An open-label study to investigate the safety, tolerability and efficacy of a single 6-hour intravenous infusion of AMO-01 to treat adolescents and adults with Phelan-McDermid Syndrome (PMS) and co-morbid epilepsy
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Protocol Identifying Number: AMO-01

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Confidentiality Statement:

The trial will be carried out in accordance with Good Clinical Practice (GCP) as required by the following: United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812), ICH E6

All key personnel (all individuals responsible for the design and conduct of this trial) have completed Human Subjects Protection Training.

SPONSOR PROTOCOL SIGNATURE PAGE

Protocol Version and Date:

Version 3.0 Dated June 1, 2019

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial/study will be conducted according to all the stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable federal regulations and ICH guidelines.

Name of Sponsor: Alexander Kolevzon, MD

Signature: _____ Date:_____

CO-INVESTIGATOR PROTOCOL SIGNATURE PAGE

Protocol Version and Date:

Version 3.0 Dated June 1, 2019

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Name of Co-Investigator: Jimmy Holder, MD, PhD

Signature: _____ Date:_____

i. Protocol Synopsis

Title:

An open-label study to investigate the safety, tolerability and efficacy of a single 6-hour intravenous infusion of AMO-01 to treat adolescents and adults with Phelan-McDermid Syndrome (PMS) and co-morbid epilepsy.

Rationale:

Phelan-McDermid Syndrome (PMS) is a neurodevelopmental disorder characterized by a chromosomal deletion or mutation at 22q13.3 that contains the *SHANK*3/ProSAP2 gene (Bonaglia et al., 2001; Anderlid et al., 2002; Wilson et al., 2003). *SHANK3* is a synaptic scaffold protein within dendrites. Neuronal activity-dependent synaptic plasticity and function is impaired with loss of function of the *SHANK3* gene. PMS individuals commonly present with hyposensitivity to pain, poor eye contact, stereotypic movements, deficits in social communication, hyperactivity, altered sensorimotor function and aggressive behavior. A key comorbidity in PMS is the presence of epilepsy (Soorya et al, 2013).

Currently there are no approved treatments for PMS. Furthermore, there has been relatively little clinical study of pharmacological interventions for PMS.

A knockout mouse model of PMS has been developed in which the *Shank3b* gene is curtailed. These mice exhibit behavioral alterations similar to those seen in PMS patients, including enhanced audiogenic seizure activity. Preclinical testing of AMO-01 in the *Shank3b* $^{-/-}$ knockout murine model indicates that AMO-01 reverses several of the key elements of the aberrant phenotype in this rodent model, including seizure potential. The effects of knockout of the *Shank 3* gene in mice may be compared to induced pluripotent stem (IPS) cell lines derived from PMS patients (Shcheglovitov et al., 2013). In PMS IPS cells, post-synaptic AMPA and NMDA responses are significantly impaired. These changes reflect fewer excitatory synapses and relate to decreased levels of the longest isoform of the *Shank3* protein.

AMO-01 is a Ras-ERK pathway inhibitor, and may also have potential therapeutic utility as an anticonvulsant. The Ras-ERK pathway has been implicated in seizure generation according to a number of converging lines of evidence in different experimental models, and in the use of Ras-ERK pathway inhibitors. Aberrations in the genes that regulate the activity of the MAPK/ERK pathway are also strongly associated with neurodevelopmental disorders including autism (Wen et al., 2016; Garg et al., 2017; Tylee et al., 2017). Further, it is known that *SHANK3* sequesters the Ras enzymatic protein, thereby modulating its activity (Lilja et al., 2017), and it has been shown that ERK activity is increased at least threefold in the cortex of *Shank3b* $^{-/-}$ knockout mice (AMO Pharma Internal Report AMO-01-PC-001). Importantly, administration of AMO-01 to the *Shank3b* $^{-/-}$ knockout model of PMS reversed behavioral abnormalities in these mice and reduced seizure activity after a single dose. Therefore, AMO-01 may provide benefit to

PMS patients exhibiting behavioral abnormalities and seizures.

Study Design:

This is an open-label study to investigate the safety, tolerability and efficacy of a single 6-hour intravenous infusion of AMO-01 to treat adolescents and adults with PMS and co-morbid epilepsy.

The subjects in this study will be administered the experimental study medication and then be followed up to ascertain safety and tolerability, and to determine whether their seizure frequency, as well as their signs and symptoms of PMS, are improving, using a caregiver-completed seizure diary as well as accepted rating scales and functional measures. In addition, pre- and post-treatment clinical EEGs will be obtained to determine whether AMO-01 diminished the prevalence of abnormal EEG findings that were evident prior to treatment. Approximately 15 subjects will be screened and up to 10 subjects will receive study medication.

The study will have 4 phases:

- Screening (Weeks -4 to -1): Subjects will be screened to ensure adherence to eligibility criteria and to assess pre-medication seizure frequency
- Baseline (Day 0): Subjects will complete baseline assessments in-clinic prior to study drug administration
- Study Drug Administration (Day 0): Eligible subjects will receive a single 6-hour intravenous infusion at a single dose of 120 mg/m²
- Follow-up (Weeks 0-4): follow-up visits will occur at 1 day after and 1, 2, 4, and 8 weeks after receiving study medication.

Objectives:

Primary Objective:
To evaluate the safety and tolerability of a single 6-hour intravenous infusion of AMO-01, from Baseline through Week 8.

Secondary Objectives:

- To evaluate the efficacy of AMO-01 in reducing seizure frequency by at least 25 percent as measured by a caregiver completed seizure diary
- To evaluate the efficacy of AMO-01, from Screening through Week 2, in reducing clinically significant EEG abnormalities
- To evaluate efficacy of AMO-01, from Baseline through Week 4, as measured by clinician-completed rating scales, caregiver completed diaries, functional assessments and biomarker assessments.
- To evaluate maintenance of efficacy from Week 4 to Week 8, as measured by telephone questionnaire

Endpoints:

Primary Endpoints:

- The incidence of Adverse Events (AEs) including Serious Adverse Events (SAEs) between Baseline and Week 8.

- The incidence of abnormal findings in objective assessments (e.g. laboratory values, ECGs, vital signs)

Secondary Endpoints:

- Seizure frequency as measured by a caregiver-completed seizure diary
- Syndrome-specific Clinical Global Impressions Improvement Scale (CGI-I)
- Clinician-completed PMS domain specific causes for concerns visual analogue scale (VAS)
- Top 3 caregiver Concerns VAS
- Aberrant Behavior Checklist (ABC) score
- Repetitive Behavior Scale- Revised (RBS-R) score
- Percentage of eye-tracking time on relevant target
- Change in phosphorylated ERK levels
- Telephone follow-up questionnaire

Exploratory Endpoints:

- Electroencephalography (EEG) – clinically significant abnormalities summarized using the SCORE-EEG format
- Sensory Assessment for Neurodevelopmental Disorders (SAND)
- Auditory Event Related Potential (AEP) – amplitude (N1), attenuation, and latency analyses
- Visual Evoked Potential (VEP) – P60 and N75 amplitude and frequency domain analyses

Population:

Diagnosis and main criteria for Inclusion:

Inclusion Criteria:

1. Subjects under study must have a diagnosis of Phelan McDermid syndrome (PMS) with genetic confirmation of pathogenic *SHANK3* deletion or mutation.
2. Subjects must be post pubertal males or females aged ≥12 years and ≤45 years at Screening.
3. Subject must have a diagnosis of epilepsy.
4. Subjects must have a syndrome-specific Clinical Global Impression- Severity Score of 4 or greater at Screening
5. Subject's parent or legally authorized representative (LAR) must provide written informed consent before any study related procedures are conducted. Where a parent or LAR provides consent, there must also be assent from the subject (as required by local regulations).
6. Subject's caregiver must be willing and able to support the subject's participation for the duration of the study.
7. Subject's caregiver is able and willing to maintain an accurate and complete daily written seizure diary for the entire duration of the study.

Exclusion Criteria: Subjects are excluded from the study if any of the following are met:

1. Receiving medications/therapies not stable (i.e. changed) within 4 weeks prior to Screening. For each enrollee, every effort should be made to maintain stable regimens of allowed concomitant medications and allowed non -medicine based therapies throughout the course of the study, from Screening until the last study assessment.
2. Known hypersensitivity to farnesylated dibenzodiazepinone or any of the formulation components.
3. Subjects with a history of uncontrolled hypotension or hypertension (Polysorbate 80 is a major constituent of AMO-01 and can cause hypotension).
4. Subjects that have received Coumadin or heparin in the 2 weeks preceding Screening.
5. Medical illness or other concern which would cause the investigator to conclude that the subject will not be able to perform the study procedures or assessments or would confound interpretation of data obtained during assessments.
6. Females who are pregnant, lactating or not willing to use a protocol-defined acceptable contraception method if sexually active and not surgically sterile.
7. Males, engaged in sexual relations with a female of child bearing potential, not using an acceptable contraception method if sexually active and not surgically sterile.
8. Clinically significant abnormalities in safety laboratory tests, vital signs or ECG, as measured at Screening (may repeat to confirm).
9. Current clinically significant (as determined by the investigator) neurological, cardiovascular, renal, hepatic, endocrine or respiratory disease that may impact the interpretability of the study results.
10. Current clinically significant (as determined by the investigator) lymphedema that may compromise venous access and/or may have an adverse impact on study drug distribution and clearance.
11. Judged clinically to be at risk of suicide by the investigator.
12. Average QTcF value of >450 msec at Screening (may repeat to confirm).
13. Subjects in whom an indwelling intravenous line could not be established or maintained.

Pharmacokinetic Assessments

Blood levels of AMO-01 will be assessed at approximately 2 hours and approximately 6 hours after the commencement of the infusion of the study medication, as well as approximately 1 hour after the cessation of administration of the study medication. The central tendency values (and ranges) for AMO-01 plasma concentrations and imputed exposures (AUC) will be measured.

Statistical Methods:

Safety and tolerability aspects of the data will be tabulated. An adverse event (AE) will be defined as any untoward medical occurrence in a study subject, temporally associated with the use of the experimental medication, whether or not considered related to the medication. An

adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of the experimental medication. A serious adverse event (SAE) will be defined as an AE that meets any of the following criteria:

- results in death;
- is life threatening;
- requires inpatient hospitalization;
- results in a persistent or significant disability/incapacity;

any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the outcomes listed above

For the assessment of efficacy, to test the hypothesis of no differences in the mean between baseline, week 1, and week 4 we will examine the data distributions of the primary and secondary efficacy variables. The primary analysis and study evaluation will be based on the 10 subjects recruited for this study. All tests of statistical hypothesis will be done on the two-sided 5% level of significance and presented together with the associated point estimate and two-sided 95% confidence interval.

Since the study participants are expected to have very differing degrees of baseline signs and symptoms, the evaluation of treatment effects will include an adjustment for the pre-treatment baseline level in each treatment period. For this purpose, the differences against baseline will be analysed.

Since this is an initial proof-of-concept study that is exploratory in nature, no multiplicity-related adjustments will be made in the reported p-values.

The SAS software, proc npar1way or similar R software will be used to fit the statistical models.

Phase: Phase IIa

Number of Sites enrolling participants: 2

Description of Study Agent: AMO-01 (INN: diazepinomicin), formerly known as ECO-4601 and TLN-4601, is a low molecular weight, brain penetrating, structurally novel, farnesylated dibenzodiazepinone.

The study drug is prepared from a sterile bulk formulation containing 32.8 mg/mL (3.15% w/w) of the active drug substance. Each drug product vial contains 70 mg of AMO-01 drug substance in 2.134 mL of formulation (plus 2% overage). This corresponds to a total drug content of 71.4 mg of active drug substance per 2.176mL of formulation. The

storage condition for the bulk study drug is refrigeration (5±3^{O}C).

This bulk IMP is provided in a 30-mL sterile, pyrogen-free borosilicate clear glass vial (USP Type I vial), closed with a sterile Teflon-coated butyl stopper, and crimp sealed with an aluminium seal. The bulk IMP is a yellow to brownish yellow viscous liquid that is required to be diluted in sterile 0.9% saline solution to produce an isotonic final dosing formulation prior to administration to study subjects.
Prior to administration, the study drug (sterile bulk formulation) must be diluted with 0.9% sterile saline solution to obtain the final dosing formulation. The study drug requires a reconstitution kit to include the following:

i. Each single-dose vial of sterile drug product contains 71.4 mg of AMO-01 drug substance in 2.176 mL of excipients (including a 2% overfill). This corresponds to a drug content label claim of 70 mg per vial and a drug product volume of 2.134 mL.

ii. An EVA infusion bag (250-, 500- or 1000-mL, depending on the dose to be administered).

iii. An administration set consisting of TOTM-plasticized PVC line with a pump connector.

iv. An extension set consisting of PVC line that is internally coated with PE, an in-line 1.2 micron filter, and an anti-siphon valve.

v. A pre-filled syringe or a bag of sterile 0.9% saline.

vi. An instruction sheet for the preparation of the dosing formulation and for assembly of the infusion kit for administration.

Study Duration: Estimated first subject screened: October 2017
Estimated last subject last visit: June 2019

Participant Duration: Up to 12 weeks, including a 4-week screening period

Table 1: Schedule of Events

Visit	Screening	Baseline[8]	Follow-up 1	Follow-up 2	Follow-up 3	Follow-up 4	Follow-up 5
	V1	V2	V3	V4	V5	V6	V7
Week	*-4 to -1*	*0*	*0+1 day*	*1*	*2*	*4*	*8*
Informed Consent/Assent	X						
Eligibility Criteria	X	X					
Diagnosis of Phelan McDermid Syndrome & confirmatory genotyping	X						
Medical/Psychiatric History	X						
Physical Examination	X	X		X	X	X	
Vital Sign Measures[1]	X	X		X	X	X	
ECG	X	X		X	X	X	
Clinical Labs[2]	X	X[a]		X[a]	X	X	
Urinalysis	X	X		X	X	X	
Pregnancy Test [3]	X	X		X	X	X	
Sample for Pharmacokinetic Analysis [4]		X					
Sample for Assessment of Phosphorylated ERK levels [5]		X		X		X	
Administer Study Medication [6]		X					
Seizure Diary Dispense/Review	X	X		X	X	X	
CGI-S	X	X		X	X	X	
CGI-I		X		X	X	X	
Clinician Completed PMS Domain Specific Causes for Concern VAS	X	X		X	X	X	
Sensory Adjustment for Neurodevelopmental Disorders (SAND)		X		X	X	X	
Caregiver Top 3 Concerns		X		X	X	X	
Aberrant Behavior Checklist (ABC)		X		X	X	X	
Repetitive Behavior Scale-Revised (RBS-R)		X		X	X	X	
Electroencephalography	X		X	X	X		
Computerized eye tracking	X	X	X	X	X	X	
Auditory Evoked Potential	X	X	X	X		X	

Visual Evoked Potential	**X**	**X**	**X**	**X**		**X**	
Adverse Events[7]	**X**	**X**		**X**	**X**	**X**	**X**
Concomitant Medications	**X**	**X**		**X**	**X**	**X**	**X**
Telephone questionnaire							**X**

1. Vital Sign Measures include Heart Rate, Respiratory Rate, Blood Pressure, Temperature, Height, and Weight
2. Clinical labs standardly include i) Full Blood Count with differential, ii) Biochemistry panel. When marked with an "a", a lipid panel will also be collected
3. Urine or serum pregnancy test for female subjects of child bearing potential
4. Pharmacokinetic sample (6 mL) will be taken at approximately 2 hours and approximately 6 hours after the start of the infusion as well as 1 hour after the cessation of the study drug.
5. 6 mL blood sample required for assessment of phosphorylated ERK levels
6. A single 6-hour intravenous treatment will be given to subjects, with total dose administered 120 mg/m^2
7. Adverse Events (AEs) and Serious Adverse Events (SAE's) will be collected from the time of informed consent.
8. Baseline procedures outlined in Table 2, Baseline Visit Schedule of Events

Table 2: Baseline Visit Schedule of Events

Baseline Procedures	Pre-Infusion[10]	During Infusion	Post-Infusion[9]
V2			
Week 0			
Eligibility Criteria	X		
Physical Examination[1]	X	X	X[a]
Vital Sign Measures[2]	X[b]	X	X
ECG	X	X[3]	
Clinical Labs[4]	X		
Urinalysis	X		
Pregnancy Test [5]	X		
Sample for Pharmacokinetic Analysis [6]	X	X	X
Sample for Assessment of Phosphorylated ERK levels [7]	X	X	
Administer Study Medication [8]		X	
Seizure Diary Dispense/Review	X		
CGI-S	X		
CGI-I			X
Clinician Completed PMS Domain Specific Causes for Concern VAS	X		
Sensory Adjustment for Neurodevelopmental Disorders (SAND)	X		
Caregiver Top 3 Concerns	X		
Aberrant Behavior Checklist (ABC)	X		
Repetitive Behavior Scale- Revised (RBS-R)	X		
Electroencephalography			
Computerized eye tracking	X		
Auditory Evoked Potential	X		
Visual Evoked Potential	X		X
Adverse Events	X	X	X
Concomitant Medications	X	X	X

1. When marked with a) a brief physical examination is acceptable
2. Vital Sign Measures include Heart Rate, Respiratory Rate, Blood Pressure, Temperature. When marked with b) Height, and Weight are also measured
3. ECG during infusion to be completed between 4-6 hours after infusion start
4. Clinical labs standardly include i) Full Blood Count with differential, ii) Biochemistry panel
5. Urine or serum pregnancy test is sufficient
6. Pharmacokinetic sample (6 mL) will be taken pre-infusion and at approximately 2 hours and approximately 6 hours after the start of the infusion as well as 1 hour after the cessation of the study drug.

7. 6 mL blood sample required for assessment of phosphorylated ERK levels will be taken pre-infusion and at approximately 2 hours and approximately 6 hours after the start of the infusion as well as 1 hour after the cessation of the study drug.
8. A single 6-hour intravenous treatment will be given to subjects, with total dose administered 120 mg/m^2
9. Post-infusion assessments will occur one hour after infusion prior to discharge with the exception of EEG which occurs the following day in the AM
10. If required for scheduling, pre-infusion assessments can be completed one day prior to the infusion

ii. Table of Contents

LIST OF ABBREVIATION

ABC	Aberrant Behavior Checklist
AE	Adverse Event
AERP	Auditory Event Related Potential
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AUC	Area Under the Curve
CFR	Code of Federal Regulations
CGI	Clinical Global Impression
EEG	Electroencephalography
EKG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EEG	Electroencephalogram
ERK	Extracellular signal-regulated kinases
FDA	Food and Drug Administration
GCP	Good Clinical Practice
IB	Investigator's Brochure
ICH	International Council on Harmonization
IMP	Investigational Medicinal Product
IP	Intra Peritoneal
LAR	Legally Authorized Representative
NMDA	N-methyl-D-aspartate Pharmacokinetic
PK	Pharmacokinetics
PMS	Phelan McDermid Syndrome
PVC	Polyvinyl Chloride
QTcF	QT interval, Fridericia's method
RBS-R	Repetitive Behavior Scale- Revised
SAE	Serious Adverse Event
SAND	Sensory Assessment for Neurodevelopmental Disorders
SUSAR	Suspected Unexpected Serious Adverse Event
VAS	Visual Analog Scale
VEP	Visual Evoked Potential

iii. Table of Tables

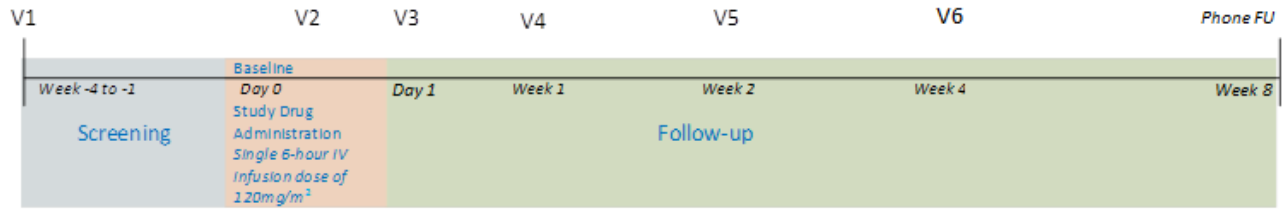
iv. SCHEMATIC OF STUDY DESIGN

This is an open-label study to investigate the safety, tolerability and efficacy of a single 6-hour intravenous infusion of AMO-01 to treat adolescents and adults with PMS and co-morbid epilepsy. The subjects in this study will be administered the experimental study medication and then be followed up to ascertain safety and tolerability, and to determine whether their seizure frequency, as well as their signs and symptoms of PMS, are improving, using a caregiver-completed seizure diary as well as accepted rating scales and functional measures. Approximately 15 subjects will be screened and up to 10 subjects will receive study medication.

