

**Outcome Measures for Clinical Trials with Children with Fragile X Syndrome
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Hyatt Regency Bethesda
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This meeting was sponsored by the Obstetric and Pediatric Pharmacology Branch (OPPB), Center for Research for Mothers and Children (CRMC), *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD), National Institutes of Health (NIH), U.S. Department of Health and Human Services (HHS) in support of the Best Pharmaceuticals for Children Act (BPCA).

Welcome

Donald R. Mattison, M.D., Captain, U.S. Public Health Service; Senior Advisor to the Directors of NICHD and CRMC; Chief, OPPB, CRMC, NICHD, NIH, HHS

Dr. Mattison welcomed the meeting participants and briefly reviewed the history of BPCA and fragile X syndrome (FXS). OPPB was created to (1) identify issues in pediatric and obstetric pharmacology and (2) promote and coordinate research, clinical trials, and drug development activities to improve the safety and efficacy of drugs to treat disease during pregnancy, infancy, and childhood. BPCA drives most OPPB activities. BPCA was passed in 2002 and reauthorized as part of the Food and Drug Administration Amendments Act of 2007 (FDAAA). BPCA's role in treating FXS began in July 2006 with information-gathering activities focused on studies of both pharmacologic and nonpharmacologic treatment approaches.

Since 2006, collaborative research activities have identified potential drug treatments for FXS. Efforts are underway to begin clinical trials of a class of compounds—the metabotropic glutamate receptor (mGluR) antagonists. In anticipation of clinical trials, preclinical data will need to link with and stimulate the design of clinical studies. Specific considerations include preclinical study design, developmental endpoints to evaluate safety and efficacy in preclinical studies, and endpoints for clinical trials.

Currently, there are about 20 BPCA clinical trials, ranging from pediatric oncology, attention-deficit disorder, and mania in bipolar disorder to the treatment of infectious diseases and sedation of children in intensive care units. The results of these studies have been unexpected and surprising. An analysis of recent pediatric trials of antihypertensive agents provides an example of the surprising results. About half the trials failed, but it is not clear whether the trials failed because of dosing or pharmacodynamics. Therefore, the design of pediatric trials should be further explored.

Goals for the Meeting

Tiina K. Urv, Ph.D., Health Scientist Administrator, Intellectual and Developmental Disabilities Branch, Center for Developmental Biology and Perinatal Medicine, NICHD, NIH, HHS

The goals for the meeting were to:

- Describe outcome measures for safety and efficacy when treating children with FXS

- Assess validation of those measures for clinical studies
- Describe approaches for preclinical toxicology studies to define efficacy and safety.

The desired outcome of the meeting was to develop recommendations related to outcome measures for clinical trials with children with FXS.

Mechanisms of Action

Robert Riddle, Ph.D., Program Director, Neurogenetics Cluster, Extramural Research Program, National Institute of Neurological Disorders and Stroke (NINDS), NIH, HHS

Dr. Riddle provided a brief overview of FXS:

- FXS is caused by the loss of expression of the FMR1 gene.
- Fragile X mental retardation protein (FMRP) is expressed in many tissues, including the central nervous system (CNS).
- FMRP functions, at least in part, as an mRNA binding protein that is a component of translational complexes.
- An abnormally large number (>200) of triplet repeats (CGG) in the 5' UTR of the FMR1 gene causes methylation, which leads to gene silencing.
- FMRP absence causes behavioral, cognitive, and emotional deficits that become apparent during early childhood.
- Changes in dendritic morphology and brain structure are apparent in FXS patients.
- *In vitro* and *in vivo* models (for example, *Drosophila* and mouse) have helped to understand FMRP function, disease progression, and treatment development.

According to the current model for the pathogenesis and correction of FXS, metabotropic glutamate receptor subtype 5 (mGluR5) and FMRP regulate translation of mRNA at the synapse in a functionally opponent manner—mGluR5 activation initiates protein synthesis and FMRP suppresses it. In the absence of FMRP, mGluR5-dependent protein synthesis proceeds unchecked, and consequent excessive translation leads to the diversity of clinical features that make up FXS. Research results demonstrate that this progression can be corrected by genetic reduction of mGluR5 activity.

Many NIH Institutes and Centers—including NINDS—support basic FXS research and have a shared interest in treating FXS. Although they have the common goal of curing FXS, the approaches of the Institutes and Centers vary. For example, NINDS focuses on the neurological basis of normal neurodevelopment and neurodegeneration. Through cooperation, coordination, and agreement in FXS research, basic research translates into clinical research and hopefully an eventual cure for FXS. Trans-NIH collaborations supporting research on FXS include:

- Research on the etiology, pathophysiology, and treatment of FXS, autism, and autism spectrum disorders
- Development of mGluR5 antagonists to treat FXS and autism
- NIH Roadmap
- NIH Blueprint for Neuroscience Research.

Levels of Evidence in Pediatric Trials

Dr. Mattison

NICHHD's mission focuses on human developmental trajectories, not on specific organ systems or diseases. The mission includes research to understand normal and abnormal developmental trajectories and how abnormal trajectories can be redirected. Many of NICHHD's programs involve therapeutics. Under BPCA, the focus has shifted to identifying gaps in therapeutics—which include drugs, biologics, and devices—and understanding how the gaps can be closed. Evidence-based approaches used for adult therapeutics will be applied to pediatric therapeutics.

Drug development begins with a disease model to help understand mechanisms of action and potential intervention strategies. In the discovery phase, candidate molecules are identified and assayed for therapeutic efficacy. Once a molecule has shown potential value, it is tested in nonhuman models to understand how it actually works, how the intact animal handles the drug, and what the drug does to the animal in terms of both disease and unexpected consequences. Preclinical data help describe outcome measures for characterizing drug safety and efficacy.

