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Citation	Berry-Kravis, E. M., D. Hessler, B. Rathmell, P. Zarevics, M. Cherubini, K. Walton-Bowen, Y. Mu, et al. "Effects of STX209 (Arbaclofen) on Neurobehavioral Function in Children and Adults with Fragile X Syndrome: A Randomized, Controlled, Phase 2 Trial." <i>Science Translational Medicine</i> 4, no. 152 (September 19, 2012): 152ra127–152ra127.
As Published	http://dx.doi.org/10.1126/scitranslmed.3004214
Publisher	American Association for the Advancement of Science (AAAS)
Version	Author's final manuscript
Citable link	http://hdl.handle.net/1721.1/95776
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Effects of STX209 (Arbaclofen) on Neurobehavioral Function in Children and Adults with Fragile X Syndrome: A Randomized, Controlled, Phase 2 Trial

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Abstract

Research on animal models of fragile X syndrome suggests that STX209, a γ -aminobutyric acid type B (GABA_B) agonist, might improve neurobehavioral function in affected patients. We evaluated whether STX209 improves behavioral symptoms of fragile X syndrome in a randomized, double-blind, placebo-controlled crossover study in 63 subjects (55 male), ages 6 to 39 years, with a full mutation in the *FMRI* gene (>200 CGG triplet repeats). We found no difference from placebo on the primary endpoint, the Aberrant Behavior Checklist—Irritability (ABC-I) subscale. In the other analyses specified in the protocol, improvement was seen on the visual analog scale ratings of parent-nominated problem behaviors, with positive trends on multiple global measures. Post hoc analysis with the ABC—Social Avoidance scale, a newly validated scale for the assessment of fragile X syndrome, showed a significant beneficial treatment effect in the full study population. A post hoc subgroup of 27 subjects with more severe social impairment showed improvements on the Vineland II—Socialization raw score, on the ABC—Social Avoidance scale, and on all global measures. STX209 was well tolerated, with 8% incidences of sedation and of headache as the most frequent side effects. In this exploratory study, STX209 did not show a benefit on irritability in fragile X syndrome. Nonetheless, our results suggest that GABA_B agonists have potential to improve social function and behavior in patients with fragile X syndrome.

Introduction

Targeted treatments are therapies that address the specific molecular pathophysiology of a disease. Until very recently, neurobiological knowledge was not adequate to allow the development of targeted treatments for neurodevelopmental disorders. Instead, psychopharmacologic agents were developed on the basis of fortuitous observations [for example, psychostimulants for attention deficit/hyperactivity disorder (1)] or by extension from their use in distinct adult conditions [for example, antipsychotics for the treatment of irritability in autism (2)]. Now, science has advanced to the point that drug development for several neurodevelopmental disorders can proceed within a rational neurobiological framework (3, 4). Fragile X syndrome (FXS) provides a key example of this approach (5).

FXS is the most common known inherited cause of autism and of intellectual disability (6), and social impairment is among its core features (7, 8). It is caused by an expansion of the CGG triplet repeat in the *FMRI* gene on the X chromosome. The discovery that the FXS phenotype is a result of the transcriptional silencing of the *FMRI* gene (9) led to the creation of animal models in which this gene has been knocked out. Study of these animals led, in turn, to the mGluR theory of fragile X (10), which posits that the neurobehavioral abnormalities in FXS result from dysregulation of neuronal signaling through group 1 metabotropic glutamate receptor-activated (mGluR1 and mGluR5) pathways and excessive dendritic protein synthesis. Pharmacologic and genetic rescue

experiments show that negative modulators of mGluR5 correct many of the abnormal phenotypes in animal models of FXS, including disruptions of neural plasticity and excitability in the hippocampus, neocortex, and amygdala; deficits in learning and memory; and altered neuronal morphology (11–15).

