A Pilot Treatment Study of Insulin-Like Growth Factor-1 (IGF-1) in Autism Spectrum Disorder
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Electrophysiological Markers for Interventions in Phelan-McDermid Syndrome and Idiopathic Autism

Protocol Identifying Number: Electrophysiological markers in PMS and iASD

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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.

Effective Date: 10/1/2021
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Introductory Statement and Investigational Plan

The genomic revolution in autism spectrum disorder (ASD) has positioned the field to study personalized approaches to treatment. Recent work suggests that up to 2% of individuals with ASD and intellectual disability (ID) have deletions or point mutations in the *SHANK3* gene, resulting in Phelan-McDermid syndrome (PMS), and approximately 65% of people with PMS meet criteria for ASD. *SHANK3* codes for a master scaffolding protein that forms a key framework in the postsynaptic density of glutamatergic (excitatory) synapses and plays a critical role in synaptic function. *SHANK3* and glutamate pathways are implicated in multiple forms of ASD and this convergence implies shared biochemical pathways with potentially overlapping therapeutic targets. Thus, targeting *SHANK3* deficiency in PMS as a specific genetic cause of ASD allows us to inform treatment in broader idiopathic ASD (iASD). Our ongoing studies provide in-depth phenotyping and natural history of PMS, pointing toward a specific clinical and electrophysiological (EEG) profile. However, the potential of EEG biomarkers as a measure of treatment response remains to be determined.

In PMS, our preliminary data using visual evoked potentials (VEPs) to examine cortical excitatory postsynaptic potentials demonstrate promising links to disease mechanisms. Biomarkers and novel clinical measures for evaluating response to intervention have been piloted successfully at our site in cohorts of patients with PMS and iASD. We have identified a unique VEP profile of excitatory deficits (markedly reduced P_{60}-N_{75} transient VEP amplitude) in PMS that is also present in a subset of children with iASD in order to stratify individuals with iASD and select those we predict will show response to growth hormone. Importantly, our pilot work showed clinical benefit of both insulin-like growth factor-1 (IGF-1) (Kolevzon et al. 2014) and growth hormone (which triggers release of IGF-1) in PMS, and significant improvement in gamma band activity on our primary VEP measure post-treatment with IGF-1. VEP biomarkers may therefore be harnessed as an indicator of brain engagement during intervention.

The overall objective of this proposal is to use clinical and electrophysiological features of PMS to characterize subtypes of iASD and inform treatment development. We will enroll children age 2-12 years, specifically recruiting intellectually disabled and minimally verbal children given the prominence of this profile in PMS and the critical need to address this group in broader ASD research. Our short-term goal is to show that select electrophysiological markers in PMS are relevant to iASD and predictive of treatment response. Our long-term goal is to optimize treatment selection in iASD by establishing biological signature(s) derived from PMS that are: a) useful for predicting treatment responders, and b) responsive to intervention

Aim 1: Validate the ASD phenotype within PMS to select clinical targets for intervention. This Aim has been completed. Measures were selected based on: a) our preliminary data showing a unique profile of sensory reactivity in PMS, and b) their focus on core symptoms of ASD. We validated our phenotypic profile of PMS, showed that it overlaps with a subset of patients with iASD, and established clinical targets and appropriate outcome measures for both groups.

Aim 2: Establish electrophysiological markers of PMS and iASD using VEPs. This aim has also been completed. We established the stability of VEP amplitudes across two time points, in order to validate this tool as a reliable stratification biomarker capturing excitatory activity. We also showed that individual variability detected by electrophysiological measures was associated with clinical symptoms of PMS and iASD measured in Aim 1, including sensory reactivity. Our data suggest a unique VEP profile of excitatory deficits in PMS that overlaps with a subset of children with iASD. Neural responses show strong test-retest reliability, associations with clinical metrics in PMS and iASD, and in Aim 3 we will now test whether they are useful in stratifying patients with iASD with excitatory deficits.

Aim 3. Evaluate the feasibility of electrophysiological markers as a measure of treatment response. Using a double-blind, placebo-controlled crossover design, we will evaluate the use of electrophysiological markers to detect neural response to growth hormone treatment in 30 children (15 PMS; 15 iASD), all of whom show characteristic VEP waveforms reflecting deficits

in glutamatergic activity pre-treatment. Based on our preliminary data, approximately 50% of patients with iASD show reduced P_{60}-N_{75} responses comparable to PMS; this subset will be targeted for the trial. We expect gamma band VEP activity will be sensitive to growth hormone and will track with clinical response on sensory measures. We will also explore the relation between electrophysiological response, growth hormone dose, and IGF-1 level, by examining VEP activity by time point.

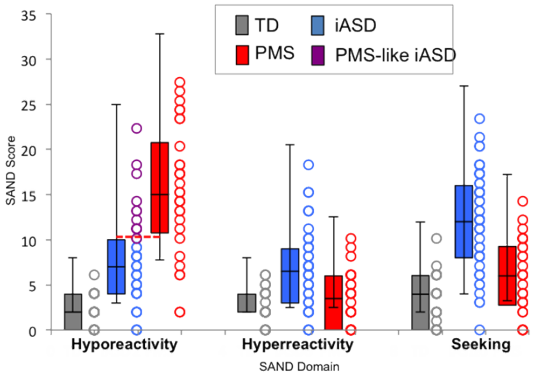
We have completed Aims 1 and 2 of this study and this revised protocol will focus on Aim 3. The expected outcome of this Aim 3 is to establish the feasibility of electrophysiological biomarkers for use in clinical trials in PMS and iASD and define a subset of patients with iASD likely to show clinical response to treatment.

RESEARCH STRATEGY

Significance: Overall Scientific Premise

Development of effective treatments for autism spectrum disorder (ASD) is hampered by several barriers: 1) ASD is a heterogeneous disorder in which etiology and neuropathology is unknown in most cases, 2) existing parent-report and clinician-administered assessments often lack the reliability, objectivity, sensitivity, and precision necessary to be confidently translated to clinical trials as outcome measures, and 3) many individuals with ASD who are most severely affected are often excluded from research. Gene discovery approaches, followed by functional analysis of model systems, have elucidated the neurobiology of several genetic subtypes of ASD and intellectual disability (ID) and led to important opportunities for developing novel, disease-modifying therapeutics. Phelan-McDermid syndrome (PMS) is a common monogenic form of ASD, accounting for 0.5-2% of ASD cases, and resulting from haploinsufficiency of *SHANK3* due to 22q13.3 deletions or mutations in the gene (Durand et al., 2007; Moessner et al., 2007; Gauthier et al., 2009; Marshall et al., 2008; Leblond et al., 2014; Cooper et al., 2011; Bonaglia et al., 2006; 2011). *SHANK3* codes for a key scaffolding protein in the postsynaptic density of glutamatergic synapses and plays a critical role in synaptic function (Boeckers et al, 2006). Indeed, recent evidence suggests that the SHANK3 and glutamate signaling pathway is common to multiple forms of ASD and that many different genetic causes of ASD and ID, including tuberous sclerosis and Fragile X syndrome (FXS), converge on common pathways, including SHANK3 (Darnell et al., 2011; Sakai et al., 2011). Thus, studies in PMS offer a unique opportunity to constrain ASD heterogeneity using a subset of individuals in which the neuropathology is better understood and can more readily be targeted for treatment. Importantly, the ratio of affected males to females is approximately equal in PMS and therefore provides critical opportunities for comparison between sexes.