After this preclinical phase, these molecules are ready to be studied in humans. Before clinical trials, investigators need to determine how the human body handles the drug, identify the role metabolites play in extending or modifying the drug's action, and define the endpoints that will characterize the molecules' effectiveness. Clinical trials should ideally be conducted in the youngest population with the disease or condition, and treatment should begin at the earliest age possible. The next step is to determine whether long-term or short-term treatment is required. Treatment may be required throughout the period of brain development and perhaps into young adulthood. For women, treating during reproductive periods may be important. Treating across the continuum of life may be necessary.

A strong line of evidence from animal models suggests that a particular class of receptor antagonists may have use in treating FXS. Emerging data from knockout animals suggests that antagonism of the mGluR5 receptor may not have long-term adverse development consequences. However, data from other drug development domains indicate that targeted drugs often inform about the roles that specific receptors play across development. Longer term treatment over critical developmental periods may have unforeseen consequences.

NICHHD is currently involved in a study of drug treatment of children with sickle cell disorder. The study has recruited children as young as 6 months of age. Imaging studies have revealed that even at the time of recruitment, these children already have silent CNS infarcts. These results and results of phenylketonuria studies have shown the need to begin treatment as early as possible. The goal of NICHHD is to provide necessary resources to evaluate potentially beneficial molecules in both preclinical and clinical studies.

Outcome Measures for Clinical Trials with Children with FXS

Linda Brady, Ph.D., Director, Division of Neuroscience and Basic Behavioral Science, National Institute of Mental Health (NIMH), NIH, HHS

Dr. Brady provided an overview of outcome measures for clinical trials with FXS based on data in the NIH clinical trials database. There have been relatively few clinical trials of pharmacologic interventions to treat FXS, especially trials in children. The goals of research in basic neuroscience are to find (1) new ways to assess preclinical safety and efficacy for trials in children and adolescents and (2) new ways to assess optimal dosing and drug exposure. There is a pressing need for developmentally sensitive outcome measures to assess efficacy in domains of dysfunction (for example, cognition, anxiety, and social behavior). Researchers hope that some of these measures will be applicable to other developmentally based disorders. Ideally, the objective measures should have a dynamic range, be sensitive to differing levels of ability, and have test–retest reliability and validation.

Completed and ongoing FXS clinical trials include:

- Effects of CX516 (an AMPA receptor positive modulator) on functioning in FXS and autism
- An open-label trial of donepezil (an acetylcholinesterase inhibitor) in FXS
- Aripiprazole in FXS (an atypical antipsychotic with partial agonist activity at D2 and 5-HT1A and antagonist activity at 5-HT2 receptors)
- An open-label study investigating safety and efficacy of NPL-2009 on prepulse inhibition (PPI) tests and continuous performance tasks in adults with FXS
- Memantine (an NMDA receptor antagonist) treatment in fragile X ataxia/tremor syndrome
- Study of fenobam in FXS
- Add-on pilot trial of lithium in FXS
- Protein synthesis in the brain of patients with FXS.

The objectives of FXS-related NIMH activities are to:

- Promote discovery in the brain and behavioral sciences to provide the foundation for understanding the pathophysiology of developmental disorders
- Define the developmental trajectory to determine when, where, and how to intervene
- Develop innovative interventions and designs for clinical trials that will allow the identification and integration of biomarkers and behavioral indicators such as cognitive, behavioral, and anxiety endpoints that are appropriate to developmental stage.

Basic research is leading to new targets for pharmacologic and behavioral interventions.

Although there are new opportunities for early intervention for treating FXS, there is a need to assess new safety and efficacy paradigms for preclinical tests and clinical trials.

Translational Research Case Study

Randall L. Carpenter, M.D., Co-Founder, President, and Chief Executive Officer, Seaside Therapeutics

There are several approaches for developing pharmacologic treatments for FXS. The safety and efficacy of marketed therapeutics can be defined in patients with FXS, and dosage forms of

drugs without patent protection can be improved to treat FXS, particularly in pediatric populations. Novel therapeutics for which there are no relevant preclinical models require empirical testing in humans. Preclinical models provide insight regarding therapeutic targets, which can lead to translation of research findings into novel therapeutics.

The promise of molecular medicine is developing rational treatments based on understanding of disease. Dr. Carpenter reviewed the background of the development of novel therapeutics for FXS. The pedigree of X-linked disabilities was described in 1943, the fragile X site was identified in 1970, and the fragile X test was developed in 1980. In 1991, the FMR1 gene mutation was discovered. Subsequently, the FMR1 knockout mouse disease model was developed. Basic neurobiological research led to a better understanding of FXS pathophysiology and development of the mGluR theory of fragile X mental retardation. According to this theory, FXS is a disease of the dendritic synapses. Understanding how synaptic signaling has gone awry holds great potential to suggest novel therapies that do not require reactivation of silenced genes or correction of mutated genes. The challenge is translating research discoveries into novel therapeutics for FXS.

Dr. Carpenter compared the financing of drug discovery with the funding of translational research. He described Seaside Therapeutics' funding to develop mGluR antagonists, which includes venture philanthropy seed funding, grant applications to augment seed funding, and a collaborative business model. The mGluR antagonist STX107 is in the advanced stages of preclinical development and has demonstrated profound efficacy in animal models of FXS. Dr. Carpenter presented results from STX107 dose–response studies in animals, including dose–receptor occupancy, audiogenic seizure, total distance in open field, marble burying behavior, duration of behavioral efficacy, and PPI. Although these results are encouraging, there are differences between human and animal brain circuitry, and the molecular pathology that yields specific behavior in humans might have distinct effects in other species.

The next steps in developing STX107 as a novel therapeutic for FXS included submitting an Initial New Drug (IND) application to the Food and Drug Administration (FDA), defining the safe dose range in normal volunteers, assessing effects in patients with FXS, and assessing efficacy in other developmental disorders. Development of mGluR5 backup compounds and discovery of GluR5 modulators continue. Dr. Carpenter offered the following conclusions:

- Translational medicine and translational research require deep expertise and coordinated efforts in multiple disciplines.
- Single gene disorders provide insights on molecular perturbations and therapeutic opportunities.
- The translation of promising basic research discoveries into treatments will meaningfully improve the lives of individuals with brain development disorders.