Deficiencies in γ -aminobutyric acid (GABA)–mediated inhibitory neurotransmission also have been implicated in FXS. In *Fmr1*-knockout mice, decreased GABAergic inhibition occurs in many areas of the brain, including the hippocampus, striatum, somatosensory cortex, and amygdala (16, 17). Humans with FXS show overactivation of the amygdala when asked to perform a face-processing task (18). Excessive activation in the amygdala and elsewhere in the limbic system is hypothesized to be a basis of the social anxiety and social avoidance that characterize FXS (19–21). GABAergic compounds reverse phenotypes in the FXS *Drosophila* model (22), and pharmacological stimulation of GABA receptors has therefore been suggested as a therapeutic strategy for FXS (17, 23). Treatment with a GABA agonist could work directly, by augmenting the deficiencies in inhibitory neurotransmission, or indirectly as a negative modulator of the mGluR pathway, by decreasing the synaptic release of glutamate, a known effect of presynaptic GABA type B (GABA_B) receptor stimulation (24).

STX209 (arbaclofen, *R*-baclofen) is a GABA_B receptor agonist and is the active enantiomer of racemic baclofen. In *Fmr1*-knockout mice, STX209 rescues the increased susceptibility to seizures and corrects the excessive dendritic spine density and protein synthesis believed to be pathogenic in FXS (25). In healthy human subjects, STX209 shows pharmacokinetic properties similar to those of racemic baclofen, with high bioavailability and a terminal half-life of 4 to 5 hours. STX209 undergoes renal elimination with no significant metabolism (26). Here, we have examined the safety and efficacy of STX209 in the treatment of neurobehavioral symptoms in humans with FXS.

Results

Subjects, disposition, and dosing

Sixty-three subjects were randomized into the study. Their demographic and baseline characteristics are shown in Table 1. Fifty-six (89%) subjects completed the entire study. Subject disposition and the composition of the primary analysis populations are shown in Fig. 1.

There were no withdrawals related to drug tolerability. Each subject's optimal titrated dose (OTD) was defined as either the highest tolerated dose or the lowest dose at which a subject was judged to be “very much improved” on the CGI-I, the Clinical Global Impression of Improvement. This instrument is a seven-point Likert scale that requires the clinician to evaluate overall improvement (or worsening), considering the entirety of the data available on the subject. Among subjects for whom an OTD was reported, the OTD was the maximum allowed dose for 91% of subjects aged 6 to 11 years [10 mg, twice daily (BID)] and 63% of subjects aged 12 to 40 years [10 mg, thrice daily (TID)]. Pharmacokinetic analyses confirmed correct treatment assignment for all subjects.

	ITT population	Subjects with ABC-L/SW ≥ 8
Total	63	27
Age (years)		
6–11	24	11
12–17	22	9
18–40	17	7
Sex (M/F)	55/8	24/3
Race (>1 response permitted)		
Caucasian	55	25
African-American	3	0
Other	5	2
Ethnicity: Non-Hispanic/Hispanic	58/5	25/2
Comorbid autism (DSM-IV and ADI-R criteria)	37	19
Concomitant psychoactive medication (any)	54	27
Antipsychotics	30	16
Psychostimulants	18	9
SSRI/SNRI	25	12
Antiepileptics	12	10
IQ (mean \pm SD)	46 \pm 8	47 \pm 11

Table 1

Baseline characteristics of study subjects. Included are the ITT population and those assessed in the secondary exploratory analyses. ADI-R, Autism Diagnostic Interview-Revised; SSRI/SNRI, selective serotonin reuptake inhibitor/serotonin-norepinephrine reuptake inhibitor.

Prespecified efficacy analyses

The primary outcome measure was the Irritability subscale of the Aberrant Behavior Checklist (ABC-I), which measures irritable mood, aggression, and self-injury. No treatment effect was found on the ABC-I in the intent-to-treat (ITT) study population. A possible period effect was seen, with greater improvement in period 1 (-5.5 ± 0.84) than in period 2 (-3.2 ± 0.87 , $P < 0.10$). No explanation for a period effect was evident in the data, and the treatment \times period interaction was not significant, failing to support a possible carryover effect. In the analyses of the secondary outcome measures, STX209 was associated with significant improvement on the visual analog scale (VAS) problem behavior ratings (Table 2), in which each parent or guardian provided severity ratings for the three most problematic behaviors for their child. Parents or guardians nominated a

variety of problems for VAS rating, including both externalizing (for example, aggression and outbursts) and internalizing (for example, anxiety, self-injurious behavior, and stereotypes) behaviors. Consistent but not statistically significant trends toward STX209 effects were found on several global measures, including the CGI-I and the blinded treatment preference (Table 2 and Fig. 2A). No significant treatment effects or period effects were found on the summary scores for other prespecified analyses. No significant age-related effects were found on any of the prespecified analyses.

