A Double-Blind Placebo-Controlled Crossover Trial of Insulin-Like Growth Factor-1 (IGF-1) in Children and Adolescents With 22q13 Deletion Syndrome(Phelan-McDermid Syndrome)
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RESEARCH STRATEGY

Significance. Several of the ASD genes identified to date, including *SHANK3*, are involved in the neuroligin-neurexin pathway at glutamatergic synapses (Bonaglia et al, 2011; Durand et al., 2007; Gauthier et al., 2009; Guilmatre et al., 2009; Moessner et al., 2007; Rosenfeld et al., 2010; Schaaf et al., 2011; Sebat et al., 2007). Glutamate signaling is also highly relevant to these pathways and to various forms of ASD. *SHANK3* is a protein found in glutamatergic synapses that helps form the postsynaptic density, and upon which glutamate receptors are clustered (Sheng and Kim 2000; Boeckers 2006; Kreienkamp 2008). There is now abundant evidence that *SHANK3* is a key regulator of synapse development and function and a critical protein for many synaptic processes. Most importantly, large-scale analyses show that *SHANK3* and associated pathways are implicated in multiple forms of ASD, including tuberous sclerosis and Fragile X syndrome (FXS) (Darnell et al., 2011; Sakai et al., 2011). According to the Interagency Autism Coordinating Committee 2011 Strategic Plan, there is a need for translational research that takes advantage of genetic findings in order to: 1) develop animal models to understand the effects of these genetic variants on brain function, 2) inform which molecular signaling pathways are affected in ASD, and 3) discover specific targets for the development of novel therapeutics. Following this approach, our group developed *Shank3*-deficient mice and discovered that AMPA-receptor signaling and long-term potentiation (LTP) were impaired, with no significant change in long-term depression (LTD) (Bozdagi et al., 2010). Treatment with IGF-1 was then tested and found to reverse the electrophysiological and behavioral deficits in *Shank3* heterozygous mice (see Figure 1). The *SHANK3* / glutamate signaling pathway is highly relevant to various forms of ASD; the link between deficits in synapse function and ASD suggest that treatment with IGF-1 may have implications for ASD associated with disruptions in common underlying pathways, and this is supported by ongoing studies of IGF-1 in Rett syndrome. *This contribution will be significant because we aim to pilot a potentially disease-modifying treatment which has the potential to shed light on pathways more broadly relevant to ASD treatment.*

Innovation. To date, the development of pharmacological treatments in ASD has mainly relied on strategies only loosely related to what is known about the neurobiology of the disorders, using etiologically heterogeneous samples, and delivering intervention broadly with mixed success. More recently, genetic discovery and model systems have led to important opportunities for developing novel, disease-modifying therapeutics. The proposed project represents a unique effort between clinical and basic science resources and draws upon the experience of clinicians with recognized expertise in *SHANK3* deficiency and ASD. This work is based on preclinical evidence that establishes proof-of-concept with IGF-1 reversing electrophysiological deficits in *Shank3*-deficient mice. A recent example of a similar approach is the large-scale clinical trial with arbaclofen currently underway in Fragile X syndrome (FXS) and in ASD. FXS accounts for approximately 1-2% of ASD cases and detailed analysis of mouse and other models of FXS gave rise to a hypothesis that the metabotropic glutamate receptor in the synapse represents a potential target in the disorder. Drugs that targeted this receptor were tried in mice and other model organisms that had mutations that mimic FXS (Bear et al., 2004; Yan et al., 2005). These drugs were shown to correct some of the cellular and behavioral deficits observed in the mice and are now being studied in human trials. Trials using comparable strategies of discovery are likewise underway in Rett syndrome and tuberous sclerosis, both known causes of ASD. *The proposed project is innovative, in our opinion, because it is based on the combination of a previously tested drug development strategy, convincing preclinical evidence with IGF-1 in a mouse model system, and a safe approved drug. In addition, it is innovative in that we introduce objective measures of language and social attention in the context of experimental therapeutics.*

Approach.

Aim: Evaluate safety, tolerability, and feasibility of IGF-1 vs. placebo targeting social withdrawal using the ABC-SW subscale as a primary outcome measure and additional secondary and exploratory outcome measures including objective assessments of language and social attention.