Discovering the function of the SHANK3 protein has led us to translational work to develop novel therapeutics for ASD. Studying *Shank3*-deficient mice, we have identified specific deficits in synaptic function and plasticity in glutamate signaling (Bozdagi et al, 2010). Insulin-Like Growth Factor-1 (IGF-1) reversed electrophysiological and behavioral deficits in the mice (Bozdagi et al., 2013) and, critically, improved social withdrawal and restricted behaviors in children with PMS (Kolevzon et al., 2014). IGF-1 has also shown benefit in human, mouse, and neuronal models of Rett syndrome (Khwaja et al., 2014; Tropea et al, 2009; Marchetto et al., 2010), providing evidence of the potential for this treatment to have significant benefit not only in PMS, but also in other forms of ASD with heterogeneous genetic etiologies. However, IGF-1 is difficult and costly to obtain and associated with significant risks of hypoglycemia. Since IGF-1 can be increased intrinsically by growth hormone administration, we will use recombinant human growth hormone (rhGH) instead and our pilot data demonstrate significant benefit with rhGH in children with PMS across a wide range of clinical symptoms.



Because PMS has constrained genetic heterogeneity and the SHANK3 and glutamate signaling pathway underlies other forms of ASD, this project represents an excellent opportunity to develop biomarkers and test a novel therapy. Our preliminary data

demonstrate promising electrophysiological results with links to glutamatergic dysregulation in PMS using visual evoked potentials (VEPs). These data show that VEPs are objective, stable, linked to ASD symptoms, and sensitive to treatment effects. This study will be significant in further probing VEP and other electrophysiological measures with links to glutamatergic functioning, in children with PMS and idiopathic ASD (iASD).

Scientific Premise for Aim 3. Our preliminary work and results from data collected as part of Aims 1 and 2 suggests some elements of the ASD phenotype in PMS that are unique. Although approximately 80% of both children with PMS and iASD show sensory reactivity abnormalities, even in lower functioning samples, children with PMS have significantly less overall sensory sensitivity but more weak/low-energy symptoms as compared to children with iASD (Mieses et al., 2016). Sensory symptoms are now included in core DSM-5 diagnostic criteria for ASD; they are especially important to this project based on relation to VEP neural responses in both PMS and iASD populations, and may represent a phenotypic profile in PMS that informs which subset of individuals with iASD are most likely to benefit from intervention with IGF-1. Our preliminary data also show a unique pattern of repetitive behaviors and sensory symptoms that overlaps with that seen in iASD but has some key defining features. Assessment of sensory aspects of the ASD phenotype has been particularly hampered by the lack of objective, observational measurements of functioning in this domain.

Figure 1: Increased hyporeactivity, hyperreactivity, and sensory seeking behaviors are seen in iASD vs. TD. Children with PMS show significantly more hyporeactivity compared to both iASD and TD. Children with PMS also show less hyperreactivity and seeking behaviors compared to children with iASD. Intellectual ability did not significantly impact any of these findings.

To address this gap, we developed the Sensory Assessment for Neurodevelopmental Disorders (Siper et al., 2017), which is a clinician-administered assessment and corresponding caregiver interview, not reliant on verbal or cognitive capacity and therefore appropriate for severely affected populations. The SAND captures hyperreactivity, hyporeactivity, and seeking behaviors across visual, tactile, and auditory domains. In an initial sample of 45 children with iASD and 33 TD controls, the SAND showed high internal consistency (Cronbach's α=.90), strong inter-rater reliability (ICC's=.87-.91), strong test-test reliability (ICC's=.82-.97), and good convergent validity with the Short Sensory Profile (SSP; Dunn, 1999) (r=-.82, *p*<.0001). Across sensory modalities, increased hyperreactivity, hyporeactivity, and seeking behaviors all characterized children with iASD compared to TD controls (*ps*<.0001), highlighting the prominence of widespread sensory reactivity abnormalities in ASD. In comparison to the broader iASD population, children with PMS (n=24) are unique in their display of more visual, auditory, and tactile *hyporeactivity* as compared to both iASD (*p*=.001) and TD (*p*=.001), as well as less sensory hyper-reactivity and seeking behavior compared to iASD (*p*=.04). Thus, sensory hyporeactivity may be a key feature of the ASD profile in PMS and is an important treatment target in PMS. Sensory hyporeactivity, and in particular, high pain tolerance in PMS can complicate injuries in PMS and in the absence of functional communication, lead to unnecessary infection. Furthermore, results suggest that the SAND can identify sensory reactivity subtypes that are a hallmark of PMS and also characterize a subset of iASD (Fig.1). Our results suggest that approximately 30% of children with iASD display SAND hyporeactivity scores within one standard deviation of the PMS mean (Fig.1; purple iASD circles above red dotted line), reflecting "PMS-like" sensory reactivity. It is also important to note that both SAND and SSP scores are significantly correlated with our electrophysiological marker (Aim 2).

Further, our electrophysiological measures of interest are sensory-related, based on the premise that sensory abnormalities are common in ASD (APA, 2013) and significantly impact daily functioning. Furthermore, our preliminary data demonstrate that individuals with PMS show a relatively consistent pattern of sensory hypo-reactivity in response to visual, auditory, and tactile input. 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 occipital scalp locations, is extracted from ongoing

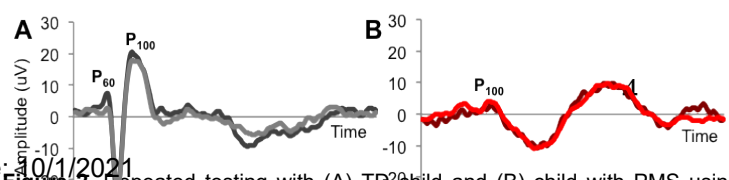


Figure 2: Repeated testing with (A) TD child and (B) child with PMS using contrast-reversing checkerboard stimulus at two time points. Across the epoch, test-retest correlation in amplitude was >.93 in both groups