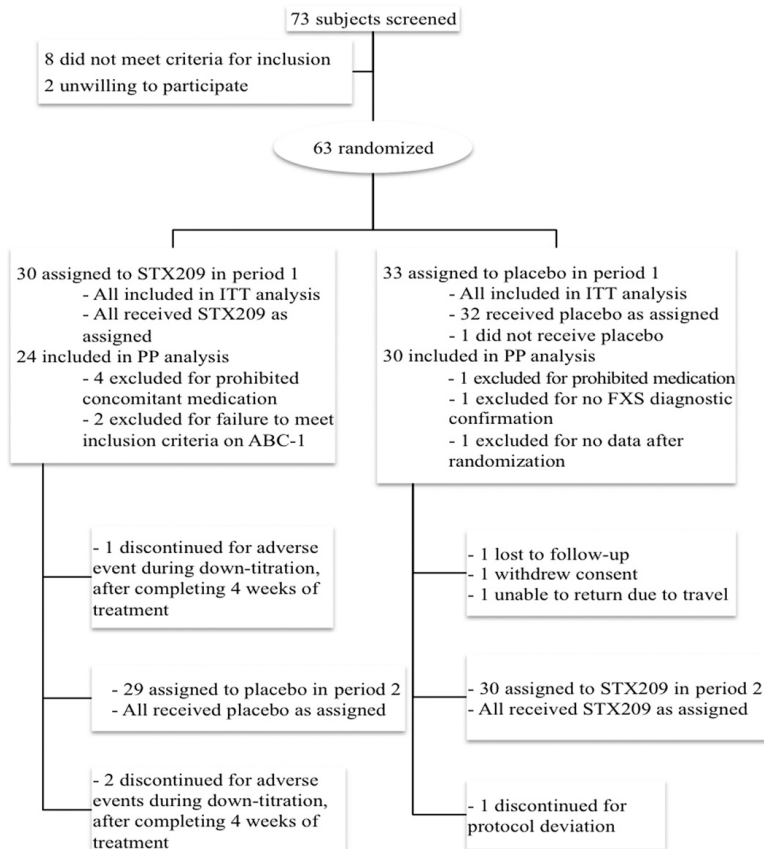


Fig. 1

Flow diagram of subject disposition. The ITT population consisted of all subjects who received at least one dose of study medication and had at least one post-baseline assessment on the ABC-I scale. The per-protocol (PP) population consists of all subjects in the ITT population who did not have a major protocol deviation, as determined before unblinding.

Newly validated ABC scoring algorithm

We performed post hoc analyses with the recently validated, FXS-specific scoring algorithm for the ABC (27). This scoring algorithm was developed through factor analysis on data from 630 individuals with FXS, resulting in six reconstituted subscales instead of the five in the standard Aberrant Behavior Checklist—Community (ABC-C) scoring algorithm. A sixth subscale emerged primarily because the original ABC—Lethargy/Social Withdrawal (LSW) split along two separate dimensions of physical lethargy/social unresponsiveness and active social avoidance. On the new ABC—Social Avoidance (ABC-SA) scale, STX209 was associated with an overall beneficial effect in

the ITT study population ($P = 0.01$), with no period effect observed (Table 2). No significant effects were found on the other validated FXS-specific factors.

Subjects with ABC-LSW greater than or equal to 8

Additional analyses were conducted in the subgroup with more severe social impairment at baseline as measured by the original ABC-LSW scale (Table 1). These analyses were motivated by the post hoc ABC-SA result and spontaneous investigator reports of social and communicative improvement in many subjects. We reasoned that benefits to social function might not be apparent in subjects whose baseline social impairments were rated as less severe. The criterion for this post hoc subgroup ($\text{ABC-LSW} \geq 8$) was chosen because it represents the upper half of severity on this subscale among the general population of males with FXS (27). In this subgroup, we noted significant improvement on the validated ABC-SA scale ($P = 0.04$), as well as significant treatment effects on the Vineland-Socialization measure of adaptive function, and on multiple global assessments, with a trend on the ABC-LSW itself (Table 3 and Fig. 2B). A responder analysis showed that significantly more subjects improved on STX209 than on placebo.