Challenges Faced in Running Clinical Trials with Children with FXS

Randi Hagerman, M.D., Medical Director, M.I.N.D. Institute, Department of Pediatrics, University of California, Davis

Clinical trials with children with FXS often require that patients stop taking their medications in order to participate. This is a difficult requirement for FXS patients, who are often taking several concomitant medications (for example, stimulants, antidepressants, and mood stabilizers). Cessation of medications can lead to rapid decline in these patients. Their behaviors may become uncontrollable. Conducting psychophysiological studies becomes extremely challenging, and measuring behaviors becomes difficult. Children with FXS often have interfering behaviors such as severe anxiety, tactile defensiveness, impulsivity, attention deficit, and hyperactivity. They can be aggressive and violent, and they may throw or break equipment. Researchers need to be careful with expensive equipment, pay attention to subjects' emotional and behavioral problems, and make the testing environment comfortable. Researchers need to know that some parents may have cognitive impairment or denial of their child's problems or even their own. These parents may not be able to provide reliable information on questionnaires and checklists. In summary, conducting clinical trials with children with FXS can be challenging.

Hurdles to Clinical Trial Design in FXS

Elizabeth Berry-Kravis, M.D., Ph.D., Departments of Pediatrics, Neurological Sciences, and Biochemistry, Rush University Medical Center

Dr. Berry-Kravis reviewed a clinical trial of FXS treatment with the ampakine CX516. The results of the CX516 safety and efficacy study (phase II; double-blind, placebo-controlled study for adults >18 years) showed no significant improvement in memory (primary outcome measure) or any individual cognitive or behavioral measure. The completion rate for both safety and efficacy measures was excellent, proving that rigorous studies can be done in FXS. The safety profile was good, which should encourage further trials with ampakines. Outcome measures were evaluated, and some were validated for FXS. Some measures worked well; some were not good in the FXS population. Cotreatment with antipsychotics suggested improvement in the CX516 group. Investigators should re-evaluate this strategy with a stronger molecule. There are several potential reasons for poor efficacy:

- CX516 may be insufficiently potent to see an effect.
- The treatment may not have been long enough.
- A different dosing scheme may be needed.
- The outcome measures may not measure effect adequately.
- Ampakines may not be good for subjects with FXS.

Although none of the outcome measures in FXS were analyzed for reproducibility prior to the CX516 study, the following outcome measures in adults with FXS were validated:

- Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) List Learning, List Recognition and Story Memory (verbal memory), Digit Span (memory and concentration)
- Peabody Picture Vocabulary Test (PPVT) (receptive vocabulary)

- Preschool Language Scale, Fourth Edition (PLS-4), Clinical Evaluation of Language Fundamentals (CELF) (global language)
- Woodcock-Johnson (W-J) Memory for Words (verbal memory)
- Behavioral forms—SNAP-IV, Aberrant Behavior Checklist Community Scale (ABC-C), Visual Analog Scale (VAS) (behavior and cognition).

The following outcome measures were not good in adult with FXS:

- Test of Visual-Perceptual Skills (TVPS) and other RBANS subtests—Visual and Visuospatial Memory and Long-Term Recall (too hard)
- Integrated Visual and Auditory Continuous Performance Test (IVA CPT) (too confusing and difficult)
- Gilliam Autism Rating Scale (GARS), Childhood Autism Rating Scale (CARS) (no variability, did not assess current function well)
- Autism Diagnostic Observation Schedule (ADOS) (all subtests except communication only moderate reproducibility).

Dr. Berry-Kravis reviewed an FXS add-on lithium trial that used the following outcome measures:

- Primary: ABC-C
- Secondary: Clinical Global Impression (CGI), VAS for parent-defined behavior, Vineland Adaptive Behavior Scale
- Exploratory cognitive battery: PPVT, RBANS List Learning and Story Memory, NEPSY Tower, Visual Memory Card Task, NVALT, North Carolina FX Project CPT (all tested for reliability in prior outcome measure or CX516 study)
- Exploratory biophysiological measures of autonomic function/auditory processing/eye gaze
- Exploratory blood biomarker: extracellular signal-regulated kinases (ERK) activation rate.

Conclusions from the lithium study are as follows:

- Lithium appears to be effective in FXS in behavioral and adaptive measures and a single cognitive measure in an add-on pilot trial in both younger and older subjects.
- For exploratory biophysiological measures and some cognitive tasks, there was either no effect or the task was difficult to do.

There are numerous challenges to clinical trial design in FXS. Cognitive outcome measures in clinical trials of FXS need to test broad ranges of ability. Higher or lower groups can be off the scales. Few measures cross the entire range of mental functioning in FXS, and few are validated. It is not clear how well measures assess core deficits. Because it is difficult to get FXS participants off their medications, new trials should consider allowing baseline medications. All FXS trial participants may want to receive the study drug. Recruitment is easier for crossover designs, and extension designs may make recruitment easier, but it is not known whether these designs are acceptable. Travel and frequent visits to the clinic are of special concern. If frequent testing is required, more test sites or home visits may be useful. Because FXS is a developmental disorder, children with FXS should be treated as young as possible. Recruitment is better at younger ages, and assessment may be better at younger ages. Mechanisms to improve recruitment and assessment at younger ages should be explored.

Disease-Specific Pharmacological and Behavioral Interventions for FXS

*Allan L. Reiss, M.D., Director, Center for Interdisciplinary Brain Sciences Research,
Department of Psychiatry and Behavioral Studies, Stanford University*

Strategies for measuring disease-specific pharmacological and behavioral intervention outcomes in clinical trials of FXS should:

- Focus on biological and behavioral traits that best distinguish FXS subjects from appropriately matched healthy control subjects and subjects with idiopathic developmental disabilities
- Use multiple levels of measurement (for example, brain structure and function, neuroendocrine, psychophysiology, cognition, and behavior)
- Focus on outcome measures that reflect naturalistic or adaptive behaviors and that are developmentally appropriate
- For pharmacologic trials, provide opportunities to demonstrate cognitive or behavioral change as opposed to “passive testing.”