EEG through signal averaging, and reflects the sum of excitatory and inhibitory postsynaptic potentials occurring on apical dendrites, which modulate excitatory and inhibitory signals received by pyramidal cells (Zemon et al., 1986). The major positive and negative peaks and troughs in VEP waveforms reflect different cellular events (Zemon et al., 1986; Creutzfeldt et al., 1973). This study focuses on a conventional transient VEP response, produced by abruptly modulating the contrast of a spatial pattern at low frequencies. The contrast-reversing checkerboard stimulus used in this study produces a positive peak at approximately 60ms (P_{60}), reflecting activation of the primary visual cortex from the lateral geniculate nucleus. A negative peak at approximately 75ms (N_{75}) reflects depolarization and glutamatergic postsynaptic activity spreading to the superficial layers of primary visual cortex, and a positive peak at approximately 100ms (P_{100}) reflects superficial hyperpolarization and GABAergic activity (Zemon et al., 1980). Coherence of high-frequency oscillatory responses, reflected in magnitude-squared coherence (MSC), can be quantified from VEPs and provide a critical metric of excitatory activity (Zemon et al., 2009). VEPs have been used in a variety of disorders for diagnostic and prognostic purposes (e.g., epilepsy, schizophrenia; Schechter et al, 2005; Sheppard et al., 2013; Zemon et al., 2008), and pharmacological studies provide evidence for VEP electrogenesis. When GABA-blocking agents are topically applied to cortex, there is an enhancement in N_{75} and attenuation or elimination of P_{100}. In contrast, when GABA is applied to cortex, N_{75} is attenuated and P_{100} enhanced (Zemon et al., 1980; Purpura et al., 1959; Purpura et al, 1953). VEPs are used throughout the lifespan, mature early, and are stable across development (Garcia-Quispe et al., 2009; Moskowitz et al., 1983).

Our preliminary findings demonstrate strong test-retest reliability of VEP recordings in TD, iASD, and PMS samples using repeated measures within a single recording session and across multiple time points (Fig. 2). We have established feasibility of our VEP battery in both individuals with PMS and iASD. Results indicate that while children with iASD (on average) and TD controls both demonstrate the characteristic waveform, VEP amplitudes are significantly smaller in iASD for both P_{60}-N_{75} (p=.005) and N_{75}-P_{100} (p=.009; Fig.3). Moreover, results from frequency domain analyses indicate that children with iASD showed significantly weaker activity in the 30-36 Hz range (p<.01) and 38-48 Hz range (p=.034), corresponding to gamma band activity. **Cognitive ability has no effect on the results**. In PMS, we see distinct VEP waveforms with smaller P_{60}-N_{75} amplitudes reflecting deficits in glutamatergic activity (Fig. 2B & 3). All PMS participants displayed reduced excitatory responses (absence of P_{60}-N_{75}), compared to both TD and iASD (p's<.001). Across groups, reduced P_{60}-N_{75} amplitude corresponded to greater sensory hypo-responsiveness on the SAND (n=32, r=-.529, p=.001) and SSP (n=32, r=.403, p=.022), suggesting VEP response marks variability in clinical symptoms. P_{60}-N_{75} amplitude also correlated with *SHANK3* deletion size (rho = .414; Fig.3 inlay) where there was significantly reduced amplitude in patients with larger deletions as compared to point mutations in *SHANK3* (p=.03). Yet, all patients with PMS display the expected GABAergic positive peak at P_{100}, indicating a specific, dissociable, glutamatergic VEP signature. These results are consistent with work in animal (Bozdagi et al., 2010) and human neuronal (Shcheglovitov et al., 2013) model systems demonstrating the deleterious effects of *SHANK3* deficiency on glutamatergic system function. In an effort to examine the use of VEPs as a stratification biomarker to aid personalized medicine approaches among an otherwise heterogeneous group of children with iASD (n=27), our results suggest that approximately 30% display P_{60}-N_{75} amplitudes within one standard deviation of the PMS mean (Fig.4; purple iASD circles below red dotted line), reflecting "PMS-like" excitatory

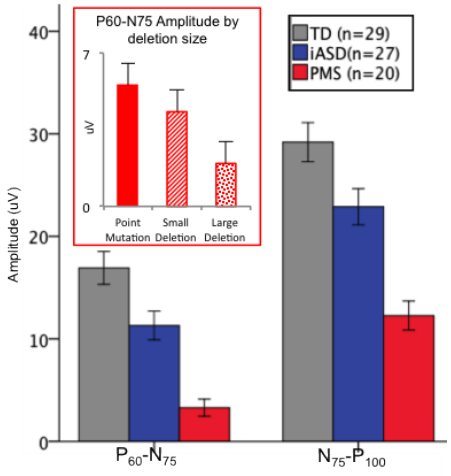


Figure 3. PMS shows significantly smaller VEP amplitudes relative to TD and iASD. Inset: P_{60}-$N7_5$ amplitude is further reduced with increased size of *SHANK3* deletion.

deficits.

Work with IGF-1 in PMS at our Center began with *Shank3*-deficient mice showing a reduction in basal neurotransmission reflecting decreased glutamate (i.e., AMPA receptor-mediated) transmission. Long-term potentiation was impaired with no significant change in long-term depression (Bozdagi et al., 2010), and intraperitoneal injection of IGF-1 reversed the electrophysiological and motor deficits seen in the *Shank3*-deficient mice (Bozdagi et al., 2013). Subsequently, we began a controlled trial of IGF-1 in PMS using the design proposed herein, and providing evidence of improvement in core ASD symptoms (Kolevzon et al., 2014) using the Aberrant Behavior Checklist (ABC; Aman et al., 1985) and the Repetitive Behavior Scale-Revised (RBS-R; Bodfish et al., 2000). IGF-1 also reversed phenotypic and electrophysiological changes in human neuronal models of PMS (Shcheglovitov et al., 2013) and iASD (Marchetto et al., 2016), providing additional support for this intervention.

IGF-1 enters the brain from the circulation where it is released mainly by the liver upon growth hormone stimulation. Blood-borne IGF-1 is found in the CNS and promotes brain vessel growth (Lopez-Lopez et al., 2004), neurogenesis, and synaptogenesis (O'Kusky et al., 2000). Once IGF-1 binds to the IGF-1 receptor, activation of the PI3K/mTOR/AKT1 and MAPK/ERK pathways induces its downstream effects (Costales & Kolevzon, 2016). A recent study in Rett syndrome provides evidence of IGF-1 brain penetrance: cerebrospinal fluid and serum analysis revealed significant increases in IGF-1 levels after treatment (Khwaja et al., 2014).