	STX209 (least squares mean ± SEM), <i>n</i> = 60*		Placebo (least squares mean ± SEM), <i>n</i> = 62*		<i>P</i>
	Baseline	End of treatment	Baseline	End of treatment	
Prespecified analyses					
ABC-I	21.0 ± 1.14	16.4 ± 0.95	21.8 ± 1.29	16.2 ± 0.95	0.89
CGI-I	—	3.1 ± 0.16	—	3.5 ± 0.16	0.15
CGI-S	5.1 ± 0.13	4.5 ± 0.12	5.1 ± 0.14	4.8 ± 0.12	0.09
Blinded treatment preference (clinician)	—	26 (57%)	—	13 (28%)	0.05
Blinded treatment preference (parent)	—	27 (59%)	—	15 (33%)	0.09
VAS problem behaviors	2.2 ± 0.22	4.2 ± 0.32	1.9 ± 0.20	3.1 ± 0.33	0.04
Post hoc analysis					
ABC—Social Avoidance	4.5 ± 0.46	3.3 ± 0.44	3.9 ± 0.43	3.6 ± 0.41	0.008

Table 2

Prespecified and post hoc efficacy analyses. *Numbers reflect n for analysis of ABC-I. n differs slightly for other variables because of missing data.

Safety

The safety and tolerability of STX209 was good. Three subjects (two placebo, one STX209) discontinued from the study because of adverse events. All three of these cases were reported to have “increased irritability” during the planned drug taper at the end of the first treatment period (Fig. 1). The STX209 case was the only serious adverse event in the study, requiring hospitalization for behavioral management in a subject with two previous similar hospitalizations. Most other adverse events in this study were mild or moderate in severity. All adverse events occurring in $\geq 5\%$ of subjects on either STX209 or placebo are listed in Table 4. There were no differences between the STX209- and placebo-treated groups on clinical laboratory assessments or physical examination.

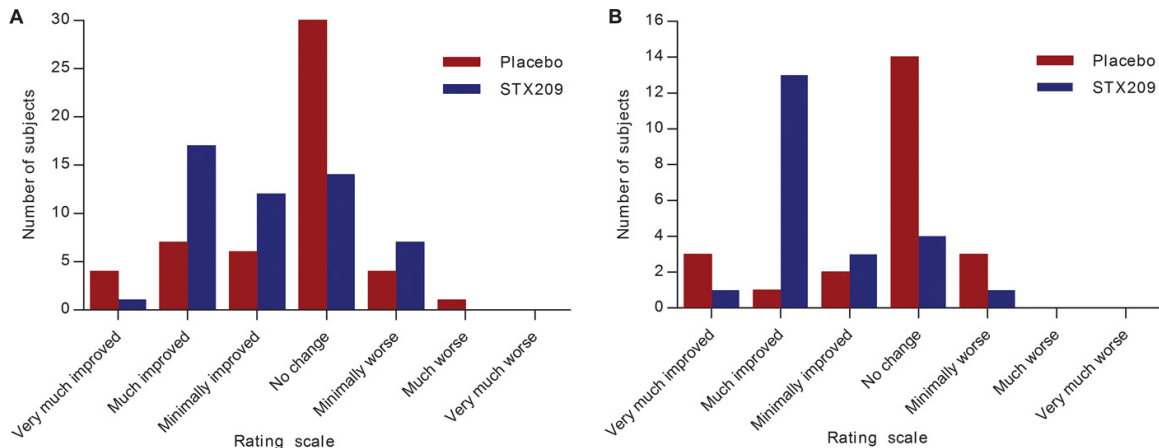


Fig. 2

Distribution of CGI-I scores. (A) PP population ($P = 0.15$). (B) Subjects with ABC-LSW ≥ 8 at screening and baseline ($P = 0.03$).

Discussion

This double-blind, placebo-controlled study evaluated STX209, a GABA_B agonist, in patients with FXS. The STX209-treated group was not different from the placebo group on the study’s primary endpoint, the ABC measure of irritability. Improvement was seen, however, on the prespecified analysis of VAS-rated problem behaviors, and multiple global assessments of neurobehavioral function showed trends in favor of STX209. Our post hoc analyses were designed to identify the nature of the possible beneficial drug effects that contributed to the trend for global functional improvement but would not be accounted for by a change in irritability and aggression as measured by the ABC-I.