Because FXS has a well-defined disease pathway, treatments can target multiple levels of pathogenesis: FXS-specific biological interventions, FXS-specific environmental interventions, and symptomatic treatments. Based on knowledge of FMRP’s function, researchers can identify potential downstream targets for intervention such as mGluRs, the hypothalamic–pituitary–adrenal axis, and the GABA system.

Individuals with FXS have difficulty modulating their stress response, particularly in socially demanding situations. Responses may include anxiety, averting gaze, and fleeing. Several lines of evidence implicated an aberrant response in the hippocampal–hypothalamic–pituitary–adrenal axis, which is reflected in high cortisol levels. Mifepristone is a potent blocker of glucocorticoid receptors and can modulate stress responses. Dr. Reiss reviewed a mifepristone trial of seven males with FXS (mean age = 15.5 years). Outcome measures were selected because they were considered robust, applicable to the FXS population, able to be used at three times, without floor or ceiling effects, and without practice effects. Four domains were included:

- Behavioral (Child Behavior Checklist (CBC), ABC, CGI, Social challenge)
- Cognitive (Paired Associate Learning, Hopkins Verbal Learning)
- Cardiovascular (heart rate during social challenge)
- Endocrine (salivary cortisol).

The results showed decreases in total scores for the CBC, the ABC, and the CGI.

Three lines of evidence suggest possible functional cholinergic deficits in FXS. In a functional magnetic resonance imaging (fMRI) study of 12 females with FXS and 16 controls (ages 7–22 years), the FXS group showed less activation in basal forebrain and hippocampus in response to novel or repeated visual stimulation. Second, the basal forebrain and hippocampus show high FMR1 mRNA expression during fetal development. Third, mouse and *Drosophila* models suggest cholinergic abnormalities. Donezepil, which increases cortical acetylcholine in

Alzheimer's disease, was studied in four males and two females with FXS. Cognitive and behavior outcome measures were:

- Contingency Naming Task
- Hopkins Verbal Learning Test
- ABC
- CBC/Adult Behavior Checklist.

The results showed improvement in working memory and decrease in aberrant behavior.

In the brain, oxytocin is involved in social recognition and bonding and may be involved in the formation of trust between people and in generosity. The results of several studies suggest that oxytocin might be used to treat FXS. Dr. Reiss reviewed an oxytocin trial of six males (mean age = 21.8 years) with FXS. Outcome measure domains were:

- Behavioral (Social Responsiveness Scale, ABC, CGI, approachability task, social challenge, eye gaze training)
- Cardiovascular (heart-rate during social challenge and eye gaze training)
- Endocrine (salivary cortisol).

The results showed decreases in total scores for the Social Responsiveness Scale, the ABC, and the CGI.

A behavioral eye gaze training study of six males with FXS (mean age = 13 years) showed that mean eye contact time could be improved from about 1.5 seconds to about 5 seconds after 200 "discrete trials" of shaping.

With regard to FXS treatments, Dr. Reiss advised that researchers use currently available behavioral, pharmacological, and educational interventions if data support their use for target symptoms. Researchers must collect individual data with standardized as well as subjective measures and use single-case designs with clinical patients. Treatment efficacy should focus on clinical symptoms. New research should focus on disease-specific targets at multiple levels.

Overview of FDA Regulations

William J. Rodriguez, M.D., Ph.D., Pediatric Science Director, Office of Pediatric Therapeutics, FDA, HHS

On September 27, 2007, the President signed into law the FDAAA of 2007, which reauthorizes some existing parts of the law, including three pediatric initiatives:

- Title IV: Pediatric Research Equity Act of 2007 (PREA)
- Title V: BPCA
- Title III: Pediatric Medical Devices.

Dr. Rodriguez reviewed the legislative details of PREA and BPCA and summarized as follows:

- | <u>PREA</u> | <u>BPCA</u> |
|-----------------------|---------------------|
| ▪ Drugs and biologics | ▪ Drugs only |
| ▪ Studies mandatory | ▪ Studies voluntary |

- Required studies only on drug/indication under review
- Studies for orphan indications exempt
- Standard review
- Pediatric studies must be labeled
- Sunsets October 1, 2012
- Studies on entire active moiety
- Written Request may be issued for orphan indications
- Priority review
- Pediatric studies must be labeled
- Sunsets October 1, 2012

As of March 2008, there have been 147 labeling changes under BPCA:

- Specific dosing change/adjustment (26)
- New or enhanced safety data (42)
- Efficacy and safety not established (46)
- Expanded age to younger pediatric population (92).

For 20–33 percent of the 140 products studied under BPCA, there was new dosing information, the product was deemed not effective, or the product had a new pediatric safety issue. Many of the studies have raised more issues. Long-term safety and effects on growth, learning, and behavior continue to be understudied. Focused pediatric postmarketing safety reviews will further identify pediatric-specific safety issues. Neonates remain mostly unstudied as to the safety and efficacy of the therapies being used to treat them.

As of June 2007, there have been 64 new labels that were not associated with BPCA and were subject to PREA. Important components under PREA are making sponsors think of how products will be used in the pediatric population early in the drug development process. There is a difference in the quantity and, in some aspects, the quality of the studies obtained under PREA compared with studies obtained under BPCA.

Outcome Measures in Clinical Trials

Roberta L. Glass, M.D., Senior Medical Officer, Office of New Drugs, Center for Drug Evaluation Research, FDA, HHS

Dr. Glass reviewed some of FDA's general guidelines for outcome measures in clinical trials, which focus on treatment, not specific methods. In the FXS population, treatment might target specific behaviors. Specific symptoms must be identified, and the primary efficacy variable must be stated. Treatments are not always considered a total cure. For example, drugs for schizophrenia focus on certain symptoms but do not cure this disorder. An advantage to treating FXS is that it has specific genetic testing, whereas most psychiatric disorders are characterized by clusters of symptoms but cannot be identified through blood tests or biomarkers. Once the target symptom is identified, a spectrum of severity should be established and points of intervention determined. Instruments for measuring outcomes should be tested for validity. The threshold of treatment must be established for the inclusion criteria. This threshold would be used by investigators across different sites. With regard to labeling, the difficulty is in a new indication. Currently there is no recognized standard instrument for measuring FXS treatment outcomes. Applications to FDA must provide a clear rationale for the instrument to be used for

outcome measures. The chosen instruments must have the potential to show improvement in symptoms.