Introduction. The *objective* of this aim is to pilot the use of IGF-1 treatment in 18 patients with *SHANK3* deficiency in order to evaluate safety, tolerability, and feasibility for a core deficit of ASD – social impairment. We will *approach* this aim by employing a placebo-controlled, double-blind, crossover design with three months of treatment with IGF-1 and three months of placebo in random order, separated by a four week wash-out period. The *rationale* is that successful completion of the proposed research will establish feasibility and contribute pilot data from the first treatment trial of *SHANK3* deficiency to the field. It will also advance knowledge about developing targeted treatments for additional causes of ASD associated with impaired synaptic development and function. It is our *expectation*, based on our rodent model, that the knowledge gained provide evidence for a novel therapeutic that has specific and disease-modifying effects on ASD

associated with *SHANK3* deficiency and pave the way for more trials in other subtypes of ASD with disruptions in shared pathways. We expect to add significantly to data from ongoing trials with IGF-1 in Rett syndrome and will be collaborating closely with the PI, Dr. Walter Kaufmann (see Letter of Support), on dosing and safety.

Justification and feasibility. There have been no controlled treatment trials in *SHANK3* deficiency to date. Our clinical experience comprehensively evaluating 32 affected families provides numerous anecdotal reports of medication trials that borrow from the broader ASD literature and target associated symptoms of attention deficit, impulsivity, hyperactivity, irritability, and repetitive behavior. An exhaustive literature review revealed only two published reports of medication treatment in *SHANK3* deficiency, one case series with intranasal insulin (Schmidt et al., 2008) and one case study with risperidone (Pasini et al., 2009), both with reported improvement. In the intranasal insulin study, six children received up to 12 months of treatment with "extremely positive development" based on sum of scores from a parental questionnaire in most domains, including cognitive, speech and language, and motor skills (Schmidt et al., 2009). Insulin was hypothesized in these cases to improve neuronal function by increasing central nervous system (CNS) glucose uptake and enhance synaptic plasticity via glutamatergic receptors. Results from the case study with risperidone were similarly optimistic but equally uncontrolled: an 18 year old girl with *SHANK3* deficiency was treated with risperidone 0.5 mg BID with significant improvement on the Clinical Global Impression Scale (CGI; Guy 1976) in anxiety, aggression, and insomnia after one month that was sustained after six months. The hypothesized mechanism of action of risperidone in *SHANK3* deficiency was to promote NMDA transmission via dopamine 2 receptor blockade. Our studies, however, indicate that reduced basal neurotransmission of *Shank3*-deficient mice at glutamatergic synapses may be AMPA receptor-mediated (Bozdagi et al., 2010).

Results from treatment with recombinant human (rh) IGF-1 in our *Shank3*-deficient mouse model are extremely intriguing (see Preliminary Studies). Importantly, IGF-1 has also been explored in Rett syndrome, another monogenic cause of ASD. Rett syndrome is caused by mutations in the genes coding for methyl CpG-binding protein 2 (MeCP2) and Tropea and colleagues (2009) used mice deficient in MeCP2 to show that treatment with N-terminal peptide IGF-1 reverses some of the phenotype, including extending lifespan, improving locomotion, improving respiration, and decreasing heart rate irregularities, all deficits found in both humans and mice with the condition. MeCP2 mutant mice have also been shown to have profound reductions in synaptic activity in slices of sensorimotor cortex (Chao et al., 2007; Dani et al., 2005), and this reduction was partially but significantly reversed by IGF-1 peptide (Tropea et al., 2009). In addition, Marchetto and colleagues (2010) developed induced pluripotent stem cells (iPSCs) from Rett patients" fibroblasts and found that derived neurons had fewer glutamatergic synapses, a phenotype that was rescued with rhIGF-1.

