, the patient will be sent for an Ophthalmology exam for further evaluation. Results of all funduscopic examinations will be documented in the source documents and the CRFs. Significant abnormalities of the funduscopic exam (not expected based on the diagnosis of FXS itself) that are present prior to the start of the study drug will be listed as a prior diagnosis in the medical history as current medical condition in the source documents and CRFs. Significant findings identified after the start of study drug which meet the definition of an adverse event must be recorded as an adverse event in the source documents and CRFs.

Autism Diagnostic Observation Scale – 2 (ADOS-2) will be performed at any point between Screening and Baseline (i.e. visits 1, 2 or 3), but must be done prior to randomization to classify children formally as having a diagnosis of autism spectrum disorder (ASD) or not, for the purposes of cohort description, and for analyses designed to determine if ASD co-diagnosis has an impact on response either to the language intervention or AFQ056.

The ADOS-2[67] is an instrument for diagnosing and assessing ASD, with the 2nd Edition (ADOS-2) updated so that scoring of the instrument will produce ASD diagnoses consistent with the revised definition of ASD in DSM-5. The ADOS-2 consists of a series of structured and semi-structured tasks that involve social interaction between the examiner and the subject. The examiner observes and identifies segments of the subject's behavior and assigns these to predetermined observational categories. Categorized observations are subsequently combined to produce quantitative scores for analysis. Research-determined cut-offs identify the potential diagnosis of ASD, allowing a standardized assessment of autistic symptoms. Scores on the ADOS stereotypic behavior/restricted interest and social interaction subscales and overall scores as well as ASD classification based on these scores will be recorded in the source documents and CRFs.

Alternatively, if the subject is using personal protective equipment resulting in the inability to perform the ADOS-2, the following procedure may be followed:

The ADOS-2 rater will complete the following evaluation to establish ASD diagnosis:

1) Clinical interview with parent
2) Review of available records
3) Semi-structured observation of child in clinic with institutional requirements for personal protective equipment or via telehealth (using ASD-DIAL)
    a. The site must submit a post-observation provider survey for QI
4) Score the CARS
5) Attempt hypothetical ADOS-2 scores
    a. This is optional, and scores will not be recorded in the database. If decide to score hypothetical ADOS-2 scores, sites must indicate on the DSM-5 checklist that these scores were used to inform diagnosis.
6) Complete the DSM-5 checklist

Administration time: 45 minutes with subject

Adverse Event Review will be done at all study visits and during dose titration reviews. Safety monitoring will be based on close observation of the child by the family. Children in the age range proposed who will, based on inclusion criteria, have developmental delay with a mental age of approximately 1-5 years, will not be able to spontaneously communicate all (if any) side effects. Parents will be queried about any differences in the child's behavior or wellbeing.

Other studies in children have to rely on parent report and based on prior experience in the Pediatric PK study with AFQ056, families were quite vigilant about reporting any changes in the child and this was not felt to be problematic. The parent/caregiver of the subject will be queried and interviewed about any illnesses, medical problems, unusual behaviors, sleep problems, potential side effects or any other problems that have occurred since the last study visit or call. All new symptoms or medical problems will be documented in an adverse event CRF containing information on the type of problem, severity (mild, moderate, severe), potential relationship to study medication (not related, possibly related, probably related, definitely related), date on onset, date of resolution, whether problem is ongoing and any intervention needed to treat the problem.

Suicidality Review will be done at all study visits. Children with a mental age of 1-5 do not understand the concept of suicide and are often not even clear on the concept and permanence of death. They thus cannot be asked questions about suicide. Suicidality monitoring will be completed by asking the caregivers two questions: whether the child has said anything about harming him- or herself and whether the child has done anything to harm his- or herself, with intent to cause injury.

Young children with FXS frequently have self-injurious behaviors that are reactions to being upset, such as hand biting or head banging and these would not be considered suicidal behaviors, and frequency and severity of these behaviors will simply be tracked during the study, and will not be considered adverse events if present at enrollment and if they do not worsen substantially from past levels of severity. Answers to the questions to the family about self-harm will be recorded in the CRF.

Administration time: 5 minutes with caregiver.

Inclusion/Exclusion Review will be done at all study visits. The investigator will review the inclusion/exclusion criteria and determine if there are any new problems such that the patient would no longer meet the inclusion/exclusion criteria and would require that the patient be withdrawn from the study.

Standard 12-lead EKG will be performed at Screening, 2, 8, 10, and 16 month visits (visit 1, 5, 9, 11, and 14). EKGs will be obtained at the study site through the EKG department and will be read by a Cardiologist, Internist or qualified physician at the site as per standard clinical EKG testing.

Clinically significant abnormalities prior to study medication dosing that are not exclusionary will be recorded as part of the medical history as current medical conditions in the source documents and CRFs. New significant abnormalities identified after the start of study medication will be recorded as adverse events in the source documents and CRFs.

The CTCAE (version 4.0) grading system for clinically significant abnormal EKG findings (laboratory adverse events) will be used. Clinically significant findings at screening will be confirmed with the Cardiologist at the site and then discussed with the PPI prior to enrolling the patient in the study. Clinically significant findings after starting study medication will be confirmed with the Cardiologist at the site and then discussed with the PPI determine whether the subject should continue in the study.

Clinical Global Impression – Improvement[68] (CGI-I) will be rated by the designated investigator (licensed physician investigator or nurse practitioner) at all study visits after Screening. After the AFQ baseline visit, it will be rated relative to performance at the AFQ baseline visit (visit 3). Completion of this scale requires the clinician to rate how much the patient's illness has improved or worsened relative to a baseline state.

Two sets of CGI-I will be administered at each applicable visit – one associated with Language/Communication, and the other to Overall Function. The Language/Communication CGI-I will be anchored to language development, i.e. to the amount of improvement in language function and milestones and nonverbal communication seen relative to baseline language ND communication abilities. The Overall Function CGI-I will be anchored to the amount of improvement or worsening in age-related functioning including behavior relative to baseline. The Overall Function CGI-I will take into account all areas of function including cognition, adaptive behavior, and maladaptive behavior and will be anchored to the child's overall functioning in the home and environments outside the home relative to a typical child of the same age.
The CGI-I is a 7-point scale is used that includes the following ratings: 1 = very much improved, 2 = much improved, 3 = minimally improved, 4 = no change, 5 = minimally worse, 6 = much worse, 7 = very much worse. The CGI-I (and CGI-S) are gold standard global measures of severity and change with treatment in placebo-controlled pharmacotherapy trials in developmental disabilities and have been used extensively in prior clinical trials in FXS. [69-72] CGI-I scores will be recorded in the CRFs.

The CGI-I should be administered by the same rater for a given subject at all applicable visits throughout the trial. If the same rater cannot administer the assessment, the site must contact the Study Team prior to proceeding and/or scheduling. The CGI-I may be administered via phone if needed.

Administration time: 15 minutes with caregiver.

Clinical Global Impression – Severity (CGI-S) will be rated by the designated investigator (licensed physician investigator or nurse practitioner) at all study visits. The CGI-S is a 7-point scale that requires the clinician to rate the severity of the patient's illness at the time of assessment, relative to the clinician's past experience with subjects who have the same diagnosis. Considering the rater's total clinical experience, a patient is assessed on severity of illness using the following classifications: 1 = normal, not at all ill; 2 = borderline ill; 3 = mildly ill; 4 = moderately ill; 5 = markedly ill; 6 = severely ill; or 7 = extremely ill. CGI-S scores will be recorded in the CRFs.

Two sets of CGI-S will be administered at each applicable visit – one associated with Language/Communication, and the other to Overall Function. The Language/Communication CGI-S will be anchored to language development, i.e. language and communication skills present at baseline and achieved during the study. The Overall Function CGI-S will be anchored to the child's functional level and behavior at baseline and ongoing functional level including behavior throughout the study. The Overall Function CGI-S will take into account all areas of function including cognition, adaptive behavior, and maladaptive behavior and will be anchored to the child's overall functioning in the home and environments outside the home relative to a typical child of the same age.

The CGI-S should be administered by the same rater for a given subject at all applicable visits throughout the trial. If the same rater cannot administer the assessment, the site must contact the Study Team prior to proceeding and/or scheduling. The CGI-S may be administered via phone if needed.

Administration time: 15 minutes with caregiver (same 15 minutes as for CGI-I, not additional time).

Weighted Child Intentional Communication Score (the primary outcome measure) will be derived from a 22- minute semi-structured examiner/child play session administered at Screening, AFQ Baseline and 2, 4, 6, 8, 10, 12, and 16 month and Follow Up visits (visit 1, 3, 5, 7, 8, 9, 11, 12, 14 and 15). The play session will be administered by designated staff (Speech and Language Pathologists and Psychologists preferred) trained to fidelity on administration of the measure. The sessions involve 12 minutes of structured play prompts designed to elicit a range of communicative behaviors (e.g., requesting, sharing of affect) and 10 minutes of free play with a standard set of toys. The weighted child intentional communication score will be coded from digital videos of the sessions centrally, according to standard coding methods by coders trained to reliability. The weighted communication score reflects child initiated communication that involves the child's use of gestures, eye contact, vocalizations, and/or words and word combinations to communicate a message to a listener. This measure has been shown to grow in a linear fashion across the stages of development that will be displayed by the children enrolled in this study.[73]

Coding of child intentional communication will be based on the occurrence of three classes of behavior:

1. Gestures or nonword vocalizations during which the child coordinated attention between the message recipient and an object or salient event.
2. Conventional gestures (e.g., distal points, head nods, pantomime) with attention to an adult
3. Symbols (i.e., spoken words or signs) that were used in a non-imitative manner.

The Weighted Frequency of Intentional Communication will be obtained by multiplying each intentional communication act by the following weights: nonverbal = 1; single symbol = 2; and multiple symbols = 3. Previous research has indicated that the weighted variable is more sensitive to change over time than the unweighted variable and that growth in the weighted variable (but not the unweighted variable) was related to later level of social impairment in younger siblings of children with ASD [74] and to communication development in FXS. Thus, this measure is objective and expected to be very sensitive to changes in communication ability as the result of the intervention.

There is no maximum score except as artificially constrained by the 22-minute duration of dyadic play session; however, in previous studies with children with autism between the ages of 15 to 25 months[75] the range of the score was 5.55 (SD 6.29) at the preintervention visit and 18.91 (SD 20.50) 9 months later at the post-intervention visit. Similarly, Yoder et al.[74] examined growth over time in weighted communication scores for younger siblings of children with autism. Weighted communication scores ranged from 14.4 (SD 10.2) at the initial visit at 14 months to 22.1 (SD 17.9) at the Time 4 visit at 30 months.

The overall weighted communication score will be recorded centrally in the source documents and in the CRFs. 10% of all sessions will be coded by a second independent, blinded coder to determine inter-rater agreement, with agreement above 90% expected as per previous research.

The Weighted Child Intentional Communication Score should be administered by the same rater (SLP or Psychologist) for a given subject at all applicable visits throughout the trial. If the same rater cannot administer the assessment, the site must contact the Study Team prior to proceeding and/or scheduling.

If the subject uses an augmentative communication device, he/she will be permitted to use it if the device serves as the primary form of communication and a SLP has prescribed it. If the subject brings their augmentative communication device to the visit they will be permitted to use it. The scoring of the WCS has been adapted to allow the inclusion of communication acts generated by the child through the use of an augmentative communication device.

Administration time: 22 minutes with subject.

Mullen Scales of Early Learning (MSEL)[76] will be administered to all subjects at Screening, AFQ Baseline, 2, 8, and 16 month visits (visits 1, 3, 5, 9, 14). This assessment can be administered by designated and trained staff (Psychologists, and Speech and Language Pathologists preferred).

The MSEL includes five subscales: gross motor, fine motor, visual reception, expressive language, and receptive language, and an early learning composite (ELC). The subscales yield alpha coefficients between 0.75 and 0.83. Test-retest values range from 0.76 to 0.96 across ages and scales, and inter-rater reliability from 0.91 to 0.96. The MSEL was standardized for children ages 3 months to 68 months but in addition to standard scores also gives age levels of function.

In this study, raw scores and age equivalent scores will be used. Instead of using the ELC, which is not valid in children over age 68 months and not necessarily accurate in populations with intellectual disability, DQ will be calculated from age equivalent scores. Subjects must have a DQ lower than 75% of their chronological age at Screening to be eligible for the study. The

MSEL has been utilized in studies with young children with FXS for many years to track developmental progress [77-79]. It can be used in children with FXS over 68 months as long as age equivalent scores are used because most children with FXS have significant developmental delays that result in an age equivalency of less than 68 months even when several years older than 68 months. Test booklets with scores will be maintained with the source documents. Raw and age equivalent subscale scores will be entered in the CRFs.

The MSEL should be administered by the same rater for a given subject at all applicable visits throughout the trial. If the same rater cannot administer the assessment, the site must contact the Study Team prior to proceeding and/or scheduling.

Administration time: 60 minutes with subject.

Preschool Language Scales – 5th Edition – the PLS-5[80] will be administered to all subjects at Screening, AFQ Baseline, 2, 8, and 16 month and Follow Up visits (visits 1, 3, 5, 9, 14). This assessment can be administered by designated and trained staff (Psychologists and Speech and Language Pathologists preferred). This is a comprehensive developmental language assessment that is normed for use with children from birth to 7 years, 11 months of age.

The test requires pointing to or verbally responding to items and yields raw scores, percentile ranks and standard scores for auditory comprehension and expressive communication as well as a total language scores. The PLS-5 was standardized on 1,400 children in 45 states in the U.S. and matches the U.S. Census figures for region, race, ethnicity, and level of caregiver education. Norms are reported for 6-month intervals for children aged 12-months to 7 years, 11 months. Split half reliabilities range from .80 to .97. Sensitivity and specificity for the Total Language score is .83 and .80, respectively. Test booklets with scores will be maintained in the source documents. Raw scores for expressive and receptive language subscales and total language will be recorded in the source documents and entered in the CRFs.

The PLS–5 should be administered by the same rater for a given subject at all applicable visits throughout the trial. If the same rater cannot administer the assessment, the site must contact the Study Team prior to proceeding and/or scheduling.

Administration time: 30-45 minutes with subject.

6.3.10 Laboratory Evaluations

Standard of care laboratory assessments for safety will be done at Screening, 2, 8, 10, and 16 month visits (visits 1, 5, 9, 11, and 14). All laboratory assessments will include: a TSH CBC with Hgb, Hct, RBC, WBC, MCV, MCH, MCHC, PLT, RDW-CV, neutrophils, lymphocytes, monocytes, eosinophils, and basophils, comprehensive metabolic profile (CMP) including sodium, glucose, potassium, BUN, creatinine, bicarbonate, chloride, total calcium, total bilirubin, ALP, AST, ALT, total protein, albumin, and cholesterol, and urine dipstick testing to test for glucose, bilirubin, ketones, specific gravity, blood, pH, protein, urobilinogen, nitrite, and leucocytes. If the safety blood samples cannot be collected at the screening visit, sites may collect them at the -2 month or baseline visits (v2 or v3) as long as it is prior to randomization. If the safety blood samples cannot be obtained at a visit occurring post-randomization (v5, 9, 11 and 14), then the sample may be collected at the next visit to the site.

When blood is drawn at the above visits the safety testing will be done first, followed by PK, followed by biomarkers in order of importance to subject protection and study conduct. Drawing of the blood tests will be ordered in this way because it may be challenging to get enough

volume for all the blood samples from children this age and the order will ensure the most important samples for patient safety are collected first.

Urine dipstick testing will be done by the coordinator or designated staff at the site. Urine dipstick testing will be done rather than a full urinalysis because there were no significant abnormalities in U/A values in any of the other Novartis studies in FXS, children tend to have less issues with kidney functions than the adults studied previously, and the children in this study will mostly not be toilet trained (larger volumes of urine which has to be collected in a urine bag may be challenging to obtain). If the urine dipstick indicates there may be a urinary infection, a full U/A and urine culture may be sent to the local lab at the site at the discretion of the investigator based on clinical assessment. In the event that a urinalysis is done by the local lab, it would be considered a clinical assessment, and not a study procedure. Urine dipstick supplies will be provided to sites by the PPI site as needed.
Urine dipstick testing should be completed during the screening visit when possible. If the urine dipstick testing cannot be completed at the screening visit, sites may collect them at the -2 month or baseline visits (v2 or v3) as long as it is prior to randomization. If the urine dipstick testing cannot be obtained at a visit occurring post-randomization (v5, 9, 11 and 14), then the sample may be collected at the next visit to the site. For more details please refer to the Manual of Operations.