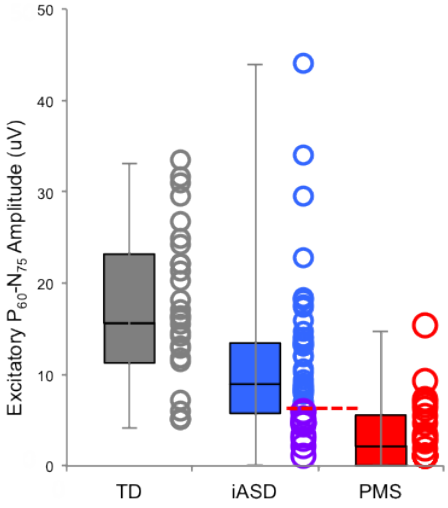
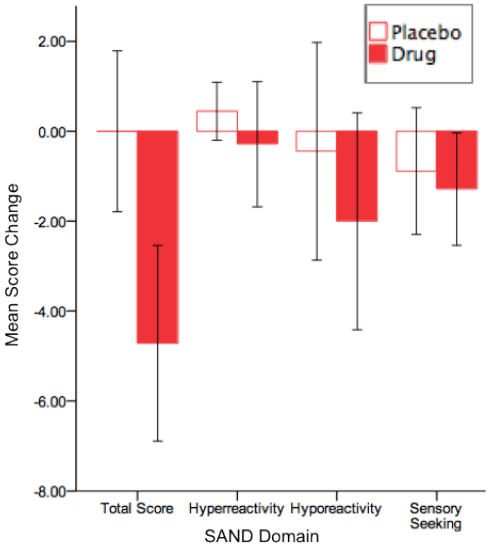
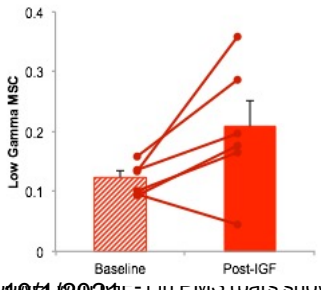


Figure 4. A subset of 50% of iASD patients fall within 1 SD of the PMS P_{60}-N_{75} mean and are defined as "PMS-like" for Aim 3.

Because of the exorbitant cost, limited availability due to manufacturing challenges, and burdensome safety monitoring required with IGF-1, we performed a pilot study with rhGH to demonstrate feasibility in a) elevating IGF-1 levels reflected in peripheral blood assays, and b) showing clinical improvement in symptom domains. We enrolled six children (2 males, 4 females) between 3.2 to 11.4 years of age in an open-label study of rhGH. At baseline, all children were of normal weight (-0.85 to 1.15 SD), height (-1.38 to 0.75 SD) and BMI (-0.82 to 1.27SD). Bone ages ranged between -0.5 to +1.5 years and were all within the normal range. Baseline IGF-1 Z-scores varied between -2.4 to 2.3 SD. Growth hormone doses were initiated at 0.14 to 0.16 mg/kg/week for two weeks and then increased to 0.27 to 0.31 mg/kg/week once tolerated. Further titrations were based on IGF-1 Z-scores with a target of 2 SD. During the course of the trial, IGF-1 Z-scores increased in all six participants, reaching a maximum of 6 SD, and demonstrating feasibility of this approach. Further, using the Wilcoxon signed rank test, rhGH was associated with statistically significant improvement in social withdrawal using the Aberrant Behavior Checklist – Social Withdrawal subscale (ABC-SW; p = 0.028), hyperactivity using the ABC - Hyperactivity subscale (p = 0.027), sensory reactivity using the Sensory Profile (SP; p = 0.042), and global improvement using the Clinical Global Impressions Improvement scale (CGI-I; p = 0.024).

We will use VEPs as our primary electrophysiological outcome. VEPs have already been used as outcome measures in clinical trials: gabapentin (Conte et al., 2009) and an infant formula (O'Connor et al., 2001) received FDA approval based in part on positive findings from VEP studies. In patients with epilepsy, augmentation with both gabapentin and vagus nerve stimulation were associated with VEP changes that reversed on discontinuation of treatment (Conte et al., 2009). These results suggest that VEP is sensitive to change with treatment and a viable outcome measure for clinical trials.





group averages; lines are individual patients (n=6; *p*=.048).

Our preliminary results suggest that VEP amplitude is a robust marker of

glutamatergic dysfunction in PMS and that approximately 50% of children with iASD display deficits in the glutamatergic P_{60}-N_{75} VEP response (Fig.4) akin to those seen in PMS. Given that VEP amplitude is associated with sensory symptoms and that IGF-1 improves clinical symptoms of PMS (Kolevzon et al., 2014), **we hypothesize that P_{60}-N_{75} VEP can be used as a stratification biomarker to predict which individuals with iASD have alterations in neural pathways overlapping with PMS and are therefore likely to respond to rhGH, a treatment that stimulates the release of IGF-1, and consequently, glutamatergic pathways**. Data from ongoing clinical trials at our center provide preliminary support for the use of transient VEPs as a biomarker of target engagement and treatment response. We piloted VEPs in a subset of patients in the context of our IGF-1 clinical trial in PMS. Results from frequency-domain analyses using MSC statistics indicated that IGF-1 consistently increased low gamma power (Fig.5) as measured by change in the band which includes frequencies from 30-36 Hz. There was a significant increase in low gamma MSC following IGF-1 relative to baseline (n=6; *p*=.048); when data were inspected individually for consistency of directional trends, MSC increased in 5 of 6 patients (Fig. 5). Increase in high frequency responses from baseline reflects stronger, more consistent excitatory contributions of the VEP, confirming glutamatergic pathway enhancement as a result of treatment, and *suggesting that MSC in the low gamma range represents an ideal biomarker for target engagement and treatment response for rhGH in a 12-week trial.*

Critically, we also saw clinical improvement in sensory hyporeactivity on the SAND with IGF-1 in the same six patients (*p*=.037; Fig.6), which itself is significantly correlated with VEP response. Given that a greater number of visual hyporeactivity symptoms is significantly correlated with smaller P_{60}-N_{75} (r=-.561, *p*=.024), in combination with our results showing treatment response on both measures, VEPs and the SAND may represent robust electrophysiological and behavioral measures for evaluating effects of rhGH.

Figure 6. Children with PMS show significant improvement in sensory symptoms following 12 weeks of treatment with IGF-1. Greatest improvement was noted in the hyporeactivity domains (n=6;

Significance of the Expected Research Contribution.

Through completion of Aims 1 and 2, we have: 1) validated the ASD phenotype within a sample of individuals with PMS, 2) established electrophysiological biomarkers of PMS, and 3) used biomarkers associated with PMS to identify an overlapping subset in iASD. In Aim 3, we will evaluate the feasibility of our biomarkers in a clinical trial with rhGH. Results will demonstrate evidence of our biomarkers' utility for: 1) stratifying subsets within iASD, 2) detecting target engagement with rhGH via neural responses, and 3) predicting treatment responders based on electrophysiological profiles. We have demonstrated feasibility of our biomarkers in individuals with ID who are minimally verbal, and this study will specifically focus on this underserved population in ASD research. *Upon successful completion of the proposed research*, we expect to clarify an underlying neural mechanism in PMS common to subsets of iASD, validate methods to predict treatment response for future clinical trials, and demonstrate efficacy of rhGH for individuals with PMS and iASD with glutamatergic dysfunction.