An effect of treatment on social function was suggested by several post hoc analyses. Most notably, improvement was found in the full study population on the ABC-SA score, a measure recently validated for FXS (27). Furthermore, subjects with more severe impairments in social function showed improvement on two independent measures in this domain (the ABC-SA and the Vineland-Socialization) and on multiple global assessments. Analysis of those who responded to treatment suggested that global improvement was closely associated with improvement in social function. These mutually reinforcing results are consistent with the hypothesis that social impairments in FXS may be related to GABA deficiencies or exaggerated glutamatergic signaling. It has been suggested that the ABC-SA scale may reflect anxiety-driven behaviors in FXS, which would be consistent with the observations of GABA deficiency and amygdala overactivation in FXS. However, the scale itself measures manifest social behavior (for

example, “withdrawn; prefers solitary activity”), as does the Vineland-Socialization scale, and no drug effect was found on the Child and Adolescent Symptom Inventory (CASI) anxiety scale. Development and validation of other outcome measures that accurately define the FXS phenotype, including the anxiety phenotype in FXS, will facilitate discovery of effective treatments for specific domains of function, as indicated by the results of this study.

	STX209 (least squares mean \pm SEM), <i>n</i> = 27		Placebo (least squares mean \pm SEM), <i>n</i> = 27		<i>P</i>
	Baseline	End of treatment	Baseline	End of treatment	
ABC-LSW	16.2 \pm 1.21	12.4 \pm 1.43	16.0 \pm 1.30	15.9 \pm 1.45	0.07
ABC—Social Avoidance	6.8 \pm 0.60	4.6 \pm 0.54	5.9 \pm 0.62	6.4 \pm 0.53	0.04
Vineland-Socialization raw score*	80.1 \pm 8.10	99.6 \pm 3.38	83.1 \pm 8.65	87.8 \pm 3.19	0.03
CGI-I	—	2.5 \pm 0.24	—	3.6 \pm 0.25	0.02
CGI-S	5.4 \pm 0.22	4.4 \pm 0.21	5.3 \pm 0.22	5.2 \pm 0.22	0.009
Blinded treatment preference (clinician) [†]	—	16/20 (80%)	—	4/20 (20%)	0.01
Blinded treatment preference (parent) [†]	—	16/20 (80%)	—	4/20 (20%)	0.01
Responders [‡]	—	10/21 (47.6%)	—	2/23 (8.7%)	0.04

Table 3

Efficacy analyses in subjects with ABC-LSW ≥ 8 at baseline. *Vineland-Socialization raw score only had data at the end of treatment and baseline; there were no data in the middle of period. [†]This table reports on a subgroup of 27 subjects; only 20 subjects had data on these particular variables. Thus, seven subjects had missing data on these variables. [‡]Responders were defined by a rating of “very much” or “much improved” on the CGI-I and improvement of at least 25% on the ABC-LSW. Not all 27 subjects had ABC-LSW raw score and CGI-I at the end of each treatment.

Translating from animal research to human pharmacotherapy is inherently difficult, and the effects of STX209 in FXS animal models have not pointed clearly to any specific endpoint for a clinical trial focused on neurobehavioral function. In particular, no robust phenotype related to social behavior has been established in FXS animal models, and thus, STX209 has not been tested on such an endpoint in animals. Moreover, although the evidence strongly suggests that the core pathophysiology of FXS is evolutionarily conserved (28), it should not be assumed that FXS pathophysiology will manifest as similar behavioral abnormalities in animals and humans, as there are profound differences in brain complexity. Thus, in this exploratory study, we cast a broad net to

find behavioral responses to treatment in humans. The designation of the ABC-I as the primary endpoint here was driven by a U.S. Food and Drug Administration (FDA) precedent. Our study's failure on this primary endpoint could be a result of either a mistaken choice of endpoint or a true lack of drug effect.

Concomitant use of antipsychotic medications in the study also may have blunted the effects of STX209 on irritability, the primary endpoint, because antipsychotics are known to diminish such symptoms in autism (6, 29). Possible effects on social function were less likely to be confounded by concomitant medications, because no medication is known to enhance social function.