Laboratory assessments will be drawn in standard specified tubes in the Study Lab Manual and samples shipped to the CLIA-certified CCC Central Laboratory at the University of Rochester where assays will be performed as per standard clinical testing. The CCC Central Laboratory will provide the specimen collection kits for safety tests (phlebotomy supplies, collection tubes, aliquot tubes, barcoded labels) and IATA compliant shipping materials for the sites to ship to the CCC Central lab. Study specific test requisitions with instructions for collecting, documenting, processing, and shipping will also be provided by the CCC Central lab. Fed Ex labels for shipping on a CCC Central lab account number will be provided to sites, and will allow tracking of shipments by the CCC Central lab to ensure timely delivery of samples and reporting of results. Instructions for obtaining, processing and sending safety labs to the CCC Central Laboratory will be provided in the manual from the CCC Central Laboratory at study initiation. All safety lab result reports will be sent to the sites where these will be maintained with the source documents.

The DCC will provide all relevant laboratories with online forms and/or electronic data exchange mechanisms - depending on their capabilities and needs - to enter, update and obtain relevant data. When a blood sample has been obtained, the clinical study site study coordinator will send the sample (participant ID, site ID, and protocol ID numbers will be used) to the University of Rochester Central Laboratory. Results will be sent via a secure system to University of Rochester laboratory with no individual-identifying information on the report. The laboratory will electronically communicate the test results to the respective clinical study sites in a secure manner. The laboratory will also transfer test results electronically to the DCC via an established data transfer mechanism.

The site investigator or an authorized sub-investigator must sign and date all laboratory results, indicating whether any out of range lab values are clinically significant (CS) or not clinically significant (NCS) on the lab report. The CTCAE (version 4.0) grading system for clinically significant abnormal lab values (laboratory adverse events) will be used. Clinically significant findings at screening will be discussed with the PPI and Study Team prior to enrolling the patient in the study. Clinically significant findings after starting study medication will be discussed with the PPI and Study Team to determine whether the subject should continue in the study.

6.3.11 Pharmacokinetic Studies

Pharmacokinetic assessment will be done for AFQ056 for all patients at screening, 2, 8, 10, and 16 month visits (visits 1, 5, 9, 11, and 14) by collecting blood for PK after safety labs are drawn. Blood samples will be drawn prior to dosing of the PM AFQ056 dose. The date and time of blood collection will be recorded on the PK blood collection page in the source documents and CRF, together with the date and time for the past three doses and the time of the last meal immediately preceding the PK sampling time.

If the PK blood samples cannot be collected at the screening visit, sites may collect them at the -2 month or baseline visits (v2 or v3) as long as it is prior to randomization. If the PK blood samples cannot be obtained at a visit occurring post-randomization (v5, 9, 11 and 14), then the samples may be collected at the next visit to the site.

PK Samples from all sites will be batched and shipped twice, in shipments midway through the study, and at the end of the study. The study sites will be notified by the Study Team site when to prepare and ship samples. The CCC Central Lab will provide tubes and materials for PK collection from subjects, shipping materials, labels, and a Fed Ex account number for shipping PK tubes from sites to the CCC Central lab. One batch of tubes will be shipped to the CCC Central Laboratory at University of Rochester in batches from each study site midway through the study and at the end of the study. The second batch of tubes will be held at the site until the CCC Central Laboratory notifies the site that the first batch of tubes have arrived and been inventoried and stored. After the notification, the sites will then ship the corresponding second batch of tubes to the CCC Central Laboratory.

After all samples have been collected and received from the sites, the CCC Central Laboratory will ship them internationally to Veeda lab analysis of AFQ056 levels. Veeda was identified by Novartis and has been used for PK measurements to quantify AFQ056 in plasma samples for previous Novartis studies.

All samples will be given a unique sample number (NeuroNEXT Subject ID) as listed in a PK log. Documentation of PK sample logs and shipping will be maintained in the source documents at study sites and in the lab records for the study at the CCC Central Laboratory. After assay, PK values will be sent directly from Veeda Laboratory to the NeuroNEXT DCC via an established data transfer mechanism. These values will not be visible to the sites to avoid unblinding.

6.3.12 Other Laboratory Studies

Blood will be collected for cellular biomarkers at Screening, 2, 8, 10, and 16 month visits (visit 1, 5, 9, 11, and 14). Biomarkers to be analyzed will include ERK, AKT, S6 kinase, APP, and MMP9. DNA and plasma leftover from the 3 ml samples noted above will be banked for future studies related to FXS.

If the biomarker blood samples cannot be collected at the screening visit, sites may collect them at the -2 month or baseline visits (v2 or v3) as long as it is prior to randomization. If the biomarker blood samples cannot be obtained at a visit occurring post-randomization (v5, 9, 11 and 14), then the samples may be collected at the next visit to the site.

Blood will also be obtained (as possible) for RNA and protein banking at Screening, 2, 6, 10, 16 month visits (visit 1, 5, 9, 11, and 14). Every attempt will be made to get blood for biomarkers

from all subjects, however this will be challenging due to the volume of blood to be obtained. Biomarker samples are expected to be able to be obtained from at least 75% of subjects. Processing and shipping of blood samples for biomarkers and banking will be done according to procedures described in the Study Lab Manual. Materials for sample collection, processing and shipping (not including Fed Ex labels and account number) for the biomarkers will be provided to the study sites by the PPI site.

ELISA will be utilized for measuring APP levels. The sandwich ELISA for APP will use antibodies immobilized in a microtiter plate that bind to target antigens consisting of total APP, APP alpha or APP beta, after an overnight incubation with plasma samples from participants. A specific detection antibody against a different epitope of either of the APP proteins is used to form a complex that will be detected after addition of a chromogen reagent. The fluorescence signal will be detected at 450 nm immediately after addition of a stop solution. An Iluminex assay will be used for measuring MMP9 levels The Iluminex assay will use a mixture of specific antibodies, each bound to a magnetic microsphere or bead that will allow the simultaneous measurement of MMP9 and MMP2 in the samples from participants. The unbound beads will be washed out and a labeled secondary antibody added. The complex will be detected by addition of a Streptavidin-PE conjugate that acts as a reporter molecule when the microspheres are exited using lasers. The fluorescent signal will be digitally recorded for each MMP9 and MMP2. Expression levels of ERK-S6K and AKT (total and phosphorylated forms) will be measured by Western Blot analysis as described in Hoeffer e al.[62]. Biomarker data will be sent to the NeuroNEXT DCC via an established data transfer mechanism. These values will not be visible to the sites to avoid potential unblinding.

6.3.13 Questionnaires

MacArthur-Bates Communicative Development Inventory The MacArthur-Bates Communicative Development Inventory (Words and Sentences version) will be administered at Screening, AFQ Baseline and 2, 4, 6, 8, 12, 16 month and Follow Up visits (visit 1, 3, 5, 7, 8, 9, 12, 14, and 15) to follow language development. This assessment can be administered by a Coordinator, Psychologist or Speech and Language Pathologist. The assessment consists of two parts: a) Part 1 (Words Children Use) is a 680-word vocabulary checklist in which the parent indicates those vocabulary words the child regularly produces in spoken language; and, b) Part 2 (Sentences and Grammar) assesses several aspects of grammar and word endings. This parent-report measure has been well-validated against direct observation and assessment measures of child language status and growth. Test booklets with scores will be maintained with the source documents. Parents will use the same test protocol at each time point using different color ink to indicate newly acquired words or grammatical constructions. Raw scores for each time point will be computed and recorded in the source documents and entered in the CRFs.

The MacArthur Bates Communicative Development Inventory should be administered by the same rater for a given subject at all applicable visits throughout the trial. The parent/legal guardian completing the assessment with the study staff member should also remain the same at all applicable visits throughout the trial. If the same rater cannot administer the assessment and/or the same parent/legal guardian cannot complete the assessment, the site must contact the Study Team prior to proceeding and/or scheduling.

Administration time: Caregiver completes independently at own pace, but typically requires no more than 45 minutes.

The Vineland Adaptive Behavior Scales, Third Edition (Vineland-3) will be administered as an interview to a parent/caregiver by trained study staff authorized to perform the interview at Screening, AFQ Baseline and 2, 4, 6, 8, 12, 16 month and Follow Up visits (visit 1, 3, 5, 7, 8, 9, 12, 14, and 15). This assessment can be administered by a Coordinator, Psychologist or Speech and Language Pathologist.

The Vineland-3 is a valid and reliable measure of a person's adaptive level of functioning from birth to 90 years of age.[82] It is commonly used in clinical care and research to measure the development and functioning of individuals with and without disabilities, including studies of natural development and clinical trials in FXS. The Vineland is an informant-based measure that yields an adaptive behavior composite score and domain standard scores in domains of: Communication (receptive, expressive, and written adaptive language functions), Daily Living Skills (personal, domestic, and community skills), Socialization (interpersonal relationships, play and leisure time, and coping abilities), and Motor Skills (gross and fine motor skills). This structure corresponds to the three broad Domains of adaptive functioning recognized by the American Association of Intellectual and Developmental Disabilities [83].

The Survey Interview Edition will be administered to parents or caregivers using a semi-structured interview format. Interview booklets with raw and standard scores should be maintained in the source documents at the site if utilized; alternatively, the Q-global application can be used during the assessment to input responses directly in to the system. Sites should use the Q-global application to calculate the composite and individual domain scores, and produce a score report. The score report must be printed from the Q-global application and filed in the subject's binder. The composite standard score and all Vineland-3 domain raw and standard scores will be entered into the CRFs.

The Vineland-3 should be administered by the same rater for a given subject at all applicable visits throughout the trial. The parent/legal guardian completing the assessment with the study staff member should also remain the same at all applicable visits throughout the trial. If the same rater cannot administer the assessment and/or the same parent/legal guardian cannot complete the assessment, the site must contact the Study Team prior to proceeding and/or scheduling.

The Vineland-3 can be administered via phone following completion of the study visit if there is not enough time to complete the assessment during the visit.

Administration time: 45-60 minutes with caregiver.

Visual Analog Scale (VAS) for Language and Communication will be completed at Screening and all subsequent study visits (visits 1 – 15). In an attempt to measure the general level of language difficulty experienced by the parent/caregiver with respect to the child with FXS, the VAS will allow parents to mark on a visual line measuring 100 mm with one side marked "worst language/communication" and the other side marked "best language/communication for age". Families will rate the subject's overall language/communication which will include receptive language and non-verbal communication as well as expressive language. The vertical marks are measured in millimeter distance where they fall from the "worst language" side (left end of the line) so that improvements or worsening of language/communication over the treatment period can be evaluated. The VAS has been successfully used in a FXS-specific clinical trial.[84] Copies of the VAS with lines and measurements will be kept in the source documents. Scores in terms of centimeter distance

from the “worst language” end of the line will be recorded in the source documents and entered into the CRFs.

The VAS for Language and Communication should be administered by the same rater (parent/legal guardian) for a given subject at all applicable visits throughout the trial. If the same rater cannot administer the assessment, the site must contact the Study Team prior to proceeding and/or scheduling.

Administration time: 5 minutes for caregiver.

The Aberrant Behavior Checklist- Community Edition (ABC) scored using the FXS-specific factoring system (ABC-FX) will be completed by the parent/caregiver at Screening, AFQ Baseline and 2, 4, 8, 10, 12, 16 month and Follow Up visits (visit 1, 3, 5, 7, 8, 9, 11, 12, 14, and 15). This parent/caregiver report measure is the gold standard measure of problem and interfering behaviors in clinical trials in DD.[85]

The ABC is actively used in over 70 countries and has been translated into over 30 languages including Spanish. The ABC asks responders to rate behaviors from 0 “not a problem at all” to 3 “the problem is severe in degree” across 58 questions. Its use has been validated in a variety of clinical populations, including in ASD and FXS, has been used extensively in clinical trials, and is a FDA-vetted endpoint utilized in the FDA approvals for use of risperidone and aripiprazole targeting irritability in youth with ASD.[70,86] It has been subjected to utility analysis in FXS[87] and linked to caregiver stress in families.[88]

Scores will be analyzed using the FXS-specific factor structure such that 54 of the items resolve into 6 subscales (Irritability, Lethargy, Social Avoidance, Stereotypic Behavior, Hyperactivity, and Inappropriate Speech).[89] Questionnaires with raw data and the six FX-factor subscale scores will be kept in the source documents. Scores for the six FX-specific factors will be recorded in the CRFs.

The ABC-FX should be administered by the same rater (parent/legal guardian) for a given subject at all applicable visits throughout the trial. If the same rater cannot administer the assessment, the site must contact the Study Team prior to proceeding and/or scheduling.

Administration time: 10-15 minutes for caregiver

Visual Analog Scale (VAS) for Behavior will be completed at Screening and all subsequent study visits (visits 1 – 15). In an attempt to measure the general level of behavioral difficulty experienced by the parent/caregiver with respect to the child with FXS, the VAS will allow parents to mark on a visual line measuring 100 mm with one side marked “worst behavior” and the other side marked “best behavior”. Families will pick and rate the subject’s three behaviors. The vertical marks are measured in millimeter distance where they call from the “worst behavior” side (left end of the line) so that improvements or worsening of behavior over the treatment period can be evaluated. The VAS has been successfully used in a FXS-specific clinical trial[61]. Copies of the VAS with lines and measurements will be kept in the source documents. Scores in terms of centimeter distance from the “worst behavior” end of the line will be recorded in the source documents and entered into the CRFs.

The VAS for Behavior should be administered by the same rater (parent/legal guardian) for a given subject at all applicable visits throughout the trial. If the same rater cannot administer the assessment, the site must contact the Study Team prior to proceeding and/or scheduling.

Administration time: 5 minutes for caregiver.

Children's Sleep Habits Questionnaire (CSHQ) will be completed at Screening, AFQ Baseline, 1, 2, 8, 9, 10, and 16 month and Follow Up visits (visits 1, 3, 4, 5, 9, 10, 11, 14, 15). The CHSQ is a parent report, sleep screening instrument that has been used in FXS to characterize sleep problems in the population,[90] and which will be utilized to screen for the presence and impact of sleep problems that may occur as a side effect of AFQ056.

The CSHQ should be administered by the same rater (parent/legal guardian) for a given subject at all applicable visits throughout the trial. If the same rater cannot administer the assessment, the site must contact the Study Team prior to proceeding and/or scheduling.

Administration time: 10 minutes for caregiver.

6.3.14 Subject Adherence Assessments and Drug Dispensing

Drug accountability/compliance will be assessed at every 2-month in-person visit after Baseline (even if there is a visit in between the two months). A medication diary will be provided to all parents/caregivers of subjects at each visit from baseline to the 16 month visit (visit 14). The parent/caregivers will record all doses given and the time given in the dosing diary. To assess compliance the diary will be reviewed with the study coordinator at each visit and missed doses noted.

All bottles provided at the last study visit will be returned and empty bottles will be counted and volume remaining in partially used and unused bottles determined by approximation. Bottle counts and expected/actual bottle use will be recorded in source documents. Correspondence between study drug used and logs will be determined and discrepancies reconciled with the parents/caregivers at each study visit. If 7 or more consecutive days (14 consecutive doses) or 14 or more total days (28 total doses) are missed between any two study visits, the subject may be withdrawn from the study. If any doses are missed compliance will be reinforced with the family at the visit.

Dispensing of Study Drug will be done every 2-month in-person visit except Follow Up (visit 15). Amount of drug dispensed will depend on the subject's dose at the time of the visit and, for the AFQ Baseline, 8, and 16 month visits (visits 3, 9, 14), any potential allowed dose titration or weaning that may be done before the next drug dispensing visit. Sufficient study drug will be dispensed to cover all dosing until the projected next study visit at which drug will be dispensed, including the maximum length of time allowed before return by the visit window.

In the case that a visit becomes delayed outside of the visit window due to weather or other unforeseen circumstances, additional study drug can be mailed to the subject to cover dosing until the subject is able to attend the study visit, so as not to interrupt dosing.

Dose Titration will be a flexible dose forced-titration per the protocol between the Baseline and 2 month visits (visits 3 and 5) in the placebo-controlled period of the study and again between the 8 and 10 month (visits 9 and 11) in the open-label extension. The initial dose at baseline and the 8 month visits will be 25 mg BID and dose titration will be done every week based on safety reports and side effects alone (efficacy will not be a factor in dose titration due to known strong early placebo effects in prior FXS studies). If the subject has no side effects at each call, the dose will be force titrated to the next level, 50 mg BID, 75 mg BID and 100 mg BID in order.

Please refer to the Manual of Operations for more details regarding how the dose titration reviews should be done.

If there is concern for side effects due to drug the dose may be left the same for an additional week or reduced. If the side effect has resolved or substantially improved after a week of holding the dose the same as the prior week or reducing the dose, the dose can then be increased. If there are side effects after the first week of treatment, the 25 mg dose will be allowed to be reduced to 12.5 mg BID. Patients who cannot tolerate 12.5 mg BID will be withdrawn from the study. The dose can be adjusted weekly through the 7th week after baseline (visit 3) and the 7th week after the 8 month visit (visit 9). The dose will be stable from the 2 month visit (visit 4) through the 8 month visit (visit 9) and from the 10 month visit (visit 11) through the 16 month visit (visit14).