Innovation

To date, the development of treatments in ASD has mainly relied on strategies only loosely related to what is known about the neurobiology, using etiologically heterogeneous samples, and delivering intervention broadly and with mixed success. More recently, genetic discovery and model systems have elucidated the neurobiology of several subtypes of ASD, including PMS, and led to opportunities for developing biomarkers and novel therapeutics. *This proposal is innovative, in our opinion, on several technical and conceptual levels*. First, this study will leverage existing work in animal models of PMS and include electrophysiological tests. Second, we have the largest known cohort of individuals with PMS and have identified both a unique clinical phenotype and a striking VEP deficit that characterizes this population. Thus, we are uniquely situated to systematically assess the reliability of both clinical and electrophysiological measures in PMS. Third, the phenotype and electrophysiological profile identified in PMS is critically relevant to ASD. We will use the knowledge gained in an ASD-related syndrome to inform stratification of a subset

of iASD with constrained heterogeneity based on a biological marker. Fourth, given our strong preliminary data in PMS and iASD and existing infrastructure and pipeline for conducting clinical trials, we will test the feasibility and robustness of electrophysiological markers in the context of treatment with rhGH, a step which has yet to be taken successfully in the ASD literature. Fifth, we expect to predict a subset of iASD with high potential to respond to treatment with rhGH based on biological profiles related to PMS, making progress toward a more personalized medicine approach for ASD.

Approach

Aim 3: Evaluate the feasibility of electrophysiological markers as a measure of treatment response.

Introduction. The objective of this aim is to administer rhGH to participants with PMS and iASD to evaluate the feasibility of select electrophysiological biomarkers to: 1) detect change in neural response (engagement) and 2) predict treatment responders on clinical assessments (efficacy). We will also explore the relationship between rhGH dose, IGF-1 level, and electrophysiological response. Participants will be selected based on electrophysiological profiles with reduced P_{60}-N_{75} VEP amplitudes, reflecting impaired glutamatergic function. *Approach:* We will employ a randomized, placebo-controlled, double-blind, crossover design with a four-week wash-out period between phases. *Rationale:* Successful completion of this aim will establish feasibility of select biomarkers for detecting treatment response and advance knowledge about developing targeted treatments for PMS and related subsets of iASD with impaired glutamatergic activity. We *expect*, based on preliminary data, that the knowledge gained will provide evidence of targeted brain engagement with rhGH, a mechanistic pathway, and evidence of clinical efficacy.

Research Design. The proposed intervention will recruit 15 children with PMS and 15 children with iASD (2-12 years old). Treatment will follow a randomized, placebo-controlled, crossover format with 12 weeks in each treatment phase (rhGH and placebo). This duration was selected based on evidence with IGF-1 in PMS suggesting our clinical outcomes will be sensitive to change within this time frame (Kolevzon at al., 2014). The primary electrophysiological outcomes will be VEP gamma band activity as our preliminary data with PMS in IGF1 suggests it is sensitive to drug effects (Fig. 5). The primary clinical outcome will be the SAND because: a) our preliminary studies suggest improvement on this measure following IGF-1 (Fig. 6), b) it accurately reflects a sensory phenotype characteristic of PMS and is relevant to a substantial subset of iASD, c) it is associated with electrophysiological measures, and d) it has been validated in ASD and ID.

Inclusion criteria: All participants with PMS will have pathogenic *SHANK3* deletions or sequence variants confirmed in clinical genetics laboratories approved by the Clinical Laboratory Improvement Amendments (CLIA). Genotyping will also be used to confirm the absence of known pathogenic copy number variants in the iASD group. Participants with iASD will meet gold standard diagnostic criteria for ASD, confirmed by the Autism Diagnostic Observation Schedule – 2nd Edition (ADOS-2; Lord et al., 2012) and the Autism Diagnostic Interview-Revised (ADI-R; Lord et al., 1994). All iASD subjects will have a reduced neural response defined by P_{60}-N_{75} amplitudes within one standard deviation of the PMS mean. Subjects will be on stable medication and psychosocial treatment regimens for at least three months prior to enrollment.

Exclusion criteria: 1) closed epiphyses; 2) active or suspected neoplasia; 3) intracranial hypertension; 4) hepatic insufficiency; 5) renal insufficiency; 6) cardiomegaly/valvulopathy; 7) allergy to growth hormone or any component of the formulation; 8) comorbid conditions which prevent participation; 8) visual problems which preclude use of VEP.

Drug: Growth hormone is secreted by the somatotrophs of the anterior pituitary gland in a pulsatile manner. It binds to its receptor, signaling a cascade of intracellular events which directly stimulated the synthesis and release of IGF-1 (Ashpole et al; 2014, Grimberg et al; 2016). Growth hormone is FDA approved for use in children with growth hormone deficiency (including idiopathic growth hormone deficiency), Turner syndrome, Noonan syndrome, Prader-Willi syndrome, short stature homeobox-containing gene (SHOX) deficiency, chronic renal insufficiency, idiopathic short

stature and children small for gestational age (https://www.fda.gov/Drugs/DrugSafety/ucm237839.htm).

Drug Administration: Growth hormone is commercially available for subcutaneous injection as a 10 mg vial to be reconstituted with in-package syringe of 1 mL of bacteriostatic water (preserved with 0.33% metacresol) and a 25G reconstitution needle. We have previously received an IND exemption from the FDA to conduct trials in PMS (#142585) and will submit this revised protocol for an exemption in ASD. Growth hormone will be administered subcutaneously once daily. Approved doses range from 0.16 - 0.25 mg/kg/week for growth hormone deficient patients (Grimberg et al.; 2016) and up to 0.47 mg/kg/week for patients born small for gestational age. In our proposed trial, we expect a majority of our patients to be growth hormone sufficient and therefore similar to the population of children with idiopathic short stature. We will use a starting a dose of 0.15 mg/kg/week divided daily for 2 weeks to ensure safety and tolerance. The dose will then be increased to 0.3 mg/kg/week for 10 weeks, an average growth hormone dose in children with idiopathic short stature. Dose will be titrated based on IGF-1 levels monitored every four weeks up to a maximum dose of 0.45 mg/kg/week based on the package insert. Drug dose is calculated on a mg/kg/week basis. Using Center for Disease Control average Weight-for-age statistics in typically developing children ages 2 to 12 years-old (www.cdc.gov/growthcharts/cdc_charts.htm), the overall average weight across a representative sample of males and females in this age range is 24.65kg. Therefore, average growth hormone dose for weeks 1-2 (0.15 mg * 24.65 kg per week) is 3.7 mg per week, and for weeks 3-10 (0.3 mg * 24.65 kg per week), is 7.4 mg per week. We anticipate each participant to require about 81.4 mg of growth hormone for 12 weeks, a total of 2442 mg for 30 participants, or 250 vials (10 mg per vial).