Although open-label studies are acceptable, the gold standard for clinical trials is the placebo control-group approach. No medicine has yet proven to be efficacious with FXS. The ideal approach for this population is for subjects to be off all medications and then use a placebo control-group design. The FXS population is small with regard to the number of potential subjects for clinical trials. However, information gathered from small clinical trials must be generalized to the larger population. Trials need to establish a baseline for the target symptom as well as a timeline for improvement. Dosing—both amount and frequency—is a issue in clinical trials. Another issue with the FXS model is addressing symptoms that are unique to the disorder, not those found across pervasive developmental disorders. Besides efficacy, clinical trials need to demonstrate safety. The FXS population is unique because the subjects may not be able to communicate when they are experiencing adverse events. Most FXS subjects cannot always speak for themselves and generally cannot give consent or assent. FDA and investigators have a responsibility to protect this population.

Designing Nonclinical Studies

Karen Davis-Bruno, Ph.D., Supervisory Pharmacologist, Division of Metabolic and Endocrine Products, Center for Drug Evaluation and Research (CDER), FDA, HHS

Dr. Davis-Bruno presented an overview of general design and principles of standard nonclinical testing. She discussed nonclinical development of pediatric products and described the differences between development programs for these products and standard programs. She briefly described what has been learned from the recent clinical pediatric initiatives and identified some of the data gaps. She described the design of two CDER juvenile animal studies—one for development and behavioral assessments and one for a safety assessment. Dr. Davis-Bruno reviewed how juvenile animal studies can begin to address the data gap and how FDA has applied juvenile animal study data to assess risk.

With regard to drug information for pediatrics, drug development historically has not required the same level of evidence for pediatrics as for adults. Pediatric studies have shown that children are more dynamic and variable than predicted. Pediatric initiatives have identified several gaps: unnecessary exposure to ineffective therapies, ineffective dosing, overdosing of effective drugs, and new pediatric adverse events. To address these gaps, juvenile animal studies may provide added hazard identification and adequate clinical monitoring in a trial. Juvenile animals may be useful to assess safety concerns that are not adequate, ethical, or safely studied in pediatrics. Juvenile animals may be appropriate for predicting postnatal developmental toxicities in children. The developmental age of animals relative to the indicated pediatric population needs to be considered. Juvenile animal studies can assess safety issues with long-term exposure during critical developmental periods. However, not every pediatric product requires juvenile animal studies. Juvenile animal data can be applied to clinical risk assessment to ensure adequate clinical monitoring (for example, identification of safety risks) and to inform label considerations. Dr. Davis-Bruno concluded that juvenile animal studies are useful, especially

when performed to address a concern, and are not prohibitively challenging to conduct. However, the current database of juvenile animal studies is limited.

Cognition: Executive Function and Memory in Children with FXS

Steve Hooper, M.D., Associate Director, Clinical Center for the Study of Development and Learning, Child Development Institute, University of North Carolina

Significant research has been devoted over the past two decades to characterizing the basic neurocognitive phenotype of FXS. This research has focused on school-age and adult populations. Research on FXS children from birth to 5 years of age is limited, although several studies suggest that the suspected general deficits and delays can be assessed and detected during this period. For the full mutation, studies suggest different profiles for males versus females, mostly with level of functioning. The areas of executive functioning and memory have proven to be fruitful in phenotypic descriptions and align with the pathophysiology of FXS. The literature on executive functioning in FXS has pointed to problems in attention modulation, impulsivity/inhibition, problems managing transitions, perseveration, sequencing problems, and working memory.

Dr. Hooper reviewed a study of executive function in boys with FXS. There were two research questions/hypotheses:

- Do boys with FXS perform more poorly on executive function domains compared with mental-age matched typically developing children at baseline? Hypothesis: Boys with FXS would demonstrate disproportionate deficits across each of the domains/measures.
- How will these cognitive functions change over time compared with mental-age matched typically developing children? Hypothesis: Boys with FXS would show a slower rate of development for each of the domains/measures.

The following domains and measures were included:

- Problem solving/cognitive flexibility/planning
 - NEPSY Tower
 - WJ-III Planning
- Inhibition
 - Auditory CPT
 - Visual CPT
 - Day–Night Task
- Working memory
 - Leiter-R Reverse Memory
 - WJ-III Auditory Working Memory
 - WJ-III Memory for Words.

The study's findings support the available literature identifying executive function deficits in children and adults with FXS. These deficits appear to be pervasive and manifest primarily in (1) problem solving/cognitive flexibility/set-shifting, (2) inhibition, and (3) working memory. These functions are disproportionately low even when compared with mental age–matched typically developing children. Longitudinal findings suggest that many of the executive functions of boys

with FXS develop at a significantly slower rate over a 3-year span. These findings suggest severe and pervasive deficits in the domain of executive functions, with the gap between their development and typical development becoming wider over time.

Dr. Hooper reviewed a study of memory skills of boys with FXS. Three questions were posed:

- How does the performance of children with FXS compare with mental age–matched typically developing children?
- Are they equivalent on some types of memory measures?
- How does their memory change over time?

Basic capacity and laboratory-based memory measures were included:

- Verbal tasks
 - Auditory Working Memory (from WJ-III)
 - Memory for Words (from WJ-III)
- Visual tasks
 - Spatial Memory (from the Leiter R)
 - Picture Recognition (from WJ-III)
- Incidental event
 - Mock health check
 - Children not told to remember
 - Immediate interview
- Deliberate object memory
 - Children play with items
 - Items hidden
 - Told to remember.