The dose titration will depend on the side effects and their severity. If the side effect is moderately severe and possibly related, per the PI's discretion, the dose may be held or increased. Any clinically significant new problems or worsening of conditions or behavior outside the range of known pre-enrollment fluctuation for the patient in question, will prompt a dose decrease, and will result in an Adverse Event as well.

A subject's dose may be decreased at any point during the open label extension if the investigator determines any adverse events may be related to study drug.

Compliance with the language intervention will be assessed for the parents/caregivers in three ways.

- First, clinicians will rate parent engagement and competence with the intervention during the coaching sessions. We will use a rating system adapted from that used successfully by Kasari et al.[66] in their research on parent-implemented interventions for children with autism.
- Second, clinicians will rate parent engagement and competence with the intervention during the parental homework sessions; again, using the adaptation of the Kasari et al.[66] coding system.
- Finally, clinicians will query the parent about mastery and ease of use of the taught strategies and the frequency of use during the past week. These queries will occur during feedback sessions.

These three quantitative measures will be analyzed separately and in combination to determine parental mastery and use of the targeted strategies and thus the effective "dose" of the language intervention actually received by the subject.

Parents will have a worksheet (Language Intervention Diary) they can use as a tool to keep a chart of their use of the intervention strategies to include the number of times and the length of time each day the intervention was used. The time chart will be formatted like a daily planner with each day divided into blocks of time in columns on the chart and the family will color in the time period when the intervention was used. One week of time will be represented on each page of the time chart. Based on times filled in by the family, the number of times the intervention is used can be tracked by the family to help them report this information systematically to the SLP during ratings of caregiver fidelity. This Language Intervention Diary will be reviewed at study visits and kept in the source documents but not entered into CRFs. The CRFs will contain only the bulleted information above. The Language Intervention Diary may serve as a guide for site staff to review intervention compliance with the family and encourage additional use when needed.

Clinician fidelity in implementing the didactic, coaching, and homework sessions will also be rated following a rubric developed previously by Abbeduto and colleagues. It is expected that scores of 80-90% correct will be achieved. Fidelity will be assessed for up to 10% of sessions per clinician.

The parent/legal guardian administering the language intervention for a given subject must remain the same throughout the trial. If the same parent/caregiver cannot administer the assessment, the site must contact the Study Team as soon as they become aware.

6.3.15 Additional Evaluations

Auditory ERP can be administered by a trained study staff member. ERP has been evaluated in FXS in a few pilot studies[91,92], and provides a badly needed direct index of brain function. Auditory ERP is known to be abnormal with a larger magnitude of response in FXS due to sensory oversensitivity to sound.[93] This is thought to relate to CNS circuit dysfunction from increased dendritic spine density in sensory cortices and lack of pruning leading to hyperactivation of sensory circuits.

A model for this has been described for the *Fmr1* knockout mouse (model of FXS) in the barrel cortex neurons that respond to whisker sensation.[94] Thus auditory ERP may be a direct window into the synaptic dysregulation in FXS, and is similarly abnormal in the FXS mouse model and humans with FXS, making it a highly attractive measure that could be used for direct translation of drug effects from mouse to man, a gap area in outcome measure development.
ERPs are inherent components of an electroencephalography (EEG) signal, and therefore must be extracted from an EEG through signal processing techniques. An EEG signal provides a coarse measure of brain activity, which makes it difficult to use it to assess highly specific neural processes or isolate individual neuro-cognitive processes. In contrast, ERPs are a class of potentials that display time relationships to a definable event such as a sensory, a cognitive, or a motor event.[95]

One of the main advantages of using ERPs to analyze cognitive activity is that they provide a continuous measure of processing between a stimulus and a response, which makes it possible to identify and quantify the response and stages of processing that are affected by a specific experimental manipulation.[95] This is especially useful when studying individuals with FXS because they often have hyper reactive responses to stimuli that are auditory, tactile, visual, and olfactory.[96] Van der Molen et al. showed that the N1 and P2 components of sensory evoked potentials from sixteen males with the FXS full mutation and twenty age-matched controls were significantly enhanced in the FXS patients.[92] By focusing on the changes in the N1, P2, and N2 (relative to the common average reference) components of subject ERP signals, between the first and last 45 stimuli presented, Schneider et al. showed that there was abnormal electrocortical habituation to auditory stimuli in patients with FXS, and this improved at N1 and P2 in individuals with FXS who were given minocycline treatment compared to a control group.[91]

The ERP will be administered in an EEG recording session during a single clinic or research visit for each participant at visits 1, 3, 5, 7, 9, 14, and 15. Dense array electrode recording equipment will be used. Stimuli will be presented on a separate computer using Presentation software by NeuroBehavioral Systems, Inc. Event markers synchronized with stimuli will be conveyed to the EEG system over a parallel digital interface.

If the auditory ERP cannot be administered at the screening visit, sites may administer them at the -2 month visit (v2).

When the EEG recording system is set up and the head cap is properly secured on the subject, EEG recording will begin. The EEG signal can be viewed on the designated digitization computer (Figure 1). An Alpha Block Test will be performed to make sure that alpha waves are minimized and do not distort the EEG recording and to get an Alpha Reactivity Index. Two 1 min segments of eyes-open resting EEG and two 30 sec segments of eyes-closed resting EEG will be recorded with segments marked in the EEG data. Eyes closed versus eyes open data will be compared to ensure suppression of alpha with the eyes open. The EEG will then be recorded for 10 min with the passive auditory oddball paradigm using Presentation software that will serve as an auditory stimulus. Total recording time for each subject will be about 15 minutes.

The auditory stimuli that will be used by the Presentation software will consist of 480 sinusoidal tones of frequencies 1000 Hz (standard tone) and 2000 Hz (target tone) that will be emitted from the speakers of the simulation computer.[59] These tones will be generated using Matlab software (The Mathworks, Natick MA). The order of the tones will be randomized, consisting of six 1000 Hz standard tones and then one different 2000 Hz target tone at either the 7th, 8th, 9th, or 10th position, with 1000 Hz standard tones filling in the remaining positions.[59] The stimulus onset-to-onset time (stimulus onset asynchrony, or SOA) will be 1 second. A bandpass filter will be set at .1 Hz-100 Hz on the EEG signal during recording of all data and will be digitized at 1024 Hz.[59] Offline, EEG data will be filtered .5-30 Hz for ERP data analyses. This is done to remove any large electrical artifacts that may be present. Moreover, this allows for an efficient way to eliminate most noise, since ERPs of interest are usually found at less than 30 Hz.[64]

The EEG data for each patient will first be segmented according to event type, standard or target tone[59] using a 1000 ms window with 100 ms before the event (the tone) and 900 ms after the event.[59] The purpose of this segmentation is to generate an easy reading frame to analyze the data both before and after a tone. In order to obtain each individual's ERPs, the EEG signal must be averaged. Each participant's EEG will be averaged and baseline corrected using the signal data from the 100 ms interval before an event (before a tone).[59] When the ERP is successfully separated from the original EEG signal for each participant, the significant auditory ERP components will be identified at each of the electrode positions. Although there are several components to an ERP, the most significant known auditory ERP components in the age range studied are P1, P2, and N2.[59] Only the electrode sites that display the largest voltage deflections for these three components with respect to the appropriate reference for that component will be selected for statistical analysis and comparisons. Amplitudes for P1, P2 and N2 components, habituation of these components between the first 45 and last 45 stimuli presented, a mismatched negativity measure (MMN) which is the earliest ERP indicator of cortical auditory processing in a passive listening paradigm, and resting state measures with eyes open and closed will be obtained from the ERP software. All ERP data will then be uploaded to SharePoint (University of Oklahoma) for central analysis.

The ERP portion of the trial will not occur at all sites. It is expected that only a subset of subjects – approximately 50% – will complete this procedure as part of the study. This is due to concerns over site equipment as well as difficulty in terms subject cooperation in this particular population.

Administration time: 20-30 minutes with subject, depending on set up time, recording will be 15 minutes.

Computerized Eye-Tracking will be done with the subjects at Screening, AFQ Baseline and 2, 4, 8, 16 month and Follow Up visits (visit 1, 3, 5, 7, 9, 14, and 15). This assessment can be

administered by a trained study staff member. Gaze avoidance is a hallmark behavioral feature of FXS and individuals with FXS have difficulty in establishing and maintaining eye-gaze during social interactions. Studies have shown that pupillary responses are regulated by the autonomic nervous system in part in response to level of emotional arousal. A computerized eye-tracking device (Tobii) will be used employing a standardized protocol that is suitable for individuals of varying ages with FXS.[97]

If the eye tracking cannot be administered at the screening visit, sites may administer them at the -2 month visit (v2).

Subjects will be required to look at a screen for approximately 10 minutes while 60 images of human faces with differing emotional content (happy, calm, fearful) will be presented alternating with scrambled faces. Automated assessments of time spent looking at and number of fixations on regions of interest (e.g. eyes, nose, mouth) in face images will be recorded. Changes in pupil diameter will be measured associated with viewing of faces as an index of autonomic arousal. Data from the Tobii eye tracker will be analyzed according to the method of Farzin et al. [98] and output including time looking at and fixations to regions of interest during each type of face presentation, as well as pupil diameter changes relative to scrambled face background for each type of face presentation will be collected. All eye tracking data will then be uploaded to SharePoint (UC Davis) for central analysis.

It is expected that only a subset of subjects – approximately 50% – will complete this procedure as part of the study. This is due to concerns over difficulty in terms subject cooperation in this particular population.

Administration time: 15-30 minutes with subject.

7 MANAGEMENT OF ADVERSE EXPERIENCES

Expected side effects include those observed in the prior adult/adolescent phase 2b trials in FXS and the pediatric PK study. These include dizziness, insomnia, headache, decreased appetite, vomiting, diarrhea, and behavioral “activation” with irritability, aggression, hyperactivity, anxiety, and impulsive behavior. These side effects are expected to be relatively infrequent, mild-moderate in degree, and insomnia and other side effects will in most cases be identified in the flexible dosing period and managed by dose adjustments during the flexible dosing period.

Insomnia may be managed by moving the dose from evening to afternoon as early as 3 PM, though doses must remain at least 4 hours apart. If the subject’s dose is reduced to 12.5 mg BID during the flexible dosing period and the subject still has intolerable side effects, that subject will be withdrawn from the study. This is not expected to occur frequently based on past experience with AFQ056 in the above-noted studies. Subjects requiring dose reduction for presumed side effects after the flexible titration when the dose is to remain fixed in the placebo-controlled period (between the 2 and 8 month visits) will be withdrawn from the study. Dose reductions for side effects during the fixed dose period of the open label extension will not be encouraged but may be allowed after review of the situation.

No clinically significant laboratory or EKG abnormalities have been seen in other studies of AFQ056 outside of non-drug related abnormalities that may have occurred during intercurrent illness. If laboratory or EKG abnormalities that are deemed to be clinically significant by the site investigator occur, these can be repeated and if still abnormal will require specific follow up testing depending on the nature of the abnormality and medical standard of care for the type of

abnormality observed. The subject may be withdrawn from the study depending on investigator and medical monitor assessment of likelihood of relationship to the study treatment with AFQ056.

Adverse events seen in the prior adolescent and adult placebo-controlled studies of AFQ056 in FXS more frequently in the group treated with AFQ056 than with placebo include the following list arranged in alphabetical order. No side effect exceeded a frequency of 20%, even at the highest doses of AFQ056 (see section 2.2 above for exact frequencies). In the Pediatric PK study, side effects all fell into the below categories, with vomiting and insomnia being the most frequent side effects. These were typically short-lived occurring in the first day or two of dosing and seemingly related to advancing to the full dose right from the outset of doing rather than titrating the dose up over time. This was evident in that vomiting after the initial dose was seen in 50% of the 5-11 year old cohort studied first all of whom were dosed from the outset at the full dose, but not seen in the 3-4 year old cohort after dosing was advanced over the course of a day (half dose for one day, followed by the full dose).

- Abdominal pain
- Agitation
- Aggression
- Anxiety
- Decreased appetite
- Diarrhea
- Dizziness
- Fatigue
- Headache
- Insomnia
- Irritability
- Nausea
- Pyrexia
- Self-injurious behavior
- Vomiting

Although not seen in prior trials in humans with FXS, including after AFQ056 exposure for over two years in the open-label extension studies with adults and adolescents (2278 and 2279), learning and memory impairment have been noted in preclinical testing of AFQ056. Learning was, however, enhanced in preclinical testing in the FXS mouse model, presumably due to the AFQ056 targeting the abnormal signaling pathway in the FXS mouse. Risk of learning and memory impairment will be monitored closely using assessments that are being administered as part of the protocol. Raw scores on the MSEL and VABS-II assessments will be monitored at the sites to assess global developmental functioning. If there is a decrease in raw scores on both measures by 10% incrementally at two consecutive visits where both assessments are administered (e.g. 10% decrease from screening to baseline, baseline to month 2, month 2 to month 8, or month 8 to month 16 as described in the schedule of assessments), subject data will be reviewed by the Medical Monitor and it will be decided if the subject should be withdrawn from the study. If there is decrement in these scores without any obvious alternative cause the participant will be withdrawn from the study.

8 CRITERIA FOR INTERVENTION DISCONTINUATION

Discontinuation criteria for individual subjects will be as follows.

Study treatment must be discontinued [and the subject withdrawn from the trial] under the following circumstances and according to Investigator discretion:

- The legal guardian withdraws informed consent.
- A serious AE occurs that is in the opinion of the investigator suspected to be related to the study medication.
- A severe CNS-related AE occurs that is in the opinion of the investigator suspected to be related to the study medication.
- A subject has intolerable side effects on the lowest dose of AFQ056 allowed in the study, 12.5 mg BID, that subject will be discontinued from the study.
- The investigator feels continuation in the study would be detrimental to the subject's well-being.

A subject may be withdrawn from the study, if:

- Clinically significant changes of laboratory parameters and/or vital signs occur that are in the opinion of the investigator suspected to be related to the study medication. The clinical significance of laboratory abnormalities and other abnormal assessments will be assessed and interpreted by the investigator in the context of the overall clinical presentation.
- The subject is lost to follow-up and is late for a study visit by over 1 month.
- ≥ 7 consecutive days of study medication are missed or a total of ≥ 14 days of study medication are omitted once the subject has begun dosing between study visits at any time during the study.
- A protocol deviation occurs that in the opinion of the Site PI or Study Team may alter the study results (e.g. administration of concomitant medication).

In cases in which it is unclear if the patient should be withdrawn from the study, the site investigator will discuss the case with the PPI, Study Team and medical monitor if needed, and a decision will be made regarding whether the subject can stay in the study and if so under what conditions. Subjects discontinued from the study after randomization will complete a Termination visit at which they will undergo the procedures listed for the 16 month visit (visit 14) and then a Follow Up visit at which they will undergo the procedures listed for that visit (visit 15). Subjects will not be maintained in follow up activities through the study after completion of the Follow Up visit.

Subjects will be monitored for AEs from the time they sign consent until the final Study visit. If an AE is ongoing or discovered at a subject's final Study visit, the AE will be followed until resolution, or for a minimum of 30 days, whichever comes first. Resolution means that the subject has returned to status at enrollment. If the AE is still ongoing after 30 days, it will be resolved with sequelae. All AEs monitored past the final visit can be followed via telephone – a repeat Termination/Final Follow-up visit does not need to occur.

All SAEs must be followed until resolution throughout the trial. SAEs that are discovered less the 60 days prior to the subject's final Study visit will be followed until resolution or for a minimum of 60 days, whichever comes first, even if it is past the final visit. If a new SAE is discovered at the subject's final Study visit, it must be followed until resolution, or for a minimum of 60 days, whichever comes first. If the SAE is still ongoing after 60 days, it will be resolved with sequelae. All SAEs monitored past the final visit can be followed via telephone – a repeat Termination/Final Follow-up visit does not need to occur.

9 STATISTICAL CONSIDERATIONS

The NeuroNEXT Data Coordinating Center will develop a formal statistical analysis plan, in collaboration with the protocol principal investigator and protocol steering committee.