Outcome measures: The primary electrophysiological outcome will be VEP gamma band activity as measured by MSC, which includes frequencies between 30-36 Hz. The secondary electrophysiological outcome will be VEP P_{60}-N_{75} amplitude. *VEP stimuli, data preprocessing, and variable extraction.* The primary stimulus condition will be a contrast-reversing checkerboard (100% contrast) of one-minute duration to elicit a transient VEP. VEPs will be analyzed in both time and frequency domains. Waveforms will be standardized into z-scores, segmented to evoking stimuli, filtered 1-100Hz with a 60Hz notch, and averaged across epochs. For time-domain analyses, VEP amplitudes over occipital cortex (Oz) will be measured peak-to-trough (P_{60}-N_{75}, N_{75}-P_{100}) and latency (P_{60}, N_{75}, P_{100}) will be measured by time-to-peak. Frequency-domain analyses will be conducted using MSC statistics (Zemon et al., 2009, Siper et al. 2016) to quantitatively assess the integrity of overall responses in different frequency bands. MSC reflects response reliability by providing an estimate of signal power relative to the combined power of signal+noise. As such, MSC measures consistency from one trial to the next at a given frequency. A pure signal produces a value of 1 and no signal produces a value of approximately 0.1 (bias level for pure noise); thus, higher MSC values reflect stronger and more consistent oscillatory activity in a given frequency band. Six distinct frequency mechanisms for the tVEP waveform include: Band 1, 6-10 Hz, Band 2, 12-28 Hz, Band 3, 30-36 Hz, Band 4, 38-48 Hz, Band 5, 50-64 Hz (60 Hz electrical noise omitted), Band 6, 66-84 Hz (Siper et al., 2016; Zemon et al, 2009, Zemon & Gordon, in prep.). These MSC frequency bands meet criteria for reliable components regardless of sample size as demonstrated by Monte Carlo computer simulations (Guadagnoli & Velicer, 1988; Stevens, 1996). Based on preliminary data, we expect both P_{60}-N_{75} amplitude and MSC values in bands encompassing gamma wave activity (Bands 3 and 4) to be decreased in PMS relative to iASD.

Primary clinical outcome: SAND hyporeactivity subscale (Siper et al., 2017). *Secondary and exploratory outcomes targeting ASD symptom domains*: ABC-SW (Aman et al., 1985) and other measures employed in Aim 1 (Table 1) based on results from Aim 1. Clinical outcomes will be measured by an independent evaluator who is blind to side effects to prevent the risk of bias, and, within subjects, the same evaluator will complete assessments across all visits. Electrophysiological markers and clinical measures will be collected at baseline, weeks 4, 8, and 12 of each phase. Safety and tolerability will be measured after two weeks in each phase and then

every four weeks thereafter during monitoring visits and phone calls using an adapted semi-structured interview, the Safety and Monitoring Uniform Report Form (SMURF); extensive clinical and laboratory assessments will occur every four weeks during visits (see Protection of Human Subjects).

Table 1: Clinical Outcome Measures

Domain	Instrument	Measurement modality
Social	Aberrant Behavior Checklist - Social Withdrawal Subscale	Caregiver report
Language /Communication	MacArthur-Bates Communication Development Inventory	Caregiver report
	Vineland Adaptive Behavior subscales	Caregiver report
	Mullen Scales for Early Learning subscales	Direct assessment
Repetitive Behavior	Repetitive Behavior Scales-Revised	Caregiver report
	Aberrant Behavior Checklist - Motor Stereotypies Subscale	Caregiver report
	Sensory Profile	Caregiver report
	Sensory Assessment for Neurodevelopmental Disorders	Direct assessment
Adaptive Behavior	Vineland Adaptive Behavior Scales-3	Caregiver report

Language testing will include standardized measures reliant on caregiver report and objective standardized assessments. Specifically, caregivers will report on a child's expressive and receptive verbal and non-verbal communication repertoire on the MacArthur-Bates Communication Development Inventory (MCDI; Fenton et al., 2007), which is a valid assessment of young children's language (Charman et al., 2003; Luyster et al., 2007). Although not normed in children above 30 months of age, preliminary data collected on this measure suggest that raw scores on the MCDI are useful in capturing the range of language functioning in our minimally verbal and intellectually disabled sample. The MSEL and Vineland will provide standardized assessments of receptive and expressive language, by direct assessment and parent interview, respectively.

The repetitive behavior domain will be evaluated using the RBS-R (Bodfish et al., 2000) and the ABC-Motor Stereotypies subscale. Sensory sensitivity will be measured using the SP (Dunn, 1999) and the SAND (Siper et al., 2017). The SP is a widely used caregiver questionnaire with 125 items investigating daily life sensory experiences. The SAND includes both a clinician-administered observation, involving standardized presentation of 15 sensory stimuli, with 5 sensory toys per modality (visual, auditory and tactile), and a corresponding caregiver interview. Behavioral responses are rated by a trained examiner on an algorithm measuring specific, discrete sensory hyperreactivity, hyporeactivity, and seeking behaviors across domains. The corresponding caregiver interview consists of 36 items and indicates whether a given sensory behavior is present (1) or absent (0). For any domain with behaviors coded "present," caregivers rate the severity of symptoms within that domain. Total SAND scores are based on the combination of the observation and interview, including scores by sensory modality and by DSM-5 symptom domain (hyperreactivity, hyporeactivity, seeking). Domain scores range from 0-30 and higher scores represent a higher level of sensory symptoms.

Behavioral assessments will be performed using the Aberrant Behavior Checklist (ABC; Aman et al., 1985) as a clinical outcome measure for Aim 3. The ABC is a parent checklist that has been well validated in children with ASD and ID and is frequently used as an outcome measure in ASD clinical trials. As part of studies in Aim 1, we compared 25 PMS patients with ID 57 participants with iASD and ID and found no significant differences between groups on any of the ABC Lethargy, Stereotypies, or Hyperactivity subscales. These results highlight commonalities across the PMS and broader iASD behavioral phenotype and support the ABC as a relevant measure.

Data Analysis. Following the procedures of a 2x2 cross-over design, each subject is randomized to one of two treatment sequences. First we will use graphical and numerical statistics to characterize treatment-, period- and cross-over effects (Jones & Kenward, 1989). Under the assumption of no cross-over effects and data following an approximate normal distribution, we will fit generalized linear mixed models (GLMMs) to the data examining the effects of group (PMS, iASD), condition (placebo, drug), time (baseline, week-12). Interactions on clinical and VEP measures will be checked for differential treatment effects, including subject level random

intercepts, to control for within subject correlations. Next, we will add baseline VEP gamma band activity, along with its interactions, to the models for clinical outcomes. In particular, a VEP by group by time interaction would suggest that the electrophysiological marker is predictive of differential treatment response. We will repeat these analyses with secondary electrophysiological markers and any derived composites. To examine whether electrophysiological markers track changes in clinical profiles, we will fit an additional GLMM with VEP values included as a time-varying rather than baseline covariate. If treatment effects become less significant it will suggest that rhGH is acting on clinical outcomes through the biomarker. Finally, we will directly correlate week-12 change scores for EEG and clinical measures. In case of major cross-over effects, we will base our primary analysis on phase 1 data only as a parallel group study. All tests will use a two-sided α of .05.