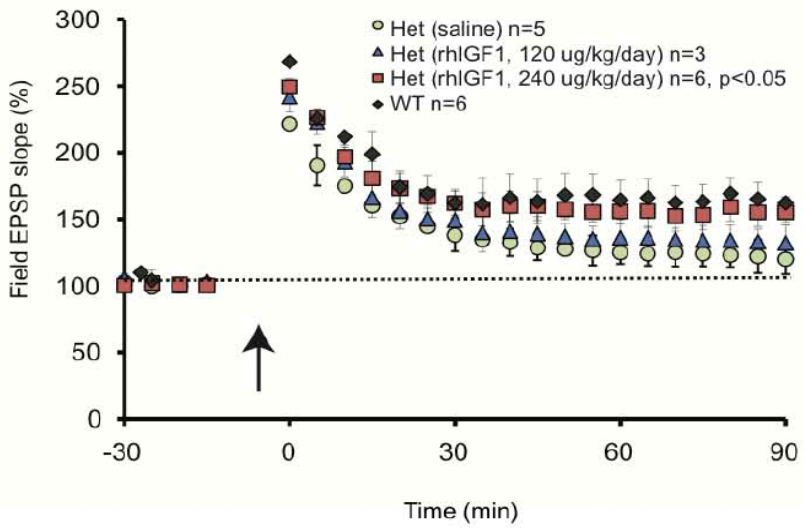
IGF-1 enters the brain from the circulation where it is released mainly by the liver upon growth hormone stimulation. Blood-borne IGF-1 is found in the CNS where it promotes brain vessel growth (Lopez-Lopez et al., 2004), neurogenesis, and synaptogenesis (O"Kusky et al., 2000). The mechanisms that trigger IGF-1 entry through the BBB are proposed to occur by diffusible messengers and depend on neuronal activity, which regulates neurovascular coupling and BBB permeability (Nishijima et al., 2010). These messengers stimulate matrix metalloproteinase-9 (MMP9) and lead to cleavage of the IGF binding protein-3 (IGFBP-3). Cleavage of IGFBP-3 increases free IGF-1 and allows passage of circulating IGF-1 into the CNS through an interaction with the endothelial transporter lipoprotein-related receptor 1 (LRP1) (Nishijima et al., 2010).

Preliminary Studies. Our group studied mice with a targeted disruption of *Shank3,* in which exons coding for the ankyrin repeat domain (ARD) were deleted and expression of full-length *Shank3* was disrupted (note that a point mutation in the ARD has been described in a child with autism, Moessner et al., 2007). We examined synaptic transmission and plasticity by multiple methods and results indicate a reduction in basal neurotransmission in *SHANK3* heterozygous mice (Bozdagi et al., 2010). Studies with a specific AMPA receptor antagonist and NMDA receptor antagonist demonstrated that the decrease in basal transmission reflected reduced AMPA receptor-mediated transmission. LTP was impaired in *SHANK3* heterozygous mice, with no significant change in LTD (Bozdagi et al., 2010 and Figure 1). In concordance with the LTP results, persistent expansion of spines was observed in control mice after theta burst pairing-induced LTP; however, only transient spine expansion was observed in *SHANK3* heterozygous mice. Behaviorally, male *SHANK3* heterozygotes also displayed less social sniffing and emitted fewer ultrasonic vocalizations during interactions with estrus female mice, as compared to wildtype littermate controls (Bozdagi et al., 2010).

Based on our hypothesis that reduced AMPA receptor levels reflected less mature synapses, and the emerging story with IGF-1 in Rett syndrome, we examined whether IGF-1 treatment could reverse deficits in *Shank3*-deficient mice. Our data show that intraperitoneal injection of IGF-1 at 240 µg/kg/day for 2 weeks reverses the electrophysiological deficits seen in the *Shank3* heterozygous mice (Figure 1). *Shank3*

heterozygotes no longer demonstrated reduced AMPA receptor-mediated transmission and showed normal LTP like that seen in wildtype animals (Bozdagi et al., unpublished and Figure 1).

Figure 1. Reversal of electrophysiological deficits in *Shank3*-deficient mice (Het) as compared to wildtype mice (WT) after treatment with recombinant human IGF-1 (rhIGF1)



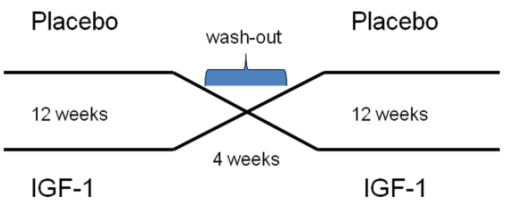
240 µg/kg/day was selected because it represents the maximum dose according to the current FDA label. This finding informs our dose titration schedule and supports the approach to titrate to 120 µg/kg twice daily (240 µg/kg/day) as rapidly as safety and tolerability allows. If positive effects are seen, future studies will determine the minimal effective dose.