The study will have 4 phases (Figure 1, Study Design):

- *Screening (Weeks -4 to -1)*: Subjects will be screened to ensure adherence to eligibility criteria and to assess pre-medication seizure frequency
- *Baseline (Day 0)*: Subjects will complete baseline assessments in-clinic prior to study drug administration
- *Study Drug Administration (Day 0):* Eligible subjects will receive a single 6-hour intravenous infusion with a total dose of 120 mg/m²
- *Follow-up (+1 day – Week 8)*: follow-up visits will occur at 1 day after and 1, 2 and 4 weeks after receiving study medication. A phone call will occur 8 weeks after receiving study medication.

Figure 1: Study Design



1. KEY ROLES

Alexander Kolevzon, M.D., PI
Dr. Kolevzon is a Professor of Psychiatry and Pediatrics and the Clinical Director of the Seaver Autism Center at Mount Sinai. Dr. Kolevzon will oversee all aspects of this study. He will also serve as the liaison with the national PMS Foundation to maintain ties with the community and ensure adequate recruitment. Dr. Kolevzon will be responsible for ensuring the integrity of data collected and will be performing psychiatric and medical evaluations of all patients seen. Dr. Kolevzon will have ultimate responsibility for overseeing the conduct and quality of all aspects of the proposed project, including supervising the treating MD, study coordinators/research assistants, and raters in their daily tasks and being readily available for consultation. Site-specific regulatory issues will be reviewed by Dr. Kolevzon and he will ensure that all reportable events are promptly disclosed per protocol. Additionally, Dr. Kolevzon will ensure recruitment timelines are met and will work in collaboration with the study team to help plan and strategize recruitment activities as needed. He will take responsibility for hiring, training, and managing project staff, and will oversee the quality of all data collection. Dr. Kolevzon has been thoroughly trained in Good Clinical Practice (GCP) standards. He will also lead regularly scheduled research meetings to oversee the conduct of the study. In collaboration with the other Co-Investigators, Dr. Kolevzon will participate in the analyses and interpretation of the data and writing and submission of research findings.

Jennifer Foss-Feig, PhD, Co-I
Dr. Foss-Feig is an Assistant Professor in the Seaver Autism Center. She is a clinical psychologist who has completed specialized ASD-focused pre- and post-doctoral clinical fellowships. Her research interests are in the brain bases of ASD and related neurodevelopmental disorders, with a particular emphasis on neural mechanisms subserving sensory and cognitive processing. Dr. Foss-Feig has specific expertise in using task-based EEG and functional neuroimaging in clinical populations, including both children and adults with ASD. With EEG, she has expertise with both visual and auditory event-related potential (ERP) experimental design and data analysis and interpretation. Clinically, she is also experienced in administering and interpreting direct assessment measures of social, language, and sensory functioning with children and adults with ASD across a wide range of functioning levels. Dr. Foss-Feig will contribute to auditory and visual evoked potential EEG experimental design, data collection, data analysis, and manuscript preparation. She will also assist the PI in data analysis and manuscript preparation/submission.

Paige Siper, PhD, Co-I
Dr. Siper is a licensed clinical psychologist and Chief Psychologist at the Seaver Autism Center for Research and Treatment at Mount Sinai. Her research is focused on sensory processing and biomarker discovery in children with neurodevelopmental disorders using visual evoked potential (VEP). She is particularly interested in the neural and psychological correlates of PMS and oversees the psychological assessment process for all PMS families participating in clinical trials and longitudinal natural history studies at the Seaver Autism Center. Dr. Siper is also the co-developer of a sensory assessment used in this study, which aims to understand the sensory domain as it relates to DSM-5 criteria for ASD. Dr. Siper will be responsible for administering and overseeing all aspects of VEP collection and data analysis, as well as training and scoring for the sensory assessment. She will also assist the PI in data analysis and manuscript preparation/submission.

Pilar Trelles, MD, Co-I
Dr. Pilar Trelles is a board certified child and adolescent psychiatrist and will be primarily responsible for performing psychiatric evaluations throughout the study. She will be a treating physician responsible for monitoring the overall well-being of participants. The treating physician will also determine whether each enrolled participant meets clinical diagnostic criteria for study entry. She will review any relevant medical history of study participants and assess the subjects at visits for any treatment emergent adverse events. She will meet with the participant and his/her caregiver to review vital signs, parent questionnaires and assess for

adverse events at the regularly scheduled clinic visits, thus monitoring safety at the individual participant level. She will also attend regularly scheduled conference calls to discuss any clinical matters as they arise. Dr. Trelles will ensure proper protocol procedures are followed and all reportable events are communicated to the site study team (site PI and study coordinator) as well as the lead site. She will also assist the PI in data analysis and manuscript preparation/submission.

Danielle Halpern, PsyD, Co-I
Dr. Halpern is an Assistant Professor of Psychiatry and a clinical psychologist in the Seaver Autism Center. Dr. Halpern conducts neuropsychological and diagnostic evaluations as well as performs ratings for Seaver Center projects. She also provides supervision for the clinical assessment team and conducts training for clinical raters in addition to ensuring reliability of training procedures. Dr. Halpern will also be the independent evaluator/rater during the clinical trial and will complete assessments, such as the CGI, Specific Causes for Concern VAS, Caregiver Top 3 Concerns, RBS, and ABC with the caregiver.

Sven Sandin, PhD, Co-I
Dr. Sandin is an Assistant Professor of Psychiatry at Mount Sinai, a statistician, and an epidemiologist with more than 25 years of experience. He has broad experience from clinical trials in the pharmaceutical industry, phases I to IV, and has played a role as statistical project lead as well as in analyzing individual studies in different areas (e.g., cardiovascular disease and pain relief) and using different study designs. He has extensive experience with epidemiological data and using register based studies where he has followed individuals from national populations and analyzed data from the Swedish national registers. He has extensive knowledge of variable measurement, data management, statistical analysis, and reporting.

Jimmy L. Holder, MD, PhD, Co-I
Dr. Holder is an Assistant Professor of Pediatrics, Division of Neurology at Baylor College of Medicine and Texas Children's Hospital. He is a board-certified neurologist who serves as the director of the Synaptopathy clinic at the Blue Bird Circle Clinic for Pediatric Neurology. He is also an Investigator at The Jan and Dan Duncan Neurological Research Institute at Texas Children's Hospital. He will serve as site PI for Texas Children's Hospital. He has extensive experience treating children with Phelan-McDermid Syndrome with a focus on epilepsy and abnormalities identified on electroencephalogram. He will evaluate EEGs pre and post treatment for changes following administration of the investigational drug. Dr. Holder will also serve as an independent evaluator/rater during the clinical trial and will complete assessments, such as the CGI, Specific Causes for Concern VAS, Caregiver Top 3 Concerns, RBS, and ABC with the caregiver.

Sarah Risen, MD, Co-I
Dr. Risen is an Assistant Professor of Pediatrics, Division of Neurology at Baylor College of Medicine and Texas Children's Hospital. She is board-certified in Pediatrics, Neurology with special qualifications in Child Neurology and Developmental Pediatrics. Dr. Risen specializes in evaluations of children with developmental disabilities. She will serve as an independent evaluator/rater during the clinical trial and will complete assessments, such as the CGI, Specific Causes for Concern VAS, Caregiver Top 3 Concerns, RBS, and ABC with the caregiver.

Elaine Seto, MD, PhD, Co-I
Dr. Seto is an Assistant Professor of Pediatrics, Division of Neurology at Baylor College of Medicine and Texas Children's Hospital. She is a board-certified neurophysiologist and director of the outpatient EEG lab at Texas Children's Hospital. She will evaluate all pre- and post- treatment EEGs to determine if there are changes in the prevalence of abnormalities following treatment.

2. INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

2.1.1 Phelan McDermid Syndrome (PMS)

Phelan McDermid Syndrome (PMS) is a rare neurodevelopmental syndrome caused by a chromosomal deletion at the 22q13.3 chromosomal location that contains the *SHANK3*/ProSAP2 gene (Bonaglia et al., 2001; Anderlid et al., 2002; Wilson et al., 2003). This gene plays a crucial role in the structural stability of dendritic connections in regions of the brain such as the neocortex. Loss of function of this gene parlays into diminished synaptic strength and, consequently, compromised brain functioning.

The cardinal signs and symptoms of PMS occur in the wake of haploinsufficiency of *SHANK3*. The central nervous system is adversely affected as are somatic tissues. The clinical features of PMS include severe neonatal hypotonia (>97% individuals), global developmental delay (>98% individuals), normal to accelerated somatic growth (95%), absent to severely delayed speech (>98%), and minor dysmorphic features (Phelan 2008). Behaviorally, PMS individuals commonly present with hyposensitivity to pain, poor eye contact, stereotypic movements, deficits in social communication, hyperactivity, altered sensorimotor function and aggressive behavior. Common comorbid psychiatric diagnoses include autism spectrum disorder and intellectual disability, while individuals with PMS are prone towards gastrointestinal problems (including reflux, diarrhea, and constipation), as well as lymphedema. As a consequence of this high degree of medical and neuropsychiatric comorbidity, the quality of life for individuals with PMS, and for their families, is significantly compromised.

A key comorbidity in PMS is the presence of epilepsy. Approximately 40% of affected individuals will have seizures (febrile and/or non-febrile) (Soorya et al, 2013). There is a trend towards increased seizure susceptibility with age, and seizure onset often correlates with regression of functional skills (Soorya et al., 2013). Individuals with PMS may experience generalized tonic–clonic, myoclonic, focal, and absence seizures at various points during development. In addition, the EEGs of individuals with PMS are characterized by a wide range of EEG abnormalities during interictal states, including slow occipital dominant rhythm and/or focal spike and slow wave activity.

The diagnosis of PMS is based on cytogenetic, molecular cytogenetic, and/or molecular demonstration of loss or disruption of chromosome region 22q13.3 which contains the *SHANK3*/ProSAP2 gene. Genetic evaluation is typically triggred in early childhood by clinical recognition of the syndromal features described above.

There are currently no pharmacological treatments approved for use in treatment of PMS.

2.1.2 AMO-01 and Rationale for Clinical Investigation

AMO-01 is a structurally novel, farnesylated dibenzodiazepinone (4,6,8-trihydroxy-10-(3,7,11-trimethyldodeca -2,6,10-trienyl)-5,10 dihydrodibenzo[b,e][1,4]diazepin-11-one). AMO-01 for injection is supplied as a sterile bulk formulation which requires dilution in sterile 0.9% saline prior to patient administration.

AMO-01 was initially developed by Ecopia and later by Thallion (now Bellus Health) as an oncology agent. However, development was halted due to insufficient efficacy as a treatment for glioblastoma.

AMO-01 has potential therapeutic utility in PMS by two possible modes. AMO-01 has demonstrated neurobehavioral rescue activity in a preclinical model of PMS, and mechanistically, it acts as a Ras-ERK pathway inhibitor, which may be important for both the neurobehavioral activities, as well as in dampening or eliminating seizures.

SHANK3 is a post synaptic scaffold protein that is considered to be highly relevant with regard to the medical and neuropsychiatric morbidity manifested in 22q13 deletion syndrome, also known as PMS. *SHANK3* contains a Ras-association domain (SPN) through which it sequesters active Ras and limits its presence at the plasma membrane (Lilja et al., 2017), a position that is required for activation of the Ras-ERK pathway (Palsuledesai and DiStefano, 2015). Reductions in *SHANK3* would, therefore, be expected to increase activation of the Ras-ERK pathway. A *Shank3b* $^{-/-}$ homozygous knockout mouse model (*Shank3b*$^{-/-}$) has been developed in which homozygous deletion mutants exhibit aberrant behaviors, such as self-injurious repetitive grooming and deficits in social interaction, and these mice have increased seizure activity (Peça et al., 2011).

Homozygous *Shank3b*$^{-/-}$ knockout mice were treated with a single intraperitoneal dose of AMO-01 followed by several behavioral tests including assessments of anxiety mediated behavior, excessive grooming behavior, social novelty, beam walking behavior, marble burying behavior, and audiogenic seizure threshold. In each test, the intraperitoneal dose of 30 mg/kg of AMO-01 rescued (i.e., markedly ameliorated) the aberrant behavior compared with vehicle treated knockout animals, and in most cases returned knockout mouse behavior to a similar level as in wild type counterparts.

Related to its farnesyl moiety and the role of farnesyl in the proper intracellular trafficking of Ras (Palsuledesai and Distefano, 2015), the effect of AMO-01 was assessed on the Ras-ERK signaling pathway (additional information is provided in the Investigator's Brochure, Appendix 1). AMO-01 was shown to be a potent inhibitor of this pathway in several cell lines as evidenced by reductions in phosphorylation of Raf-1 and ERK. This effect was mediated through a reduction in the generation of activated Ras (Ras-GTP) and in membrane-associated Ras after stimulation by EGF. AMO-01 did not inhibit Ras farnesylation, nor did it directly inhibit phosphorylation of Raf-1 or ERF.

Aberrations in the genes that regulate the activity of the MAPK/ERK pathway are strongly associated with neurodevelopmental disorders including autism (Wen et al., 2016; Garg et al., 2017; Tylee et al., 2017). Wen et al. (2016) systematically evaluated gene pathways related to 667 genes implicated in autism spectrum disorder, and identified the Ras-ERK pathway as a pathway likely to make a major contribution to the pathophysiology of autism spectrum disorder (ASD). In a blood microarray study of ASD patients, Ras-ERK was one pathway demonstrated to be misregulated in this population (Tylee et al., 2016). Several rare diseases have been linked to defects in the Ras-ERK pathway, including Noonan, cardiofaciocutaneous (CFC), and Costello syndromes (Garg et al., 2017). Garg et al. (2017) showed a high prevalence of ASD children with two of these disorders (Noonan and CFC), supporting a link between Ras-ERK pathway defects and ASD behaviors.

The activation of the Ras-ERK pathway has also been implicated in seizure generation based on a number of converging lines of evidence in different experimental models, and on the preclinical and clinical use of Ras-ERK pathway inhibitors. Ras-ERK pathway activation (as indexed by increased levels of the phosphorylated ERK (pERK)) is seen during various forms of adaptation in synaptic mechanisms that underlie excitability in the brain. For example, Davis et al., (2000) showed that induction of hippocampal LTP in the rat is accompanied by a rapid but transient increase in pERK. Giordano et al., (2016) reported on a model that uses 6 Hz corneal stimulation to induce seizures in the mouse. In this model, seizure susceptibility was paralleled by sustained increases in pERK, i.e., activation of the Ras-ERK pathway.

Nateri et al., (2007) developed a transgenic mouse that over-expresses MEK1, which is an upstream activator of ERK. Over-expression of MEK caused a 2 to 3 fold increase in total brain pERK and was associated with development of spontaneous and electrophysiological seizures in these mice beginning at 6 to 8 weeks of age.

The proposed role for activation of Ras-ERK in seizure activity is supported by studies with Ras-ERK pathway inhibitors. Glaznova et al., (2015) studied the role of Ras-ERK activation in the development of audiogenic seizures in seizure prone rats. The MEK inhibitor SL-327 blocked seizures in this model and inhibited ERK activation in hippocampus and temporal cortex in correlation with anti-seizure activity.

These data are consistent with the potential for Ras-ERK pathway inhibitors to reverse neurobehavioral deficits related to PMS and to have anti-convulsant properties. Therefore, they support the investigation of AMO-01 as a therapy for PMS subjects with co-morbid epilepsy. Given the above information, this study is designed to investigate the safety and efficacy of AMO-01 in adolescents and adults age 12-45 years affected by PMS with co-morbid epilepsy. For further information on the rationale to study AMO-01 in PMS see section 2.2 Rationale.

Refer to the current Investigator's Brochure for additional information on AMO-01.

2.1.3 Pre-Clinical Research

A comprehensive GLP- and ICH-compliant pre-clinical program has been conducted with AMO-01. Summary information is described in this section and additional information on the non-clinical studies conducted to date are contained within the current version of the Investigator Brochure.

2.1.4 Non-Clinical Pharmacology

The pharmacological properties of AMO-01 were assessed *in vitro* and *in vivo,* on the Ras signaling pathway. Additionally, mechanism of action studies relating to the Ras-MAPK signaling pathway were conducted in a mouse model of PMS (*Shank3b*$^{-/-}$ knockout).

In Vitro Primary Pharmacodynamics

The Ras protein undergoes prenylation as a post-translational modification, which is required for attachment of Ras to the cell membrane and proper enzyme activity (Palsuledesai and DiStefano, 2015). In this process, a farnesyl moiety is attached to Ras at a C-terminal consensus sequence (CaaX), catalyzed by farnesyltransferase (FTase). In another form of prenylation, a geranylgeranyl isoprenoid can also be attached to CaaX sites, through action of GGTase-1.

Because AMO-01 contains a farnesylated moiety, it was hypothesized that AMO-01 could interfere with the Ras signaling pathway, and this activity was assessed in a series of *in vitro* experiments.

AMO-01 was first evaluated for its ability to interfere with Ras processing by monitoring FTase and GGTase-I activities. MCF7 cells were treated for 24h with semi-log increasing concentrations (3-30 μM) of AMO-01 or lovostatin (a non-specific inhibitor of protein prenylation). The data show that while lovostatin treatment resulted in the expected mobility shift indicative of inhibition of prenylation, AMO-01 had no effect, demonstrating that AMO-01 does not inhibit prenylation.

The effect of AMO-01 on downstream events of Ras signaling *in vitro* was subsequently examined by monitoring the phosphorylation levels of c-Raf-1 and ERK1/2 by Western blot analysis in various cell lines. In all cell lines tested, AMO-01 inhibited phosphorylation of Raf-1 in a concentration-dependent manner at concentrations ranging from 1 to 30 μM. It was also noted that AMO-01 not only inhibited the phosphorylation of c-Raf, but also caused a decrease in the amount of total Raf-1. Phosphorylation of ERK was reduced by AMO-01 (3 to 30 μM) dependent on the assay methodology and the cell system utilized. It was, therefore, demonstrated that the inhibitory activity of AMO-01 on the Ras-MAPK signaling pathway is post Ras prenylation but prior to Raf-1 phosphorylation/degradation.

Unlike current Ras signaling pathway inhibitors, AMO-01 is not a direct kinase inhibitor. This was documented by evaluating the effect of AMO-01 on human EGFR, c-RAF, MEK1, MAPK1 (ERK1) and MAPK2 (ERK2) kinase-activity (Upstate Kinase Profiler™ Service; Dundee, UK).

In a pull down assay for active Ras in MCF-7 cells stimulated with EGF, concentrations of AMO-01 in the range of 1 to 10 μM prevented activation of Ras and phosphorylation of ERK, and reduced the level of activated Ras bound to cellular membranes.

In *ex vivo* studies, AMO-01 (30 mg/kg, IP) also significantly reduced phosphorylation of ERK in lymphocytes isolated from a mouse model of fragile X syndrome (*Fmr1* KO), a condition which is also associated with activation of the Ras pathway.

Taken together, the *in vitro* and *ex vivo* data suggest that AMO-01 inhibits Ras signaling and activation of downstream targets by inhibiting Ras membrane anchorage and activation. However, AMO-01 is not a FTase inhibitor and does not directly inhibit EGFR, c-Raf, MEK1, ERK1 or ERK2 kinase activities. Considering results from all assays, the minimum efficacious concentration (MEC) was considered to be ~ 3µM *in vitro*.

***In Vivo* Efficacy in Animal Models**

In PMS, deletion or mutation in a region of 22q13.3 that contains the *SHANK3*/ProSAP2 gene results in activity-dependent synaptic plasticity loss of function of the *SHANK3* gene.

A *Shank3* homozygous knockout mouse model (*Shank3b*$^{-/-}$) of PMS has been developed in which homozygous deletion mutants exhibit self-injurious repetitive grooming, deficits in social interaction and increased seizure activity (Peça et al., 2011). Compared with wildtype littermates, the *Shank3b*$^{-/-}$ mice also have enhanced activation of the Ras-ERK pathway (indexed as increased pERK levels) in the cortex.

The effects of AMO-01 on neurobehavioral deficits and seizure activity were investigated in the homozygous *Shank3b*$^{-/-}$ knockout mice (n=10, aged 2 months, -/-C57BL/6 background, AMO Pharma Internal Report AMO-01-PC-001). In this experiment, a single dose of 30 mg/kg AMO-01 was administered IP to *Shank3b*$^{-/-}$ mice and various behaviors were investigated on consecutive days as follows:

Day 1: Increased anxiety in the light dark box test

Day 2: Excessive grooming leading to skin lesions

Day 3: Abnormalities in social recognition and response to social novelty

Day 4: Sensory motor function as assessed by beam walking tests

Day 5: Activities of daily living / species typical behaviors as assessed by marble burying

The single administration of AMO-01 rescued each behavioral abnormality compared with vehicle treated knockout animals, and in most cases returned knockout mouse behavior to a level similar to that of wild type littermates. These effects were apparent within 4 hours of the single intraperitoneal administration of 30 mg/kg, and persisted through 5 days post-treatment. Additionally, AMO-01 administration ameliorated the susceptibility of *Shank3b*$^{-/-}$ knockout mice to audiogenic seizures. This dose corresponded to a C_{max} of 3602 ng/mL (~8 µM) and a sustained concentration through 8 hours of ~700 ng/mL (~1.5 µM).