The results were as expected. The memory capacity of boys with FXS was lower than that of their mental age–matched peers. On the laboratory tasks, the performance was partially determined by how the question was asked. FXS children had a harder time with yes/no questions and were better at free recall. Yes/no questions ease the burden on memory but require impulse control. Group x time interactions were present in nearly all of the memory composites.

Dr. Hooper explained that there are a number of possible effects of various treatment strategies. These strategies include pharmacological, behavioral therapy, academic, and environmental approaches. Treatment can directly affect ecological outcomes such as adaptive, social, behavioral, emotional, academic, and other cognitive measures. Treatment strategies can be targeted at neurocognition such as memory processes, attention, regulation, inhibitory controls, working memory, and set-shifting/flexibility. Treatments that improve neurocognition will affect ecological outcomes. Composite measures will be needed to measure these outcomes.

Dr. Hooper listed the following lessons learned:

- Test standardization procedures for many tests, as published, may hinder obtaining a more reliable score.
- These single-test measures/designs should be lessened in an effort to increase reliability of measurement.

- The use of measures in longitudinal designs, particularly in intervention trials, may require alternate forms, a better understanding of test–retest reliability for the FXS population at different ages, and inclusion of change-over-time indices (for example, Raush-Wright Scaling).
- Behavioral observation schemes should be used or developed (for example, attention, memory, use of strategies, inhibitory control, and set-shifting), particularly in ecological settings, or other test variables (for example, variability score and error patterns) should be used.

Outcome Measures for Clinical Trials with Children with FXS: Language Development

Leonard Abbeduto, Ph.D., Director, University Center on Excellence in Developmental Disabilities, Waisman Center, University of Wisconsin–Madison

Dr. Abbeduto explained that measures of language are “low tech” by design. The goal is to understand language problems in children and adolescents with FXS but also to create measures and assessment protocols that can be used by speech/language clinicians and teachers in classroom settings. Existing measures are used where applicable, and new measures are created when necessary. The specific aims of Dr. Abbeduto’s research are to (1) distinguish the language phenotype of FXS from those of other neurodevelopmental disorders, (2) describe and explain within-syndrome variation in the language phenotype of FXS, (3) understand genetic and environmental contributions to the trajectory of the language phenotype of FXS, and (4) understand the language learning process in FXS.

Language is a complex system. It is not a unitary ability. Language can be dissected into a number of conceptually distinct, but interrelated components: phonological, lexical, syntactic, pragmatic, and receptive versus expressive. The use of a single broad measure (for example, verbal intelligence quotient [IQ]) does not provide an adequate characterization of language.

Dr. Abbeduto described findings from recent studies of language phenotype and syndrome specificity and the measures used. Group comparisons were used to determine the profile of strengths and weaknesses in language for FXS and to determine the specificity of the profile. FXS subjects were compared with younger typically developing children matched on one or more “benchmark” abilities and youths with cognitive disabilities due to other causes matched on age and one or more benchmark abilities. Measures included tests for auditory comprehension of language, receptive vocabulary and syntax, expressive vocabulary and syntax, expressive vocabulary diversity, expressive syntax, pragmatics (talking about referents), signaling noncomprehension, and frequency of noncomprehension signaling. Dr. Abbeduto summarized the findings:

- Acquisition of lexical and syntactic skills is delayed but keeps pace with (nonverbal) cognitive development.
- Pragmatics is generally more problematic and “splintered,” with some domains being especially delayed (relative to nonverbal cognitive development).

Dr. Abbeduto described findings from recent studies of within-syndrome variation in language phenotype. The studies focused on factors associated with within-syndrome variation in language and related abilities such as autism status, gender, and FMRP levels. Outcome measures included receptive language, verbal perseveration, receptive vocabulary, expressive vocabulary, and receptive syntax. The findings were as follows:

- Receptive language is especially impaired in those with comorbid autism even after controlling for cognitive differences.
- Females with low IQs are less impaired in language than males, and variations across dimensions are not fully explained by IQ.
- Differences in vocabulary and syntax among individuals with FXS are related to variation in FMRP levels, but the relationship may be mediated by cognitive ability.

Dr. Abbeduto's current research focuses on a longitudinal study of language development in FXS, assessment of early word learning ("online" vocabulary learning), and the acquisition of new language skills.

Behavior and Psychiatric/Emotional Features of FXS

David Hessel, Ph.D., M.I.N.D. Institute, Department of Psychiatry and Behavioral Sciences, University of California, Davis

Executive functioning is a significant problem in people with FXS, affecting inhibitory control, attention, organization, planning, and working memory. Autistic behaviors are also a significant problem, particularly stereotypic behaviors, restricted and intense interests, deficits in reciprocal social interaction, and communication problems. About 33 percent of full mutation males meet DSM-IV criteria for autistic disorder. Autism symptoms have correlated with FMRP in some studies but not in others. Aggression and self-injury are other prominent behaviors in FXS. Secondary genes (for example, serotonin transfer alleles) have been shown to moderate aggressive and self-injurious behaviors. Another facet of the behavioral phenotype is the Prader-Willi syndrome. Psychotic symptoms of FXS have been understudied. Although it is estimated that 12 percent of FXS males have these symptoms, few cases appear in the literature.

Findings on sensory processing and gating abnormalities in FXS provide a rationale for PPI study in FXS. There is a need for a feasible, objective, reproducible, and biologically based measure to reflect FXS psychophysiological deficits. An ideal measure would be mediated by mGluR5 and/or GABAergic systems. The measure could be used in both animal (FMR1 knockout) and human studies. PPI is a unique measure that satisfies these criteria. Dr. Hessel described a protocol of modulation of PPI of startle in 61 FXS subjects and 63 controls. The protocol used startle-alone trials and PPI trials. The results showed highly significant, highly robust PPI deficits in both males and females with FXS. Test-retest reliability was demonstrated. This outcome measure is ready for treatment trials.

Social anxiety, specific phobias, generalized anxiety, selective mutism, and obsessive-compulsive symptoms are other characteristic FXS behaviors. Dr. Hessel and other investigators have attempted to establish objective physiologic measures associated with anxiety in FXS. A fear-potentiated startle protocol was used as a biobehavioral probe of amygdala response. Recent

findings show that impaired fear potentiated startle corresponds with reduced fMRI amygdala response in men with FMR1 premutation.