In additional preliminary studies, male wildtype and Shank3 heterozygous mice were treated with saline or recombinant human IGF-1 and tested for motor performance and motor learning by measuring time latencies to fall off a rotating rod. Mice were challenged with three 2-minute trials (each separated by 15 minutes) where the rotation was gradually increased from 0 to 45 rpm. Heterozygous mice injected with saline exhibited reduced latencies (time) to fall off as compared to wildtype mice (p=0.017). After IGF-1 treatment, heterozygous mice exhibited significantly longer latencies in comparison to saline-injected mice of the same genotype (p=0.003) (Bozdagi et al., unpublished).

In addition, our clinical group has evaluated 32 patients with *SHANK3* deficiency using a comprehensive assessment battery. Eighty-three percent of these patients meet criteria for ASD using gold standard instruments, including the Autism Diagnostic Interview-Revised (ADI-R; Lord et al., 1994), the Autism Diagnostic Observation Schedule-Generic (ADOS-G; Lord et al., 2000), and the Diagnostic and Statistical Manual for Mental Disorders-IV (DSM-IV; APA, 1994).Ninety-seven percent of patients had intellectual disability and 50 percent were profoundly intellectually disabled. Three patients have already been enrolled in a clinical trial with IGF-1 using the proposed design to begin collecting data on the safety, tolerability, and feasibility of IGF-1 vs. placebo targeting social withdrawal and additional secondary and exploratory outcome measures. Although it is too soon to evaluate efficacy, there have been no significant adverse events.

In an ongoing clinical trial in humans with Rett syndrome previously conducted at Boston Children"s Hospital by Dr. Omar Khwaja and now assumed by Dr. Walter Kaufmann (see letter of support), 12 patients have completed a four-week safety study with IGF-1 and 24 patients are in ongoing treatment. Dr. Kaufmann will serve as a consultant on this project. Among the 12 treated girls with Rett syndrome who completed four weeks of treatment, there were no serious adverse events (AEs) related to IGF-1. Two AEs were determined not related to IGF-1 and one AE was determined possibly related to IGF-1: gingival hyperplasia in a patient also on phenytoin. Dr. Kaufmann"s safety protocol uses a dose escalation design beginning at 0.04 mg/kg BID and titrating to 0.12 mg/kg BID in week three and four. *A positive signal is found with IGF-1 in Rett syndrome at 0.12 mg/kg BID within four weeks* (Kaufmann, personal communication).

Research design. Treatment will follow a randomized, placebo-controlled, crossover format with 12 weeks in each treatment arm (IGF-1 and placebo), separated by a four-week wash-out phase. The trial duration was selected based on preliminary evidence with IGF-1 in Rett syndrome and our primary outcome, the ABC, is sensitive to change within this time frame (Aman, 2010).



Inclusion criteria: The proposed pilot will recruit 18 children between 5 and 12 years-old with *SHANK3* deficiency previously evaluated as part of ongoing studies in our Center. All subjects will have a minimum raw score of 12 on the ABC-SW based on the decision to use this scale as the primary outcome measure (see Outcome Measures section). The minimum raw score was selected by adding approximately one standard deviation to the mean ABC-SW subscale score derived from a normative sample of 601 children aged 6-17 with intellectual disability (Brown et al., 2003). Subjects will also be on stable medication regimens for at least

three months prior to enrollment. Exclusion criteria: Cases will be excluded if any of the following are applicable: 1) closed epiphyses; 2) active or suspected neoplasia; 3) intracranial hypertension; 4) hepatic insufficiency; 5) renal insufficiency; 6) cardiomegaly/valvulopathy; 7) allergy to IGF-1; 8) patients with comorbid conditions deemed too medically compromised to participate.