Power Analysis: Power is calculated for drug group differences in baseline to week 12 change scores in VEP gamma power since we are primarily interested in an overall treatment effect rather than a differential effect by group. These correspond to paired t-tests, taking advantage of the crossover design. With α at .05, we have 80% power to detect moderate to large effects (d=.77 within each group, d=.53 across groups), a reasonable expectation based on the potentially disease modifying effects of IGF-1 and our pilot study, which found a large effect (d=1.05) of IGF-1 in PMS on a different primary outcome (ABC-SW).

Potential pitfalls and alternative strategies. We expect loss of some electrophysiological data due to the severely affected nature of the population; however, this study is well powered and our proposed sample size can accommodate attrition and still detect meaningful differences across a range of responses. We have been successful in recording VEP in more than 70% of patients with PMS seen in our center. We will implement a sensory desensitization and behavioral training protocol and have extensive experience collecting these data from children with ASD, PMS, and severe ID. Moreover, for the VEP, short-duration stimulus conditions are being utilized. In addition, crossover trial designs carry risks of contamination effects, so we have provided a four-week washout period to minimize this risk. We have not observed significant carryover effects in our preliminary data. We believe this design is important because all patients will be afforded the opportunity to receive active medication. Second, the burden of frequent monitoring visits and subcutaneous injections risks increase the risk of subject withdrawal. Given the rigid control of the study procedures, relatively simple measurement techniques, and based on our experience to date, we do not foresee any major loss of data due to drop-outs or missing values. Still, in case of drop-outs we will use the intent to treat principle so all randomized patients will be included in the data analysis. Third, because of concerns about recruitment feasibility, we have established an excellent working relationship with the national PMS Foundation. Fourth, because we are only enrolling patients with iASD with PMS-like VEP amplitudes, we will not be able to test the *specificity* of target engagement and successful treatment response. Finally, if VEPs cannot be established as a biomarker for stratification or efficacy, we still have a wealth of other electrophysiological and clinical outcomes that will provide critical information.

Expected outcomes. We expect to provide evidence for the feasibility of using VEPs to detect neural change related to glutamatergic activity in response to treatment with rhGH. Specifically, we expect rhGH will be associated with increased low gamma band activity as compared to placebo in both PMS and the PMS-like iASD subset. We further anticipate enhanced gamma band activity to be associated with changes on clinical outcome measures, particularly those that reflect sensory reactivity given our preliminary data. Finally, we expect to demonstrate that rhGH treatment will result in changes on primary and secondary outcomes of sensory reactivity, social impairment, repetitive behavior, and functional outcomes of adaptive behavior. *If positive, in addition to demonstrating efficacy of rhGH for PMS, results from this clinical trial will demonstrate the feasibility of using electrophysiological markers to stratify patients with iASD and identify early biomarkers of potentially disease-modifying effects on core symptoms of ASD.*

Timeline

It is anticipated that 30 months will be required to complete Aim 3. Months 0-4 will be dedicated to study startup, regulatory, and Review Board approval. Months 5-20 will be required to enroll 30 participants (15 PMS; 15 iASD) at a rate of 1 participant per group per month. Months 21-27 will be required to complete data collection and months 28-30 will be reserved for data analysis. This timeline is considered feasible based on our experience and assessment of current interest among families on our waiting list and registered with the PMS Foundation.

Protection of Human Subjects

Risks to the Subjects

Side effects of recombinant human growth hormone (rhGH) include: headaches, muscle pain, extremity stiffness, and swelling of arms and legs due to retaining water in body. Less common side effects include bruising, lumps under the skin, gynecomastia (enlarged breast tissue in males), progression of previously present curvature of the spine, resistance to insulin hormone and low thyroid hormone levels, allergic reaction (such as hives, itching, breathing difficulty, swelling of face/lips), slipped capital femoral epiphysis (a fracture through the growing part of the bone at the hip), and benign intracranial hypertension (increased pressure in the brain with headaches and blurring of sight). Cancer has been found to be associated with long-term treatment with rhGH only in children with a previous history of cancers or with certain syndromes or genetic conditions which are predisposed to cancers. Because rhGH is administered subcutaneously, there is risk of pain and irritation at the injection site. There is also risk of inflammation and increased growth of fat cells in the skin at the injection site. It is recommended to alternate injection sites (upper arm, thigh, buttock or abdomen) with each injection to reduce these risks.

In terms of somatic effects, rhGH is expected to have significant positive effects on growth. However, growth improvement is not the goal of therapy in this study. Moreover, the rate of growth in subjects who have an exuberant response is unwanted. Anthropometric measurements, including height, weight, body mass index (BMI) and head circumference will be collected throughout the study. Growth will be defined as the height velocity and change in height SD score during treatment.

Another risk to participating in this project is the blood drawing procedures. The risks of a blood draw include pain, bruising, and the slight possibility of infection at the needle insertion site. Some children may also feel dizziness following the blood draw. Blood sampling may be

particularly uncomfortable for children with developmental disabilities and will be kept to a minimum to reduce risks. The procedures for minimizing risk include assisting parents in keeping their child still, the use of a special lounge chair to help the child relax during the procedure, the use of numbing cream if needed to reduce pain, and the use of professionals in the Mount Sinai Clinical Research Center who are trained and certified in blood drawing procedures in children with developmental disabilities.

The risks in participating in the electrophysiology aims of this study are minimal. The physical contacts with the instruments are limited to the standard EEG electrodes placed on the scalp. Electrodes are disinfected between participants. It is possible that as a result of participating in electrophysiological testing, participants may experience fatigue or anxiety. We will attempt to minimize potential discomfort from testing/evaluation by providing frequent breaks during the visits. If the participant finds any discomfort during the EEG intolerable, he or she will be withdrawn from the study. If the participant feels uncomfortable, the participant or his/her parents can tell the investigators and ask to stop the EEG at any time. We will implement a sensory desensitization and behavioral training protocol in which participants will have the opportunity to practice mock EEG procedures over the course of visits. Participants will also receive a social story describing the components of their visit as needed. Visual schedules and positive reinforcement systems (e.g., token economies) will be used throughout testing.

A Data and Safety Monitoring Board (DSMB) has been convened and has primary responsibility for developing the oversight necessary to promptly identify and act upon any adverse events. The DSMB is made up of three physicians who will meet routinely every six months and immediately should a severe adverse event occur (please see attached DSMB Charter).