9.1 General Design Issues

Trial Design: The trial will be a phase II double blind placebo-controlled parallel-group flexible-dose forced-titration study that will use a novel design to address the impact of AFQ056 on language learning in 3-6 year old children with FXS. The flexible dose design will mimic practice, take into account differential responsiveness and the known inter-child variability in drug levels with AFQ056, and allow use of the maximum tolerated dose (MTD) where MTD is defined as the maximum tolerated dose of the available dose levels in the protocol, which is felt likely to be most effective. There will be a 4-month single-blind placebo lead in after screening assessments during which subjects will have therapy treatments as usual to control for the effects of the language intervention. The placebo lead-in will also control for placebo effects which are prominent in clinical trials in FXS. This will be followed by an 8-month placebo-controlled phase in which 100 subjects with FXS will have AFQ056 baseline assessments and be randomized 1:1 to AFQ056 or placebo. Dose titration to MTD starting at 25 mg AFQ056/matching placebo will occur over 2 months and then subjects will have language intervention baseline assessments and initiate the language intervention, remaining on a stable AFQ056/placebo dose for the next 6 months. After 8 months of treatment in the placebo-controlled phase (6 months of language intervention), all subjects will have final assessments and will enter the extension and be treated with active drug according to the same schedule as in the placebo-controlled phase with 2 months of flexible dose titration to the MTD followed by a period of stable treatment. The duration of the stable treatment will depend on the month the subject is consented into the study. As such, some subjects will have an acute dose decrease if they were on a dose of 50-100 mg BID of active drug in the initial phase but experience from the Pediatric PK study conducted previously suggests that children can stop AFQ056 without withdrawal symptoms and the dose decrease and titration in the open label extension are unavoidable to maintain the blind of the study through the completion of the placebo-controlled period by the final participant. The open-label extension will allow all children a period of active drug exposure to make recruitment feasible, despite the long placebo period, and will allow additional collection of long-term safety data. Subjects will continue the language intervention through the extension phase to gain additional data on long-term effects of the combination of AFQ056 and the language intervention on developmental trajectory and the ERP biomarker.

Both treatment groups will receive an intensive language intervention for 6 months after 2 months of dose optimization, to drive learning effects proposed to be enhanced by the AFQ056, during the blinded treatment exposure. A post-intervention follow up visit will be conducted one month after the final assessment visit of the trial (month 16, visit 14). At that point, all subjects will be weaned off of AFQ056 for at least a week. Most assessments, including the biomarkers, will be completed at the follow-up visit in order to determine if there have been changes from coming off AFQ056. The language intervention will continue through the end of the study and parents may continue to apply it after the study is completed but patients will not be followed further after the end of the follow-up visit.

Goals and Hypotheses: The overall goals are to change the paradigm for development of mechanism targeted pharmacotherapy in neurodevelopmental disorders and provide a definitive test of the mGluR theory in humans by determining whether AFQ056, an mGluR5 negative modulator, can enhance neural plasticity in the form of language learning during an intensive language intervention in very young children with fragile X syndrome. This trial therefore will use an innovative but exploratory trial design to examine efficacy of an agent with substantial support

as a drug targeting CNS plasticity in preclinical models of a developmental disorder. If the design is successful, this trial can serve as a model for future trials of mechanistically-targeted treatments operating on neural plasticity in other neurodevelopmental disorders.

The primary objective is to demonstrate greater improvement in language in young children with FXS treated with AFQ056, using an overall weighted communication score, in combination with an intensive standardized parent-implemented language learning intervention. The improvement in mean overall weighted communication scores over 6 months in the intervention group will be compared to placebo. This comparison provides a marker of drug effect on neural plasticity, the core problem in this disorder.

9.2 Factors Influencing Choice of Study Design

Studying Core Features of FXS: Cognition and Learning as a Surrogate for Neural Plasticity: This study was designed to incorporate features that would address problems that have been observed in prior trials in FXS and uncertainties about whether prior trials targeting behavior in adults and adolescents with FXS have provided adequate surrogate outcomes for the normalization of synaptic morphology/plasticity and learning observed with mGluR5 blockers in the mouse model of FXS. The general consensus in the field is that it is unclear whether behaviors that have been fixed by many years of development in older individuals with FXS can actually be modified by targeted treatment in a time frame compatible with a placebo-controlled trial and whether behavior and functional skills will necessarily improve concurrently and be treated by the same interventions. Thus, to truly address the potential of mGluR5 blockers to modify disease in humans with FXS, effects on learning and cognition, the core problem in FXS, need to be studied and these effects need to be evaluated at the youngest age possible. Given this, the age group for this study was chosen to be the youngest possible children (age 3-6 years) for whom PK studies had been completed and in whom an mGluR5 blocker (AFQ056 has been used at the youngest ages currently) could be used and the key outcomes chosen for study will address learning and cognition.

Language Learning Intervention: To mimic the plasticity studies in the animal model it was critical to embed a learning intervention in the trial. The language learning intervention was chosen because in the young FXS age group to be studied, language is emerging and this is the main developmental focus for this age group. Children with FXS at this age are not typically able to do currently available cognitive or academic interventions, thus the language intervention chosen is optimal because it targets skills that are in a critical developmental period and can be carried out with the age group to be studied.

Parallel Group Design: Given the complexity of running a study in which a learning intervention is embedded, and the lack of knowledge about how much carry-over effects might be seen in a study directed at plasticity and learning a crossover study was considered too difficult to interpret, a parallel group design was chosen. As there are no approved treatments for FXS, an equivalency or non-inferiority study could not be done. Likewise, as there is no approved comparator drug for treatment of FXS, the comparator for AFQ056 will be placebo.

9.3 Rationale for Specific Design Features

Length of Placebo-Controlled Treatment Period: A concern in prior trials of targeted treatments for FXS has been inadequate length of the placebo-controlled treatment period to see effects emerge in a developmental disorder. In particular, to see an improvement in learning take place, a relatively long exposure to drug will be necessary, even as long as one or several

years. Although the learning intervention is expected to speed progress so that effects can be seen in a shorter time period, at least a 6-month placebo-controlled active treatment period in the presence of the language intervention is deemed necessary even in young children, and was incorporated to have the best chance of seeing learning effects. A longer period would likely be better for measuring learning progress; however, this must be balanced against the difficulty of maintaining children with FXS on placebo for such an extended period of time during the critical assessment period of the study. In order to avoid the potential attrition that might be associated with a 1 year placebo-controlled period, the 6-month period was chosen for the active/placebo treatment coupled with language intervention.

Number of Arms: The number of arms to be employed in this trial was carefully considered, including possible 2-, 3- and 4-arm designs. The 3-arm design would include an AFQ056 group with language intervention, a placebo group with language intervention and a placebo group with treatment-as-usual. Although this would have resulted in information about the effectiveness of the language intervention alone, families would know if they are getting a language intervention, and it would have been very hard to keep such a trial blinded. A 4-arm design was considered modeled after Compton et al.[123] in a study of SSRI treatment and behavioral therapy for OCD, with treatment-as-usual and language intervention groups for both placebo and AFQ056 groups, however in the Compton study the pharmacotherapy and behavioral therapy were considered potentially equivalent interventions from an efficacy standpoint, ***while in this trial the main hypothesis is that the AFQ056 will enhance learning in young children with FXS and the language intervention is being employed to push learning rate and "plasticity" and provide a substrate for the drug to act upon*.** Thus, this trial is conceptually different from that in the Compton study and the 4-arm design is not critical to the hypothesis. Given the hypothesis and the need to increase the trial size to at least 160 participants for a 4-arm trial, the 2-arm trial with the language intervention with placebo and AFQ056 groups was chosen to maximize power to evaluate the key conditions, minimize cost and to maximize feasibility in a rare disease. However, given the value of understanding the effects of the language intervention itself, a placebo lead-in incorporated into the trial will allow comparison of 6 months of placebo treatment with and without the language intervention in the group randomized to placebo (see paragraph below). Further, all outcomes including the primary outcome will be evaluated at visit 5, two months after baseline when subjects are at MTD, before starting the language intervention, to determine if the AFQ056 has had an early independent effect on outcomes in the absence of language learning intervention.

Placebo Lead-In: A major issue in prior FXS trials has been large placebo effects. Placebo lead-in periods have been used in some cases to try to control for placebo effect. While these have not eliminated placebo effects, they do allow for an understanding of the magnitude of placebo effects that are operating on the measures they are impacting. Placebo lead-in periods thus far employed in FXS trials have been 2-4 weeks. Based on the above considerations, a 4-month placebo-lead in period was chosen for this trial. This should be long enough to ensure placebo effects are stabilized before the initiation of the placebo-controlled active treatment period. An additional motivation for the placebo lead-in and determinant of the placebo lead-in length is the potential to use the placebo lead-in period to study the benefits of the language intervention alone in the placebo-treated cohort. This cohort will receive placebo with treatment-as-usual for 6 months followed by placebo with the language intervention for 6 months, followed by 8 months of treatment with both the language intervention and AFQ056, allowing comparison of rate of progress in this cohort in the first two periods to define the effects of the language intervention.

Open-Label Extension: The prolonged placebo treatment period of 4 months (lead-in) and 6 months during the placebo-controlled treatment period could result in difficulties with subject retention, even with provision of the language intervention which will be an attraction for recruiting. Retention is expected to be far less of a problem if an open-label treatment period follows the placebo-controlled period. An open-label extension for all subjects at the end of the trial will also allow for acquisition of 1) more long-term safety data (Note: If AFQ056 were ever to be used for treatment of FXS based on this and other subsequent studies, patients would be treated for life or at least for many years until something better is developed. Although there is safety data over long periods in older patients, there is no extended safety data for young children. Even though we are not predicting specific safety issues, this extended treatment of up to 16 months will help build an important chronic safety profile for future use in very young children.); 2) more long-term data describing the trajectory of progress on the drug for the group getting AFQ056 during the placebo-controlled period, will allow more time for developmental progress on the AFQ056 to correlate with the auditory ERP and eye tracking primary and secondary biomarkers, to get better longitudinal data about how well these biomarkers predict long-term clinical outcomes; 3) will allow comparison of progress in the group treated with placebo in 3 treatment conditions for at least 6 months each (treatment-as-usual, language intervention, language intervention + AFQ056); 4) will allow analysis of biomarker responses during each of these conditions, adding to data on long-term predictive value of the biomarkers for both a behavioral and pharmacological intervention; and 5) will allow a greater length of time to determine if tolerance to the AFQ056 occurs based on repeated assessments.

Flexible Dose Design: Variability in maximal tolerated dose (MTD) has also been seen in numerous prior FXS studies, as expected based on the high interpersonal variability in optimal dosing for most psychoactive medications. This problem is compounded by the fact that the most common side effects seen with most targeted medications in FXS trials including mGluR blockers and AFQ056, are behavioral/CNS in nature and thus can at times be confused with the typical behavioral ups and downs seen in individuals with FXS. Since a fixed dose assignment may assign a patient to a suboptimal dose or a dose on which he or she has side effects, a patient assigned to a suboptimal dose may have less improvement in the primary outcome than if on a higher dose and a patient assigned to a dose producing side effects is likely to have side effects that get in the way of assessing cognitive, language, behavioral or adaptive functioning, and thus less improvement than if assigned to a lower dose. A beneficial drug effect may thus be partially masked by the wrong dose even within an expected therapeutic range. The range of doses required for similar blood levels was quite variable for AFQ056 in the Pediatric PK study conducted by Novartis, and side effects have not been particularly correlated to drug levels in any of the AFQ056 studies. Because of all of these concerns, a flexible dose design was chosen over a fixed dose design. The flexible dose design will also best mimic clinical practice.

Forced Titration Design with Sufficient Time and Flexibility to Optimize Dosing: Given the flexible dose design, it is important to ensure the study participants are on the MTD when they start the language intervention to ensure it is delivered at the presumed most effective dose. In order to achieve the MTD a dose titration period is thus required before starting the language intervention. In prior FXS studies with flexible dosing, it has been clear that because of the frequent day-to-day behavioral ups and downs in patients with FXS that often overlap with potential drug-induced CNS side effects, it is important to have adequate time at each dose to make decisions about dose escalation, and also sufficient flexibility to allow doses to be moved up or down if it becomes clear with time whether a side effect such as hyperactivity or behavioral activation is actually occurring or not. Also, given week-to-week behavioral variability in FXS it is important to avoid leaving the patient on too low of a dose because the family feels

he or she is having a good week and thus titrating to a suboptimal dose and discovering later that transient behavioral improvement had nothing to do with the dosing. Given these considerations, a forced (mandatory) titration strategy was chosen, such that the dose will be titrated up to the next level if there are no side effects, and a 2-month dose titration period with dose adjustments no more frequently than weekly was chosen to ensure plenty of time for careful assessment of side effects and provide enough flexibility to hold or move up or down on doses if it takes more than a week to ascertain whether a dose is producing side effects.

Tolerance Issues and Assessment: Concerns have been raised about whether tolerance occurs in previous trials of targeted treatments in FXS; this concern has been raised with respect to AFQ056 treatment although it has not been substantiated. To evaluate for tolerance effects, most outcome measures will be repeated multiple times during treatment period including assessments during the placebo-controlled period at 2 months, 4 months, and 8 months of drug exposure and subsequently during the open-label extension at 2 months, 4 months, and 8 months. This should allow determination of whether tolerance effects are occurring with respect to cognitive language and biomarker measures.

9.4 Randomization

In order to prevent age imbalance, randomization will be stratified by age with two strata, 3-4 years and 5-6 years. Block randomization will be used with random block sizes, and age (coded as a two-level factor) will be used as a covariate in all statistical analysis. The stratification will ensure that all individuals enrolled will have a Mullen ELC (equivalent to Developmental Quotient) of <75. This will ensure sufficient symptoms to measure learning improvement and will result in enrollment of only significantly affected females, most likely excluding at least the higher functioning half of females with a full mutation (because of this, females are expected to comprise about 20% of study participants). Likewise, very high functioning males (the best functioning 10-20%) will be excluded. Thus, the males and females enrolled are expected to show heavily overlapping clinical phenotypes and developmental function and stratification is expected to be unnecessary. Further, there is no reason to expect a priori a difference in response in lower versus higher functioning individuals within the impaired group with delays enrolled, and thus even if the females are on average higher functioning within the impaired range than males, it is not clear they will respond sufficiently differently to justify stratification in a randomized design.

9.5 Outcomes

9.5.1 Primary Outcome (Including Definition)

The primary objective of this study is to demonstrate greater improvement in language in young children with FXS treated with AFQ056 in combination with an intensive standardized parent-implemented language intervention, relative to those treated with the language intervention and placebo, after 6 months of intervention, as a marker of drug effect on neural plasticity, the core problem in the disorder. This will be assessed using a primary language endpoint involving a weighted child intentional communication score coded from a 22-minute structured task which is an examiner/child dyadic play session, including a structured and unstructured play session.

Because the language intervention will specifically be targeting increased communicative behavior and increased use and production of language, the weighted communication measure

was chosen as the primary outcome because it will specifically measure child initiated communication that involves the child's use of gestures, eye contact, vocalizations, and/or words and word combinations to communicate during a standardized play session, the precise skills which are directly being taught by the language intervention. The measure is thus ideal for measuring the type of learning expected to take place from the language intervention and which potentially would be enhanced by the concomitant AFQ056 treatment. Although this measure is less well-validated than standardized language measures, it was chosen to be the primary outcome because it is so optimally matched to measure the skills the intervention is delivering and will be more sensitive to skills that are likely to improve from the language intervention than other standardized cognitive, adaptive, or language measures that have more extensive validation. Because the FXS phenotype varies, participants will range from having minimal language/infrequent communication to using phrases or sentences and/or be more talkative. Because the WCS reflects both linguistic sophistication and frequency of communicative attempts regardless of form, it has the potential to capture change in the full range of FXS participants. This measure will not have floor effects for non-verbal individuals seen with standardized measures. It is likely that an important effect of the interventions (both LLI and AFQ056) will be to increase social engagement and the frequency of participant communicative attempts and thus, improvement is possible even for participants who enter with sophisticated language. In addition, it is possible to extend the upper bound of the score if we find that the complexity of multiword combinations participants use changes (e.g., by adding a number rating point for communicative attempts consisting of more than three words), Thus ceiling effects are not expected.

The Weighted Communication Score coded from a standardized play session has been used in cohorts with autism spectrum disorder (ASD) and has been able to measure change in language skills with age[74] and change in language skills in response to an intervention.[75] Although there is not specific data on this measure in FXS, it is not possible to validate every outcome specifically for FXS given the rarity of the disorder. Communication and language issues are similar in FXS and ASD so the measure is likely to perform similarly in response to an intervention in children with FXS.

9.5.2 Key Secondary Outcome(s)

Functional Key Secondary Outcomes: One key secondary objective of this study is to show greater improvement in specific standardized language, cognitive, and adaptive measures (see Endpoints Derived from Evaluations Section). To address this objective, a number of key secondary functional endpoints will be obtained (using all raw scores, except as noted):

- Mullen Scales of Early Learning (MSEL)
  - Development Quotient (DQ)
  - Expressive Language Subscore
- Vineland Adaptive Behavior Scale – Version 3
  - Composite Score
  - Communication Subscore
- Preschool Language Scale – 5th Edition
  - Expressive Communication Score
- McArthur-Bates Communication Development Index
  - Number of Spoken Words
- Clinical Global Impression-Improvement (CGI-I)
  - Overall Function

Although the primary outcome was chosen to be the likely most sensitive measure to the intervention, albeit less validated, the additional key secondary functional outcomes were chosen to be the measures most validated, accepted and utilized in the FXS population, albeit potentially somewhat less sensitive to change. This should allow exploration of both novel and sensitive strategies for measuring outcome as well as standard strategies. The secondary measures chosen will most reliably assess broad domains of cognitive, language, adaptive and maladaptive functioning. It is anticipated that gains in language will result in broader improvement in overall functioning in multiple domains, and it is possible the AFQ056 will target learning in domains outside of those covered by the language intervention, although it is hard to predict which domains will be most impacted. Thus, the mostly broadly used and well-validated general standardized measures for the cognitive, language, adaptive and maladaptive domains were chosen as key functional secondary outcomes.