Drug Administration: IGF-1 (Increlex) is an aqueous solution for injection containing human insulin-like growth factor-1 (rhIGF-1) produced by recombinant DNA technology. We have received an IND exemption from the FDA (#113031) to conduct the trial. Based on the package insert, dose titration will be initiated at 0.04 mg/kg twice daily by subcutaneous injection, and increased, as tolerated, every week by 0.04 mg/kg per dose to a maximum of 0.12 mg/kg twice daily. This titration is justified based on results from the trial in Rett syndrome and our preclinical data which suggests that 0.24 mg/kg/day is effective in reversing electrophysiological deficits whereas 0.12 mg/kg/day is not as effective. We aim to reach the therapeutic dose as quickly as is safe and tolerated in order to allow maximum time for clinical improvement. Doses may be decreased according to tolerability by 0.04 mg/kg per dose. Medication will be administered twice daily with meals, and preprandial glucose monitoring will be performed by parents at treatment initiation, prior to each injection, and until a well tolerated dose is established. Parents will be carefully trained in finger stick monitoring, symptoms of hypoglycemia, and medication administration.

Outcome measures (Table 1): Efficacy measurements will be taken at baseline of each treatment phase, and at weeks 4, 8, and 12 of each treatment phase. Safety and tolerability will be measured every two weeks throughout the trial during monitoring visits and phone calls (see Adverse Events). All safety and efficacy measurements will be repeated four weeks post-treatment to ensure safety and explore maintenance of treatment effects. Primary and secondary outcomes will be measured by an independent evaluator who is blind to side effects to prevent the risk of bias. All evaluators will maintain a minimum of 80 percent reliability through ongoing training and routine reliability assessments. *Primary outcome*: ABC-SW (Aman et al., 1985). The ABC is a rating scale used to monitor an array of behavioral features, including social withdrawal (i.e., Lethargy subscale). It was chosen as the primary outcome measures because our preliminary data suggest that it accurately reflects the phenotype and because the ABC has been well validated in both intellectually disabled and ASD patients and is currently accepted as an appropriate outcome measure within the field of pediatric psychopharmacology research (Aman et al., 2010). *Secondary outcomes*: Clinical Global Impression-Improvement and Severity Scales (CGI-S; CGI-I; Guy, 1976). The CGI-S and CGI-I are 7-point rating scales commonly used to measure symptom severity and treatment response in studies of patients with intellectual disability and ASD (Aman et al., 2002; RUPP, 2005a; 2005b). *Exploratory outcomes targeting core symptom domains of ASD:* Language using the Macarthur-Bates Communicative Development Inventory (MCDI; Fenson et al., 1993; 2007), Vineland Adaptive Behavior Communication Domain (Sparrow, 1984), and Language Environment Analysis (LENA; Warren et al., 2010); social attention using the Social Orienting Task (Dawson et al., 2004); repetitive behavior using the Repetitive Behavior Scale-Revised total score (RBS-R; Bodfish et al., 2000); Sensory sensitivity and sensory seeking behavior using the Sensory Profile (Dunn, 1999); Adaptive functioning using the Vineland Adaptive Behavior Composite (Sparrow, 1984); Caregiver strain using the Caregiver Strain Index (CSI; Brannan et al., 1997); All of the selected exploratory measures have been previously validated as measurement tools in ASD populations and we aim to assess their utility in detecting change with IGF-1 treatment in *SHANK3* deficiency.

Language Testing will be performed with a combination of standardized measures that rely on parent/caregiver report, and objective, naturalistic assessments. Dr. Helen Tager-Flusberg (see Letter of Support) co-chaired a working group assembled by the National Institute on Deafness and Other Communication Disorders (NIDCD) which recommended using a combination of measures for improving language assessment and developing benchmarks of expressive language for use as treatment outcome measures (Tager-Flusberg et al., 2009). Two objective approaches can be taken with expressive natural language samples: verbatim transcription followed by coding with analysis using supporting software (e.g., Systematic Analysis of Language Transcripts; Miller & Chapman 2008) or automated technologies such as LENA. A recent study compared vocal production in 26 young children diagnosed mainly with Autistic Disorder to 78 typically developing controls using a digital language processor (DLP) and LENA (Warren et al., 2010). Audio processing algorithms measured the amount of vocalizations produced during a 12-hour period in the children"s natural environment. Significant differences in vocal production were found between groups and this study provides proof of concept that automated measurement can capture and distinguish speech-related and non-speech vocalizations in children with language delays and ASD. In addition, LENA measurements were positively correlated with previously validated parent report measures (Warren et al., 2010), including the MCDI

(Fenson et al., 1993; 2007). In the proposed study, LENA sampling will occur pre- and post-treatment during the semi-structured ADOS-G in the clinic and during a 12-hour time period in the child"s naturalistic home environment. Software quantifies and analyzes language output to count the number of times a child vocalizes during a given period and then filters out vegetative sounds such as respiration and fixed signals like cries.