Problems of eye contact and gaze avoidance are often seen in people with FXS. A potential objective measure for this social behavior is eye tracking. A protocol using images of facial expressions and an infrared eye tracker is being developed. The tracker provides information on where the subject is looking on the image as well as pupil size, which is regulated by the sympathetic nervous system. One of the hypotheses of the protocol is that, compared with controls, FXS subjects will show a greater pupillary response when they look at the eyes of the facial images.

Defining Outcome Measures for Clinical Trials in FXS

Dr. Berry-Kravis

Dr. Berry-Kravis discussed a recent study to evaluate new outcome measures in FXS. The goal of the study was to develop a paradigm for evaluating outcome measures before using them in clinical trials. Outcome measures have been used with no supporting data on whether the measure (1) could be used consistently in the FXS population in a clinical trial setting, (2) was reproducible in test–retest, and (3) had the ability to measure drug effects. Another important aspect of an outcome measure is whether FXS subjects can successfully perform the test or task. Success may depend on age, IQ, or developmental quotient.

The study cohort was composed of 46 full-mutation FXS subjects (39 males and 7 females). The age range was 5–47 years. The IQ range was 30–89. The mental age range was 2–10 years. Age, IQ, and mental age ranges were intentionally wide. The subjects took tests at the initial visit and then 5–8 weeks later. Medications were not changed in the interim. Reproducibility was analyzed with ICC, weighted Kappa.

The following measures were evaluated:

- RBANS List Learning and Story Memory (validation data for FXS < 18 years)
- W-J Spatial Relations (easier visuospatial test than TVPS)
- S-B Symbol Search (processing speed)
- NEPSY Tower (executive, problem solving)
- Computerized Card Task (visual memory)
- North Carolina FXS Project CPT (executive, attention, impulsivity, separate auditory and visual components, less complicated than IVA)
- NVALT Learning and Reversal (visual associative learning)
- Biophysical measures—heart rate variability, eye tracking (anxiety, failure of sympathetic inhibition)
- Blood biomarkers—ERK activation.

The study results are as follows:

- There was no correlation of performance with age on any measures.
- Performance correlated with IQ and mental age on all measures except the NVALT Continuous Performance Test.

- Because there were different mental age cutoffs to perform different tests, a series of tests may be needed to cover the entire range of FXS function.
- Low-functioning FXS subjects (IQ < 40) were hard to test because of a lack of results on all tests.
- Refusal was a problem but was less in adults (11 percent of the total refusals were for subjects > 18 years).
- RBANS List learning and Story memory, W-J Spatial Relations, NEPSY Tower, and CPT should be sufficiently reliable (ICC or Kappa > 0.7) for use in clinical trials as cognitive measures for FXS.
- S-B Symbol Search and Computerized Card Task had poor reproducibility.
- There was a NVALT practice effect in higher functioning individuals.

Using Neuroimaging to Assess Treatment Response in FXS

Dr. Reiss

Reduced FMRP in individuals with FXS leads to deficits in synaptic maturation and plasticity, which has significant effects on how the brain develops and functions. Neuroimaging can reveal brain structure and function. Neuroimaging is a tool for investigating pathophysiology of FXS, a potential modality for tracking or predicting treatment, and even possibly a treatment itself. Dr. Reiss briefly reviewed the strategies for measuring disease-specific pharmacological and behavioral intervention outcomes in clinical trials of FXS.

Neuroimaging involves multiple modalities, including:

- Imaging of brain structure (for example, volumetric, voxel-based morphometry, shape analyses, cortical anatomy, and diffusion tensor imaging [DTI])
- Imaging of brain function (for example, fMRI, positron emission tomography, and single photon emission computed tomography)
- Imaging of brain biochemistry (for example, magnetic resonance spectroscopy [MRS]).

Structural neuroimaging can be used to date when a brain abnormality occurred during embryological or fetal development. Recent research has shown significant enlargement of the caudate nucleus in FSX. The caudate nucleus is part of several modulatory systems involved with cognition, language, emotion, and motor activity. Greater caudate nucleus volume has been correlated with lower FMRP levels, lower IQ, higher AuBC scores, and higher AbBC stereotypy scores. Findings of caudate nucleus enlargement are robust in the 1–3 years age group of children with FXS. Caudate nucleus volume may offer the best outcome measure for monitoring response to early intervention. Long-term follow-up would be required.

DTI can be used to image white matter anatomy and connectivity of the brain. DTI has shown abnormalities in caudate nucleus connectivity to the ventral prefrontal cortex, which is an area that is vitally important for cognition and executive function. Abnormalities in connectivity may reflect FMRP effects on white matter development.

fMRI uses an MRI scanner to visualize dynamic brain activity during movement, perception, language, learning, emotions, memory, mathematical reasoning, and socially mediated cognition.

fMRI can relate brain activation to anatomical measures, task performance, learning, behavioral and cognitive profiles, and disease symptomatology. fMRI suggests possible functional cholinergic deficit in FXS and has shown deficits in the activation of the prefrontal cortex–striatal pathway, as well as a lack of coordination between these two structures.

Recent studies have shown abnormal activation in brain areas associated with emotion processing and autonomic nervous system output in FXS. One study demonstrated left insula hyperactivation in FXS subjects during a directed gaze task. In another study, the amygdala failed to show normal adaptation to direct gaze tasks.

MRS offers the capability of using MRI to noninvasively study tissue biochemistry at a single point in time. In MRS, either ^1H atoms in molecules or other atoms such as ^{31}P , ^{23}Na , K , ^{19}F , or Li are imaged. Within a given brain region (also called a *voxel*), information about these molecules is available to identify and quantify the amount of a compound. MRS can provide data on glutamate and choline and can potentially provide data on GABA. MRS has limited spatial and temporal resolution and is restricted to the study of mobile magnetic compounds.