The effects of a single dose of AMO-01 represented a significant normalization of function in the anxiety and audiogenic seizure domains, and a complete normalization of function in the grooming, social, sensori-motor and species typical behaviors, and effects persisted for at least five days.

The *in vitro* activity of AMO-01 as a Ras-ERK inhibitor, and its *in vivo* efficacy in the mouse model of PMS support the hypothesis that AMO-01 has potential to rescue the clinical phenotype of PMS in human subjects. The persistence of effects for up to 5 days in the *Shank3b*$^{-/-}$ mice supports the single administration dosing regimen proposed for the planned clinical trial in PMS patients.

2.1.5 Non-Clinical Pharmacokinetics, Metabolism and Interactions

Absorption, Distribution, Metabolism and Excretion (ADME) studies in mice, rats, and monkeys have shown distribution of AMO-01 in several organs and elimination in urine and feces. Human microsome stability of AMO-01 was studied, as well as its metabolism *in vitro* in hepatocytes from several species. Metabolite identification was also performed in mice, rats, and monkeys. A simulation of the pharmacokinetic profile of

AMO-01 in humans is presented from data generated in several species following IV bolus or continuous IV infusion.

In mouse studies, the presence of measurable quantities of AMO-01 in brain tissue suggests that the drug crosses the blood-brain barrier. In all species, a single IV bolus injection of AMO-01 resulted in a high C_{max} and a subsequent rapid elimination from the plasma (less than 4 hours in monkeys). Effective *in vitro* concentrations for Ras-ERK pathway inhibition were in the range of 1 to 10 μM, and were similar to the 2 μM target concentration threshold identified for the previous cancer indication. The rapid clearance after IV bolus administration prevented sustained concentrations above the target concentration threshold; therefore, subsequent 7- and 14- day continuous infusions dose studies were conducted in rat and monkey, and infusions were also used in the clinical oncology program. An analysis was performed to derive allometric equations for AMO-01 pharmacokinetic parameters using AMO-01 plasma concentration-time data from three species, including mouse, rat, and monkey, and to estimate human pharmacokinetic parameters from those allometric equations can be found in the Investigator's Brochure.

2.1.6 Non-Clinical Toxicology

Studies have been performed in rodents and non-rodents (monkeys) to characterize the toxicity of AMO-01. AMO-01 toxicity was evaluated following single bolus administration in mice, rats, and monkeys and following continuous IV infusion for 7 or 14 days in rat and monkey.

When AMO-01 was administered by continuous infusion for 14 days to cynomolgus monkeys, the NOAEL was 15 mg/kg/day based on reductions in red cell mass at 30 mg/kg/day, and 30 mg/kg/day was considered a non-severely toxic dose. At the 30 mg/kg/day dose level, effects included: 1) minor clinical signs, including inappetance and unidentified material in the cage pans; 2) a moderate degree of regenerative (reversible) anemia; 3) elevations in serum cholesterol and triglycerides, and a decrease in serum albumin; and 4) diffuse vacuolization of hepatocytes and accumulation of foamy histiocytes (macrophages) in the spleen, which was interpreted to reflect clearance of the hydrophobic components of the vehicle for AMO-01. At the next lowest dose level of 15 mg/kg/day (NOAEL), test article-related anemia and changes in serum chemistry parameters were not observed in the monkeys. No degenerative changes were observed in any organs (including the infusion site), and there were no effects on body weight, ocular condition, electrocardiographic activity or any other parameters assessed in the monkeys. The monkeys used in the 14-day toxicity study were 24 to 31 months of age at start of treatment. This corresponds to ~8 to 10 years of age in humans (Weinbauer et al., 2011) and, therefore, supports the proposed enrolment age of 12 years and above in the planned clinical trial in PMS patients.

In the rats, infusion of AMO-01 at dose levels of 25, 50 and 75 mg/kg/day was associated with deteriorating condition of animals from all groups, including the vehicle control group, which led to the premature sacrifice or death of a significant percentage of the study animals. Examination of the study findings revealed that the poor condition stemmed from necrotizing, inflammatory lesions at the infusion site (i.e., at the location of the catheter tip in the femoral vein) that were caused by the vehicle for AMO-01. The reason for the development of this lesion in rats, but not in monkeys, was attributed to the smaller size of the infusion vessel (i.e., lesser dilution of the vehicle) and to a concurrent catheter tract infection that was highly prevalent in the study animals. As such, the results of the 14-day studies in rats are not considered clinically relevant, and dose selection for the clinical studies in the oncology indication was based on the safety profile characterized in monkeys.

The primary components of the vehicle chosen for AMO-01 are polysorbate (Tween) 80, PEG 400 and ethanol, the quantities of which are included as a fixed ratio to the amount of AMO-01. These same vehicle components have been used together in other clinical formulations for approved products and have been found to be well tolerated across a range of exposures. Additionally, no evidence of injection site reactions were noted in clinical subjects administered AMO-01 at doses up to 480 mg/m^2 for up to 14 day infusion cycles, as described below.

Dose selection for the proposed clinical study in the PMS indication is based on clinical safety information from Phase I and Phase II studies evaluating AMO-01 as an anticancer agent (see below), nonclinical toxicity studies in monkeys, and results of pharmacology studies in the *Shank3b*$^{-/-}$ knockout mouse model of PMS.

2.1.7 Clinical Experience

AMO-01 has been evaluated in two clinical trials, a Phase I study in patients with a range of solid tumors and a Phase II study focused only on patients with glioblastoma (GBM).

Study ECO-4601-101: A Phase I, Dose Finding, Pharmacokinetic, and Safety Study of Continuous IV Infusion of ECO-4601 in Patients with Advanced Cancer including an Extension Portion to obtain Safety Data at the Highest Tolerable Dose. This study was conducted in two centers in Canada in the period March 2006 until August 2007. Subjects were enrolled into the study in a sequential manner starting with the dose escalation portion of the study with 1 to 6 patients per dose level according to the appearance of a dose limiting toxicity (DLT). Subsequently, a cohort of 14 subjects was enrolled in the dose extension portion of the study at the maximum dose (MD) as determined in the dose escalation portion. A total of twenty-six subjects with late stage (stages 2-4) colorectal, duodenal, glioma, lung, ovarian or pancreatic primary tumors were exposed to AMO-01 at intravenous doses of 30, 60, 120, 180 270, 360 or 480 mg/m^2/day.

No dose-limiting toxicity (DLT) was reached at the highest dose level of 480 mg/m^2/day in the dose escalation portion of the study. A decision was made by the Clinical Trial Coordinating Committee to use the maximum dose (MD) dose of 480 mg/m^2/day as the dose for the extension portion of the study considering that the plasma concentration of AMO-01 at this dose is two-fold higher than the estimated therapeutic plasma threshold and that a dose of 480 mg/m^2/day using a 1-L bag permits the average subject to be treated for 4 days before having to return to the clinic for an intravenous bag change. Therefore, the dose of 480 mg/m^2/day (MD) was used for the dose extension portion of the study, as well as recommended for subsequent Phase II studies.

One Phase II study has been conducted to date.

Study TLN-4601-201: A Phase 2 multicentre, single arm, open-label study of AMO-01 monotherapy designed to evaluate efficacy (as measured by 6-month progression-free survival), safety, tolerability and biomarkers at a dose of 480 mg/m^2/day in adults (>=18 years old) with a histologically confirmed diagnosis of glioblastoma multiforme (GBM) at first progression.

Up to 40 patients were to be enrolled into the study, however a planned interim analysis following the recruitment of 20 subjects (10 males and 10 females) revealed insufficient efficacy and the study was halted.

2.1.8 Safety Summary

2.1.8.1 Phase I Study (ECO-4601-101)

All twenty-six patients who were enrolled and received AMO-01 in the Phase I study (Study ECO-4601-101) were included in the safety analysis. Twenty-five of the 26 subjects who received study drug reported 322 events during the study. Six events started prior to study drug dosing (pre-existing conditions) and are excluded from the analysis and reporting of adverse events. Approximately 40% of all adverse events (n =124 events) were reported in the group of 14 subjects that received the highest dose (representing 54% of the total study population). Overall, the most frequently reported adverse events were fatigue (58%), pyrexia (39%), nausea (35%), vomiting (31%), decreased appetite (31%), diarrhea (27%) and asthenia (27%). Catheter-associated adverse events were reported in 6 subjects and included erythema (n = 3, 12%), complication (n = 1, 4%), bruise (n = 1, 4%), discharge (n = 1, 4%), pain (n = 1, 4%), pruritus (n = 1, 4%), reaction (n = 1, 4%), infection (n = 1, 4%) and sepsis (n = 1, 4%). The majority of events were mild or moderate in severity. Approximately 50% of all adverse events reported were unrelated to the study drug. Sixteen subjects reported a total of 30 adverse events that were 'severe' in intensity. Severe adverse events with a relationship of either unlikely, possibly, probably or definitely to the study drug were reported in 8 subjects; anaphylactoid reaction, catheter

sepsis, pneumonia, dyspnea, restlessness, hydronephrosis, general physical health deterioration, decreased hemoglobin, and asthenia. Discontinuations due to adverse events were observed in 7 subjects. A total of 18 serious adverse events (SAEs) were reported in 15 subjects with 2 SAEs definitely related to the study drug, categorized as anaphylactoid reaction and rash. Both related SAEs occurred at the 30 mg/m^2/day dose. At study completion, 7 SAEs were associated with death, 1 remained persistent and the remaining 10 SAEs resolved without sequelae, including the 2 SAE's related to the study drug.

With regard to objective assessments of safety in this study, the clinical laboratory findings were generally unremarkable. Vital signs and ECG findings were also unremarkable.

2.1.8.2 Phase II Study (TLN-4601-201)

All twenty patients who were enrolled and received AMO-01 in the Phase II GBM study (TLN-4601-201) were included in the safety analysis.

Of the twenty subjects that were enrolled into the study and who received study medication, sixteen patients had debulking surgery at diagnosis and four had biopsies. All patients received radiotherapy with temozolomide as part of initial management. All patients had measurable disease at the time of first progression. Thirteen patients were receiving corticosteroids at the time of study entry and an additional five patients commenced steroids during the study. At study entry, Eastern Co-operative Oncology Group (ECOG) performance status was zero in 11 patients, one in four patients and two in five patients.

The most frequently reported adverse events, reported in four or more patients, included fatigue (50%, n= 10), headache (30%, n= 6), catheter site erythema (25%, n= 5), muscular weakness (25%, n= 5), anxiety (20%, n= 4) and seizures (20%, n= 4). A total of 11 serious adverse events (SAEs) were reported in five patients.

During the trial, 16 deaths were reported and all were due to disease progression that was documented by imaging criteria in 13 subjects and by clinical deterioration in three subjects.

Two SAEs led to study drug discontinuation: one patient developed diabetic ketoacidosis (not study drug related) and the other developed sepsis (not study drug related but central line related) that was associated with moderate disseminated intravascular coagulation that resolved without sequelae.

During treatment, changes in patients' biochemistry and hematologic profiles were minimal and observed changes had no clinical significance.

In summary, the administration of AMO-01 was generally shown to be generally safe and well tolerated.

2.2 Rationale

Currently there are no approved treatments for PMS. Furthermore, there has been relatively little clinical study of pharmacological interventions for PMS. A key comorbidity in PMS is the presence of epilepsy (Soorya et al, 2013). Holder and Quach (2016) cite the data of Shcheglovitov et al., (2013) in relation to the increased seizure susceptibility seen in PMS by referring to the IPS cells studied as having increased input resistance compared to control neurons, potentially related to reduced expression of subunits of hyperpolarization-activated cyclic nucleotide–gated (HCN) channels.

AMO-01 is a structurally novel, farnesylated dibenzodiazepinone that has demonstrated efficacy in an *in vivo* model of PMS, the homozygous *Shank3b*$^{-/-}$ knockout mouse. In this mouse model, AMO-01 rescued some behaviors relevant to PMS including anxiety-mediated behavior, excessive grooming, response to social novelty, beam walking, marble burying behavior, and also a lowered audiogenic seizure threshold.

Furthermore, related to its farnesyl moiety and the role of farnesyl in the proper intracellular trafficking of Ras (Palsuledesai and Distefano, 2015), the effect of AMO-01 was assessed on the Ras-ERK signaling pathway. AMO-01 was shown to be a potent inhibitor of this pathway in several cell lines as evidenced by reductions in

phosphorylation of Raf-1 and ERK. This effect was mediated through a reduction in the generation of activated Ras (Ras-GTP) and in membrane-associated Ras after stimulation be EGF. AMO-01 did not inhibit Ras farnesylation, nor did it directly inhibit phosphorylation of Raf-1 or ERF.

Aberrations in the genes that regulate the activity of the MAPK pathway are strongly associated with neurodevelopmental disorders including autism (Wen et al., 2016; Garg et al., 2017; Tylee et al., 2017). Wen et al. (2016) systematically evaluated gene pathways related to 667 genes implicated in autism spectrum disorder, and identified the Ras-ERK pathway as a pathway likely to make a major contribution to the pathophysiology of autism spectrum disorder (ASD). In a blood microarray study of ASD patients, Ras-ERK was one pathway demonstrated to be misregulated in this population (Tylee et al., 2016).

Several rare diseases have been linked to defects in the Ras-ERK pathway, including Noonan, cardiofaciocutaneous (CFC), and Costello syndromes (Garg et al., 2017). Garg et al. (2017) showed a high prevalence of ASD children with two of these disorders (Noonan and CFC), supporting a link between Ras-ERK pathway defects and ASD behaviors.

The activation of the Ras-ERK pathway has also been implicated in seizure generation based on a number of converging lines of evidence in different experimental models, and on the use of Ras-ERK pathway inhibitors. Ras-ERK pathway activation is indexed by levels of the phosphorylated form of ERK (pERK). This activation is seen during various forms of adaptation in synaptic mechanisms that underlie excitability in the brain. For example, Davis et al., (2000) showed that induction of hippocampal LTP in the rat is accompanied by a rapid but transient increase in pERK. Giordano et al. (2016) reported on a model that uses 6 Hz corneal stimulation to induce seizures in the mouse. In this model, seizure susceptibility was paralleled by sustained increases in hippocampal pERK, indicating activation of the Ras-ERK pathway. Nateri et al. (2007) developed a transgenic mouse that over-expresses MEK1, an upstream activator of ERK. Over-expression of MEK caused a 2 to 3 fold increase in total brain pERK. Additionally, these mice developed spontaneous and electrophysiologically-induced seizures beginning at 6 to 8 weeks of age, thereby demonstrating an association between ERK activation and epileptogenesis.

The proposed role for activation of Ras-ERK in seizure activity is supported by studies with Ras-ERK pathway inhibitors. Glaznova et al., (2015) studied the role of Ras-ERK activation in the development of audiogenic seizures in seizure prone rats. The MEK inhibitor SL-327 blocked seizures in this model and inhibited ERK activation in hippocampus and temporal cortex in correlation with anti-seizure activity. SL-327 had no effect on GABA mechanisms, but increased levels of the vesicular glutamate transporter. These data are consistent with the potential for Ras-ERK pathway inhibitors to have anti-convulsant properties.

AMO-01 is a Ras-ERK pathway inhibitor, and may also have potential therapeutic utility as an anticonvulsant. *SHANK3* is a synaptic scaffold protein within dendrites. The Ras-ERK pathway has been implicated in seizure generation according to a number of converging lines of evidence in different experimental models. Additionally, evidence in the literature (Davis et al., 2000, Giordano et al, 2016, Nateri et al 2007, Sava et al 2012, Glaznova et al 2015) suggests that Ras-ERK pathway inhibitors, such as AMO-01, have anti-convulsant properties. Therefore, AMO-01 may be of additional therapeutic benefit to PMS subjects with seizure disorders.

AMO-01 has potential therapeutic utility by two possible modes. AMO-01 has demonstrated neurobehavioral rescue activity in a preclinical model of PMS and it also acts as a Ras-ERK pathway inhibitor.

In studies with homozygous *Shank3b* $^{-/-}$ knockout mice, treatment with a single intraperitoneal dose of AMO-01 was followed by several behavioral tests including assessments of anxiety mediated behavior, excessive grooming behavior, social novelty, beam walking behavior, marble burying behavior and audiogenic seizure threshold. In each test AMO-01 rescued (i.e. markedly ameliorated) the aberrant behavior compared with vehicle treated knockout animals, and in most cases returned knockout mouse behavior to a similar level as in wild type counterparts.

Taken together, the potential ability of AMO-01 to not only alleviate behavioral disorders associated with PMS but also to relieve seizures experienced by a high proportion of PMS patients supports the investigation of AMO-01 as a therapy for PMS patients.

2.2.1 Dose:

A summary of clinical and nonclinical exposures in shown in the table below.

Table 3: AMO-01 Nonclinical-Clinical Exposure Comparisons

	Route, dose, duration	**C_{max}** (ng/mL) **[µM]**	**$C_{sustained}$** (ng/mL) **[µM]**	**AUC** (ng.h/mL) **[µM.h]**
Mouse *Shank 3b* $^{-/-}$	IP, 30 mg/kg, single	3,602 [**7.8**]	~700 through 8 hr [**1.8**]	5,104 [**11.0**]
Monkey NOAEL	IV, 15 mg/kg/day, 24 hr infusion, 14 days	1173 [**2.5**]	1173 [**2.5**]	400,116 [**865**]
Clinical 480 mg/m^2	IV, 480 mg/m^2, 24 hr infusion	2732 [**5.9**]	2732 [**5.9**]	870,000 [**1881**]
- Over 6 hrs	IV, 120 mg/m^2, 6 hr infusion	2732 [**5.9**]	2732 [**5.9**]	217,500 [**470**]
Clinical 360 mg/m^2	IV, 360 mg/m^2, 24 hr infusion	1325 [**2.9**]	1325 [**2.9**]	512,000 [**1107**]
- Over 6 hrs	IV, 90 mg/m^2, 6 hr infusion	1325 [**2.9**]	1325 [**2.9**]	128,000 [**277**]
Clinical 270 mg/m^2	IV, 270 mg/m^2, 24 hr infusion	1164 [**2.5**]	1164 [**2.5**]	383,000 [**828**]
- Over 6 hrs	IV, 67.5 mg/m^2, 6 hr infusion	1164 [**2.5**]	1164 [**2.5**]	95,750 [**207**]
Clinical 180 mg/m^2	IV, 180 mg/m^2, 24 hr infusion	707 [**1.5**]	707 [**1.5**]	239,000 [**517**]
- Over 6 hrs	IV, 45 mg/m^2, 6 hr infusion	707 [**1.5**]	707 [**1.5**]	59,750 [**129**]

As described above, in monkey toxicology studies, test article-related effects on hematology parameters occurred at a dose of 30 mg/kg/day, and the NOAEL in the 14-day study was 15 mg/kg/day. The C_{ss} at the monkey NOAEL was 1173 ng/mL (2.5 µM), corresponding to the C_{ss} in the clinical trial at the 270 mg/m^2 dose (1164 ng/mL, 2.5 µM). It was presumed that monkeys were less sensitive to injection site reactions than rats due to their larger infusion site vessel diameter; therefore, the monkey was considered more relevant than the rat for predicting human injection site reactions.

A Phase I dose finding, pharmacokinetic, and safety study using a 14-day continuous IV infusion of AMO-01 was administered to 26 patients with histologically confirmed solid tumors (high grade glioma, colorectal, lung, breast, ovarian, pancreatic and prostate) for whom no standard therapy was available, or who had failed standard therapy ECO-4601-101. The results indicated that that AMO-01 was safe and well-tolerated up to the maximum dose tested of 480 mg/m^2/day. Adverse events potentially related to AMO-01 were nonspecific and common in this type of patient population. Only two serious adverse events related to the treatment were observed in the study: one anaphylactoid reaction (grade 4) that occurred in the first patient and was procedure-related (flushing of central line) and one rash (grade 2) that was handled by pre-medication with prednisone.