The Mullen Scales of Early Learning (MSEL) has been used to track developmental progress of children with FXS in longitudinal studies and to characterize cohorts of young children with FXS for many years [77-79]. More recently the MSEL was analyzed in a retrospective chart review to evaluate effects of early sertraline treatment on development in FXS[106] and has been used in a 6 month placebo-controlled clinical trial of sertraline in young children with FXS.[107]

The Vineland Adaptive Behavior Scale (Vineland, all editions) has been used extensively to track progress of children with FXS in large longitudinal studies[10] and to characterize cohorts of patients with FXS both for clinical trials and other studies.[61,84,114,115] The Vineland-II has been used in a phase 2 trial of arbaclofen and showed improvement in the Socialization subscore in a post-hoc analysis of the more socially impaired subgroup of FXS participants[116] and the Daily Living subscore showed significant improvement in a recent placebo-controlled phase 2 trial of metadoxine in FXS.[117]

The Preschool Language Scale, now on Version 5 (PLS-5), has been used to track language progress of children with FXS in longitudinal studies and to describe the language profile of patients with FXS for many years.[108,109]

The MacArthur-Bates Communicative Development Inventory gives an opportunity to make sure language data is acquired even for children uncooperative with assessment, as it is a parent interview that allows tracking of the child's progress in developing words, sentences, and more complex language. This involves a parent-report measure that has been well-validated against direct observation and assessment measures of typical child language status and growth. This measure has been used to track language developmental trajectory in young children with ASD[110,111] as well as other developmental disabilities[112,113], and has been shown effective in characterizing language change in populations with limited communication abilities.

The Clinician Global Impression (CGI-I) assesses clinician or caregiver impression of global functioning or a specific symptom to which the CGI-I is anchored. Collection of global clinician and parent impressions allow mapping of specific responses to more general perception of function, and give a sense of whether changes on more specific measures are meaningful. The CGI-I has been used in virtually all prior trials in FXS to assess global change in various pre-specified target behaviors or function.[115] Standardized training modules exist for training investigators to reliability on this measure. The CGI has been sensitive to change in the ITT population in a placebo-controlled trial of minocycline in FXS[121] and in post-hoc subgroup analyses of placebo-controlled trials of arbaclofen)[84] and AFQ056.[122]

9.5.3 Other Secondary & Exploratory Outcomes

Safety Endpoints
The number of adverse events (AEs) and serious adverse events (SAEs) will be tracked over time. Counts will be statistically compared among the AFQ056 and placebo groups to evaluate safety in regards to whether levels are statistically equivalent over time. This will be looked at separately during the main study phase and extended study phase. In order to evaluate safety, the following will be assessed.

- Safety measures including clinically significant laboratory values and adverse events tabulated for descriptive comparison between the placebo and active drug groups.

- Tolerability, defined as the ability to at least continue treatment after the dose has been reduced to the lowest allowed dose (12.5 mg) due to side effects. The necessity to discontinue treatment after dose reduction to 12.5 mg would be considered lack of tolerability.

Biomarker Exploratory Outcomes: Another key secondary objective of this study is to show improvement in specific measures from ERP biomarkers in the combination of the AFQ056/language intervention group relative to those in the language intervention/placebo group. To address this objective, a number of key secondary biomarker endpoints will be obtained:

- ERP
  - Auditory habituation first 15% versus last 15% for amplitude of pediatric p50/N1 (P1) peak occurring at approximately 100 ms post-stimulus
  - Amplitude of auditory response at three components: pediatric p50/N1, N2, and P2
- Eye Tracking
  - Percent of looking time to eyes (all conditions)
  - Number of fixations to eyes (all conditions)

The major key secondary biomarker outcome was chosen as auditory habituation on ERP given the test is abnormal in FXS, it directly measures cortical activity which is what the drug is proposed to change, auditory processing is linked to language functioning, and it has shown improvement in a prior trial of minocycline.[91] Abnormal enhanced cortical auditory responses are seen in the FXS mouse.[93] Further, MPEP (mGluR5 NAM) was shown to reduce N1 auditory EP amplitudes in a mouse model of autism, suggesting mGluR5 involvement in modulation of this response.[103] Habituation of N1, P2, N2 component s of auditory ERP has been studied in FXS and these components were shown to be significantly enhanced in 16 males with the FXS full mutation relative to 20 age-matched controls.[92] By focusing on the changes in the N1, P2, and N2 components (relative to the common average reference) of subject ERP signals, between the first and last 45 stimuli presented, Schneider et al. showed that there was abnormal electrocortical habituation to auditory stimuli in patients with FXS, and this improved at N1 and P2 in individuals with FXS who were given minocycline treatment compared to a control group.[91] Data from the ERP recording will also be subjected to analyses of MMN and resting state (when obtainable) as an exploratory marker as no data currently exists on these measures in FXS.

Eye tracking was chosen as an additional key secondary biomarker outcome because it is abnormal in FXS,[97,98] it corresponds well to a key phenotype of gaze aversion in FXS, it has strong feasibility and test-retest reliability[97], it might be expected to improve if language improves, as eye

gaze is often used to initiate communication, and most importantly, it improved in the phase IIb placebo-controlled trial of AFQ056 in adolescents and adults[125] suggesting it is sensitive to treatment with and may provide an index of disease reversal by mGluR5 NAMs. Eye Tracking has good feasibility and reproducibility in FXS[97] and has been used in several targeted treatment trials for older individuals with FXS.

Other Functional, Biomarker and Exploratory Outcomes

A number of additional secondary and exploratory outcomes will be collected and assessed. These objectives include, but are not limited to the following:

(1) Explore whether greater improvement occurs in additional language, cognitive, adaptive, and behavioral measures in the combination AFQ056/language intervention group relative to the language intervention/placebo group.
(2) Examine long-term safety and tolerability of AFQ056 in young children with FXS.
(3) Explore whether improvement can be shown in multiple additional measures from the ERP and eye tracking in the combination AFQ056/language intervention group relative to the language intervention/placebo group.
(4) Explore whether normalization of eye tracking/pupillometry biomarkers is correlated with improvement in clinical outcome markers including language.
(5) Explore whether normalization of blood biomarkers of translational pathways regulated by FMRP and mGluR5 receptors is associated with language, functional, and/or biomarker improvements from AFQ056 treatment.
(6) Explore whether blood levels of AFQ056 correlate with improvement in language, functional, or biomarker measures.
(7) Explore whether longitudinal analysis shows an improvement in the rate of change of language, functional, or biomarker responses with the language intervention alone, and further improvement in rate of change when AFQ056 is added to the language intervention.

In order to explore these additional objectives, the following outcomes will also be assessed:

- Additional Functional Outcomes
  - Weighted Communication
    - Structured play score
    - Nonstructured play score
  - Preschool Language Scale – 5th Edition (PLS-5)
    - Auditory Comprehension Subscore
    - Total Language Score
  - Mullen Scales of Early Learning (MSEL)
    - Receptive Language Subscore
    - Visual Reception Subscore
    - Gross Motor Subscore
    - Fine Motor Subscore
  - Vineland Adaptive Behavior Scale – Version 3
    - Communication
    - Daily Living Skills
    - Socialization
    - Motor
  - Aberrant Behavior Checklist – Fragile X Validated Version (ABC-FX)
    - Irritability Subscore
    - Stereotyped Behavior Subscore
    - Abnormal Speech Subscore

        - Hyperactivity Subscore
        - Social Avoidance Subscore
        - Lethargy Subscore
    - Visual Analog Scale (VAS) of Target Behavior (1, 2, & 3 sum)
    - Visual Analog Scale (VAS) of Language
    - Clinical Global Impression-Improvement (CGI-I)
        - Language
    - Clinical Global Impression-Severity (CGI-S)
        - Overall Function
        - Language

- Additional Biomarker Outcomes
    - ERP
        - Auditory Habituation first 15% versus last 15% for amplitude of each of N2 and P2 peaks
        - Resting State – Power in Alpha band, power in Gamma band, and Alpha Reactivity Index (Eyes Open vs. Eyes Closed)
        - Mismatched Negativity Amplitude
    - Pupillometry
        - Maximum Relative Pupillary Diameter Change after each type of emotional stimulus (Calm, Happy, and Fearful)
    - Eye Tracking
        - Percent Looking Time for each of three Individual types of emotional stimulus (Happy, Calm, and Fearful)
        - Number of Fixations for each of three Individual types of emotional stimulus (Happy, Calm, and Fearful)

The Aberrant Behavior Checklist- Community Edition (ABC-C) has been used in virtually all prior trials in FXS to assess maladaptive behavior.[115] This assessment has been validated in populations with intellectual disability and improvements in this scale were used to support FDA approval of Risperdal[118] and Abilfy[119] for management of irritable behavior in ASD. The ABC-C was subjected to validation and factor analysis based on over 600 ratings in FXS and found to factor into 6 domains instead of 5.[89] Since that time the ABC using the FX-specific factor analysis (ABC-FX) has been used in FXS trials. The ABC-FX has been sensitive to change in post-hoc subgroup analyses of placebo-controlled phase 2 trials of arbaclofen (Berry-Kravis 2013) and AFQ056 (Jacquemont 2013) and in the ITT group at the highest dose in a phase 3 trial of arbaclofen.[120]

Visual Analog Scales (VAS) have been used in multiple clinical trials in FXS to measure parent assessment of the level of various problems behaviors or specific functional skills[115], and the VAS for problem behaviors was sensitive to change in a phase 2 study of arbaclofen in FXS.[84]

Exploratory biomarker outcomes represent key proteins in the signaling pathways regulated by FMRP and thus may normalize with reduction in mGluR5 activity by AFQ056. It is recognized that mGluR5 receptors may not regulate signaling cascades similarly in blood cells and brain, and this is a major reason these measures are considered exploratory. If there were a correlation between one of these markers and clinical response, however, this finding would be extremely helpful to the field, since currently no proven pathway-based cellular or molecular biomarkers of treatment response exist. Hence this trial has included the most promising of the measures that interrogate the mGluR5 pathway being targeted in the trial.

9.6 Statistical Analysis Plan

9.6.1 Primary Hypothesis

Primary Efficacy Hypothesis: ***Children treated with AFQ056 will show greater improvement in language and its nonverbal communicative precursors as reflected in on change in a weighted communication score designed to assess both the proclivity to engage in communication and the sophistication of the means employed for communication from baseline (visit 3 assessment) to 8 months (visit 9 assessment) than those treated with placebo.***

The study design is longitudinal involving unequally spaced data collections over the placebo-controlled phase at 0 (baseline), 2, 4, and 8 months (see Figure 2). Focus is on the difference in the rate of change among the placebo (PL) and treatment (TX) groups. To account for the model design and potential dropout, linear mixed models (LMMs) will be used for the analysis. Suppose that Y_{ij} is the outcome (e.g., language score) for the i^{th} participant ($i = 1, \ldots, N$) at the j^{th} time point ($j = 1, \ldots, T_i$). Assuming linear change over time, the LMM can be written as:

$$Y_{ij} = \beta_0 + \beta_1 t_{ij} + \gamma_0 g_i + \gamma_1 t_{ij} g_i + (b_{0i} + b_{1i} t_{ij} + e_{ij}) \quad (1),$$

where $g_i = 1$ if the participant is in TX and 0 if in PL, t_{ij} is time in months, b_{0i} and b_{1i} are random effects, e_{ij} is random error, and the remaining parameters are fixed effects. We make the typical normality and related assumptions for the random effects and random error.[99] Due to randomization, there is no average intercept difference and we set $\gamma_0 = 0$. The mean longitudinal change is then: for PL is then:

$$\text{For PL: } E(Y_{ij})_{PL} = \beta_0 + \beta_1 t_{ij} \quad (1a),$$

$$\text{For TX, } E(Y_{ij})_{TX} = \beta_0 + (\beta_1 + \gamma_1) t_{ij} \quad (1b).$$

The target of inference is γ_1 that represents the mean difference in rate of change among the groups. For the primary language endpoint, treatment efficacy occurs when $\gamma_1 > 0$, because language is acquired at a faster rate for TX (cf. Equations 1a and 1b). Therefore, the primary null hypothesis for the analysis is:

$$H_0: \gamma_1 = 0.$$

The LMM is estimated with maximum likelihood, which provides unbiased estimates under an ignorable missing data mechanism.[100] The likelihood ratio test (LRT) will be used to evaluate H_0 by comparing the full model of Equation 1 with a reduced model that omits γ_1 (cf. Equations 1a and 1b). There is a one-parameter difference among the models, and the LRT test statistic, χ^2_1, has an asymptotic chi-squared distribution. Evidence of treatment efficacy is provided when $\chi^2_1 > \chi^2_{1,1-\alpha}$, the latter being the upper $1 - \alpha$ chi-squared quantile, which leads to the rejection of H_0. An alternative index of efficacy relevant for this proof-of-concept proposal is the difference in Akaike's information criterion (AIC) among the models.[101] The AIC is an unbiased estimator of -2 times the mean expected log likelihood, and the difference in AIC among two models is their statistical distance [102]. Suppose AIC(full) is the sample AIC for the estimated full model of Equation 1, and AIC(reduced) is that of the reduced model that omits γ_1. Smaller AIC values indicate better statistical fit, so evidence of efficacy is provided when ΔAIC = AIC(reduced) $-$ AIC(full) > 0 because the model with $\gamma_1 > 0$ has better fit than the model with $\gamma_1 = 0$.

The open label phase of the study has both groups receiving treatment (see Figure 2). Change during this phase will also be analyzed using a separate LMM. Focus will again be on the PL and TX slope difference, but the intercept difference will be non-zero ($\square_0 \neq 0$) due to the possibility of a treatment effect in the treatment phase (i.e., an inequality at the end of the treatment phase). The group slope difference might not be statistically significant if the PL group catches up to the TX group during the open level phase. Therefore, the open label phase analysis is considered exploratory.

In the placebo group, LMM analyses will be used to compare trajectory of developmental progress during the 6 months on placebo alone and during the 6 months receiving the language intervention while on placebo. This will generate information about the efficacy of the language intervention alone. Trajectory of developmental progress in these two periods will be compared with trajectory in the 8 months after the placebo group begins AFQ056 in the open label extension.

9.6.2 Key Secondary Hypotheses

For the key secondary hypotheses, a high level statistical analysis plan is described below. Further details and specifications regarding the assessment of each hypothesis will be provided in a formal statistical analysis plan prior to data lock.

Functional Key Secondary Hypotheses: ***Children treated with AFQ056 will show greater normalization of specific measures of language and one or more other domains (including cognitive, adaptive behavior, global functioning based on change from baseline (visit 3 assessment) to 8 months (visit 9 assessment) than those treated with placebo.***

All of the key secondary functional outcomes are measured on scales similar to that of the weighted communication score primary outcome. All key secondary functional hypotheses generally state that there will be greater improvement in language, communication, cognitive, developmental, and adaptive functioning in the AFQ056-treated group over the placebo group during an 8-month period [baseline (visit 3 assessment) to 8 months (visit 9 assessment)]. Each scale produces a numeric score that can be assessed in a manner similar that described for the assessment of the primary endpoint in section 9.6.1.

9.6.3 Exploratory Hypotheses

Biomarker Exploratory Hypotheses: ***Children treated with AFQ056 will show greater improvement of these specific ERP and eye tracking measures based on change from baseline (visit 3 assessment) to 8 months (visit 9 assessment) than those treated with placebo.***

For the biomarker assessments, the ERP requires data processing centrally but eventually provides a score that can be assessed in a manner similar to that described for the assessment of the primary endpoint in section 9.6.1. The eye tracker produces two important outputs relevant to these hypotheses – the percent of looking time to eyes and the number of fixations to the eyes. We expect both to be essentially continuous variables, and feel confident that they can also be assessed in a manner similar to that described in section 9.6.1.

A number of additional exploratory hypotheses can be considered using the data produced from this study. These will include, but are not limited to:

- Examine whether the same effects observed for the overall population are also observed for the group of males only enrolled into the study. With the anticipated enrollment of about 80 males and 20 females, power should be sufficient to analyze the male-only group.

- Examine whether one or more blood biomarkers will be abnormal in FXS, and will normalize in children treated with AFQ056 relative to those treated with placebo.