Real-time functional imaging feedback is based on image processing procedures developed for standard fMRI. As opposed to offline postprocessing, data are processed as fast as they are acquired. Real-time functional imaging feedback provides a method for assessing response to treatment as well as a potential treatment itself. A subject is given stimuli or a cognitive task, and feedback is provided in real time during the training. Feedback has been shown to change activation of very specific brain areas.

Functional near-infrared spectroscopy (fNIRS) can generate data output that is highly amenable to real-time paradigms and is an alternative to fMRI. This neuroimaging approach is promising but requires validation.

Neuroimaging targets for FXS treatment studies should be considered for both pharmacologic and behavioral trials. The brain is more proximal to the “site” of disease pathogenesis than is behavior. Brain changes often occur before behavioral and cognitive problems appear (for example, Parkinson’s and Huntington diseases). Using brain imaging, it may be possible to predict behavioral and cognitive changes and identify appropriate interventions. Longitudinal studies are essential for refining imaging phenotypes.

Regarding Potential Endpoints

John March, M.D., M.P.H., Professor of Psychiatry and Chief, Psychiatry Department, Duke University Medical Center

In the clinical trial framework, dependent variables have two functions: (1) inform dose escalation through dose–response curves and (2) allow signal detection, that is, determine whether treatment is effective. The same dependent variables should be used in early and later phases of clinical trials. FXS is a disorder that has no problems with diagnosis. To treat it, the dependent variable must come from the six or seven behavioral symptomatic domains relevant to FXS. Once the domains are identified, methods to capture the domains are developed. The

methods should be easy to use in clinical environments, and they must be simple enough to not impose undue burden on research subjects. Methods should have both interrater and test–retest reliability. There should be a range of symptomatology for the signal being measured. The methods must be sensitive to change.

Dr. March offered the following approach for developing an FXS rating scale:

- Establish the domains of highest importance and interest
- Identify all the rating scales that have been developed for the domains
- Q sort the scales to estimate relevance to outcome domains of interest
- Select about 20 scales for each outcome domain
- Test about 400–500 FXS subjects (about 100 in each relevant age group)
- Conduct statistical analyses to determine which tests are the most appropriate
- Test a population of matched normal subjects to reduce redundancy, thereby decreasing the number of tests and refining the rating scale.

Researchers could use both scalar and global outcome measures in clinical trials. Adverse events should be monitored with a rating scale or tool.

A cooperative clinical trials network is needed to develop the rating scale and new drug therapies for FXS. Establishing such a network is a complex endeavor, and it requires much work to build the infrastructure. Network operation requires an integrated information management system and a clearly defined governance structure. It is essential that each network site consistently adhere to study protocol.

Outcome Measure Recommendations

- There should be developmentally sensitive outcome measures to assess efficacy in different domains of dysfunction (for example, cognition, anxiety, and social behavior).
- Measures should potentially be applicable to other developmentally based disorders.
- Objective measures should have a broad dynamic range, be sensitive to differing levels of ability, and have test–retest reliability and validation.
- Cognitive outcome measures in clinical trials of FXS should test broad ranges of ability.
- Because higher or lower groups can be off the scales; there should be no floor or ceiling effects in the measures.
- Measures should span the entire range of mental functioning in FXS.
- Measures should be able to assess core FXS deficits.
- Because it is difficult to get FXS participants off their medications, new trials should consider allowing baseline medications.
- Determine whether crossover and extension designs are acceptable in FXS clinical trials.
- Travel and frequent visits to the clinic are of special concern. If frequent testing is required, more test sites or home visits may be useful.
- Mechanisms to improve recruitment of younger subjects should be explored.
- Outcome measures should
 - Focus on biological and behavioral traits that best distinguish FXS subjects from control subjects and subjects with idiopathic developmental disabilities

- Use multiple levels of measurement (for example, brain structure and function, neuroendocrine, psychophysiology, cognition, and behavior)
- Reflect naturalistic or adaptive behaviors that are developmentally appropriate
- Provide opportunities to demonstrate cognitive or behavioral change as opposed to “passive testing.”
- Outcome measures should be robust, applicable to the FXS population, able to be used at several times, without floor or ceiling effects, and without practice effects, particularly for working memory tasks.
- Collect individual data with standardized as well as subjective measures and use single case designs.
- Establish a coordinated clinical trials network or consortia to improve sample sizes, increase statistical power, and develop measurement models. Interventions with small effect size will require larger sample sizes to demonstrate efficacy.
- Develop strategies to measure outcomes in comfortable, naturalistic settings.
- Monotherapy trials should be considered to determine the true effect of interventions. For pharmacological interventions, subjects should cease all medications to determine how treatment affects pathology by itself.
- Clinical trials could be designed to include some subjects who remain on concomitant medications and some who are taking no medication.
- The design of FXS clinical trials should be targeted to meet the appropriate level of evidence for both safety and efficacy as is required for other therapeutics in children. Study data should meet the criteria of FDA and other regulatory agencies.
- Criteria for “better” (that is, the degree of efficacy) should be established and defined.
- Psychometric approaches should be applied to ensure that outcome measures in clinical trials are able to determine efficacy (that is, the extent to which increases in test scores translate into functional improvements).
- Demonstrating drug efficacy for FDA approval may require two outcome endpoints (for example, cognitive and functional), as has been done for drugs for Alzheimer’s disease.
- Experts should consider developing new outcome measures, tools, or instruments to specifically evaluate FXS domains. Composite measures across the domains and multidimensional approaches should be considered.
- Tasks selected for a clinical trial need to be tailored to the mental age of the sample. Cognitive tasks from the developmental literature can be adapted for this purpose. Strategies for testing different age groups include using different tasks, applying psychometric scaling techniques, and truncating age range for some tasks.
- With regard to cognition, outcome measures should evaluate components of function rather than a single global measure.
- Develop a standardized observational measure of perseverative language for clinical trials.
- Neuroimaging targets for FXS treatment studies should be considered for both pharmacologic and behavioral trials.
- Develop a new, composite FXS rating scale.

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