AMO-01 demonstrated preliminary evidence of antitumor activity in refractory cancer patients with 4 stable-disease out of 7 patients evaluable after 6 cycles of treatment. At the higher dosing levels (270, 360 and 480 mg/m^2/day) the human pharmacokinetic data demonstrated that AMO-01 plasma concentrations exceeded the target therapeutic threshold as determined by animal efficacy studies, but these doses were well-tolerated and a maximum tolerated dose was not achieved or identified. Additionally, the steady state plasma levels of AMO-01 suggested no drug accumulation following multiple dosing cycles. These study results in 26 cancer patients indicate that a 14-day IV infusion of AMO-01 at doses up to 480 mg/m^2/day was generally safe and well tolerated.

In a Phase II glioblastoma study (TLN-4601-201), twenty patients with glioblastoma were dosed with AMO-01. AMO-01 was shown to be generally safe and well tolerated at a dose of 480 mg/m^2/day. Adverse events were nonspecific and common for the patient population. Eleven SAEs were reported. Two SAEs led to study drug discontinuation, diabetic ketoacidosis and sepsis (central line related), neither of which were reported as study drug-related. Preliminary data showed inhibition of pERK in peripheral lymphocytes, but the study was stopped following enrolment of twenty patients as no signal of efficacy was demonstrated at the interim analysis.

Overall, the safety data from these two studies in solid tumor patients receiving doses up to 480mg/m^2/day for multiple 14-day cycles support potential further clinical investigation of AMO-01 in alternate indications.

Therefore, in summary, in clinical studies of AMO-01 in oncology patients, doses were escalated above the monkey NOAEL exposure to a top dose of 480 mg/m^2/day (2732 ng/mL, 5.9 µM). This dose was well tolerated in humans receiving CIV for one or more 14-day cycles, and dose-limiting toxicity was not reached, nor was there an increase in injection site reactions. Exposure at this dose exceeded the proposed minimal clinical efficacious exposure of 2 µM for oncology and dose escalation was halted due to a lack of efficacy in cancer rather than due to tolerability issues.

In *in vitro* studies in MCF-7 cells, activation of Ras was inhibited in a dose-dependent manner at AMO-01 concentrations of 1 to 10 µM, and phosphorylation of downstream Ras-ERK pathway targets was inhibited in a dose-dependent manner at 3 to 30 µM, with an overall *in vitro* minimum efficacious concentration (MEC) of ~3 µM. Comparable exposures were efficacious in reversing behavioral abnormalities in the *Shank 3b* $^{-/-}$ knockout mouse model of PMS for up to 5 days following a single IP administration of 30 mg/kg/day (C_{max} of 7.8 µM and a sustained concentration of ~1.8 µM through 8 hours postdose).

Based on this information, a single 6-hour infusion is proposed for PMS at the concentration used for the 480 mg/m^2 clinical dose (which was provided over a 24-hour period). This dosing regimen is anticipated to provide a total dose of 120 mg/m^2 over a 6 hour period and a sustained concentration of ~5.9 µM. This concentration is within the range of exposures anticipated to be pharmacologically active based on *in vitro* inhibition of Ras and *in vivo* reversal of behavioral effects in the *Shank 3b* $^{-/-}$ knockout mouse, and is approximately 2-fold higher than the *in vitro* MEC. This dose is also anticipated to be well tolerated in PMS patients, given that it is a single dose at the same steady state concentration and at one-fourth the daily dose that was tolerated in cancer patients for one or more 14-day cycles of continuous IV infusion. The AUC exposure at this proposed dose is also ~2-fold lower than AUC exposure in the monkey at the NOAEL dose of 15 mg/kg/day. Given that pharmacodynamic effects were sustained through 5 days after a single IP injection in the *Shank 3b* $^{-/-}$ knockout mouse, effects on efficacy endpoints would be predicted to occur in PMS patients after a single dose.

2.2.2 Safety:

In Phase I/II studies to date, 46 patients with solid tumors or Glioblastoma Multiforme (GBM) have been treated with AMO-01 for an average of 29.6 days. AMO-01 has been shown to be generally safe and well tolerated at doses of up to 480 mg/m^2/day. Importantly, as measured by biomarkers in these studies, successful inhibition of the Ras-ERK pathway in patients was achieved. The compound also selectively targets cell types expressing the peripheral benzodiazepine receptor (PBR), such as activated astroglia. AMO-01 is being

evaluated as a potential treatment for PMS, and it may have additional clinical utility in the prevention of seizures experienced by PMS patients. PMS is a neurodevelopmental disorder with clinical signs/symptoms including neonatal hypotonia, global developmental delay, along with a key comorbidity of epilepsy. Seizures affect up to 41% of PMS patients, most commonly manifested by atypical absence seizures (90%), but also other types of seizures including tonic (54%) and atonic (18%).

2.3 Potential Risks and Benefits:

2.3.1 Known Potential Risks

In clinical studies, forty-six patients with solid tumors or GBM have been exposed to AMO-01 at dose levels of up to 480 mg/m^2/day for up to 29.6 days. The AEs leading to drug discontinuation that were related to study drug included anaphylactoid shock, clot and fatigue. It is not felt that such AEs would pose significant risk to patients with PMS, and if these or other AEs do occur, it is anticipated that they will resolve readily upon discontinuation of the study drug. Additional information describing the anaphalactoid case is included in the Investigator's Brochure, pp 96-98.

The overall risk profile of AMO-01 has been shown to be acceptable in the clinical studies conducted to date. The unmet need in this population and lack of treatment options supports the investigation of AMO-01 for the treatment of PMS patients with co-morbid epilepsy.

In nonclinical studies, no target organ toxicity was identified. Slight changes in hematology parameters (regenerative anemia) and clinical chemistry parameters (elevations in cholesterol and triglycerides) were observed in monkeys at doses above the dose proposed for the clinical trial in PMS. These effects are easily monitorable and are not anticipated at the proposed clinical dose.

Therefore, the potential benefits outweigh the safety risks of a trial with AMO-01 in PMS patients with co-morbid epilepsy.

2.3.2 Known Potential Benefits

Patients with PMS experience an impaired quality-of-life with significant physical, social and psychological consequences. The clinical features of PMS include severe neonatal hypotonia (>97% individuals), global developmental delay (>98% individuals), normal to accelerated growth (95%), absent to severely delayed speech (>98%), and minor dysmorphic features (Phelan 2008). Behaviorally, PMS individuals commonly present with hyposensitivity to pain, poor eye contact, stereotypic movements, decreased social function, hyperactivity, altered sensorimotor function and aggressive behavior. The behavioral phenotype may regress with age showing a decline in social, motor or psychiatric function (Soorya et al., 2013). Physically PMS may be associated with minor dysmorphic features and lymphedema.

A key comorbidity in PMS is presence of epilepsy. Treatment currently takes the form of multiple anti-convulsants and poly-pharmacy for seizures is common.

Preclinical and clinical data indicate that AMO-01 is a Ras-ERK pathway inhibitor. Overactivity of the Ras-ERK pathway has been implicated in seizure generation and in neurobehavioral disorders according to a number of converging lines of evidence in different experimental models, and in the use of Ras-ERK pathway inhibitors. In an *in vivo* mouse model of PMS (*Shank3b*$^{-/-}$), a single dose of AMO-01 reversed behavioral abnormalities and reduced seizure activity, and effects were sustained through 5 days. The *in vitro* and *in vivo* pharmacodynamic and efficacy data provide evidence that AMO-01 could be efficacious in this patient population.

Therefore, overall anticipated benefits of AMO-01 could include control of seizures in patients with PMS, as well as syndromal improvement with regard to the neuropsychiatric manifestations of this disorder, and if this transpires, it may enhance the general quality-of-life for PMS patients and their families.

3. OBJECTIVES AND PURPOSE

Primary Objective

The primary objective of this study is to evaluate the safety and tolerability of a single 6-hour intravenous infusion of AMO-01 in adolescents and adults with a diagnosis of PMS, from Baseline through Week 8.

Secondary Objectives

Secondary Objectives of this study are to:

- To evaluate the efficacy of AMO-01 in reducing seizure frequency in adolescents and adults with PMS by at least 25 percent as measured by a caregiver completed seizure diary.
- To evaluate the efficacy of AMO-01, from Screening through Week 2, in reducing clinically significant EEG abnormalities
- To evaluate efficacy of AMO-01, from Baseline through Week 4, as measured by clinician-completed rating scales, caregiver completed diaries, functional assessments and biomarker assessments.
- To evaluate maintenance of efficacy from Week 4 to Week 8, as measured by telephone questionnaire

4. Study Design and Endpoints

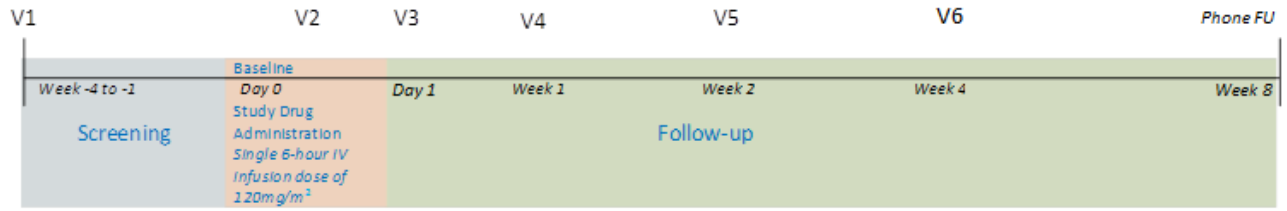
4.1 Description of the Study Design

This is an open-label study to investigate the safety, tolerability and efficacy of a single 6-hour intravenous infusion of AMO-01 to treat adolescents and adults with PMS and co-morbid epilepsy. The subjects in this study will be administered the experimental study medication and then be followed up to ascertain safety and tolerability, and to determine whether their seizure frequency, as well as their signs and symptoms of PMS, are improving, using a caregiver-completed seizure diary as well as accepted rating scales and functional measures in addition to clinical EEG. Approximately 15 subjects will be screened and up to 10 subjects will receive study medication.

The study will have 4 phases (Figure 1, Study Design):

- *Screening (Weeks -4 to -1*): Subjects will be screened to ensure adherence to eligibility criteria and to assess pre-medication seizure frequency
- *Baseline (Day 0)*: Subjects will complete baseline assessments in-clinic prior to study drug administration
- *Study Drug Administration (Day 0):* Eligible subjects will receive a single 6-hour intravenous infusion at a single dose of 120 mg/m²
- *Follow-up (+1 day – Week 8)*: follow-up visits will occur at 1 day after and 1, 2 and 4 weeks after receiving study medication. A phone call will occur 8 weeks after receiving study medication.
- .

Figure 1: Study Design



4.2 Study Endpoints

4.2.1 Primary Endpoint:

- The incidence of Adverse Events (AEs) including Serious Adverse Events (SAEs) between Baseline and Week 8.
- The incidence of abnormal findings in objective assessments (e.g. laboratory values, ECGs, vital signs)

4.2.2 Secondary Endpoints:

- Seizure frequency as measured by a caregiver-completed seizure diary
- Syndrome-specific Clinical Global Impressions Improvement Scale (CGI-I)
- Clinician-completed PMS domain specific causes for concerns visual analogue scale (VAS)
- Top 3 caregiver Concerns VAS
- Aberrant Behavior Checklist (ABC) score
- Repetitive Behavior Scale- Revised (RBS-R) score
- Eye-tracking time on relevant target
- Change in phosphorylated ERK levels
- Telephone follow-up questionnaire

4.2.3 Exploratory Endpoints:

- Sensory Assessment for Neurodevelopmental Disorders (SAND)
- Electroencephalography (EEG)
- Auditory Evoked Potential (AEP)
- Visual Evoked Potential (VEP)

5. Study Enrollment and Withdrawal

5.1 Participant Inclusion Criteria

The subject will not be considered eligible for the study without meeting all of the criteria below:

1. Subjects under study must have a diagnosis of Phelan McDermid Syndrome (PMS) with genetic confirmation of pathogenic *SHANK3* deletion or mutation.
2. Subjects must be post pubertal males or females aged ≥12 years and ≤45 years at Screening.
3. Subject must have a diagnosis of epilepsy.
4. Subjects must have a syndrome-specific Clinical Global Impression- Severity Score of 4 or greater at Screening.
5. Subject's parent or legally authorized representative (LAR) must provide written informed consent before any study related procedures are conducted. Where a parent or LAR provides consent, there must also be assent from the subject (as required by local regulations).
6. Subject's caregiver must be willing and able to support the subject's participation for the duration of the study.
7. Subject's caregiver is able and willing to maintain an accurate and complete daily written seizure diary for the entire duration of the study.

5.2 Participant Exclusion Criteria

Subjects are excluded from the study if any of the following are met:

1. Receiving medications/therapies not stable (i.e. changed) within 4 weeks prior to Screening. For each enrollee, every effort should be made to maintain stable regimens of allowed concomitant medications and allowed non -medicine based therapies throughout the course of the study, from Screening until the last study assessment.
2. Known hypersensitivity to farnesylated dibenzodiazepinone or any of the formulation components
3. Subjects with a history of uncontrolled hypotension or hypertension (Polysorbate 80 is a major constituent of AMO-01 and can cause hypotension).
4. Subjects that have received Coumadin or heparin in the 2 weeks preceding Screening.
5. Medical illness or other concern which would cause the investigator to conclude that the subject will not be able to perform the study procedures or assessments or would confound interpretation of data obtained during assessments.
6. Females who are pregnant, lactating or not willing to use a protocol-defined acceptable contraception method if sexually active and not surgically sterile.
7. Males, engaged in sexual relations with a female of child bearing potential, not using an acceptable contraception method if sexually active and not surgically sterile.
8. Clinically significant abnormalities in safety laboratory tests, vital signs or ECG, as measured at Screening (may repeat to confirm).
9. Current clinically significant (as determined by the investigator) neurological, cardiovascular, renal, hepatic, lymphatic, endocrine or respiratory disease that may impact the interpretability of the study results.
10. Judged clinically to be at risk of suicide by the investigator.
11. Average QTcF value of >450 msec at Screening (may repeat to confirm).
12. Subjects in whom an indwelling intravenous line could not be established or maintained.

5.3 Strategies for Recruitment and Retention

One challenge in studying rare diseases is the feasibility of recruitment. We have established relationships with more than 90 families through the Developmental Synaptopathies Consortium (DSC) as part of the Rare Disease Clinical Research Network across six sites. Approximately 40 percent of these children are expected to

have seizures and should meet inclusion criteria. Intractable epilepsy is a frequently discussed issue in parent online forums and we are confident that the first clinical trial specifically geared to address seizures will attract enormous interest, especially among the most severely affected families. Should recruitment difficulties arise, we have collaborated with the national Phelan-McDermid Syndrome Foundation that has an international membership of approximately 1200 families, approximately 10 percent of whom live in the Northeast US. The PMSF has been a close partner on all our studies in PMS, enable successful recruitment of the DSC, and believes epilepsy to be a critical area that is currently understudied and where treatment is poorly understood. Likewise, we are confident that the interest in this area will ensure adequate retention for follow-up visits after the initial infusion.

5.4 Participant Withdrawal or termination

5.4.1 Reasons for Withdrawal or Termination

If a subject is discontinued at any time after Baseline (V2), the investigator will make every effort to see the subject and complete the final in-clinic study visit (V6) as soon as possible, ideally within one week of discontinuation of the other scheduled study procedures. A follow-up telephone call will still be attempted 4 weeks later.

Subjects may withdraw from the study at any time without stating a reason and without prejudice to further treatment. The Investigator may withdraw a subject from the study and discontinue study treatment and assessments at any time.

Early discontinuation of any subject who has given informed consent to participate will be recorded including the reason for discontinuation. The primary reason for a subject withdrawing prematurely will be selected from the following standard categories of early discontinuations:

- Failed to meet enrollment criteria.
- Adverse Event: Clinical events occurred or laboratory results are reported that in the medical judgment of the investigator are grounds for discontinuation in the best interests of the subject.
- Withdrawal of Consent: The subject desired to withdraw from further participation in the study. The subject is not obliged to provide any reason for withdrawal of consent, but where a reason is given this will be recorded on the CRF.
- Protocol Violation: The subject failed to adhere to the protocol requirements, at the investigator's discretion
- Lost to Follow-Up: The subject stopped coming for visits and study personnel were unable to contact the subject or caregiver. Every effort should be made to re-contact the subject prior to declaring a subject as lost to follow-up, which must be at least 3 documented attempts. The 3rd must be in writing and confirmed to have been received (e.g. registered post).
- Other: The subject was terminated for a reason other than those listed above, such as termination of study.

5.4.2 Handling of Participant Withdrawals or termination

If a subject is withdrawn from active study during screening or observation phases of the study, they will be returned to the referring physician and standard care will be recommended; in addition we will request the subject's participation in an end-of-study visit.

If a subject is withdrawn from active study during active treatment, they will also be returned to the referring physician and standard care will be recommended; however, in this case, we will in addition

request the subject's participation in clinical and laboratory safety assessments and follow up per protocol schedule.

5.5 Premature Termination or Suspension of Study

The following criteria will be used to identify possible adverse treatment events, which will indicate the need to halt active participation of the subject:

- Withdrawal of Consent
- PI or any regulatory authority (Safety Monitoring Board, IRB) believe withdrawal is necessary for the subject's health, well-being, or best interests.
- Any serious related AE of any sort (clinical, laboratory) ≥ 4 will result in halting for the individual and also for the study as a whole.

Return of a subject to active study participation will not be permitted, except if a transient clinical or laboratory abnormality unrelated to study treatment has occurred and subsequent permission of the Safety Monitoring Board to return the patient to active study has been provided.

If a subject is withdrawn from active study during screening or observation phases of the study, they will be returned to the referring physician and standard care will be recommended; in addition we will request the subject's participation in an end-of-study visit

If a subject is withdrawn from active study during treatment, they will also be returned to the referring physician and standard care will be recommended; however, in this case, we will request the subject's participation in clinical and laboratory safety assessments and follow up per protocol schedule.

The study will be halted* if two patients experience stopping conditions across sites. (Exceptions for this criterion: Patients who withdraw voluntarily for reasons not directly related to or intrinsic to the study, e.g. incidental considerations such as concerns about travel time to study visits, unexpected pregnancy in the family, etc. In addition, the study will be halted if one patient experiences a serious related adverse effect (grade ≥ 4 AE).

All safety data will be reported to the Safety Monitoring Board and IRB every six months, or, in the case of any major safety concern or question, immediately. If any study stopping condition occurs, this will be reported immediately and the study halted, pending review by the Safety Monitoring Board and IRB, and until the decision by regulatory authorities to resume, suspend or close the study has been made.

*Operationally, "halting" will ordinarily mean that no further screening of new subjects and no treatment initiation will occur until the safety issue has been investigated and resolved (i.e., a final decision has been made to resume, suspend or close the study has been made). Enrolled study participants who have no new symptoms or adverse effects will ordinarily be allowed to continue study observation or treatment without interruption while the safety issue is being investigated, unless it is the contemporaneous judgment of the PI or subsequent judgment of the Safety Monitoring Board or IRB that it is unsafe to do so, in which case all observation or treatment interventions will be suspended forthwith.

6 Study Agent and Administration

6.1 Study Agent

AMO-01 Chemical Name: 4,6,8-trihydroxy-10-(3,7,11-trimethyldodeca -2,6,10-trienyl)-5,10 dihydrodibenzo[b,e][1,4]diazepin-11-one

The Investigational Medicinal Product (IMP) is AMO-01 70mg sterile bulk drug product vials. Each vial contains 70 mg of AMO-01 drug substance in 2.134 mL of formulation (plus 2% overage). This corresponds to a total drug content of 71.4 mg of active drug substance per 2.176mL of formulation.

6.1.1 Acquisition

IMP will be provided by AMO Pharma directly to the Investigational Research Pharmacy for storage, preparation, and eventually dispensation.

6.1.2 Formulation, Appearance, Packaging, and Labeling

Formulation

The bulk IMP is a homogeneous liquid in which AMO-01 is dissolved. The appearance is a yellow to brownish yellow viscous liquid. The bulk IMP must be diluted in sterile 0.9% saline solution to produce an isotonic final dosing formulation prior to administration to study subjects.

The AMO-01 bulk vial formulation components are listed in Table 4.

Table 4: Bulk Formulation Components

Ingredient	Grade	Function	Fill Content[1] (mg/vial)	Concentration (% w/w)	Reference Standard
AMO-01	cGMP	Active Ingredient	71.4	3.15	Manufacturer's Standard
Polysorbate 80	NF	Excipient	1249.5	55.15	NF
Polyethylene glycol 400	USP/NF	Excipient	357.0	15.76	USP/NF
Absolute Ethanol	USP	Excipient	282.0	12.45	USP
Water for Injection	USP	Excipient	282.0	12.45	USP
(+)-Sodium L-Ascorbate	USP	Antioxidant	23.6[2]	1.04	USP
TOTAL			2265.5	100.00	

[1]Fill content corresponds to the true content in the vial, taking into account the overage. [2](+)-Sodium L-ascorbate label claim and fill content correspond to amount initially present in bulk formulation (i.e. preservative).