Adverse event	STX209, <i>n</i> (%)	Placebo, <i>n</i> (%)
Upper respiratory tract infection	8 (13)	6 (10)
Headache	5 (8)	1 (2)
Sedation	5 (8)	1 (2)
Fatigue	4 (6)	1 (2)
Irritability	4 (6)	4 (6)
Diarrhea	3 (5)	5 (8)
Increased appetite	4 (6)	2 (3)
Vomiting	4 (6)	1 (2)
Aggression	3 (5)	4 (6)
Nasopharyngitis	2 (3)	6 (10)

Table 4

Treatment-emergent adverse events. Only those events reported in $\geq 5\%$ of subjects in either group are included.

The safety and tolerability of STX209 was congruent with the known profile of racemic baclofen. Sedation was reported at a relatively low rate of 8% and in all cases resolved without dose adjustment. In comparison, the FDA-approved label for racemic baclofen reports drowsiness in up to 63% of subjects. Increased irritability and aggression were reported to be equally common in patients given drug and placebo, suggesting that these were related to underlying pathology, rather than drug treatment effects.

The key limitation of this study is the post hoc nature of most of the positive results. This issue is mitigated by the cohesive results on multiple endpoints for the subjects with ABC-LSW ≥ 8 and by the positive result on the ABC-SA, which has been validated in FXS (27). Prospective replication of these results is essential, and the results speak to the importance of developing and using assessments that are validated in the populations under study.

Our findings can be compared with a recent double-blind, placebo-controlled crossover study that examined the effects of 3 weeks of treatment with AFQ056, an mGluR5-negative allosteric modulator, in 30 young adults with FXS. In the full study population, AFQ056 was not associated with improvement on the ABC or on global measures of behavior, although repetitive behaviors did show improvement ($P = 0.05$). Post hoc analyses on the subgroup of seven individuals with full methylation of the *FMRI* gene promoter showed treatment-related improvement on several assessments, including the ABC subscales of Stereotypy and Hyperactivity, and the CGI-I, but not the ABC-LSW or ABC-I scales (30). The seven subjects with full methylation were also more severely affected behaviorally in comparison to the 23 subjects with partial methylation, which may have been a factor in the positive response of this group, similar to the larger response in our more socially impaired subgroup.

The effort to develop targeted treatments for neurodevelopmental disorders is just beginning and its promise is large. Although existing agents can provide some relief of secondary symptoms, new targeted treatments may be able to ameliorate core impairments in cognition, language, and social function. Given our limited understanding of how to anticipate and measure treatment outcomes in these domains, the path to targeted treatments will require careful interpretation of all potentially informative data so that future study design and study endpoints can be optimized to find real and valuable treatment effects.

Materials and Methods

Participants

Individuals with a DNA-confirmed *FMRI* full mutation, aged 6 to 40 years, either male or female, were eligible for the study. Up to three concomitant psychoactive medications (including antiepileptic drugs) were permitted, but use of vigabatrin, tiagabine, riluzole, or racemic baclofen was prohibited because of their GABAergic mechanisms. Medication regimens were required to be stable for 1 month, and educational, behavioral, and other treatments for 2 months, before and for the duration of the study. Subjects with any previous seizure were required to be on anticonvulsant medication and seizure-free for 6 months. Minimum scores of 9 (if age 12 to 40 years) or 12 (age <12 years) on the Irritability subscale of the ABC-C and of 4 (moderate) on the CGI-S (Clinical Global Impression of Severity, a seven-point Likert scale that requires the clinician to evaluate overall symptom severity, considering the entirety of the data available on the subject.) were required at the screening visit and at the beginning of treatment period 1. Female subjects of childbearing potential were tested and excluded if they were pregnant.

All subjects or guardians provided voluntary informed consent or assent, as appropriate. This study (clinicaltrials.gov identifier NCT00788073) was approved by the institutional review boards governing each site.

Design

This was an exploratory, first-in-patient trial, using a randomized, double-blind, placebo-controlled, multisite, two-period crossover design, at 12 sites in the United States, between December 2008 and March 2010. Study drug was flexibly titrated every 3 to 4 days, starting at 1 mg BID, and then 2 mg BID, 3 mg BID, 5 mg BID, 10 mg BID

(maximum for age <12 years), and 10 mg TID, until the optimal tolerated dose was established. Regular phone contact was maintained throughout the titration period.