Adequacy of Protection Against Risks

Recruitment and Informed Consent:

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. 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. 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 parents/guardians.

Assent:

Our preliminary phenotyping studies and clinical trials that include characterizing cognitive and adaptive functioning in Phelan-McDermid syndrome (PMS) suggest that these children have severe to profound intellectual disability (ID). As such, these patients do not have the capacity to provide assent. We will specifically be recruiting patients with idiopathic autism spectrum disorder (iASD) who are also intellectually disabled and anticipate that they will not have capacity to provide assent.

Ethics and Regulatory Considerations:

The study will be conducted according to Good Clinical Practice (GCP), the 1996 Declaration of Helsinki, and local rules and regulations of the United States.

Confidentiality of Source Documents and Study Data**:**
A subject identification code will be used in lieu of the subject's name on all study data compiled and delivered to the secure database. All source documents and study data will be kept confidential, in accordance with all requirements of the laws.

Adverse Events
Definition of an Adverse Event
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.

Adverse Events Reporting
The Principal Investigator (PI) will be responsible for the detection and documentation of events meeting the criteria and definition of an adverse event or serious adverse event, as provided in this protocol. During the study, when there is a safety evaluation, the investigator or team member will be responsible for reporting adverse events and serious adverse events. Each subject's parents/guardians will be instructed to contact the investigator immediately should the subject manifest any signs or symptoms they perceive as serious.

Clinical laboratory parameters and other abnormal assessments qualifying as adverse events and serious adverse events
Abnormal laboratory findings or other abnormal assessments that are judged by the investigator to be clinically significant will be recorded as AEs or SAEs if they meet the definition of an AE. Clinically significant abnormal laboratory findings or other abnormal assessments that are detected during the study or are present at baseline and significantly worsen following the start of the study will be reported as AEs or SAEs. The investigator will exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

Medically attended visits
For each AE the subject experiences, the subject's parents/guardians will be asked if they received medical attention defined as hospitalization, an emergency room visit or a visit to or form medical personnel (medical doctor/doctor of osteopathy or nurse practitioner) for any reason. This information will be recorded in the CRF.

Lack of Efficacy
Lack of efficacy per se will not be reported as an AE. The signs and symptoms or clinical sequelae resulting from lack of efficacy will be reported if they fulfill the AE or SAE definition.

Time Period, Frequency, and Method of Detecting AEs
All AEs occurring from the initiation of therapy until completion 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 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.

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 CRF 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.

Follow-up of Adverse Events and Serious Adverse Events and Assessment of Outcome:
After the initial AE/SAE report, the investigator will actively follow each subject and provide further information on the subject's condition. All AEs and SAEs documented at a previous visit/contact and designated as not recovered/not resolved or recovering/resolving will be reviewed at subsequent visits/contacts. Investigators will follow-up subjects with SAEs or subjects withdrawn from the study as a result of an AE, until the event has resolved, subsided, stabilized, disappeared, the event is otherwise explained, or the subject is lost to follow-up. In the case of other non-serious AEs, until they complete the study or they are lost to follow-up. Clinically significant laboratory abnormalities will be followed until they have returned to normal, or a satisfactory explanation has been provided. The investigator may perform or arrange for the conduct of supplemental measurements and/or evaluations to elucidate as fully as possible the nature and/or causality of the AE or SAE.

Regulatory Reporting Requirements for Serious Adverse Events:
All safety data will be reported to 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 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. The protocol will be filed under Investigational New Drug (IND) exemption status with the US FDA.

Safety Measures

Participants will undergo comprehensive medical evaluation by the study physicians. Medical history, family history, physical and neurological examination (including anthropometric measurements and fundoscopic exams), routine hematology, thyroid function, and blood chemistry (including liver profile), bone X-ray for bone age, and electrocardiography will be performed to determine eligibility for participation. Patients will be monitored for safety and tolerability at weeks 2, 4, 8, and 12 in both treatment phases – see Table 1 and 2. There are a number of tertiary outcomes designed to monitor the safety of rhGH (see Tables 1 and 2) and will be reported to the DSMB (see Stopping Conditions).

Our preliminary phenotyping studies characterizing cognitive and adaptive functioning in Phelan-McDermid syndrome suggest that these children and adults have ID. This sample was significantly delayed in receptive language, expressive language, and overall functional communication skills. We will specifically be recruiting children with iASD who also have ID. As such, it is impossible to directly query these children using verbal methods with respect to safety and tolerability. Instead, we rely on parent report using open-ended methods and a modified SMURF, in addition to physical and neurological exams, and laboratory measures.

Monitoring for AEs will be conducted during scheduled and unscheduled visits per clinical and laboratory assessments. If a subject develops significant neurological signs and symptoms (e.g. cerebrovascular event), they will be seen immediately for a comprehensive evaluation, appropriate treatment, and removal from active participation in the study. We will also apply criteria for attribution of AEs by means of the following descriptors and codes.

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

Stopping Conditions for Individual Subjects

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 rhGH study

- 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.
- 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 an exploratory trial of a novel, unproven treatment, the safety of subjects is our primary *overriding* concern. If there is doubt concerning a subject's safety, the default mode will be withdrawal from treatment or active study participation, followed by close observation (safety follow up visits) and recommendation of standard treatment.

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 rhGH 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. (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. Clearly, patients who experience adverse effects during rhGH treatment would count towards this criterion for whole study stopping).

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 DSMB every six months and to the IRB annually, 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 DSMB 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.

Tables

Table 1. Safety and Screening Measures

Measures*	Baseline	Week 4	Week 8	Week 12
Physical and neurological exam	X			X
Fundoscopy	X			X
Electrocardiography	X			X
Bone X-ray for bone age	X			X
Pregnancy Test	X			X
Height	X			X
Weight	X			X
Head circumference	X			X
Side effect monitoring**	X	X	X	X

*Safety measures will be performed at these time points in *both* phases of the crossover trial.

**In addition to the above, side effect monitoring will occur at week 2 by phone, or at any point in person as needed, in both phases.

Table 2. Laboratory Safety Measures

Measures	Baseline*	Week 4*	Week 8*	Week 12*
Electrolytes (Na, K, Cl, CO2)	X			X
Complete blood count	X			X
Liver function panel	X			X
Thyroid function (TSH/T3/T4)	X			X
Renal function (BUN/CR)	X			X
Hemoglobin A1c	X			X
Albumin	X			X
Glucose (fasting)	X			X
Insulin (fasting)	X			X
Lipid profile	X			X
IGF-1 / IGF-BP3	X	X	X	X

*Laboratory safety measures will be performed at these time points in *both* phases of the crossover trial

Table 3. Dose Titration Schedule

Week	Dose
Week 1 and 2	0.15 mg/kg/week divided into daily doses
Week 3 to 12	0.30 mg/kg/week divided into daily doses

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