The bulk IMP is provided by AMO Pharma in 30-mL sterile, pyrogen-free borosilicate clear glass vials (USP Type I vial), closed with a sterile teflon-coated butyl stopper, and crimp sealed with an aluminum seal.

Each bulk vial is individually labeled. At minimum, the label shall provide the following information: drug product name, label claim for strength per vial (including dose, concentration, volume and overage), professed sterility, manufacturing lot number, storage conditions and retest/expiry date.

6.1.3 Product Storage and Stability

Bulk Product Stability

Formal stability studies indicate that the bulk drug product is chemically stable over at least 12 months when stored at 5 ± 3°C. Therefore the recommended long term storage conditions for the DP is USP refrigeration, 2-8°C.

In-Use Stability

Stability of the final dosing formulation was evaluated in both ethylene-vinyl acetate (EVA) and polypropylene (PP) infusion bags and in the final diluted drug product vial. The results obtained with the EVA or PP infusion

bags at drug concentrations of 4.49 mg/mL demonstrated stability of the final dosing formulation for 7 days when refrigerated or stored at ambient temperature (15-30°C). The final dosing formulation (4.49 mg/mL), when placed in 100mL borosilicate vials, was also stable for 7 days when refrigerated or stored at ambient temperature.

Storage and Transport

Bulk formulation vials are to be stored at 5 ± 3°C, and protected from light. Although in-use stability confirms acceptable stability of the final formulation for up to 7 days, it is preferable that the final AMO-01 formulation be prepared as close to the planned dosing time as possible in order to minimize the time that the drug is in the prepared final formulation state (ideally not more than 48 hours before dosing). Any prepared final formulation should be stored at 5 ± 3°C, and protected from light until ready to dose within 48 hours of dilution.

Special Precautions

Since AMO-01 is a cytotoxic agent, procedures for proper handling and disposal of anticancer drugs should be followed. Several guidelines on this subject have been published (US Dept of Health and Human Services, 1983; Yodaiken et al., 1986). Specific to AMO-01, the following precautionary measures are recommended for the handling and preparation of AMO-01 for injection:

1) The reconstitution and bag transfer procedure should be carried out in a flow hood.

2) The personnel reconstituting AMO-01 drug product should wear polynitrile gloves, safety glasses, disposable gowns, and masks.

3) Due to the high surfactant concentration, careful handling is required during preparation of the dosing formulation and also during administration. Those handling the final prepared AMO-01 formulation should avoid any vigorous agitation at all times as foaming can occur following dilution.

4) All vials, syringes, needles, and other materials, that have come in contact with AMO-01, should be segregated and destroyed by incineration.

6.1.4 Preparation

AMO-01 for injection is supplied as a sterile bulk formulation which requires dilution in sterile 0.9% saline prior to subject administration.

It is planned to use the same sterile bulk formulation qualitatively and quantitatively i.e. 32.84mg/ml drug concentration as developed previously. However bulk vial volumes were reduced to 2.176 ml to deliver a dose of 70mg/vial. A similar dilution factor in 0.9% saline will be performed to arrive at final dosing formulation concentration of 4.49mg/ml. As before, depending on the dose to be administered, several vials could be used for preparation of the infusion bag.

The study drug requires a reconstitution kit to include the following:

- Each single-dose vial of sterile drug product contains 71.4 mg of AMO-01 drug substance in 2.176 ml (including a 2% overfill). This corresponds to a drug content label claim of 70 mg per vial and a drug product volume of 2.134 mL.
- An EVA infusion bag (250-, 500- or 1000-mL, depending on the dose to be administered).
- An administration set consisting of TOTM-plasticized PVC line with a pump connector.
- An extension set consisting of PVC line that is internally coated with PE, an in-line 1.2 micron filter, and an anti-siphon valve.
- A pre-filled syringe or a bag of sterile 0.9% saline.

- An instruction sheet for the preparation of the dosing formulation and for assembly of the infusion kit for administration.

Instructions for final formulation preparation from sterile bulk formulation vials:

The vial content shall be diluted with 13.73 mL of sterile 0.9% saline. Upon dilution, the final volume will be 15.91 mL. This will ensure sufficient dosing formulation to allow for the withdrawal of a minimum isotonic premix volume of 15.59 mL at a concentration of containing 4.49 mg/mL AMO-01, corresponding to the label claim of 70 mg/vial. Depending on the dose to be administered, several vials could be used for the preparation of the infusion bag.

After dilution and gentle mixing (by gently swirling of the vial in a circular motion) the dosing formulation is transferred from the drug product vial into a 1-L disposable sterile bottle (all vials required to prepare an infusion bag are pooled in a single disposable sterile bottle). Using a peristaltic pump the dosing formulation in the disposable sterile bottle is transferred into a 250-mL, 500-mL or 1-L ethyl-vinyl acetate (EVA) or polypropylene (PP) infusion bag. The filled infusion bag is then connected to a pump for continuous infusion.

6.1.5 Dosing and Administration

AMO-01 will be administered as a single dose via intravenous infusions over a 6 hour period, with the total dose administered as 120 mg/m^2.

6.1.6 Route of Administration

Following dilution of the sterile bulk formulation to final formulation concentration, AMO-01 will be administered intravenously over a 6 hour period.

6.1.7 Starting Dose and Dose Escalation Schedule

Not Applicable

6.1.8 Dose Adjustments/Modifications/Delays

Not Applicable

6.1.9 Duration of Therapy

The expected duration of participation is 12 weeks. Participation for individual subjects will consist of up to 4 weeks of an initial Screening period followed by a Baseline Day 0 visit. At this visit, if the subject is eligible they will receive a single 6-hour intravenous infusion for a total dose administered of 120 mg/m^2 of AMO-01. Subjects will return for follow-up visits at 1 day and 1, 2 and 4 weeks post-dose. A follow-up phone call will be conducted 8 weeks post-dose.

6.1.10 Tracking of Dose

Not Applicable

6.1.11 Device-Specific Considerations

Not Applicable

6.2 Study Agent Accountability Procedures

In accordance with regulatory requirements, the Investigator or designated site staff must document the amount of IMP received from AMO Pharma. Following receipt they should document the amount of IMP dispensed and/or administered to study subjects and the amount returned to the pharmacy. Product accountability records must be maintained throughout the course of the study.

At the end of the study, all IMP will be reconciled and all remaining materials will be destroyed by the site according to the sponsor's instruction once it has been inventoried and drug accountability records reviewed. All destroyed IMP must have a documented proof of destruction.

7 Study Procedures and Schedule

Refer to Table 1 for an overall schedule of assessments for each visit. Refer to Table 2 for a detailed schedule of assessments to be completed on V2.

7.1 Study Procedures and Evaluations

All assessments will be performed by the investigator or appropriately delegated and trained personnel.

7.1.1 Medical/Surgical and Medication history

The investigator must record all medically and clinical relevant information regardless of the time since the date of diagnosis.

History should include (but is not limited to):

- All current and past non-pharmacologic therapies taken 1 month before the Screening Visit (V1)
- History of respiratory, cardiovascular, renal, gastro-intestinal, hepatic, endocrine, hematological, neurological, psychiatric and any other diseases

7.1.2 Physical Examination

A full physical examination will be conducted. This will be completed by a delegated physician.

A full physical examination is composed of a review of the following body systems:

- General appearance
- Skin
- Head, eyes, ears, nose and throat
- Respiratory
- Cardiovascular
- Abdomen (including liver and kidneys)
- Musculoskeletal
- Neurological

Any abnormalities that are identified at the Screening Visit (V1) will be documented in the subject's source and on the medical/surgical history CRF page. Any changes (including new and worsening findings) between the Screening Visit (V1) and end of study should be captured as AEs on the AE CRF page, as determined by the Investigator.

If an improvement/resolution of a physical examination finding documented in the subject's medical history occurs during the study, it should be recorded in the source document. If there is resolution of a physical examination finding previously noted as an AE, then the event resolution and stop date should be recorded on the AE CRF page.

7.1.3 Height

A calibrated stadiometer should be used to measure height. Height should be measured in centimeters without shoes with the subject standing on a flat surface and with their chin parallel to the floor. The body should be straight but not rigid. The subject's height should be recorded to the nearest 0.5cm.

7.1.4 Weight

The same calibrated scale should be used for all weight measurements for a subject. Weight should be measured in kilograms without shoes and recorded to the nearest 0.1 kg. Bulky items should be removed whenever possible to ensure the most accurate weight is recorded.

7.1.5 Adverse Event collection

Subjects will be questioned in a non-leading way to determine if AEs have occurred since the last visit e.g. "Have you had any health problems since your last visit?" AE's will be collected from the time of consent. Refer to section 8 Safety for additional details of AE collection and reporting.

7.1.6 Vital signs

Vital signs will include the following measures: temperature, pulse, systolic and diastolic blood pressure and respiratory rate. Blood pressure and pulse will be determined after the subject has been in the sitting position for 5 minutes.

Blood pressure should be determined by cuff (using the same method, the same arm, and in the same position throughout the study). A BP cuff appropriate for the subject's arm length and girth should be used for all BP measurements. The cuff should be approximately two-thirds the length/width of the subject's arm (from elbow to shoulder). The cuff should be calibrated and ideally the same cuff should be used on a subject throughout the study. All BP measurements should be performed by the same study site personnel (if possible) throughout the study.

Any vital signs which in the opinion of the investigator are deemed to be clinically significant are to be recorded as an AE. Any clinically significant abnormalities at the last in-clinic study visit (V6) should be followed up and repeated until they have returned to baseline or are, in the opinion of the investigator, no longer clinically significant.

7.1.7 ECG

All the 12 lead ECGs will be performed using a GE MAC 5500 ECG machine. The investigator will perform the interpretation of the ECG immediately after collection to ensure the safety of each subject.

The study site will be required to print out at least 1 copy of the original tracing of the ECG. The original ECG(s) should be signed by the investigator, and maintained at the site with the subject's medical records. The investigator will be responsible for determining the clinical significance of each ECG.

The HR, PR interval, QRS interval, and QT interval will be measured and QTcB and QTcF will be calculated for all ECGs.

Subjects will be assessed in a quiet state (after 5 minutes of rest) in the supine position. A standard ECG recording device will be used with the standard paper rate of 25 mm/second and the standard scale setting of 10 mm/volt.

Three ECGs will be obtained at the Screening Visit (V1) to determine subject eligibility. Subjects will not be eligible for inclusion in this study if they have an abnormal clinically significant ECG at the Screening Visit (V1). The eligibility of a subject will be based on the assessment of the ECG by the investigator, in consultation

with the medical monitor. The investigator will evaluate the potential impact of an abnormal, clinically significant ECG on the continued participation of the subject.

For all other visits, where an ECG is requested according to the schedule of assessments, these will also be performed in triplicate (ideally separated by at least five minutes).

7.1.8 Caregiver-Completed Seizure Diary

A diary will be provided to the subject/caregiver at each visit from Screening (V1) to Week 2 (V5) which should be completed every day. The diary should be returned at each subsequent visit and the diary should be reviewed by a delegated member of the study team. The diary will be collected at Week 4 (V6). The diary should be completed in full so that the following information can be ascertained:

- Date/time of seizure activity
- Length of seizure
- Type of seizure

A copy of the caregiver-completed seizure diary is located in APPENDIX B: Caregiver Completed Seizure Diary.

7.1.9 Syndrome-specific Clinical Global Impressions Improvement Scale (CGI-I)

The clinician administered Clinical Global Impressions of Severity (CGI-S) and Improvement (CGI-I) scale (Guy, 1976) will be performed in accordance with schedule of assessment. The CGI rating scale permits a global evaluation of the subject's improvement over time and will be administered as detailed in the schedule of assessments.

The CGI-S is a 7-point Likert type scale that requires the clinician to rate the severity of the subject's illness at the time of assessment, relative to the clinician's past experience with subjects who have the same diagnosis. Considering total clinical experience, a subject is assessed on severity of illness at the time of rating 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.

The CGI-I requires the clinician to rate how much the subject's illness has improved or worsened relative to a baseline state. A seven point Likert type scale is used from 1 = very much improved, 2 = much improved, 3 = minimally improved, 4 = no change, 5 = minimally worse, 6 = much worse, 7 = very much worse.

CGI-I ratings should be compared against the CGI-S, done at Baseline (V2).

In this study, a standard set of probes/prompts will be provided to the sites to assist the evaluator in eliciting commentary from the subject or their caregiver across each of the core domains of the affected individual's PMS and also on associated symptoms. Every effort will be made so that the same evaluator will also observe the subject at each visit to the clinic.

A copy of the CGI-S and CGI-I to be used in the study, along with instructions is included in APPENDIX C: Syndrome Specific Clinical Global Impressions- Severity and Improvement.

7.1.10 Clinician-completed PMS Domain Specific Causes for Concern Visual Analogue Scale (VAS)

The Clinician-completed PMS Domain Specific Causes for Concern VAS is a 9 item VAS completed by the clinician that scores the severity of concerns in the following domains that are clinically relevant in PMS:

- Speech and language difficulties
- Problems with thinking and learning
- Seizures

- Problems with gross motor functioning
- Restricted interests and/or repetitive behaviors
- Social communication problems
- Sensory sensitivities
- Activities of daily living
- Sleep disturbance

For each subject, the clinician is instructed to identify the top 4 or 5 items that are of particular concern and that the clinician would most like to see change during the course of treatment with the study medication. These same 4 or 5 concerns are rated again on each occasion that the clinician completes this assessment measure. The severity of the clinician's concern in each domain is scored by using a 10 cm VAS, with anchors of "not at all severe" at the left end and "very severe" at the right end. The clinician is asked to make a vertical line indicating his/her level of concern in each domain, using a time frame of the past week for reference. A score for each domain is determined by measuring the number of centimeters on the 10-cm VAS line from the anchor point on the left side of the line. A total VAS score for each subject is calculated as the sum of the scores for the 9 domains and is reported as both an absolute number and a percentage of the total possible line length (e.g. xx cm).

The Clinician-completed PMS Domain Specific Causes for Concern VAS will be administered according the schedule of assessments.

A copy of the Clinician-completed PMS Domain Specific Causes for Concern VAS is included in APPENDIX A: Clinician-Completed PMS Domain-Specific Causes for Concern VAS.

7.1.11 Top 3 Caregiver Concerns VAS

The Top 3 concerns VAS allows caregivers to identify their main three causes of concern, related to the subject's PMS, rather than these being pre-specified within a scale, and then rating how these concerns have changed at specific time-points during the study.

Caregivers will be asked to rate each of the three causes for concern by drawing a vertical mark on a 10cm long VAS with anchors of "not at all severe" at the left end and "very severe" at the right end. The 3 concerns related to the subject's PMS will be chosen and rated at baseline. These 3 signs and symptoms will be rated again according to the schedule of assessments using the same scales.

A copy of the Top 3 concerns VAS is included in APPENDIX D: Top 3 Caregiver Concerns VAS.

7.1.12 Aberrant Behavior Checklist (ABC) score

The Aberrant Behavior Checklist (ABC) is a 58-item rating scale to be completed by a caregiver. Five subscales measure the following items: a) irritability (15 items), b) lethargy/social withdrawal (16 items), c) stereotypic behavior (7 items), d) hyperactivity/noncompliance (16 items), and e) inappropriate speech (4 items). A copy of the ABC is included in APPENDIX E: Aberrant Behavior Checklist – Community (ABC).

7.1.13 Repetitive Behavior Scale- Revised (RBS-R) score

The Repetitive Behavior Scale- Revised (RBS-R) is a 44-item caregiver-report questionnaire that is used to measure the breadth of repetitive behavior in children, adolescents and adults with Autism Spectrum Disorders. RBS-R includes six subscales: sterotyped behavior, self-injurious behavior, compulsive behavior, routine behavior, sameness behavior, and restricted behavior. Caregivers will be asked to read a list of behaviors and score on a 4-point likert scale ranging from "0- Behavior does not occur" to "3- Behavior occurs and is a severe problem".

A copy of the RBS-R is included in APPENDIX F: Repetitive Behavior Scale- Revised (RBS-R).

7.1.14 Sensory Assessment for Neurodevelopmental Disorders (SAND)

The SAND is a clinician-administered assessment and corresponding caregiver interview that is not dependent on verbal or cognitive ability and is therefore appropriate for severely affected or nonverbal individuals. The algorithm measures sensory discrete hyperreactivity, hyporeactivity, and sensory-seeking behaviors across visual, tactile, and auditory domains. The SAND has also been validated in ASD with significant correlation to the previously validated Sensory Profile. Results from our preliminary data suggest the SAND can identify sensory reactivity subtypes that differentiate Fragile X syndrome and other forms of ASD.

7.1.15 Follow-up Telephone Call

The follow-up telephone call will be conducted by the study clinician and will address the following questions:

1. If applicable, is the participant's seizure activity the same, worse, or better than recorded at Week 4?
2. Are there any other clinical changes since Week 4?

7.2 Laboratory Procedures/Evaluations

7.2.1 Clinical Laboratory Evaluation

All clinical laboratory assays will be performed according to the local laboratory's normal procedures. Reference ranges will be supplied by the local laboratory and will be used to assess the laboratory data for clinical significance and out of range changes.

Biochemistry

A biochemistry sample will be collected at screening and each clinic visit. Sample collection will include: sodium; potassium; chloride; carbon dioxide; urea nitrogen; creatinine; glucose; alkaline phosphatase; gamma-glutamyl transferase (GGT); alanine aminotransferase (ALT); asparate aminotransferase (AST); lactate dehydrogenase (LD); bilirubin (total and direct). Biochemistry blood samples should be drawn with the subject in a non-fasting state.

Hematology

A full blood count with differential should be collected at screening and each clinic visit and include: red blood cells; white blood cells (including differential); hemoglobin; hematocrit; platelets.

Urinalysis

Urinalysis will be performed by sending a sample to the local laboratory. Subject/caregiver will collect sample in clean/sterile urine collection cup. If needed, a toilet hat will be used for collection. If necessary, urine may also be collected from a diaper by squeezing urine from the diaper gauze pad directly into collection cup.

Pregnancy Test

A urine or serum pregnancy test for female subjects of child bearing potential will be performed at Screening and at all subsequent clinic visits.

Lipid Panel

A non-fasting lipid panel will be collected at Baseline (V2) and Follow-up (V4) and will include cholesterol; high density lipoprotein (HDL) cholesterol; low density lipoprotein (LDL) cholesterol; triglcyerides; very low density lipoprotein (VLDL) cholesterol.

7.2.2 Other Assays or Procedures

7.2.2.1 Pharmacokinetic Assessments

Blood levels of AMO-01 will be assessed at four time-points; pre-infusion, approximately 2 hours and approximately 6 hours after the commencement of the infusion of the study medication, as well as approximately 1 hour after the cessation of administration of the study medication. The central tendency values (and ranges) for AMO-01 plasma concentrations and imputed exposures (AUC) will be measured.

7.2.2.2 Biomarker/Physiological Assessments

7.2.12 Eye-tracking time on relevant target

Eye tracking will utilize three tasks to measure attention in general and social attention specifically. (1) The Visual Paired Comparison task presents two identical stimuli (faces or patterns) side by side for familiarization. The familiar stimulus and a new stimulus are then paired on test and recognition is inferred from preferential looking to the new target. (2) The Gap-Overlap task measures the latencies of gaze shifts from a central fixation point to a peripheral target are under 2 conditions. During gap trials, the fixation cross disappears prior to the onset of the peripheral cue, whereas in the overlap condition, the fixation cross remains on the screen throughout the trial. Saccadic latencies are typically greater for overlap than for gap trials since the former requires disengagement of attention in addition to orienting to a target (gap effect). (3) Flicker measures the temporal resolution of attention. Four squares that flicker from black to white are presented against a grey background. The target square flickers 180 degrees out-of-phase from the three distractors. Flickering occurs at 1 of 4 frequencies: 0.2, 0.5, 1, or 2 Hz. A preference for the target square indicates phase individuation, which is easier at slower frequencies. Participants are directed to look at a screen for approximately 10 minutes while images of human faces are presented and automated assessments of time spent looking at socially salient features of test images are recorded.