- Biomarker (ERP, eye tracking/pupillometry, molecular/cellular) responses, if present will be evaluated for correlation with responses on clinical measures and with drug levels from PK determinations, in order to determine whether biomarkers track with and predict clinical and/or pharmacological responses to provide clinical and pharmacological validity data for future work.

- Examine whether blood levels of AFQ056 will correlate with improvement in language, functional, and biomarker measures relative to baseline throughout the study.

- Clinical responses and side effects presumed likely due to AFQ056 will be evaluated for correlation with drug levels from PK analyses.

- Longitudinal analysis to determine whether the rate of change in language, functional, or biomarker responses in the group assigned to placebo will be lowest during the first 6 months of the study (Screening to visit 5/month 2), higher in the next 6 months with the language intervention (visit 5/month 2 to visit 9/month 8), and highest in the final 8 months with both the language intervention and AFQ056 treatment (visit 9/month 8 to visit 14/month 16) – with inflection points at visit 5/month 2 when the language intervention is started and visit 9/month 8 when AFQ056 is started.

- Analyses of change from baseline at the 2 month visit (visit 5) will be evaluated for all clinical and biomarker outcomes, to determine if effects of drug alone can be identified before the language intervention is initiated. Likewise analyses of change from baseline and from the final visit before weaning treatment with AFQ056 (16 month visit, visit 14) at the Follow Up visit (visit 15) will be evaluated for all clinical and biomarker outcomes, to determine if regressive effects of coming off drug are evident. Covariates in the analyses will be medication (coded as a factor with a small number of levels), age, and additional variables that are shown to be imbalanced through unlucky randomization.

9.7 Safety Analysis

Secondary analyses to examine long-term safety of AFQ056 in this cohort of young children with FXS will be done as follows. Frequencies of adverse events occurring throughout the entire treatment period (both clinical and laboratory) will be tabulated and compared between the active drug and placebo groups during the placebo-controlled period of the study (8 months) and compared across the entire study duration divided based on treatment-assignment at the time of the AE. Low frequency AEs occurring in less than 5% of the cohort and those that are not different between the drug and placebo groups by visual inspection will not be analyzed further. Higher frequency events for which statistical comparison would be possible and that appear to be occurring at different frequencies between the drug and placebo groups for either set of comparator groups will be formally compared statistically using a chi-squared analysis. Longitudinal analyses will be done for AEs occurring more frequently in the AFQ056-treated group to determine if there is an increase over time of treatment in these AEs.

9.8 Sample Size and Accrual

Estimated required sample size was calculated for the primary endpoint of language. Data from the observational study of[126] was used, and additional estimates of effect size (no raw data) were from an unpublished pre-post randomized clinical trial (RCT). Winarni et al.[126] extracted data from chart reviews for 45 FXS children age 12-50 months. Eleven children were treated with sertraline, and the remainder of 34 were not. Variance components of the random effects and error were estimated and sample size was computed using analytic formulas for LMMs[127]. Recall that in the LMM of Equation 1, γ_1 is the difference of the slopes, and it is defined as the proportionate increase of the PL slope, β_1, due to the efficacy of TX (if there is any). We write, $\gamma_1 = \pi\beta_1$, where π is the *effect proportion*. For example, if $\pi = 1$, Equation 1a shows that the TX slope is twice the PL slope, because $\beta_1 + \gamma_1 = \beta_1 + (1)\beta_1 = 2\beta_1$. The analysis used the expressive language and receptive language subscales (age-equivalent scores) of the Mullen Scales of Early Learning[124]. The effect proportion (π) was estimated considering the on-sertraline group ($N = 11$) as a proxy for TX, and the off-sertraline group ($N = 34$) as a proxy for PL. The effect proportion was also estimated using slope estimates from unpublished data in a RCT of sertraline efficacy.[103] The RCT used a pre-post design over 6 months to study the effect of sertraline versus placebo with FXS children with enrolled age of 24-72 months. Estimates of the effect proportion for the Winarni et al. data were $\hat{\pi} = 1.27, 2.66$ for expressive language and receptive language respectively, and the estimates from the clinical trial were $\hat{\pi} = 1.43, 1.52$. For the analysis, power was set to 80%, and Type I error rate (α) was determined as the following. Because this is a proof-of-concept (Phase IIa) proposal with a novel design for a critical population (children with FXS), the α-level was set to allow for a sensitive detection of efficacy, defined as $\Delta AIC > 0$ (see statistics section above). It can be shown that $\Delta AIC = \chi^2_1 - 2$, so that using $\chi^2_{1,1-0.16}$ as a critical value for the LRT will allow for detection of $\Delta AIC > 0$. In addition to $\alpha = 0.16$, sample size was calculated with $\alpha = 0.10$ because this level is often used in Phase II studies. Figure 3 shows the function of π and α. A filled circle indicates the required N for the smallest effect proportion among the studies, $\min(\hat{\pi}) = 1.43, 1.27$ (expressive, receptive). The figure shows that for the combination of the smallest effect proportion ($\hat{\pi} = 1.27$) and the smallest α, the required single-arm $N = 50$ (expressive language). We consider this to be an upper bound on the required sample size, so that a total of $N = 100$ (50 in each group) should be more than sufficient to detect any magnitude of efficacy (i.e., $\Delta AIC > 0$). Because of high parental motivation, the dropout rate is anticipated to be very low. However, the $\alpha = 0.16$ panel of the figure indicates that with total N = 100, power will still be sufficient to detect $\Delta AIC > 0$ with as much as 20% dropout ($100 - 0.2 \times 100 = 80$ or 40 in each group for expressive language).

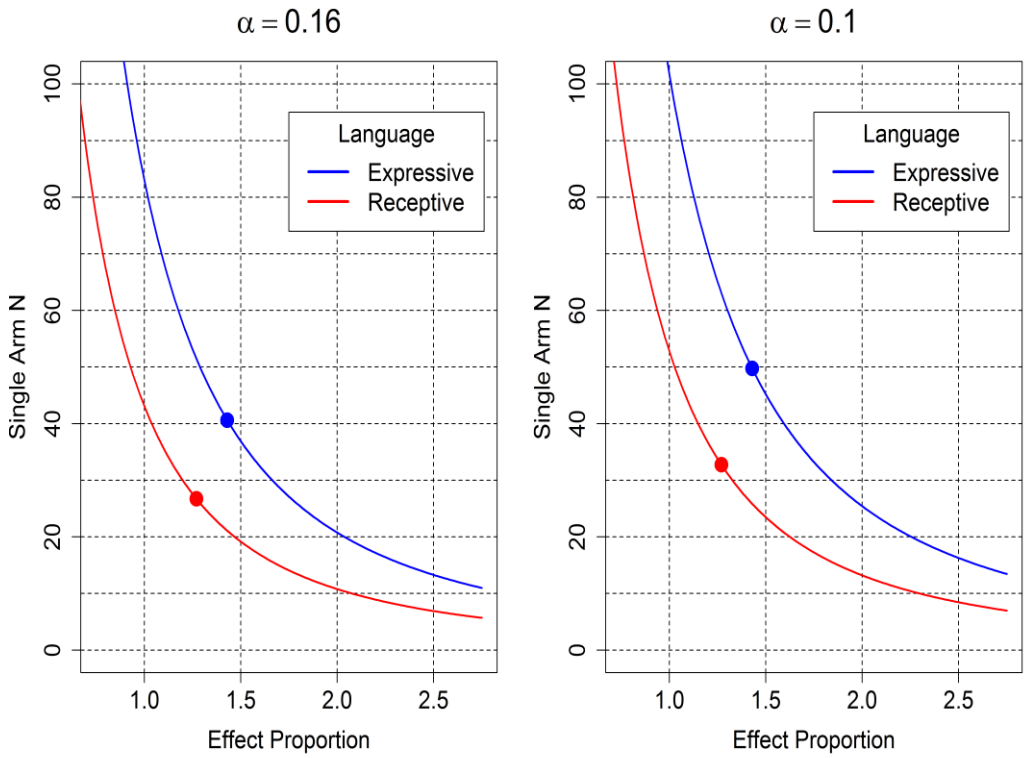


Figure 3. *Single-arm sample size as a function of effect proportion, type of language measure, and Type I error rate. Filled circles indicate N for the smallest effect proportion among studies.*

9.9 Data Monitoring

All aspects of the study will be monitored by qualified individuals designated by the sponsor. Monitoring will be conducted according to Good Clinical Practice and applicable government regulations. The investigator agrees to allow monitors access to the clinical supplies, dispensing and storage areas, and to the clinical files of the study subjects, and, if requested, agrees to assist the monitors.

Safety monitoring will include careful assessment and appropriate reporting of adverse events. Medical monitoring will include contemporaneous assessment of serious adverse events.

The monitoring of subject safety and data quality will follow the NINDS Guidelines for Data and Safety Monitoring in Clinical Trials. A Data and Safety Monitoring Board (DSMB) appointed by the NIH/NINDS will meet at six-month intervals (or as determined by the NINDS) to review partially unblinded study data provided by the study statistician. This committee will monitor rates of adverse events and endpoints in the trial and will monitor the performance of the trial. The frequency and format of DSMB meetings, reports, and guidelines for interim analysis will be agreed upon prior to study subject enrollment.

The Protocol PI will appoint an Independent Medical Monitor (IMM) to review all adverse events, in a blinded fashion, on a periodic basis. In addition, the IMM will review all events that meet the regulatory definition of a Serious Adverse Event, upon receipt of notification via the Electronic Data Capture (EDC) system.

An adverse event (AE) is any unfavorable and unintended sign (including a clinically significant abnormal laboratory finding, for example), symptom, or disease temporally associated with a study, use of a drug product or device whether or not considered related to the drug product or device. FDA, Office of Human Research Protection (OHRP), and NeuroNEXT CIRB requirements for reporting AEs will be followed. Subjects will be monitored for AEs from the time they sign consent until 30 days following permanent discontinuation of study drug. At that point, all ongoing AEs will be followed to resolution, but no new AEs will be recorded. The IMM/DSMB will review cumulative AEs; the frequency of this review will be determined by the IMM/DSMB in conjunction with the Protocol PI.

Subjects will be monitored for AEs from the time they sign consent until the final Study visit. If an AE is ongoing or discovered at a subject's final Study visit, the AE will be followed until resolution, or for a minimum of 30 days, whichever comes first. Resolution means that the subject has returned to status at enrollment. If the AE is still ongoing after 30 days, it will be resolved with sequelae. All AEs monitored past the final visit can be followed via telephone – a repeat Termination/Final Follow-up visit does not need to occur.

All SAEs must be followed until resolution throughout the trial. SAEs that are discovered less the 60 days prior to the subject's final Study visit will be followed until resolution or for a minimum of 60 days, whichever comes first, even if it is past the final visit. If a new SAE is discovered at the subject's final Study visit, it must be followed until resolution, or for a minimum of 60 days, whichever comes first. If the SAE is still ongoing after 60 days, it will be resolved with sequelae. All SAEs monitored past the final visit can be followed via telephone – a repeat Termination/Final Follow-up visit does not need to occur.

Each Clinical Study Site Investigator and research team (co-Investigators, research nurse, clinical trial coordinator) are responsible for identifying and reporting AEs and determining the relationship of the event to the study drug. Aggregate reports blinded by treatment group, detailed by severity, attribution (expected or unexpected), and relationship to the study drug, will be available from the DCC for review by the IMM. A separate report detailing protocol compliance will also be available monthly from the DCC for review by the Protocol PI, who will provide feedback to individual sites as needed. The Protocol Steering Committee (PSC) will advise the Protocol PI as to whether the protocol or informed consent document requires revision based on these reports.

10 DATA COLLECTION, SITE MONITORING, AND ADVERSE EXPERIENCE REPORTING

10.1 Data Management

Site personnel will collect, transcribe, correct, and transmit the data onto source documents, CRFs, and other forms used to report, track and record clinical research data. The DCC will monitor clinical sites to ensure compliance with data management requirements and Good Clinical Practices. The DCC is responsible for developing, testing, and managing clinical data management activities, as required, at the study sites, the CCC, and at the DCC.

The general NINDS Common Data Elements (CDE) will be used to construct data collection forms. All study data will be collected via systems created in collaboration with the DCC and will comply with all applicable guidelines regarding patient confidentiality and data integrity.

10.1.1 Registration

Registration of participants on this protocol will employ an interactive data system in which the clinical study site will attest to the participant's eligibility as per protocol criteria and obtain appropriate informed consent. NeuroNEXT CIRB approval for the protocol must be on file at the DCC before accrual can occur from the clinical study site.

The DCC will use a system of coded identifiers to protect participant confidentiality. When the participant is registered to participate in the study using the DCC-provided web-based registration, the system will assign a participant ID number. The unique ID code will include a site ID, and a unique participant ID. To confirm the correct participant ID, the data entry system will require a second entry of the unique participant ID and compare for consistency. In this fashion, no personal identifiers would be accessible to the DCC and the data will be collected on the correctly identified subject.

10.1.2 Data Entry

Data entry will occur at the enrolling clinical study sites. Data quality assurance and analyses will be performed by the DCC. The DCC, located at the University of Iowa, will coordinate all data and statistical services for the study, as well as on-site monitoring for all participating clinical study sites.

Data collection for this study will be accomplished with online electronic case report forms. Using encrypted communication links, online forms will be developed that contain the requisite data fields.

10.2 Role of Data Management

Data Management (DM) is the development, execution and supervision of plans, policies, programs, and practices that control, protect, deliver, and enhance the value of data and information assets.

All data will be managed in compliance with NeuroNEXT policies, and applicable Sponsor and regulatory requirements. The DCC will instruct site personnel to collect, transcribe, correct, and transmit the data onto source documents, CRFs, and other forms used to report, track and record clinical research data. The DCC will monitor clinical sites to ensure compliance with data management requirements and Good Clinical Practices. The DCC is responsible for developing, testing, and managing clinical data management activities, as required, at the clinical study sites (CSS), the CCC, and at the DCC.

The DCC is responsible for all aspects of clinical data management, and for properly instructing key study personnel (including the CCC, the CSS, and DCC staff) on how to collect, transcribe, correct and transmit the data onto CRFs or other data collection forms and logs.

The DCC is responsible for establishing procedures to ensure that clinical data management activities occur as required at the CCC, the CSS, and at the DCC.

10.3 Quality Assurance

By signing this protocol, the Sponsor and Investigator agree to be responsible for implementing and maintaining quality control and quality assurance systems with written standard operating procedures (SOPs) to ensure that studies are conducted and data are generated, documented,

and reported in compliance with the protocol, accepted standards of GCP, and all applicable federal, state, and local laws, rules and regulations relating to the conduct of the clinical study.

10.3.1 Development of Monitoring Plan

Onsite and remote monitoring visits will be conducted by DCC monitors according to a pre-defined Monitoring Plan. The monitoring plan will detail the frequency of on-site visits and remote review of source documentation and CRFs, the study data to be monitored, the review of any regulatory files, drug and supplies accountability (if applicable), documentation of remote reviews and on-site visit, and the resolution process for data errors that are discovered during visits and remote reviews. All participating clinical study sites will be monitored at least once after a study initiation visit and all sites will have a close-out visit for each protocol. Two remote reviews are anticipated for each clinical study site per year. All subjects will be monitored for inclusion and exclusion criteria, informed consent procedures, and adverse events. A certain percentage of data is also monitored/ source data verified against the data entered into the study database. The monitoring plan will include flexibility to revise the frequency of visits/remote reviews, or data monitored depending on clinical study site or study related issues.

10.3.2 Site Monitoring Visits

On-site monitoring visits will be conducted by DCC monitors according to a pre-defined monitoring plan for each protocol. The goal of on-site monitoring is to analyze (review) the data as it is collected, to check the validity and integrity of the data, to verify source documentation, to ensure protection of human subjects, and to ensure protocol compliance with federal regulations. During the monitoring visit, the monitor assesses the overall status of the study, staff, and facilities to determine whether the study is being conducted per protocol and in compliance with regulatory requirements. The monitor also conducts a CRF review that includes checks of all adverse event documentation, verifies the presence of all critical correspondence and records related to investigational products and clinical supplies (if applicable), and determines if protocol violations have occurred and are documented properly. After the monitoring visit, the monitor documents the results of the monitoring visit and completes a post-visit monitoring letter that conveys any issues discovered during the visit and the need for data corrections, if appropriate. Drug and supplies accountability may also be monitored during the site visit. The DCC will work closely with the CCC to monitor and document drug distribution from the manufacturers to the clinical study sites (CSS). Each CSS will be provided with a drug accountability log which will be reviewed by the DCC monitors and reconciled with distribution logs. At the study closeout visit, the monitors confirm that appropriate data have been reviewed, source documentation has been verified, and all required documents are present in the Study Regulatory File.