Subjects returned for evaluations 2 and 4 weeks after starting treatment. Study drug was then tapered down over 1 to 2 weeks. After a minimum 7-day washout, subjects entered the second treatment period. An end-of-study safety evaluation was performed 14 days after the end of the second treatment period, with telephone follow-up 28 days later.

Study drug and matching placebo were provided as 1- and 5-mg capsules. Subjects were assigned to treatment by the local (eight sites) or a central (four sites) pharmacy, according to a centrally generated randomization list. Randomization was stratified by age (6 to 11, 12 to 17, and 18 to 40 years). Treatment compliance was monitored with a dosing form, which guardians completed on a daily basis.

Assessments

Baseline assessments included the Stanford-Binet Intelligence Scales, Fifth Edition (SB-5), the Autism Diagnostic Interview-Revised, and a review of autism spectrum disorder criteria from the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV).

Global outcome measures included the CGI-I and CGI-S assessments, both rated on a seven-point Likert scale, and blinded treatment period preference. Focused assessments included the ABC-C (31), a 58-item, parent-rated questionnaire yielding five-factor scores, including Irritability, Lethargy/Social Withdrawal, Hyperactivity, Inappropriate Speech, and Stereotypy. Other measures included VAS of the three most problematic behaviors for each child, the Vineland Adaptive Behavior Scales, Second Edition (Vineland II) (32), the Social Responsiveness Scale (33), the Repetitive Behavior Scale-Revised (RBS-R) (34), the CASI anxiety scale (35), the ADHD Rating Scale-IV (36), and measures of vocabulary and short-term and working memory.

Safety assessments included physical examination, standard hematology and clinical chemistry assessments, urinalysis, electrocardiograms, and spontaneously reported adverse events.

Statistical analysis

The ABC-I was designated the primary endpoint because of regulatory precedents (2, 37). It was analyzed with a mixed-effect model repeated-measures approach in the ITT population. Unstructured within-subject covariance was used. The model included terms for treatment, time, treatment-by-time interaction, period, treatment-by-period interaction, age group, and period-specific baseline score as a covariate. The number of female subjects was too small to support the inclusion of sex as a covariate. Other appropriate variables were analyzed with a similar approach in the per-protocol population. Categorical outcomes were analyzed by sign test. For all comparisons, a nominal *P* value of 0.05 or less was required to declare significance, and no adjustments for multiplicity were made.

The study was designed to have 90% power to detect a treatment effect of size 0.6, with a *P* level of 0.05 in a crossover design. The planned sample size was 60 subjects.

Post hoc analyses

A post hoc analysis on the entire ITT population was performed with a newly validated scoring algorithm for the ABC-C recently developed in a separate study (26). Other post hoc analyses were conducted on the study subgroup with ABC-LSW scores ≥ 8 at screening and at baseline visits for both period 1 and period 2. One outlier subject was excluded from these analyses. Outcomes in this subgroup were analyzed similarly to the above efficacy analysis.

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Acknowledgments

We thank the families who participated in this study, and the FRAXA Research Foundation, the National Fragile X Foundation, and the Elwyn Institute, who assisted with study recruitment through Web site postings and other mechanisms.

Funding

This study was sponsored by Seaside Therapeutics Inc.

Author contributions

E.M.B.-K., B.R., P.Z., M.C., J.G.-H., R.L.C., and R.J.H. designed the study. B.R., P.Z., M.C., K.W.-B., P.P.W., and R.L.C. oversaw the execution of the study. E.M.B.-K., D.H., and R.J.H. led the subject testing and data collection. K.W.-B., Y.M., and D.V.N. led the statistical analysis. R.L.C. and M.F.B. provided the original insights and motivation for the study. All authors contributed to writing and revision of the manuscript.

Competing interests

P.Z.,M.C.,K.W.-B.,P.P.W., and R.L.C. are full-time employees of Seaside Therapeutics. R.L.C. and M.F.B. are co-founders of Seaside Therapeutics. J.G.-H. has consulted for Seaside Therapeutics. E.M.B.-K., D.H., and R.J.H. were compensated for their data collection efforts in this study.

Data and materials availability

This trial is registered at www.clinicaltrials.gov