7.2.13 Auditory Event Related Potential (AERP)

AERPs are useful for characterizing early processing of auditory tones and habituation to rapidly repeated stimuli as in speech processing. Tones are presented in rapid succession (e.g., up to every 500 ms) and ERP components to both initial stimulus presentations and their habituation to subsequent stimuli (i.e., reduction in amplitude) are examined. Auditory gating waveforms have been well characterized across development (Milner at l., 2014; Tremblay et al., 2014; Magnee et al., 2011) and are comprised of a fronto-central peak 100 ms after the onset of the auditory stimulus (N_1) and a subsequent positive peak (P_2) reflecting activity in auditory cortex, mesencephalitic reticular activating system, planum temporale, and auditory association cortex (Crowley et al., 2004; Godey et al., 2001). The N_1-P_2 complex reflects two distinct components: initial stimulus processing (N_1) and subsequent attention to and processing of stimulus information (P_2). P_2 also has been implicated in speech processing (Knowland et al., 2014; Klucharev et al., 2003) suggesting that its analysis may be important for understanding neurophysiological mechanisms associated with broader cognitive and language developmental dysfunctions. Recent AERP studies suggest that measures of auditory gating may provide a sensitive and selective probe of sensory physiology in neurodevelopmental disorders (Hall et al., 2009). Individuals with FXS, for example, show auditory hypersensitivities consistent with Fmr1KO mouse models of local-circuit hyper-excitability involving prolonged “UP” states in the gamma frequency range, decreased glutamatergic drive on fast-spiking GABAergic inhibitory neurons in sensory cortex, and heightened neurophysiological response to auditory stimuli (Rotschafer et al., 2013).

7.2.14 Visual Evoked Potential (VEP)

VEPs provide a noninvasive technique to evaluate the functional integrity of visual pathways in the brain from the retina to the visual cortex via the optic nerve/optic radiations. The VEP is recorded from the head’s surface, over the visual cortex, and is extracted from ongoing EEG through signal averaging. VEPs reflect the sum of

excitatory and inhibitory postsynaptic potentials occurring on apical dendrites (Zemon et al., 1986) which modulate excitatory and inhibitory signals received by the pyramidal cells. The major positive and negative peaks and troughs in VEP waveforms reflect different cellular events. The contrast-reversing checkerboard stimulus used in this study produces a positive peak at approximately 60 ms (P_0 or P_{60}), reflecting activation of the primary visual cortex from the lateral geniculate nucleus. A negative peak at approximately 75 ms (N_0 or N_{75}) reflecting depolarization and glutamatergic postsynaptic activity spreading to the superficial layers of primary visual cortex, and a positive peak at approximately 100 ms (P_1 or P_{100}) reflecting superficial hyperpolarization and GABAergic activity (Zemon et al., 1980). VEPs have been used in clinical trials; for example, an antiepileptic drug (gabapentin; Conte et al., 2009) and an infant formula (O'Connor et al., 2001); both received FDA approval based in part on positive findings from VEP studies. Data from ongoing clinical trials at Mount Sinai provide support for use of VEPs as a measure of treatment response.

7.2.15 Electroencephalography (EEG)

EEG is a non-invasive measure of brain activity. An array of 24 electrodes will be placed on the subject's head using a standard 10-20 configuration. The EEG recording will last for approximately 30 minutes and will include an assessment of the effects of intermittent photic stimulation. The EEG signals that are obtained will be amplified and sent to a recording computer for processing and on-line viewing using Nicolet Natus Software. The EEGs will be edited both through automatic artifact-detection software and hand-editing. The EEG findings for each subject will be summarized using the SCORE-EEG reporting format. Each report will include a description of modulators/procedures (e.g. intermittent photic stimulation), background activity (e.g. posterior dominant rhythm), interictal findings (e.g. epileptiform interictal activity, including spike and sharp waves), episodes (e.g. seizures), and clinically relevant confounds (EEG artifacts). Clinically relevant screenshots will also be included in the SCORE-EEG report. A single clinician will review all processed EEGs and will render the summary of findings, the diagnostic significance, and clinical comments for each EEG tracing obtained from all subjects. EEGs will be obtained at Screening/V1, and at Follow-up1/V3, Follow-up 2/V4 and again at Follow-up 3/V5.

Clinically relevant EEG findings at Screening, V3, V4 and V5 will be listed for each subject and clinically relevant changes (e.g. in the frequency and prevalence of abnormal interictal activity) will be summarized at each assessment time point.

7.2.2.3 Change in phosphorylated ERK levels

Samples of 6 ml blood will be taken pre-infusion, approximately 2 and 6 hours after the start of the infusion, approximately 1 hour after the cessation of the infusion, and at the 1 week (V4) and 4 week (V6) visits. The sample will be provided as whole blood with fixative, to be used for the analysis of levels of phosphorylated ERK and total ERK protein.

7.2.3 Specimen Preparation, Handling and Storage

7.2.3.1 PK

Instructions for collection, handling, storage, processing and shipment of the PK sample will be provided in a separate laboratory manual.

7.2.3.2 pERK

Instructions for collection, handling, storage, processing and shipment of the pERK sample will be provided in a separate laboratory manual.

7.2.4 Specimen Shipment

7.2.4.1 PK

Instructions for collection, handling, storage, processing and shipment of the PK sample will be provided in a separate laboratory manual.

7.2.4.2 pERK

Instructions for collection, handling, storage, processing and shipment of the pERK sample will be provided in a separate laboratory manual.

7.3 Study Schedule

7.3.1 Screening

The Screening visit (V1) will occur in the 4 weeks preceding the Baseline visit (V2). Participants and their families will come in to sign the informed consent and meet with members of the study team. Refer to the schedule of events (Table 1) for the assessments to occur at the Screening visit (V1).

7.3.2 Enrollment/Baseline

If all eligibility criteria are met, the subject will be booked in for their Baseline visit (V2). Pre-infusion procedures as outlined in Table 2, schedule of events, will occur prior to the administration of AMO-01. Procedures outlined as 'during infusion' and 'post infusion' will occur during and after the administration of AMO-01, respectively. Table 2 outlines the procedures to be completed during and after AMO-01 administration.

7.3.3 Follow-up

Planned follow-up visits will occur at 1 day (V3), week 1 (V4), and week 2 (V5) post-dose. A follow-up phone call will occur 8 weeks post-dose. Refer to the schedule of events (Table 1) for a list of assessments to be completed at each follow-up visit.

7.3.4 Final In-Clinic Study Visit

A final in-clinic study visit (V6) will be conducted 4 weeks post-dose. Refer to the schedule of events (Table 1) for a list of assessments to be completed at this visit.

7.3.5 Early Termination Visit

In the event of early termination or withdrawal, the subject will complete the assessments scheduled for the final in-clinic study visit (V6). The follow-up phone call will also be attempted. Refer to the schedule of events (Table 1) for a list of assessments to be completed at this visit.

7.3.6 Unscheduled Visit

Unplanned visits will be permitted if there are any concerns about safety and/or adverse events (AEs).

7.3.7 Schedule of Events Table

Refer to Table 1 and Table 2 for schedule of events.

7.4 Participant Access to Study Agent At Study Closure

As AMO-01 is not a marketed product and this study represents a pilot investigator led study, there will be no access to study agent at study closure.

7.5 Concomitant Medications, Treatments, and Procedures

Non-pharmacologic educational and behavioral interventions will be allowed. Subjects will be asked at the time of consent/assent to not change concomitant treatments for the duration of the study.

Concomitant medication will be allowed, providing that they have been stable for at least 1 month prior to Screening (V1) and every effort will be made for medications to continue to be stable during the study. Exceptions may be allowed if concomitant medication is required on a self-limited basis for the clinical management of adverse events (i.e. headache, infection).

Contraindicated medications include Coumadin or heparin administered within the two weeks prior to Screening (V1).

8 ASSESSMENT OF SAFETY

8.1 Specification of Safety Parameters

8.1.1 Definition of Adverse Events (AE)

An adverse event (AE) will be defined as any untoward medical occurrence in a study subject, temporally associated with the use of the experimental medication, whether or not considered related to the medication. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of the experimental medication.

8.1.2 Definition of Serious Adverse Events (SAE)

A serious adverse event (SAE) will be defined as an AE that meets any of the following criteria:

- results in death;
- is life threatening;
- requires inpatient hospitalization;
- results in a persistent or significant disability/incapacity;
- any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the outcomes listed above.

8.1.3 Definition of Unanticipated Problems (UP)

Unanticipated problems will be defined by three criteria:

1. The AE is unexpected (see section 8.2.3);
2. The AE is possibly related to participation in the research (see 8.2.2.);
3. The AE suggests that the research places subjects or others at a greater risk of harm than was previously known or recognized.

8.2 Classification of an Adverse Event

8.2.1 Severity of Event

Monitoring for AEs will be conducted during scheduled and unscheduled visits per clinical and laboratory assessments. General AEs will be graded by the National Cancer Institute (NCI) CTCAE scales. Any AE that the PI deems serious, although not easily categorized in the National Cancer Institute grading system, will be considered ≥ grade 3. If a subject develops significant (CTCAE AE ≥ 3 grade) neurological signs and

symptoms (e.g. cerebrovascular event or peripheral neuropathy), they will be seen immediately for a comprehensive evaluation, appropriate treatment, and removal from active participation in the study.

8.2.2 Relationship to Study Agent

We will apply NCI-CTCAE criteria for attribution of AE by means of the following descriptors and codes, following the NCI guidelines for their application.

- unrelated to treatment 1
- unlikely related to treatment 2
- possibly related to treatment 3
- probably related to treatment 4
- definitely related to treatment 5

8.2.3 Expectedness

Expectedness of AEs will be evaluated based on clinical phase I and Phase II studies (see Section 2.1.7; Clinical Experience). Unexpectedness will be defined as any AE occurring in one or more subjects where the nature, severity, or frequency of which is **not** consistent with either:

1. the known or foreseeable risk of adverse events associated with the procedures involved in the research that are described in (a) the IRB-approved research protocol, (b) investigator brochure, (c) IRB-approved informed consent document; or

2. the expected natural progression of the underlying disease or condition of the subject(s) and the subject's predisposing risk factor profile for the adverse event.

8.3 Time Period and Frequency for Event Assessment and Follow-Up

All AEs occurring from screening (V1) until the time of the follow-up phone call 8 weeks after study drug administration will be recorded on the Adverse Event form in the subject's CRF, irrespective of severity or whether or not they are considered medication-related. Onset of chronic illness (e.g. autoimmune disorders, asthma, type 1 diabetes and allergies) and conditions prompting emergency room (ER) visits or physician office visits that are not related to well-child care, injury, or common acute illnesses (e.g., upper respiratory tract infection, otitis media, pharyngitis, and gastroenteritis) will be reported during the entire study period. The investigator will inquire about the occurrence of AEs at every visit/contact during the study and throughout the follow-up phase as appropriate. All AEs either observed by the investigator or a clinical collaborator or reported by the subject's parent/guardian spontaneously or in response to a direct question will be evaluated by the investigator. AEs not previously documented in the study will be recorded in the Adverse Event form within the subject's CRF. The nature of each event, date and time (where appropriate) of onset, outcome, intensity and relationship to drug administration should be established. Details of any corrective treatment should be recorded on the appropriate page of the CRF.

8.4 Reporting Procedures

8.4.1 Adverse Event Reporting

When an AE/SAE occurs, it will be the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory, and diagnostic reports) relative to the event. The investigator will then record all relevant information regarding an AE/SAE on the eCRF or SAE Report Form as applicable. The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information.

8.4.2 Serious Adverse Event Reporting

All safety data will be reported to the PI/Sponsor, DSMB, IRB, and FDA every six months, or, in the case of any major safety concern or question, immediately. All SAEs will be reported to the PI/Sponsor within 72 hours. If any study stopping condition occurs, this will be reported immediately, and the study will be halted, pending review by these agencies. The investigator has a legal responsibility to promptly notify, as appropriate, both the local regulatory authority and other regulatory agencies about the safety of a product under clinical investigation. Prompt notification of SAEs by the investigator to the Study Contact for Reporting SAEs is essential so that legal obligations and ethical responsibilities towards safety of other subjects are met. The investigator, or responsible person according to local requirements, will comply with the applicable local regulatory requirements related to the reporting of SAEs to regulatory authorities and the IRB. This protocol will be filed under an Investigational New Drug (IND) status with the US FDA. A given SAE may qualify as an IND Safety Report if the SAE is both attributable to the investigational product and unexpected. In this case, all investigators filed to the IND (and associated INDs for the same compound) will receive an Expedited Investigator Safety Report (EISR), identical in content to the IND Safety Report submitted to the FDA.

An EISR is required for: a) development compounds (i.e. compounds not marketed), if the event is serious, unexpected and has a suspected relationship to study drug treatment. Expected adverse events for development compounds will be described; b) marketed compounds (i.e. approved in a least one market), if the event is serious, unexpected and has a suspected relationship to treatment with a drug product AND is a significant new emerging safety issue. Expected adverse events for marketed compounds will be described in the Core Safety Information (CSI). An EISR is required if an SAE was expedited to the IND in the US or to fulfill regulatory obligations in other countries. The purpose of the EISR is to fulfill specific regulatory and Good Clinical Practice (GCP) requirements, regarding the product under investigation.

8.4.3 Unanticipated Problem Reporting

All unanticipated problems will be reported to the drug manufacturer, PI/Sponsor, DSMB, IRB, and FDA every six months, or immediately in the case of any major safety concern or question. Unanticipated problems that are serious adverse events will be reported to the IRB within 1 week of the investigator becoming aware of the event. Unanticipated problems will be defined as any incident, experience, or outcome that meets **all** of the following criteria:

- unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;

- related or possibly related to participation in the research (in this guidance document, *possibly related* means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and

- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized

8.4.4 Events of Special Interest

An AE of special interest (serious or non-serious) is defined as one of scientific and medical concern specific to the AMO-01 for which ongoing monitoring and rapid communication by the investigator to the sponsor is appropriate. Such an event might warrant further investigation in order to characterize and understand it. Depending on the nature of the event, rapid communication by the investigator to the IRB, DSMB, and FDA might also be warranted. A report of events of special interest will be generated and include AEs of special interest that are unexpected as defined (see section 8.2.3). An example that may be relevant to AMO-01 would

be an anaphylactic reaction which has been reported in one patient previously when the intravenous catheter was flushed too quickly.

8.4.5 Reporting of Pregnancy

If serum pregnancy results are positive, the lead investigator will inform the subject and the caregiver with the subject's consent and it will be documented in the case report form.

8.5 Study Halting Rules

As indicated in the Synopsis, the following criteria will be used to identify possible adverse treatment events, which will indicate the need to halt active participation of the subject in the study:

- Withdrawal of Consent
- PI or any regulatory authority (DSMB, IRB, or FDA) believe withdrawal is necessary for the subject's health, well-being, or best interests.
- PI or any regulatory authority believe that withdrawal is necessary for the subject's health, well-being, or best interests
- Laboratory: any abnormality on any test with adverse event (AE, Common Terminology Criteria for Adverse Events, National Cancer Institute, scales) ≥ 3 or greater at any time in the study
- Any AE of any sort (clinical, laboratory) ≥ 4 will result in halting for the individual and also for the study as a whole.

To avoid bias, all analyses will include all subjects, including those withdrawn from the study, regardless of adherence to study protocol. Especially in a phase I/II of exploratory trial of a novel, unproven treatment, the safety of subjects must be the primary *overriding* concern. If there is doubt concerning a subject's safety, the default mode must be withdrawal from treatment or active study participation, followed by close observation (safety follow up visits) and recommendation of standard treatment.

If a subject is withdrawn from active study during screening or observation phases of the study, they will be returned to the referring physician and standard care will be recommended; in addition we will request the subject's participation in an end-of-study visit, as per the schedule of the part of the study.

If a subject is withdrawn from active study during treatment, they will also be returned to the referring physician and standard care will be recommended; however, in this case, we will in addition request the subject's participation in clinical and laboratory safety assessments and follow up per protocol schedule.

Stopping Conditions for Study as a Whole

The study will be halted* if two patients experience stopping conditions across sites. Any patient experiencing stopping conditions will be reported to the PI/Sponsor immediately (Exceptions for this criterion: Patients who withdraw voluntarily for reasons not directly related to or intrinsic to the study, e.g. incidental considerations such as concerns about travel time to study visits, unexpected pregnancy in the family, etc.). In addition, the study will be halted if one patient experiences a serious related adverse effect (grade ≥ 4 AE).

All safety data will be reported to the PI/Sponsor, DSMB, IRB, and FDA every six months, or, in the case of any major safety concern or question, immediately. If any study stopping condition occurs, this will be reported immediately and the study halted, pending review by DSMB, IRB, and FDA, and until the decision by regulatory authorities to resume, suspend or close the study has been made.

*Operationally, "halting" will ordinarily mean that no further screening of new subjects and no treatment initiation will occur until the safety issue has been investigated and resolved (i.e., a final decision has been made to resume, suspend or close the study has been made). Enrolled study participants who have no new symptoms or adverse effects will ordinarily be allowed to continue study observation or treatment without interruption while the safety issue is being investigated, unless it is the contemporaneous judgment of the PI or

subsequent judgment of the DSMB, IRB, or FDA that it is unsafe to do so, in which case all observation or treatment interventions will be suspended forthwith.

8.6 Safety Oversight

All safety data will be reported to sponsor, DSMB, IRB, and FDA every six months, or, in the case of any major safety concern or question, immediately. The Principal Investigator will assume primary responsibility for all safety oversight.

9 CLINICAL MONITORING

Subjects will be monitored for safety at baseline pre-infusion, 1 hour after infusion begins, 1 hour after infusion is discontinued, and then during follow-up visits 1-day and 1-, 2-, 4- and 8-weeks post-infusion. Safety monitoring at each time point will include physical examination, vital sign monitoring, laboratory assessments, electrocardiography, and AE/tolerability monitoring. Our preliminary phenotyping studies characterizing cognitive and adaptive functioning in Phelan McDermid syndrome suggest that these children and adults have intellectual disability. This patient population is significantly delayed in receptive language, expressive language, and overall functional communication skills. As such, it is impossible to directly query these subjects using verbal methods with respect to safety and tolerability. Instead, we rely on parent report using open-ended methods, in addition to physical exams and laboratory measures.

Clinical monitoring for efficacy will occur at baseline, 1-hour post-infusion, 1-day post-infusion, and 1-, 2-, 4-, and 8-weeks post-infusion.

10 STATISTICAL CONSIDERATIONS

10.1 Statistical and Analytical Plans

All details of the statistical analyses will be described in a separate study specific 'Statistical Analysis Plan' (SAP), which will be finalized before the clinical data base is declared clean and locked. Baseline for all comparisons will be the measurements obtained as close as possible before administration of the study drug.

10.2 Statistical Hypotheses

We hypothesize that the medication will be safe and tolerable as determined by the absence of SAEs and propose that at least 70% of participants enrolled will be able to complete the infusion.

We hypothesize that, when relevant, seizures will be reduced in frequency by at least 25% compared with 'baseline.' More explicitly, the null (H0) and alternative (Ha) hypothesis will be defined as

H0: The average number of weekly seizures at follow-up (week 4) are reduced by less than 25% compared to baseline

Ha: The average number of weekly seizures at follow-up (week 4) are reduced by at least 25% compared to baseline

We hypothesize that EEG abnormalities will be reduced in frequency by at least 25% compared with 'baseline.' More explicitly, the null (H0) and alternative (Ha) hypothesis will be defined as

H0: The average number of EEG abnormalities at follow-up (week 2) are reduced by less than 25% compared to baseline

Ha: The average number of EEG abnormalities at follow-up (week 2) are reduced by at least 25% compared to baseline

10.3 Analysis Datasets

The statistical analyses will be performed only when the data have been declared clean and the study database has been locked.

10.4 Description of Statistical Methods

All data on a continuous scale will be presented using box plots with individual data points overlaid. To examine treatment effects and to test the hypothesis of no differences in the mean between baseline, week 1 and week 4 the Wilcoxon signed-rank test will be calculated for the differences in data distributions (change over time) from baseline (day 0) to week 1 and to week 4 (follow-up 4). The Wilcoxon signed-rank test is a non-parametric test and does not require the data to follow a particular distribution; it is robust to single gross outliers and has higher power than a t-test, unless the data follow exactly a Gaussian distribution, and then the power is very close.

For test of the hypothesis of at least 25% reduction of seizures, the Wilcoxon signed rank test and associated point estimate and 2-sided 95% confidence intervals will be calculated on the log transformed data. We will conclude a 25% reduction of seizures if the upper confidence limit is lower or equal than 0.75 on the original scale.

All tests of statistical hypotheses will be done on the two-sided 5% level of significance and presented together with the associated point estimate and two-sided 95% confidence interval. Since this is an initial proof-of-concept study that is exploratory in nature, no multiplicity-related adjustments will be made in the reported p-values.

The SAS software, proc npar1way or similar R software package will be used to perform the statistical analyses.

10.4.1 General Approach

The safety and efficacy data will primarily be analyzed using summary statistics (number of values, number of missing values, mean, median, standard deviation, 1st and 3rd quartiles and minimum and maximum values) and graphs. Since the study participants are expected to have widely differing degrees of baseline signs and symptoms, the evaluation of treatment effects will include an adjustment for the baseline level by subtracting the baseline data from measurements post baseline.