10.3.3 Remote Monitoring Visits/Review

DCC monitors and data managers will also perform remote review of source documentation, CRFs and investigational product accountability to achieve the goals described in section 10.3.2. Remote review involves a range of data management procedures (queries, edit checks, review of missing data, etc.), as well as more traditional monitoring procedures conducted remotely through the exchange and review of source documentation and CRFs. DCC monitors will request access to source documentation and CRFs through the following methods:

1. Remote review of electronic medical records
2. Site upload of source documentation and CRFs to a secure portal hosted and managed by the DCC.
3. Site upload of source documentation and CRFs to a secure portal hosted and managed by the site.

DCC monitors will contact site staff as early as possible in the course of a trial to arrange remote access to source documentation and CRFs. Sites are required to comply with DCC requests for remote access.

Additionally, the DCC will conduct remote reviews of investigational product accountability and storage conditions after an initial on-site visit to each site's pharmacy. DCC monitors will request accountability records and temperature logs from CSS coordinators or study pharmacists in order to complete these remote reviews.

10.3.4 Laboratory Data Flow

Safety Monitoring Labs: The DCC will provide laboratories with online forms and/or electronic data exchange mechanisms - depending on their capabilities and needs - to enter, update and obtain relevant data. When a blood sample has been obtained, the clinical study site study coordinator will send the sample (participant ID, site ID, and protocol ID numbers will be used) to the University of Rochester Central Laboratory. The laboratory will electronically communicate the test results to the respective clinical study sites in a secure manner. The laboratory will also transfer test results electronically to the DCC.

10.4 Adverse Experience Reporting

The adverse event (AE) definitions and reporting procedures provided in this protocol comply with all applicable United States Food and Drug Administration (FDA) regulations and International Conference on Harmonization (ICH) guidelines. The Site Investigator will carefully monitor each subject throughout the study for possible adverse events. All AEs will be documented on CRFs designed specifically for this purpose. It is important to report all AEs, especially those that result in permanent discontinuation of the investigational product being studied, whether serious or non-serious.

Subjects will be monitored for AEs from the time they sign consent until the final Study visit. If an AE is ongoing or discovered at a subject's final Study visit, the AE will be followed until resolution, or for a minimum of 30 days, whichever comes first. Resolution means that the subject has returned to status at enrollment. If the AE is still ongoing after 30 days, it will be resolved with sequelae. All AEs monitored past the final visit can be followed via telephone – a repeat Termination/Final Follow-up visit does not need to occur.

All SAEs must be followed until resolution throughout the trial. SAEs that are discovered less the 60 days prior to the subject's final Study visit will be followed until resolution or for a minimum of 60 days, whichever comes first, even if it is past the final visit. If a new SAE is discovered at the subject's final Study visit, it must be followed until resolution, or for a minimum of 60 days, whichever comes first. If the SAE is still ongoing after 60 days, it will be resolved with sequelae. All SAEs monitored past the final visit can be followed via telephone – a repeat Termination/Final Follow-up visit does not need to occur.

Each clinical study site's Principal Investigator and research team are responsible for identifying adverse events and reporting them through the DCC Online Adverse Event Reporting System. Investigators are also responsible for complying with NeuroNEXT CIRB's reporting requirements for all safety reports. Copies of each report and documentation of IRB notification and receipt will be kept in the investigator's study file.

An adverse event is defined as: "…an unfavorable and unintended sign, symptom, or disease associated with a participant's participation in this research trial."

Serious adverse events include those events that: "result in death; are life-threatening; require inpatient hospitalization or prolongation of existing hospitalization; create persistent or significant disability/incapacity, or a congenital anomaly/birth defects."

Unexpected adverse event is defined as any adverse experience…the specificity or severity of which is not consistent with the risks described in the protocol.

Expected adverse events are those that are known to be associated with or have the potential to arise as a consequence of participation in the study.

On-line Adverse Event Reporting System

Upon entry of a serious adverse event by a site investigator, the DCC Online Adverse Event Reporting System will immediately notify the Independent Medical Monitor (IMM).

- Within **24 hours** (of learning of the event), investigators must report any Serious Adverse Event (SAE). Investigators should report all other AEs within **5 working days/7 calendar days** (of learning of the event).

Serious adverse events: The site investigator determines causality (definitely not related, probably not related, possibly related, probably related, definitely related) of the adverse event. The IMM will review the SAE report. The IMM may request further information if necessary. The Online Adverse Event Reporting System maintains audit trails and stores data (and data updated) and communication related to any adverse event in the study. The IMM may determine that the Serious Adverse Event requires expedited reporting to the FDA. The DCC will prepare a MedWatch safety report for submission to the FDA. If warranted, the IMM will notify the DSMB chair. The DSMB may suggest changes to the protocol or consent form to the Study Chair as a consequence of adverse events.

Non-serious adverse events: Non-serious adverse events that are reported to or observed by the investigator or a member of his research team will be submitted to the DCC in a timely fashion (within 5 working days). The events will be presented in tabular form and given to the IMM on a quarterly basis or as requested. Local site investigators are also required to fulfill all reporting requirements of their local institutions.

The DCC will prepare aggregate reports of all adverse events (serious/not serious, expected/unexpected and relationship to study drug) for the IMM and the DSMB on a quarterly basis or as requested. In addition, all adverse events will be coded using the MedDRA system. A separate report detailing protocol compliance will also be available from the DCC for DSMB and/or site review monthly or as requested. The research team will then evaluate whether the protocol or informed consent document requires revision based on the reports.

10.4.1 Definitions of Adverse Events, Suspected Adverse Drug Reactions & Serious Adverse Events

10.4.1.1 Adverse Event and Suspected Adverse Drug Reactions

An adverse event (AE) is any unfavorable and unintended sign (including a clinically significant abnormal laboratory finding, for example), symptom, or disease temporally associated with a

study, use of a drug product or device whether or not considered related to the drug product or device.

Adverse drug reactions (ADR) are all noxious and unintended responses to a medicinal product related to any dose. The phrase "responses to a medicinal product" means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility, i.e., the relationship cannot be ruled out. Therefore, a subset of AEs can be classified as suspected ADRs, if there is a causal relationship to the medicinal product.
Examples of adverse events include: new conditions, worsening of pre-existing conditions, clinically significant abnormal physical examination signs (i.e. skin rash, peripheral edema, etc.), or clinically significant abnormal test results (i.e. lab values or vital signs), with the exception of outcome measure results, which are not being recorded as adverse events in this trial (they are being collected, but analyzed separately). Stable chronic conditions (i.e., diabetes, arthritis) that are present prior to the start of the study and do not worsen during the trial are NOT considered adverse events. Chronic conditions that occur more frequently (for intermittent conditions) or with greater severity, would be considered as worsened and therefore would be recorded as adverse events.

Adverse events are generally detected in two ways:

Clinical → symptoms reported by the subject or signs detected on examination.

Ancillary Tests → abnormalities of vital signs, laboratory tests, and other diagnostic procedures (other than the outcome measures: the results of which are not being captured as AEs).

If discernible at the time of completing the AE, a specific disease or syndrome rather than individual associated signs and symptoms should be identified by the Site Investigator and recorded on the AE CRF. However, if an observed or reported sign, symptom, or clinically significant laboratory anomaly is not considered by the Site Investigator to be a component of a specific disease or syndrome, then it should be recorded as a separate AE CRF. Clinically significant laboratory abnormalities, such as those that require intervention, are those that are identified as such by the Site Investigator.

An unexpected adverse event is any adverse event, the specificity or severity of which is not consistent with the current Investigators Brochure or package insert or described in the protocol. An unexpected, suspected adverse drug reaction is any unexpected adverse event that, in the opinion of the Site Investigator or Sponsor, there is a reasonable possibility that the investigational product caused the event.

10.4.1.2 Serious Adverse Events
A serious adverse event (SAE) is defined as an adverse event that meets any of the following criteria:
1. Results in death.
2. Is life threatening: that is, poses an immediate risk of death as the event occurred.
    a. This serious criterion applies if the study subject, in the view of the Site Investigator or Sponsor, is at immediate risk of death from the AE as it occurs. It does not apply if an AE hypothetically might have caused death if it were more severe.
3. Requires inpatient hospitalization or prolongation of existing hospitalization.
    a. Hospitalization for an elective procedure (including elective PEG tube/g-tube/feeding tube placement) or a routinely scheduled treatment is not an SAE by this criterion

because an elective or scheduled “procedure” or a “treatment” is not an untoward medical occurrence.

4. Results in persistent or significant disability or incapacity.
    a. This serious criterion applies if the “disability” caused by the reported AE results in a substantial disruption of the subject’s ability to carry out normal life functions.
5. Results in congenital anomaly or birth defect in the offspring of the subject (whether the subject is male or female).
6. Necessitates medical or surgical intervention to preclude permanent impairment of a body function or permanent damage to a body structure.
7. Important medical events that may not result in death, are not life-threatening, or do not require hospitalization may also be considered SAEs when, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

An inpatient hospital admission in the absence of a precipitating, treatment-emergent, clinical adverse event may meet criteria for "seriousness" but is not an *adverse* experience, and will therefore, not be considered an SAE. An example of this would include a social admission (subject admitted for other reasons than medical, e.g., lives far from the hospital, has no place to sleep).

A serious, suspected adverse drug reaction is an SAE that, in the opinion of the Site Investigator or Sponsor, suggests a reasonable possibility that the investigational product caused the event.

The Site Investigator is responsible for classifying adverse events as serious or non-serious.

10.4.1.3 Assessment and Recording of Adverse Events

This study will utilize the CTCAE version 4.0 coding system for adverse event recording. Adverse events reported using CTCAE will be recoded into MedDRA terms by the DCC.

Assessment of Adverse Events

At each visit (including telephone interviews), the subject will be asked “Have you had any problems or symptoms since your last visit?” in order to determine the occurrence of adverse events. If the subject reports an adverse event, the Investigator will determine:

1. Type of event
2. Date of onset and resolution (duration)
3. Severity (mild, moderate, severe)
4. Seriousness (does the event meet the above definition for an SAE)
5. Causality, relation to investigational product and disease
6. Action taken regarding investigational product
7. Outcome

Relatedness of Adverse Event to Investigational Product

The relationship of the AE to the investigational product should be specified by the Site Investigator, using the following definitions:

1. Not Related: Concomitant illness, accident or event with no reasonable association with treatment.

2. Unlikely: The reaction has little or no temporal sequence from administration of the investigational product, and/or a more likely alternative etiology exists.
3. Possibly Related: The reaction follows a reasonably temporal sequence from administration of the investigational product and follows a known response pattern to the suspected investigational product; the reaction could have been produced by the investigational product or could have been produced by the subject's clinical state or by other modes of therapy administered to the subject. (suspected ADR)
4. Probably Related: The reaction follows a reasonably temporal sequence from administration of investigational product; is confirmed by discontinuation of the investigational product or by re-challenge; and cannot be reasonably explained by the known characteristics of the subject's clinical state. (suspected ADR)
5. Definitely Related: The reaction follows a reasonable temporal sequence from administration of investigational product; that follows a known or expected response pattern to the investigational product; and that is confirmed by improvement on stopping or reducing the dosage of the investigational product, and reappearance of the reaction on repeated exposure. (suspected ADR)

Recording of Adverse Events

All clinical adverse events are recorded in the Adverse Event (AE) case report form (CRF) in the subject's study binder. The site should fill out the AE CRF and enter the AE information into the online Adverse Event Reporting System within 5 working days of the site learning of a new AE or receiving an update on an existing AE.

Please Note: Serious Adverse Events (SAEs) must be reported to the NeuroNEXT Data Coordinating Center within 24 hours of the site learning of the SAE.

Entries on the AE CRF (and into the online Adverse Event Reporting System) will include the following: name and severity of the event, the date of onset, the date of resolution, relationship to investigational product, action taken, and primary outcome of event.

Subjects will be monitored for AEs from the time they sign consent until the final Study visit. If an AE is ongoing or discovered at a subject's final Study visit, the AE will be followed until resolution, or for a minimum of 30 days, whichever comes first. Resolution means that the subject has returned to status at enrollment. If the AE is still ongoing after 30 days, it will be resolved with sequelae. All AEs monitored past the final visit can be followed via telephone – a repeat Termination/Final Follow-up visit does not need to occur.

All SAEs must be followed until resolution throughout the trial. SAEs that are discovered less the 60 days prior to the subject's final Study visit will be followed until resolution or for a minimum of 60 days, whichever comes first, even if it is past the final visit. If a new SAE is discovered at the subject's final Study visit, it must be followed until resolution, or for a minimum of 60 days, whichever comes first. If the SAE is still ongoing after 60 days, it will be resolved with sequelae. All SAEs monitored past the final visit can be followed via telephone – a repeat Termination/Final Follow-up visit does not need to occur.

Adverse Events and Serious Adverse Events - Reportable Events

The following are considered reportable events and must be reported to the NeuroNEXT Data Coordinating Center within 24 hours of the site being notified of the event.

- ❖ All events that meet the above criteria for Serious Adverse Events (SAEs)

All occurrences of Serious Adverse Events (SAEs) must be reported within 24 hours of discovery of the event. All other Adverse Events (AEs) must be reported within 5 working days/7 calendar days (of discovery of the event).

Additionally, the PPI (Sponsor) shall forward to Novartis any SAEs and reports of Study Drug misuse or abuse, including initial and follow up reports, arising from the Study in subjects exposed to the Study Drug, as soon as it becomes available, but in any event within fifteen (15) calendar days of becoming aware of such information.

The PPI shall forward to Novartis any findings that might alter the current benefit-risk profile of the Study Drug or that would be sufficient to consider changes in the Study Drug's administration or in the overall conduct of the Study, as soon as it becomes available, but in any event within five (5) calendar days of becoming aware of such information, by transmitting it to Novartis

The PPI shall prepare and issue expedited safety reports for suspected unexpected SAEs ("SUSARs") in the Study in accordance with the applicable laws and regulations, this includes preparing and issuing Investigator Notifications (INs) or biannual SUSAR listings, where applicable. The PPI shall provide a copy of any PPI-generated INs to Novartis DS&E within fifteen (15) calendar days of first notification of the SUSAR or subsequent follow-up information.

For each SAE report, report of Study Drug misuse or abuse and report of drug exposure during pregnancy arising from subjects exposed to the Study Drug (both initial and follow-up reports), the Sponsor shall forward a MedWatch form completed with full information (as known at the time of forwarding). The PPI shall ensure that at a minimum the form contains:

(a) Information on the person who contacted PPI (i.e., the initial reporter);
(b) Information about the patient or clinical trial subject;
(c) Details of the suspected Study Drug
(d) Details on the safety events experienced by the subject

Adverse Event Data Management System (AEDAMS)

Upon entry of a serious adverse event by a clinical site, the DCC Online Adverse Event Reporting System will immediately notify the IMM. If warranted, the IMM will notify the DSMB chair.

Serious adverse events: The site investigator determines causality (definitely not related, probably not related, possibly related, probably related, definitely related) of the adverse event. The IMM will review the SAE report. The IMM may request further information if necessary. The DSMB may suggest changes to the protocol or consent form to the Project PI as a consequence of adverse events. The Online Adverse Event Reporting System maintains audit trails and stores data (and data updated) and communication related to any adverse event in the study.

Non-serious adverse events: Non-serious adverse events that are reported to or observed by the investigator or a member of his research team will be submitted to the DCC within 5 working days. The events will be presented in tabular form and given to the IMM on a monthly basis or as requested. Local site investigators are also required to fulfill all reporting requirements of their local institutions.

The DCC will prepare aggregate reports of all adverse events (serious/not serious and expected, unexpected) for the DSMB.

11 HUMAN SUBJECTS

Documented approval from the NeuroNEXT CIRB will be obtained for all participating centers prior to clinical trial start, according to ICH GCP, local laws, regulations and organization. When necessary, an extension, amendment or renewal of the CIRB approval must be obtained.
Evidence of training in responsible conduct of research shall be on file for each CSS PI and co-investigator.

11.1 Institutional Review Board (IRB) Review and Informed Consent

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the NeuroNEXT CIRB responsible for oversight of the study. A signed consent form, approved by the NeuroNEXT CIRB, will be obtained from the subject. For subjects who cannot provide consent for themselves, such as those below the legal age, a parent, legal guardian, or person with power of attorney, must sign the consent form; additionally, the subject's assent must also be obtained if he or she is able to understand the nature, significance, and risks associated with the study. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the subject, parent, or legal guardian, and this fact will be documented in the subject's record.

11.2 Subject Confidentiality

All laboratory specimens, evaluation forms, reports, video recordings, and other records that leave the clinical study site will be identified only by the study specific Subject Identification Number (SID) to maintain subject confidentiality. All records will be kept in a locked file cabinet. All computer entry and networking programs will be done using study specific SIDs only. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by CIRB, the FDA, the NINDS, the OHRP, the sponsor, or the sponsor's designee.