10.4.2 Analysis of the Primary Endpoint(s)

Adverse events (AEs) will be tabulated. Changes in rates of particular groups of AEs from baseline to follow-up may be formally examined calculating the McNemar test.

10.4.3 Analysis of the Secondary Endpoint(s)

Seizure frequency will be analyzed on a relative scale, examining the hypothesized percental decrease in number of seizures from baseline to week 4. For this purpose, the log-transformed data will be analyzed and the resulting point estimates and 95% confidence limits from the Wilcoxon signed-rank test will be converted back to the original scale.

10.4.4 Safety Analyses

See Analysis of Primary Endpoint (Section 10.4.2)

10.4.5 Adherence and Retention Analyses

We do not foresee any major problem with missing values. Study drop-out will be examined by interviews of the study participants (see section “Participant Withdrawal or termination”) and the reasons for drop-out will be

tabulated. If drop-outs occur due to tolerability or perceived side effects this information will be integrated into the safety analysis.

10.4.6 Baseline Descriptive Statistics

Demographic data and baseline characteristics will be presented using descriptive statistics (e.g. Number of observations, Number of missing values, Mean, Median, 1st and 3rd quartiles, Minimum and maximum values, Standard deviation).

10.4.7 Planned Interim Analyses

No interim analysis is planned. An independent DSMB will review safety data on an ongoing basis.

10.4.9 Multiple Comparison/Multiplicity

No adjustments for multiplicity of statistical tests are planned.

10.4.10 Tabulation of Individual Response Data

All individual data will be tabulated in listings and will be described in detail in the SAP.

10.5 Sample Size

The sample size was not based on any statistical criteria but what was pragmatic and achievable, given that PMS is a rare disease. See section “Strategies for Recruitment and Retention”.

10.6 Measures to Minimize Bias

Subjects will be recruited on the basis of the genetic diagnosis of PMS and epilepsy. There will otherwise be no selection bias and we anticipate a random sampling of participants within the age range and within the pre-defined severity level of seizures.

10.6.1 Enrollment/ Randomization/ Masking Procedures

Not Applicable

10.6.2 Evaluation of Success of Blinding

Not Applicable

10.6.3 Breaking the Study Blind/Participant Code

Not Applicable

11 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Source documents will be hard copies of the case report forms (CRFs) that will be entered into an electronic database. Source documents will be stored securely in accordance with GCP and the electronic database is password protected on a secure server. All data from source documents will be double entered to ensure fidelity within the electronic database. Access to source documents and the electronic database will be limited to authorized individuals (according to 21 CFR 11.10(d)). Each user of the electronic database will have an individual account. The user will log into that account at the beginning of a data entry session, input information (including changes) on the electronic record, and log out at the completion of data entry session. Authorized individuals will work only under their own password and will not share the password with others and will not login on behalf of others. When someone leaves a workstation, the authorized individual will log off and automatic log off will be triggered after idle periods in addition to an automatic screen saver to prevent data entry until a password is entered.

12 QUALITY ASSURANCE AND QUALITY CONTROL

Data will be collected on CRFs or parent self-report surveys and uploaded in double-entered fashion to a secure electronic database. Hard copy records will be stored at a secure location in locked cabinets within locked offices. Electrophysiological and eye tracking data will be automatically recorded in electronic format and subsequently entered into the database. Backup and recovery procedures will protect against data loss and records will be regularly backed up to prevent loss and ensure the quality and integrity of the data. Backup and recovery logs will be maintained to facilitate an assessment of the nature and scope of data loss in case of a system failure.

13 ETHICS/PROTECTION OF HUMAN SUBJECTS

13.1 Ethical Standard

The study will be conducted according to Good Clinical Practice (GCP), the 1996 Declaration of Helsinki (Protocol Appendix A), (US 21 CFR Part 50—Protection of Human Subjects, and Park 56—Institutional Review Boards) and local rules and regulations of the country.

13.2 Institutional Review Board

The study will be reviewed and approved by the appropriate Program for the Protection of Human Subjects, the Investigational Research Pharmacy, and by the General Clinical Research Center as indicated.

13.3 Informed Consent Process

13.3.1 Consent/assent and Other Informational Documents Provided to Participants

The investigator will describe the protocol to potential subjects' parents/guardians in person, although general information and assessment for eligibility can be carried out by phone if necessary. Each subject's signed informed consent form will be kept on file by the investigator for possible inspection by regulatory authorities. The parents/guardians will receive a copy of the signed and dated written informed consent form and any other written information provided to the subjects' parents/guardians, and will receive copies of any signed and dated consent form updates. Any amendments to the written information will be provided to subjects' parents/guardians.

13.3.2 Consent Procedures and Documentation

The Informed Consent may be read to the subjects' parent/guardians, but, in any event, the investigator or designee shall give the subjects' parents/guardians ample opportunity to inquire about details of the study and ask any questions before dating and signing the Informed Consent Form. The Informed Consent will be created with a level of language fully comprehensible to the prospective subjects' parents/guardians. Informed consent will be documented by the use of a written consent form approved by the IRB and signed and dated by the subjects' parents/guardians and by the person who conducted the informed consent discussion. The signature confirms the consent is based on information that has been understood.

13.4 Participant and Data Confidentiality

13.4.1 Research Use of Stored Human Samples, Specimens or Data

A subject identification code will be used in lieu of the subject's name on all study data compiled and delivered to the designated data collection center. All source documents and study data will be kept confidential, in accordance with all requirements of the laws.

13.5 Future Use of Stored Specimens

Not Applicable

14 DATA HANDLING AND RECORD KEEPING

14.1 Data Collection and Management Responsibilities

Upon signing informed consent, all subjects will be assigned a unique identification and only the principal investigator and authorized personnel will have access to a password protected linking document stored on a secure server. Data will be collected on case report forms (CRFs) with assigned IDs and entered into a de-identified database on a secure server. Informed consent and clinical documents will be stored separately. All non-electronic data will be stored in locked file cabinets contained within locked offices. All electronic data will be stored on a password protected secured server. The investigator is responsible for all aspects of the conduct of the study at their respective site. These would include: the dispensing and the administration of the investigational product, the implementation of the study protocol, the collection and reporting of the study data and the protection of the health and welfare of the personnel involved in the study during the study.

14.2 Study Records Retention

Study records will be retained for a period of at least 2 years following the date a marketing application is approved for AMO-01 in PMS. If no application is filed or if the application is not approved for such indication, records will be retained until 2 years after the investigation is discontinued and FDA is notified.

14.3 Protocol Deviations

Protocol deviations will be reported to the sponsor and to the IRB and documented in a note to file in the patients case report form.

14.4 Publication and Data Sharing Policy

The investigators are committed to collaborate and share data freely with other investigators. Our institution sponsors well established resources for the identification, evaluation and distribution of research tools developed with federal grant funding. The research team is committed to an open model of scientific sharing. The investigators will comply with the standard Resource Sharing Plans. We routinely disseminate our scientific discoveries through the publication of peer-reviewed manuscripts in journals, at scientific conferences, and through the internet (e.g. http://www.shank3gene.org/; https://www.rarediseasesnetwork.org/cms/DSC; http://22q13.org/j15/). Any advances that result from the proposed studies may be disseminated to the broadest community through patent protection and commercialization. We will continue to utilize these approaches to have a broad impact upon the research community and the patients who directly benefit from this treatment development.

15 STUDY ADMINISTRATION

15.1 Study Leadership

Alexander Kolevzon, MD will be the study principal investigator. Dr. Kolevzon is a board certified child and adolescent psychiatrist and Professor of Psychiatry and Pediatrics at Icahn School of Medicine at Mount Sinai. Dr. Kolevzon has served as the Clinical Director of the Seaver Autism Center since 2007. His clinical expertise revolves around autism and related neurodevelopmental disorders and his research is focused on developing new pharmacological interventions. Most recently, his group has focused on studying monogenic forms of neurodevelopmental disorders, including PMS, in order to better understand the phenotype and to explore possible targets for pharmacological intervention. He has participated in many multi-centered clinical trials and is currently the principal investigator on the PMS project for the Rare Disease Clinical Research Network entitled Developmental Synaptopathies Associated with TSC, PTEN and SHANK3 Mutations.

Jimmy Holder, MD, PhD, will be the site principal investigator at Texas Children's Hospital. Dr. Holder is an Assistant Professor of Pediatrics, Division of Neurology at Baylor College of Medicine and Texas Children's

Hospital. He is a board-certified neurologist who serves as the director of the Synaptopathy clinic at the Blue Bird Circle Clinic for Pediatric Neurology. He has extensive experience treating children with Phelan-McDermid Syndrome with a focus on epilepsy and abnormalities identified on electroencephalogram. He will evaluate EEGs pre and post treatment for changes following administration of the investigational drug.

16 CONFLICT OF INTEREST POLICY

The Conflicts of Interest Office (COI) at Icahn School of Medicine was established in 2009 and is located in the Office of the Dean. The COI Office helps to oversee Icahn School of Medicine at Mount Sinai's efforts to identify and manage potential conflicts of interest. The school encourages collaborative relationships with industry that could lead to breakthroughs in research and clinical care, and it is essential that these relationships are free of real or perceived conflicts. The COI Office educates faculty, staff and trainees about Icahn School of Medicine at Mount Sinai's COI policies, both institutional and research-specific, and provides guidance in the disclosure of financial interests. We also are responsible for institutional compliance with regulatory requirements relating to conflicts of interest. All of these components are essential to creating an environment of transparency for our relationships with industry. All investigators are required to complete an Annual Reporting of Outside Relationships Form to disclose all financial interests that investigators have outside of their employment at Mount Sinai. In addition, a Financial Interests in Research Disclosure Form is completed with each grant submission and annual continuation to determine whether any of the financial conflicts of interest pose actual or perceived conflicts with a proposed research project.

TCH and Baylor College of Medicine (BCM) require all Investigators to report Significant Financial Interests (SFIs) that reasonably appear to be related to the investigator's Institutional Responsibilities. BCM must review the matter and determine if an Investigator's Significant Financial Interest is related to the research, and if the research-related Significant Financial Interest is a Financial Conflict of Interest that should be managed, reduced or eliminated. Special precautions must be taken to avoid perceived or actual bias with respect to research involving human subjects that encompasses the evaluation of strategies or products that may affect or be affected by the financial interests of BCM or BCM Investigators. A BCM Investigator shall not ordinarily participate in any Research involving human subjects that encompasses evaluation of such a strategy or product if he/she has a Significant Financial Interest that could directly affect the design, conduct or reporting of the Research unless he/she presents a compelling justification for a waiver of this policy based on his/her unique qualifications as an Investigator. The degree of risk to human subjects and the compelling justification will be reviewed by the Research Conflict of Interest Committee (RCOIC). If compelling circumstances are found, the Research will be subject to stringent management measures to ensure the safety of the human participants and the integrity of the Research. The IRB must review and approve any management plan for human subject Research. The IRB may require additional safeguards to protect human subject participants in addition to those required by the RCOIC.

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APPENDIX A: Clinician-Completed PMS Domain-Specific Causes for Concern VAS

Date _____ **ID** _____ **Evaluator Signature**_____

Phelan-McDermid Syndrome Specific Concerns – Clinician

Please rate the areas of concern that are priority targets for change during treatment in the categories below. Your choices should be associated with the patient's Phelan-McDermid Syndrome. You can select any sign or symptom fitting the category.

1. Speech and language difficulties

The sign or symptom causing concern is:

_____

How severe has the sign or symptom been in the past week? Place a vertical mark on the line below to indicate how bad you think the sign or symptom has been in the last week.

Completely Absent Very Severe

2. Problems with thinking and learning

The sign or symptom causing concern is:

_____

How severe has the sign or symptom been in the past week? Place a vertical mark on the line below to indicate how bad you think the sign or symptom has been in the last week.

Completely Absent Very Severe

3. Seizures

The sign or symptom causing concern is:

_____

How severe has the sign or symptom been in the past week? Place a vertical mark on the line below to indicate how bad you think the sign or symptom has been in the last week.

Completely Absent Very Severe

Date _____ **ID** _____ **Evaluator Signature**_____

Phelan-McDermid Syndrome Specific Concerns – Clinician

Please rate the areas of concern that are priority targets for change during treatment in the categories below. Your choices should be associated with the patient's Phelan-McDermid Syndrome. You can select any sign or symptom fitting the category.

4. Problems with gross motor functioning

The sign or symptom causing concern is:

_____

How severe has the sign or symptom been in the past week? Place a vertical mark on the line below to indicate how bad you think the sign or symptom has been in the last week.

Completely Absent Very Severe

5. Restricted interests and/or repetitive behaviors

The sign or symptom causing concern is:

_____

How severe has the sign or symptom been in the past week? Place a vertical mark on the line below to indicate how bad you think the sign or symptom has been in the last week.

Completely Absent Very Severe

6. Social communication problems

The sign or symptom causing concern is:

_____

How severe has the sign or symptom been in the past week? Place a vertical mark on the line below to indicate how bad you think the sign or symptom has been in the last week.

Completely Absent Very Severe

Date _____ **ID** _____ **Evaluator Signature**_____

Phelan-McDermid Syndrome Specific Concerns – Clinician

Please rate the areas of concern that are priority targets for change during treatment in the categories below. Your choices should be associated with the patient's Phelan-McDermid Syndrome. You can select any sign or symptom fitting the category.

7. Sensory sensitivities

The sign or symptom causing concern is:

_____

How severe has the sign or symptom been in the past week? Place a vertical mark on the line below to indicate how bad you think the sign or symptom has been in the last week.

Completely Absent Very Severe

8. Activities of daily living

The sign or symptom causing concern is:

_____

How severe has the sign or symptom been in the past week? Place a vertical mark on the line below to indicate how bad you think the sign or symptom has been in the last week.

Completely Absent Very Severe

9. Sleep disturbance

The sign or symptom causing concern is:

_____

How severe has the sign or symptom been in the past week? Place a vertical mark on the line below to indicate how bad you think the sign or symptom has been in the last week.

Completely Absent Very Severe

APPENDIX B: Caregiver Completed Seizure Diary

<u>INSTRUCTIONS:</u> Enter the Month and Year on the line where indicated. The days of the month are already entered on each line of the log. Please enter seizure information in your diary each day. For each day that a seizure occurred enter in the code and number of seizures that occurred as demonstrated in part A. If a new type of seizure occurs, describe it. If a code has been specified for a type of seizure, please make sure to keep the same code for the same type of seizure at the following visits. Use a new code for a new type of seizure. If no seizures occurred, please check the box where indicated. Please leave the last two columns (seizure code and number of seizures of each type) blank.

A.

Example: <u>Seizure Description</u>	<u>Sample Code Defined by Study Doctor</u>
Left Arm Jerking	A
I pass out; my whole body starts to jerk	B

Enter Description of your Seizure	**Codes to be defined by your Study Doctor**
•	Code ____
•	Code ____
•	Code ____
•	Code ____
•	Code ____
•	Code ____
•	Code ____
•	Code ____
•	Code ____
•	Code ____

B.

To be completed by parent			To be completed by study staff	
Date: ___ ___ ___ (DD MM YY)	Please check the box below if **NO seizure** occurred on this day	**Patient Description of Seizures** • If new seizure type, enter description • **Example: If you had 2 code A seizures you would enter A:2**	**Seizure Code**	**Number of Seizures of Each Type**
___ ___ ___	☐	______ ______	___ ___ ___ ___ ___ ___	___ ___ ___ ___ ___ ___
___ ___ ___	☐	______ ______	___ ___ ___ ___ ___ ___	___ ___ ___ ___ ___ ___
___ ___ ___	☐	______ ______	___ ___ ___ ___ ___ ___	___ ___ ___ ___ ___ ___
___ ___ ___	☐	______ ______	___ ___ ___ ___ ___ ___	___ ___ ___ ___ ___ ___
___ ___ ___	☐	______ ______	___ ___ ___ ___ ___ ___	___ ___ ___ ___ ___ ___
___ ___ ___	☐	______ ______	___ ___ ___ ___ ___ ___	___ ___ ___ ___ ___ ___
___ ___ ___	☐	______ ______	___ ___ ___ ___ ___ ___	___ ___ ___ ___ ___ ___
___ ___ ___	☐	______ ______	___ ___ ___ ___ ___ ___	___ ___ ___ ___ ___ ___
___ ___ ___	☐	______ ______	___ ___ ___ ___ ___ ___	___ ___ ___ ___ ___ ___
___ ___ ___	☐	______ ______	___ ___ ___ ___ ___ ___	___ ___ ___ ___ ___ ___
___ ___ ___	☐	______ ______	___ ___ ___ ___ ___ ___	___ ___ ___ ___ ___ ___
___ ___ ___	☐	______ ______	___ ___ ___ ___ ___ ___	___ ___ ___ ___ ___ ___
___ ___ ___	☐	______ ______	___ ___ ___ ___ ___ ___	___ ___ ___ ___ ___ ___
___ ___ ___	☐	______ ______	___ ___ ___ ___ ___ ___	___ ___ ___ ___ ___ ___
___ ___ ___	☐	______ ______	___ ___ ___ ___ ___ ___	___ ___ ___ ___ ___ ___
___ ___ ___	☐	______ ______	___ ___ ___ ___ ___ ___	___ ___ ___ ___ ___ ___

To be completed by parent			To be completed by study staff	
Date: ___ ___ ___ (DD MM YY)	Please check the box below if **NO seizure** occurred on this day	**Patient Description of Seizures** • If new seizure type, enter description • **Example: If you had 2 code A seizures you would enter A:2**	**Seizure Code**	**Number of Seizures of Each Type**
___ ___ ___	☐		___ ___ ___ ___ ___ ___	___ ___ ___ ___ ___ ___
___ ___ ___	☐		___ ___ ___ ___ ___ ___	___ ___ ___ ___ ___ ___
___ ___ ___	☐		___ ___ ___ ___ ___ ___	___ ___ ___ ___ ___ ___
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APPENDIX C: Syndrome Specific Clinical Global Impressions- Severity and Improvement

CGI-S and CGI-I

The Clinical Global Impression – Severity of Illness (CGI-S) and Clinical Global Impression – Improvement of Illness (CGI-I) are brief, easy to administer, clinician-rated measures (Guy, 1976). The CGI-S asks the clinician to rate the patient's current severity of illness based on the clinician's total clinical experience with the relevant population. The CGI-S is rated on a 7-point scale, with a range of responses from 1 (normal, not at all ill) through 7 (amongst the most extremely ill patients). The CGI-I asks the clinician to rate the patients' total improvement since baseline, whether or not the improvement is judged to be due entirely to the experimental treatment. The CGI-I is rated on a 7-point scale, with a range of responses from 1 (very much improved) to 7 (very much worse). For both the CGI-S and the CGI-I, the clinician is allowed to use all available information at the time of the rating.

The CGI-I has emerged as a convention for bifurcating clinical trial subjects into "responders" and "non-responders". In this application, the CGI-I can be a useful tool for gaining a general overview of the therapeutic potential of an experimental treatment. One of the relative detriments of both the CGI-S and –I is the lack of the measures' assessment of specific sign or symptoms associated with the disorder under study (Busner et al., 2009). An approach that has been developed to enhance the precision and clinical meaningfulness of CGI ratings is to utilize anchor points that are specific to the signs and symptoms of the disorder under study.

References:

Busner J, Targum SD, and Miller DS (2009), The Clinical Global Impressions scale: errors in understanding and use. Comprehensive Psychiatry 50:257-262

Guy W (1976). Clinical global impressions. In: Guy W, editor. ECDEU assessment manual for psychopharmacology (Revised). Rockville, Maryland, National Institute of Mental Health: 217-221.

Clinical Global Impression – Severity of Illness Scale (CGI-S):

"Considering your total clinical experience with PMS patients, how severely ill is this patient at this time?"

1- *Normal, not at all ill*: The patient is indistinguishable from other individuals that do not have PMS. The patient has no overt PMS symptoms and no persistent dysfunction in the wake of having been diagnosed with PMS.

2- *Borderline ill*: The patient has very occasional PMS symptoms that seem modestly excessive in intensity, frequency or duration compared to individuals that have not been diagnosed with PMS. These symptoms have only a transient impact on functioning, with no need for any special intervention.

3- *Mildly ill*: The patient has occasional PMS symptoms that seem modestly excessive in intensity or duration, or the patient experiences very occasional PMS symptoms that are modestly excessive both in intensity and duration as compared to individuals without a PMS diagnosis. These symptoms have a limited impact on the patient's functioning, generally only in one setting, and require that others make some adjustments or accommodations in interacting with this patient.

4- *Moderately ill*: This PMS patient is clearly distinguishable from other individuals because of his/her PMS symptoms and the impairment that they cause. The patient's PMS symptoms are clearly excessive in frequency, intensity or duration compared to others that have not received a PMS diagnosis, and have limited impact on the patient's functioning in multiple settings or moderate impact in one setting. Caregivers, family members, teachers and co-workers make adjustments when interacting with this patient to avoid exacerbation of his/her symptoms and to deal with them when they occur.

5- *Markedly ill*: This patient's PMS symptoms occur frequently and are noticeable in intensity or duration to even casual observers or occur infrequently but are quite intense or long-lasting. There is moderate impact on the patient's functioning in multiple settings or extreme impact in one setting. Caregivers, family members, teachers and co-workers utilize interventions that are necessary in order to deal with this patient's symptoms. Special accommodations related specifically to the patient's PMS symptoms are likely necessary at home, at school or in the workplace.

6- *Severely ill*: The patient's PMS symptoms occur very frequently and are noticeable in intensity or duration to even casual observers or occur infrequently but are severely intense or extremely long-lasting. Often there is marked impairment of normal day–to–day capabilities or skills necessary to function at school or in the workplace. Multiple interventions are required to address the patient's PMS symptoms to minimize consequences.

7- *Among the most extremely ill patients*: The patient's PMS symptoms occur the majority of the time and are very disruptive to functioning in multiple areas. There are very few times, if any, of normal functioning. There are often serious concerns about the patient's ability to provide adequate care for him/herself as a consequence of the PMS symptoms. The patient requires almost constant monitoring by caregivers or others.

Subject ID: Study ID:

Clinical Global Impression- Severity of Illness Scale (CGI-S)

Date of Assessment ☐☐ ☐☐☐ ☐☐☐☐

Considering your total clinical experience with Phelan McDermid Syndrome patients, how severely ill is this patient at this time?

1- Normal, not at all ill
2- Borderline ill
3- Mildly ill
4- Moderately ill
5- Markedly ill
6- Severely ill
7- Among the most extremely ill patients

Score ☐

Evaluator Name _____

Evaluator signature _____

Date ☐☐ ☐☐☐ ☐☐☐☐

CGI—Improvement – A User's Guide

1. *Very Much Improved* designates marked improvement, across settings and/or across multiple problem areas. Although a CGI-I of 1 does not strictly require that the patient qualify for a CGI-S rating better than baseline, usually the CGI-S does also improve. Such improvement must be very substantial and is usually accompanied by considerable patient and/or caregiver enthusiasm. Such patients are usually noticeably improved in the clinic as well.

2. *Much Improved* may denote moderate improvement in a single symptom area, especially if seen across settings. Likewise, moderate improvements in several areas, even if confined to one setting, may warrant a rating of "Much improved." Durability of the change should be taken into account. For example, a change reported for the last few hours probably would not warrant such a rating. On the other hand, a change that was clearly in evidence for the last several days or longer probably would warrant a rating of 2. It is not necessary that the patient qualify for a CGI-S rating better than baseline to receive a CGI—I rating of 2, but often (not always) the CGI-S also improves.

3. *Minimal Improvement* indicates modest improvements, especially if confined to one setting. Trivial changes or changes that are possibly present or require guesswork usually would be scored as 4 (the level below this one).

4. *No Change* indicates, by definition, the absence of change in behavior or clinical presentation from baseline to subsequent assessments. Chance fluctuations and equivocal improvements or declines should be included here.

5. *Minimally Worse* indicates some worsening in symptoms that are mild to moderate or may be confined to one setting.

6. *Much Worse* designates moderate to moderately serve worsening. This may include moderate levels of worsening in a single symptom area when observed across settings. Moderately severe changes that are confined to one setting may warrant a rating of "Much Worse."

7. *Very Much Worse* designates significant worsening, across settings and/or across multiple symptoms.

N.B. The CGI-I is a rating of *change*; normalization is not necessary for a rating of 1, although if the clinical presentation is unequivocally improved and is evident across settings, it suggests an Improvement score of 1. A CGI-I of 2 is appropriate for definite, unequivocal improvement of a magnitude that makes the clinician confident that the treatment is helping. An improvement score of 3 (or 5) is appropriate if variations in ratings and other criteria appear to represent more than random chance or rating error, but are not definite and unequivocal. A score of 4 is appropriate for slight variation in either direction of a magnitude that is likely due to chance, natural history, external events, or rating error.

Subject ID: Study ID:

Clinical Global Impression- Improvement Scale (CGI-I)

Date of Assessment ☐☐ ☐☐☐ ☐☐☐☐

Compared to the baseline assessment in this study, if you consider the signs and symptoms associated with this patient's Phelan McDermid Syndrome, how much has he/she changed? Rate his/her total improvement whether or not, in your judgement, it is due entirely to the study drug.

1- Very much improved
2- Much improved
3- Minimally improved
4- No change
5- Minimally worse
6- Much worse
7- Very much worse

Score ☐

Evaluator Name _____

Evaluator signature _____

Date ☐☐ ☐☐☐ ☐☐☐☐

APPENDIX D: Top 3 Caregiver Concerns VAS

	Site no. | | | |	
	Subject no. | | | |	

Caregiver Top Three Concerns - Baseline

Date of assessment _____
dd mmm yyyy

Please rate the three causes for concern associated with your child's Phelan McDermid Syndrome that you would most like to see change during treatment. You can select any sign or symptom.

Your Concerns

Concern number 1
The sign or symptom causing concern is:

How severe is the sign or symptom? Place a vertical mark on the line below to indicate how bad you think the sign or symptom has been in the past week, including today.

Not at All Severe — Very Severe

Concern number 2
The sign or symptom causing concern is:

How severe is the sign or symptom? Place a vertical mark on the line below to indicate how bad you think the sign or symptom has been in the past week, including today.

Not at All Severe — Very Severe

Concern number 3
The sign or symptom causing concern is:

How severe is the sign or symptom? Place a vertical mark on the line below to indicate how bad you think the sign or symptom has been in the past week, including today.

Not at All Severe — Very Severe

	Site no. | | | | Subject no. | | | |	

Caregiver Top Three Concerns - Follow-Up

Date of assessment _____
dd mmm yyyy

At baseline, you rated the three causes for concern, related to your child's Phelan McDermid Syndrome that you most liked to see change during treatment. You selected the following signs or symptoms.

Your Concerns

Concern number 1
The sign or symptom causing concern was:

How severe is the sign or symptom? Place a vertical mark on the line below to indicate how bad you think the sign or symptom has been over the past week, including today.

Not at All Severe — Very Severe

Concern number 2
The sign or symptom causing concern was:

How severe is the sign or symptom? Place a vertical mark on the line below to indicate how bad you think the sign or symptom has been over the past week, including today.

Not at All Severe — Very Severe

Concern number 3
The sign or symptom causing concern was:

How severe is the sign or symptom? Place a vertical mark on the line below to indicate how bad you think the sign or symptom has been over the past week, including today.

Not at All Severe — Very Severe

ABERRANT BEHAVIOR CHECKLIST – COMMUNITY

Clients Name: _____ Rater's Name: _____

Clients Gender (circle): Male/Female

Relationship to Client (check):
- ❑ Parent
- ❑ Teacher
- ❑ Trainer/Supervisor
- ❑ Other (please specify) _____

Date of Birth ____/____/____ (Month Day Year)

Today's Date ____/____/____ (Month Day Year)

Where Was the Client Observed?
- ❑ Home
- ❑ School
- ❑ Residential Unit
- ❑ Workshop
- ❑ Other (please specify) _____

If in School, Type of Class (check one): ❑ Developmentally Handicapped ❑ Multihandicapped ❑ Severe Behavior Handicap ❑ Other _____

Ethnic Group (check):

❑ Caucasian ❑ Hispanic
❑ African-American ❑ Other (please specify) _____

CLIENT'S MEDICAL STATUS (Please circle)

a. Deafness?	No	Yes	? (Don't Know)
b. Blindness?	No	Yes	?
c. Epilepsy?	No	Yes	?
d. Cerebral Palsy?	No	Yes	?

e. Other _____

CURRENT MEDICATIONS (Please list any medication and dosage schedule)

1. _____
2. _____
3. _____
4. _____
5. _____

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INSTRUCTIONS

The ABC–Community rating scale is designed to be used with clients living in the community. Please note that the term *client* is used throughout to refer to the person being rated. This may be a child of school age, an adolescent, or an adult.

Please rate this client's behavior for the last four weeks. For each item, decide whether the behavior is a problem and circle the appropriate number:

0 = not at all a problem
1 = the behavior is a problem but slight in degree
2 = the problem is moderately serious
3 = the problem is severe in degree

When judging this client's behavior, please keep the following points in mind:

(a) Take relative *frequency* into account for each behavior specified. For example if the client averages more temper outbursts than most other clients you know or most others in his/her class, it is probably moderately serious (2) or severe (3) even if these occur only once or twice a week. Other behaviors, such as noncompliance, would probably have to occur more frequently to merit an extreme rating.

(b) If you have access to this information, consider the experiences of other care providers with this client. If the client has problems with others but not with you, try to take the whole picture into account.

(c) Try to consider whether a given behavior interferes with his/her *development, functioning,* or *relationships.* For example, body rocking or social withdrawal may not disrupt other children or adults, but it almost certainly hinders individual development or functioning.

Do not spend too much time on each item — your first reaction is usually the right one.

1. Excessively active at home, school, work, or elsewhere	0	1	2	3
2. Injures self on purpose	0	1	2	3
3. Listless, sluggish, inactive	0	1	2	3
4. Aggressive to other children or adults (verbally or physically)	0	1	2	3
5. Seeks isolation from others	0	1	2	3
6. Meaningless, recurring body movements	0	1	2	3
7. Boisterous (inappropriately noisy and rough)	0	1	2	3
8. Screams inappropriately	0	1	2	3
9. Talks excessively	0	1	2	3
10. Temper tantrums/outbursts	0	1	2	3
11. Stereotyped behavior; abnormal, repetitive movements	0	1	2	3
12. Preoccupied; stares into space	0	1	2	3
13. Impulsive (acts without thinking)	0	1	2	3
14. Irritable and whiny	0	1	2	3
15. Restless, unable to sit still	0	1	2	3
16. Withdrawn; prefers solitary activities	0	1	2	3
17. Odd, bizarre in behavior	0	1	2	3
18. Disobedient; difficult to control	0	1	2	3
19. Yells at inappropriate times	0	1	2	3
20. Fixed facial expression; lacks emotional responsiveness	0	1	2	3

21. Disturbs others	0	1	2	3
22. Repetitive speech	0	1	2	3
23. Does nothing but sit and watch others	0	1	2	3
24. Uncooperative	0	1	2	3
25. Depressed mood	0	1	2	3
26. Resists any form of physical contact	0	1	2	3
27. Moves or rolls head back and forth repetitively	0	1	2	3
28. Does not pay attention to instructions	0	1	2	3
29. Demands must be met immediately	0	1	2	3
30. Isolates himself/herself from other children or adults	0	1	2	3
31. Disrupts group activities	0	1	2	3
32. Sits or stands in one position for a long time	0	1	2	3
33. Talks to self loudly	0	1	2	3
34. Cries over minor annoyances and hurts	0	1	2	3
35. Repetitive hand, body, or head movements	0	1	2	3
36. Mood changes quickly	0	1	2	3
37. Unresponsive to structured activities (does not react)	0	1	2	3
38. Does not stay in seat (e.g., during lesson or training periods, meals, etc.)	0	1	2	3
39. Will not sit still for any length of time	0	1	2	3
40. Is difficult to reach, contact, or get through to	0	1	2	3
41. Cries and screams inappropriately	0	1	2	3
42. Prefers to be alone	0	1	2	3
43. Does not try to communicate by words or gestures	0	1	2	3
44. Easily distractible	0	1	2	3
45. Waves or shakes the extremities repeatedly	0	1	2	3
46. Repeats a word or phrase over and over	0	1	2	3
47. Stamps feet or bangs objects or slams doors	0	1	2	3
48. Constantly runs or jumps around the room	0	1	2	3
49. Rocks body back and forth repeatedly	0	1	2	3
50. Deliberately hurts himself/herself	0	1	2	3
51. Pays no attention when spoken to	0	1	2	3
52. Does physical violence to self	0	1	2	3
53. Inactive, never moves spontaneously	0	1	2	3
54. Tends to be excessively active	0	1	2	3
55. Responds negatively to affection	0	1	2	3
56. Deliberately ignores directions	0	1	2	3
57. Has temper outbursts or tantrums when he/she does not get own way	0	1	2	3
58. Shows few social reactions to others	0	1	2	3

APPENDIX F: Repetitive Behavior Scale- Revised (RBS-R)

REPETITIVE BEHAVIOR SCALE – Revised (RBS-R)

Name: _____ **ID#**: _____

Gender: female male **Date of Birth**: ___ / ___ / _____ **Today's Date**: ___ / ___ / _____

Informant's Name: _____

Instructions:
Please rate this person's behavior by reading each of the items listed and then choosing the score that best describes how much of a problem the item is for the person. Be sure to read and score all items listed. Make your ratings based on your observations and interactions with the person ***OVER THE LAST MONTH****. Use the definitions in the box given below to score each item.*

0 = behavior does not occur
1 = behavior occurs and is a mild problem
2 = behavior occurs and is a moderate problem
3 = behavior occurs and is a severe problem

When deciding on a score for each item, consider: (a) how frequently the behavior occurs (e.g. weekly versus hourly), (b) how difficult it is to interrupt the behavior (e.g. can be easily redirected versus becomes distressed if interrupted) and (c) how much the behavior interferes with ongoing events (e.g. easy to ignore versus very disruptive).

I. Stereotyped Behavior Subscale

(DEFINITION: apparently purposeless movements or actions that are repeated in a similar manner)

1	WHOLE BODY (Body rocking, Body swaying)	0	1	2	3
2	HEAD (Rolls head, Nods head, Turns head)	0	1	2	3
3	HAND/FINGER (Flaps hands, Wiggles or flicks fingers, Claps hands, Waves or shakes hand or arm)	0	1	2	3
4	LOCOMOTION (Turns in circles, Whirls, Jumps, Bounces)	0	1	2	3
5	OBJECT USAGE (Spins or twirls objects, Twiddles or slaps or throws objects, Lets objects fall out of hands)	0	1	2	3
6	SENSORY (Covers eyes, Looks closely or gazes at hands or objects, Covers ears, Smells or sniffs items, Rubs surfaces)	0	1	2	3

0 = behavior does not occur 1 = behavior occurs and is a mild problem 2 = behavior occurs and is a moderate problem 3 = behavior occurs and is a severe problem

II. Self-Injurious Behavior Subscale

(DEFINITION: movement or actions that have the potential to cause redness, bruising, or other injury to the body, and that are repeated in a similar manner)

7	HITS SELF WITH BODY PART (Hits or slaps head, face, or other body area)	0	1	2	3
8	HITS SELF AGAINST SURFACE OR OBJECT (Hits or bangs head or other body part on table, floor or other surface)	0	1	2	3
9	HITS SELF WITH OBJECT (Hits or bangs head or other body area with objects)	0	1	2	3
10	BITES SELF (Bites hand, wrist, arm, lips or tongue)	0	1	2	3
11	PULLS (Pulls hair or skin)	0	1	2	3
12	RUBS OR SCRATCHES SELF (Rubs or scratches marks on arms, leg, face or torso)	0	1	2	3
13	INSERTS FINGER OR OBJECT (Eye-poking, Ear-poking)	0	1	2	3
14	SKIN PICKING (Picks at skin on face, hands, arms, legs or torso)	0	1	2	3

III. Compulsive Behavior Subscale

(DEFINITION: behavior that is repeated and is performed according to a rule, or involves things being done "just so")

15	ARRANGING / ORDERING (Arranges certain objects in a particular pattern or place; Need for things to be even or symmetrical)	0	1	2	3
16	COMPLETENESS (Must have doors opened or closed; Takes all items out of a container or area)	0	1	2	3
17	WASHING / CLEANING (Excessively cleans certain body parts; Picks at lint or loose threads)	0	1	2	3
18	CHECKING (Repeatedly checks doors, windows, drawers, appliances, clocks, locks, etc.)	0	1	2	3
19	COUNTING (Counts items or objects; Counts to a certain number or in a certain way)	0	1	2	3
20	HOARDING/SAVING (Collects, hoards or hides specific items)	0	1	2	3
21	REPEATING (Need to repeat routine events; In / out door, up / down from chair, clothing on/off)	0	1	2	3
22	TOUCH / TAP (Need to touch, tap, or rub items, surfaces, or people)	0	1	2	3

0 = behavior does not occur 1 = behavior occurs and is a mild problem 2 = behavior occurs and is a moderate problem 3 = behavior occurs and is a severe problem

IV. Ritualistic Behavior Subscale

(DEFINITION: performing activities of daily living in a similar manner)

23	EATING / MEALTIME (Strongly prefers/insists on eating/drinking only certain things; Eats or drinks items in a set order; Insists that meal related items are arranged in a certain way)	0	1	2	3
24	SLEEPING / BEDTIME (Insists on certain pre-bedtime routines; Arranges items in room "just so" prior to bedtime; Insists that certain items be present with him/her during sleep; Insists that another person be present prior to or during sleep)	0	1	2	3
25	SELF-CARE – BATHROOM AND DRESSING (Insists on specific order of activities or tasks related to using the bathroom, to washing, showering, bathing or dressing; Arranges items in a certain way in the bathroom or insists that bathroom items not be moved; Insists on wearing certain clothing items)	0	1	2	3
26	TRAVEL / TRANSPORTATION (Insists on taking certain routes/paths; Must sit in specific location in vehicles; Insists that certain items be present during travel, e.g., toy or material; Insists on seeing or touching certain things or places during travel such as a sign or store)	0	1	2	3
27	PLAY / LEISURE (Insists on certain play activities; Follows a rigid routine during play / leisure; Insists that certain items be present/available during play/leisure; Insists that other persons do certain things during play)	0	1	2	3
28	COMMUNICATION / SOCIAL INTERACTIONS (Repeats same topic(s) during social interactions; Repetitive questioning; Insists on certain topics of conversation; Insists that others say certain things or respond in certain ways during interactions)	0	1	2	3

V. Sameness Behavior Subscale

(DEFINITION: (resistance to change, insisting that things stay the same)

29	Insists that things remain in the same place(s) (e.g. toys, supplies, furniture, pictures, etc.)	0	1	2	3
30	Objects to visiting new places	0	1	2	3
31	Becomes upset if interrupted in what he/she is doing	0	1	2	3
32	Insists on walking in a particular pattern (e.g., straight line)	0	1	2	3
33	Insists on sitting at the same place	0	1	2	3
34	Dislikes changes in appearance or behavior of the people around him/her	0	1	2	3
35	Insists on using a particular door	0	1	2	3
36	Likes the same CD, tape, record or piece of music played continually; Likes same movie / video or part of movie / video	0	1	2	3
37	Resists changing activities; Difficulty with transitions	0	1	2	3
38	Insists on same routine, household, school or work schedule everyday	0	1	2	3
39	Insists that specific things take place at specific times	0	1	2	3

0 = behavior does not occur 1 = behavior occurs and is a mild problem 2 = behavior occurs and is a moderate problem 3 = behavior occurs and is a severe problem

VI. Restricted Behavior Subscale

(DEFINITION: Limited range of focus, interest or activity)

40	Fascination, preoccupation with one subject or activity (e.g., trains, computers, weather, dinosaurs)	0	1	2	3
41	Strongly attached to one specific object	0	1	2	3
42	Preoccupation with part(s) of object rather than the whole object (e.g., buttons on clothes, wheels on toy cars)	0	1	2	3
43	Fascination, preoccupation with movement / things that move (e.g., fans, clocks)	0	1	2	3

Scoring Summary:

1. Number of subscale items endorsed: number of items in a subscale rated 1, 2, or 3
2. Total subscale score: sum of the ratings for all of the items in a subscale
3. Overall number of items endorsed: sum of the "Number of subscale items endorsed"
4. Overall score: sum of the "Total subscale scores"

Subscale	Number of subscale items endorsed	Total subscale score
I. Stereotyped Behavior		
II. Self-injurious Behavior		
III. Compulsive Behavior		
IV. Ritualistic Behavior		
V. Sameness Behavior		
VI. Restricted Behavior		

Overall number of items endorsed	Overall Score

References for RBS-R:
Bodfish, J.W., Symons, F.J., Parker, D.E., & Lewis, M.H. (2000). Varieties of repetitive behavior in autism. Journal of Autism and Developmental Disabilities, 30, 237-243.
Bodfish, J.W., Symons, F.J., Lewis, M.H. (1999). The Repetitive Behavior Scale. Western Carolina Center Research Reports.