11.3 Study Modification/Discontinuation

The study may be modified or discontinued at any time by the CIRB, the NINDS, the sponsor, the OHRP, the FDA, or other government agencies as part of their duties to ensure that research subjects are protected.

12 PUBLICATION OF RESEARCH FINDINGS

Publication of the results of this trial will be governed by the policies of the NeuroNEXT Network and procedures developed by the NeuroNEXT Data Sharing and Publication Committee. Any presentation, abstract, or manuscript will be made available for review by the sponsor and the NINDS prior to submission.

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Appendix A: PET Study Data with AFQ056

Excerpts from CSR from CAFQ056A2104 (PET Study with AFQ056)
Estimation of regional brain mGluR5 receptor occupancy following single oral doses of the mGluR5 antagonist AFQ056 with positron emission tomography (PET) of [11C]-ABP688 in healthy volunteers

Treatments administered: AFQ056
Subjects were assigned to 1 of 2 cohorts and received 2 single doses (separated by 1 week) of oral AFQ056 as follows:
Cohort 1: 25 mg AFQ056 in Period 2 and 200 mg AFQ056 in Period 3
Cohort 2: 100 mg AFQ056 in Period 2 and 400 mg AFQ056 in Period 3
Demographic data for participants Is shown in Table 1.

Table 1 Demographic summary (all subjects, n=8)

Age (years)	Mean 27.4	SD 6.25	Median 26.0	Range 21-40
Gender	n (%) Male 8 (100)			
Race	n (%) Caucasian 8 (100)			
Body frame	n (%) Medium 8 (100)			
Weight (kg)	Mean 73.94	SD 5.841	Median 75.25	Range 64.3-82.1
Body mass index (kg/m^2)	Mean 23.250	SD 1.6466	Median 23.715	Range 20.47-25.62
Height (cm)	Mean 178.4	SD 6.46	Median 178.0	Range 169-191

[11C]-ABP688 and [15O]-water
In each of the 3 treatment periods, subjects received a slow bolus i.v. injection with 200 MBq (207±28.6 MBq, mean ±SD) [11C]-ABP688 starting at 3h after AFQ056 administration. Before each PET scan with [11C]-ABP688, subjects received a slow bolus i.v. injection with 500 MBq (0.62 mSv) of [15O]-water in order to measure the cerebral blood flow in each treatment period.

Identity of investigational product(s): AFQ056
The investigational drug AFQ056 was prepared by Novartis. The study drug for the subjects and an equal number of replacement subjects was packaged, labeled and supplied to the investigator by Novartis Drug Supply Management as open labeled bulk medication. The dosage form for the early clinical studies is a dry powder in hard gelatin capsules intended for oral administration. Dosage strengths available in this clinical study were 5, 10, 25, 100 mg. The drug product is packed in high density polyethylene bottles with induction seal. The investigational products used in the study were the following. Doses consisted of a single oral dose of the following: 25 mg AFQ056 1 x AFQ056 25 mg capsule, 100 mg AFQ056: 1 x AFQ056 100 mg capsule, 200 mg AFQ056 2 x AFQ056 100 mg capsules, 400 mg AFQ056: 4 x AFQ056 100 mg capsules

PET Study Data
Table 2 shows pharmacokinetic parameters after administration of the AFQ056 at the doses used on this study.

Table 2 **AFQ056 pharmacokinetics after single administration of 25 mg, 100 mg, 200 mg and 400 mg in healthy volunteers**

Parameter	AFQ056 25mg	AFQ056 100mg	AFQ056 200mg	AFQ056 400mg
N	5	3	4	3
t_{max} (h)	2.00 (2.00 – 3.25)	3.25 (2.00 – 3.50)	2.50 (1.00 – 4.02)	2.00 (2.00 – 3.00)
C_{max} (ng/mL)	54.23 ± 39.09 (39.3)	191.6 ± 112.5 (160.0)	337.6 ± 217.6 (267.0)	1004 ± 674.1 (837.2)
AUC_{3-4} (ng.h/mL)	31.42 ± 17.84 (25.30)	156.3 ± 103.7 (130.0)	202.8 ± 99.35 (177.4)	715.0 ± 429 (597.7)
AUC_{0-12} (ng.h/mL)	200.9 ± 107.6 (169.3)	957.5 ± 524.8 (823.5)	1798 ± 946.0 (1492)	5054 ± 3409 (4156)
AUC_{0-24} (ng.h/mL)	253.3 ± 143.3 (211.5)	1221 ± 628.5 (1075)	2396 ± 1210 (1998)	6939 ± 4946 (5587)

t_{max} values presented as median (range); C_{max}, AUC_{0-t}, AUC_{0-12} and AUC_{0-24} values represented as mean ± sd (geometric mean)

Analysis of receptor occupancy as a function of exposure

Two cohorts of three subjects were examined for each of the 4 AFQ056 doses (25/200 and 100/400 mg). This included all examined brain regions. Table 3 summarizes obtained specific distribution volume (BPP). An estimate of the binding potential relative to the non-displaceable compartment (BPND) at baseline has also been given. This is calculated from the estimated rate constant parameters k3/k4 per region at baseline described in (Innis 2007)

Table 3 **Distribution volume (BP_P) and ratio k_3/k_4 (BP_{ND}) per region at baseline**

Region	Distribution volume (BP_P)					Ratio K_3/K_4 (BP_{ND})					N
	Mean	Std	Med	Min	Max	Mean	Std	Med	Min	Max	
Anterior cingulum	5.28	1.08	5.12	3.89	7.23	7.1	2.09	6.7	4.33	9.71	6
Caudate	4.99	1.18	4.79	3.62	7.18	7.02	2.46	6.48	4.22	10.1	6
Cerebellum	2.01	0.36	1.91	1.75	2.73	2.72	0.86	2.76	1.56	3.71	6
Frontal cortex	4.5	1.04	4.4	3.15	6.37	5.91	2.05	5.13	3.78	8.67	6
Lateral temporal cortex	4.52	1.07	4.4	3.23	6.44	6.05	1.92	5.76	3.56	8.63	6
Medial orbitofrontal cortex	5.24	1.11	5.21	3.75	7.11	7.13	2.33	6.5	4.22	10.7	6
Mediotemporal cortex (mainly hippocampus)	5.05	1.51	4.77	3.23	7.7	6.62	2.18	6.4	3.6	9.17	6
Occipitotemporal cortex	4.09	1.07	4	2.8	6.04	5.53	1.8	5.08	3.2	8.13	6
Orbitofrontal cortex	4.39	1.06	4.24	3.14	6.33	5.96	2.06	5.31	3.63	8.5	6
Parietal cortex	4.11	0.84	3.98	2.99	5.55	5.57	1.82	5.19	3.27	8.13	6
Posterior cingulum	4.65	0.96	4.64	3.35	6.31	6.39	2.13	5.94	3.75	9.57	6
Prefrontal cortex	4.19	0.91	4.04	3.13	5.87	5.53	1.74	5.26	3.4	8	6
Putamen	4.75	1.03	4.66	3.49	6.55	6.55	1.93	6.54	3.8	9.11	6
Thalamus	3.34	0.62	3.23	2.57	4.45	4.4	1.27	4.22	2.8	6.18	6
Ventral part of anterior cingulum	5.52	1.23	5.37	3.89	7.69	7.43	2.46	6.75	4.75	10.9	6
Visual cortex	3.77	0.92	3.61	2.93	5.53	5.12	1.87	4.7	2.7	7.5	6
White matter	1.8	0.45	1.72	1.24	2.6	2.44	0.89	2.17	1.5	3.73	6
Sum across all regions except cerebellum and white matter	5.28	1.08	5.12	3.89	7.23	7.1	2.09	6.7	4.33	9.71	6

The distribution volumes (BPP) agree very well with previously published data using ABP688 (Ametamey et al, 2007) across regions. The highest distribution volume was observed in the

anterior cingulate region. White matter (myelinated axonal sheets which are likely devoid of mGluR5 receptors and sheathed from drug absorption) and cerebellum show a low uptake also in agreement with the previously published data. Fitting an Emax model to the cerebellum and white matter data also resulted in a poor fit to data and outlying low Emax estimates (35% and 27%) vs. other regions (estimates ranging from 56% to 72%). For these reasons, these regions are not shown in Table 4 and were not used in the analysis of pooled cortical areas. The displacement measured was regressed on doses and on plasma concentrations between 3 and 4 hours after drug intake (time of the PET scan). The Saturation Binding Model was used to estimate:

- ED50 and Emax based on the dose
- EC50 and Emax based on plasma concentrations

for each individual region of the brain, and also for the pooled displacement across all regions (except cerebellum and white matter) (Table 4 and Table 5).

Table 4 Estimated ED_{50} per region of the brain

Region	Estimated ED_{50}	Std. Error
Anterior cingulum	65	18
Caudate	77	21
Cerebellum	53	53
Frontal cortex	61	18
Lateral temporal cortex	66	13
Medial orbitofrontal cortex	64	16
Mediotemporal cortex (mainly hippo)	69	18
Occipitotemporal cortex	69	22
Orbitofrontal cortex	71	22
Parietal cortex	68	21
Posterior cingulum	62	23
Prefrontal cortex	76	21
Putamen	68	21
Thalamus	72	20
Ventral part of anterior cingulum	80	23
Visual cortex	65	27
White matter	139	175
Sum across all regions except cerebellum and white matter	69	24

Table 5 Estimated EC_{50} per region of the brain

Region	Estimated EC_{50}	Std. Error
Anterior cingulum	43	14
Caudate	58	20
Cerebellum	33	33
Frontal cortex	38	15
Lateral temporal cortex	35	13
Medial orbitofrontal cortex	42	14
Mediotemporal cortex (mainly hippo)	38	17
Occipitotemporal cortex	43	18
Orbitofrontal cortex	36	16
Parietal cortex	39	16
Posterior cingulum	44	17
Prefrontal cortex	45	17
Putamen	50	16
Thalamus	38	14
Ventral part of anterior cingulum	50	18
Visual cortex	46	21
White matter	9	19
Sum across all regions except cerebellum and white matter	43	20

Table 6 Estimated RO at 3-4hr post dose for study doses

Dose in mg	25	100	200	400
Percent occupancy at 3-4 hours post-dose	27	59	74	85

The pooled displacement values (summing across all regions except cerebellum and white matter) are plotted versus doses and plasma concentrations in Figure 1 and Figure 2.

Figure 1 **Displacement at 3-4 hours post-dose vs. dose**

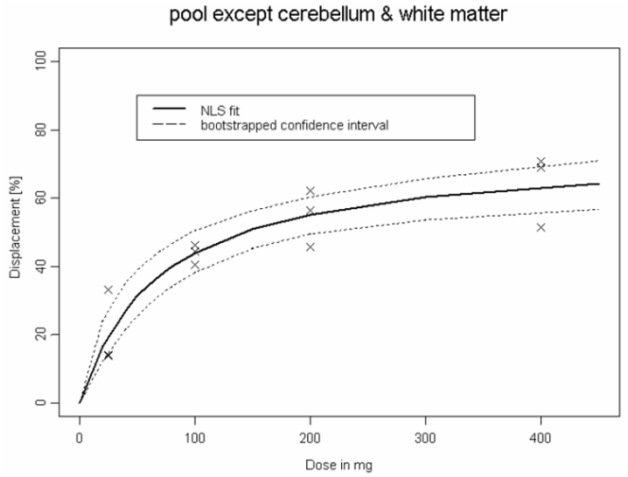
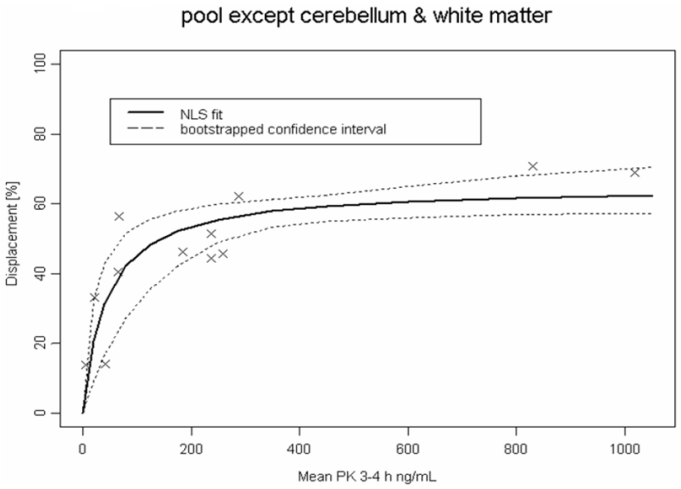


Figure 2 **Relative displacement vs PK concentration**



Then assuming that Emax corresponds to a mGluR5 receptor occupancy of 100% and that therefore the observed EC50 is the same for receptor occupancy, the prediction curve of the RO was plotted versus the dose and the mean of concentration between 3 and 4 hours (Figure 3 and Figure 4). Predicted RO at 3-4 hours post-dose was also summarized per dose in Table 5. The EC50 of striatum (caudate and putamen) does not differ from other regions of the cerebral cortex. Emax was also observed to be similar across cortical regions and striatum. An Emax was observed in cerebellum and white matter, suggesting specific binding in those areas. It is also possible that spill-over from neighboring regions with specific uptake could result in an apparent observed RO in those two regions, especially in white matter.

Figure 3 **Estimated receptor occupancy at 3-4 hours post-dose vs. dose**

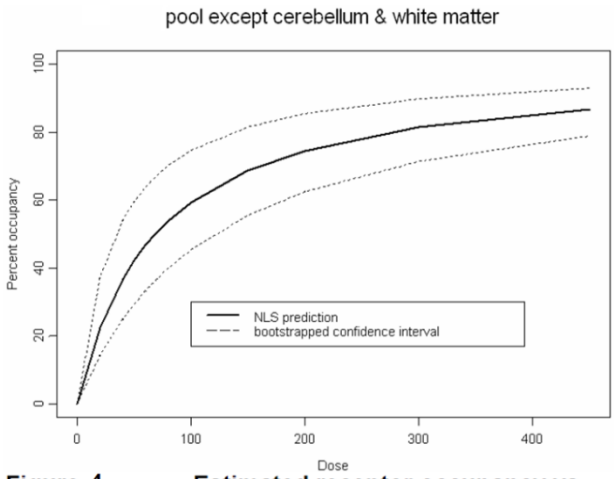
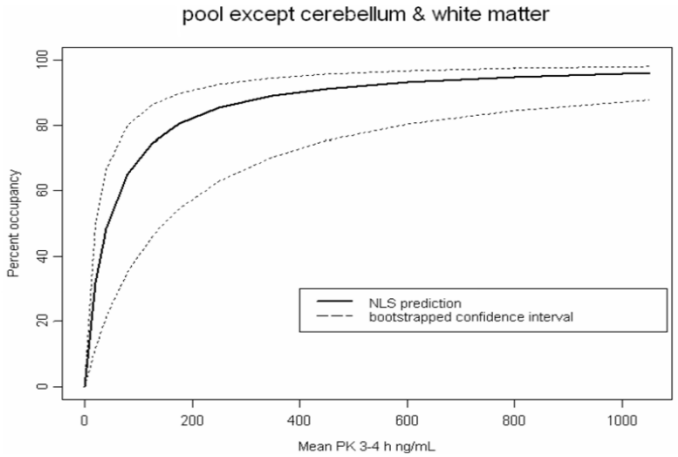


Figure 4 **Estimated receptor occupancy vs. PK concentration**



Conclusions

AFQ056 penetrates the brain and induces dose/exposure dependent displacement of [11C]-ABP688 from the mGluR5 receptors in humans in vivo. The observed EC50 (KD) for AFQ056 at the mGluR5 receptor was 43 (+/- 20) ng/mL. The single oral dose of 400 mg induced an estimated displacement of 63% at scan time of 3-4 hours post-dose, inferring a receptor occupancy estimate of 85%.

Appendix B: Prohibited Medications

Concomitant medications that are strong/moderate inhibitors or inducers of CYP1A1/2, CYP2C9/19 or CYP3A4 such as:

- Amiodarone
- Amprenavir
- Aprepitant
- Atazanavir
- Barbiturates (e.g. primidone)
- Carbamazepine
- Clarithromycin
- Cimetidine,
- Ciprofloxaxin
- Digoxin
- Diltiazem
- Erythromycin
- Fluconazole
- Fluoroquinolones
- Fluvoxamine
- Fosamprenavir
- Gemfibrozil
- Gestodene
- Glucocorticoids (except for topical or inhalation use)
- Indinavir
- Itraconazole
- Ketoconazole
- Metoclopramide
- Mibefradil
- Nefazodone
- Nelfinavir
- Nicotine
- Phenytoin
- Rifabutin
- Rifampin
- Rifapentin
- Ritonavir
- Saquinavir
- St. John's Wort
- sulphaphenazole
- Telithromycin
- Trimethoprime,
- Thioridazine
- Verapamil
- Warfarin

...are prohibited during this study.