Social Attention measurement will also be piloted using a Social Orienting Task (Dawson et al., 2004) as an exploratory outcome. Dawson and colleagues have developed the term „social orienting impairment" which refers to the failure in children with ASD of orienting spontaneously to naturally occurring stimuli in their environment (Dawson et al., 1998). Impairments in joint attention and social orienting reflect core social deficits and specifically differentiate young children with ASD from those without ASD (Ventola et al., 2007; Dawson et al., 2004). It has been suggested that early social attention deficits deprive children of social information input and lead to disrupted neural and behavioral development (Mundy & Neal, 2001). Failure of social orienting may represent one of the earliest and most fundamental social deficits in ASD (Dawson et al., 1998). In addition, social attention has been shown to be critical for the acquisition of verbal and gestural communication (Carpenter et al., 1998) and possibly intellectual functioning (Poon et al., 2011). The proposed study will replicate the social orienting task developed by Dawson and colleagues (2004). Orienting is defined as turning the head or eyes toward an auditory stimulus and a series of four social (e.g., calling the child"s name) and four non-social (e.g., phone ringing) stimuli will be delivered three times each. A standard and familiar testing room will be used and the presence, latency, and duration of orienting (e.g., turning the head or eyes) will be measured by trained coders through a one-way mirror and the use of video. We aim to explore the use of this measure and to assess its utility as an outcome measure sensitive to change with treatment.

Adverse Events (AEs). Monitoring AEs will be conducted during scheduled and unscheduled visits using an adapted semi-structured interview, the Safety and Monitoring Uniform Report Form (SMURF) every two weeks, and extensive clinical and laboratory assessments every four weeks (see Section on the Protection of Human Subjects). AEs will be carefully documented with respect to severity, duration, management, relationship to study drug, and outcome. Severity will be graded using a scale of mild, moderate, or severe.

Table 1: Outcome Measures	**Baseline** Phase 1 and 2	**Wk 4** Phase 1 and 2	**Wk 8** Phase 1 and 2	**Wk 12** Phase 1 and 2	**Post-study**
Social:					
ABC-SW subscale	X	X	X	X	X
Social Orienting Task	X	X	X	X	X
Language:					
LENA	X			X	X
MCDI	X			X	X
Vineland subscales	X			X	X
Repetitive Behavior:					
Repetitive Behavior Scale	X	X	X	X	X
Other:					
Sensory Profile	X	X	X	X	X
CGI Scales	X	X	X	X	X
Caregiver Strain Index	X	X	X	X	X
Vineland Behavior Composite	X			X	X

Data Analysis: This trial is using a randomized cross-over design, thus, time of assessment is nested (repeated) within treatment and treatment is nested within subjects. The between subject randomization variable is Order. The most appropriate analysis depends on the presence of order of treatment effects. As such, we will consider several analytic strategies, including 1) *fully within subject design; 2) within subjects design with order as a between subjects factor; 3) first phase only (between subjects design).* The within subjects design using order as a between subjects factor (2) will have 8 observations/subject, reflecting the within subject time by treatment effects. However, a between subjects Order variable would be included to evaluate the differential effect of treatment over time in the context of order. If the three-way interaction involving order (time by treatment by order) was significant, or any other order effects were detected, this would preclude the use of the fully within subject design that ignored order. Mixed effect regression is our primary analysis and we anticipate estimating an unstructured covariance matrix ("MANOVA") for the error term. However, if all order effects appear to be negligible as anticipated, we will consider the fully within subjects design (1) where the model would again focus on treatment and time, but treatment would also be a within subjects factors with a total of 8 observations/subject. Finally, as an alternative strategy, we may also use the first phase only analysis (3) that would examine data from the first 12 weeks only as a placebo-

controlled, parallel group study. The model for this design is a 4 (time) by 2 (treatment) design with repeated measures on the time factor. We would use mixed effects regression with group dummy coded as 0=placebo and 1=control and time coded naturally as weeks (0, 4, 8, and 12). Here the primary focus would be the time by treatment interaction, which estimates the differential change in the two groups on the outcome measures.

Sample Size and Statistical Power: Power estimates are based on a sample size of 18 individuals using baseline to week 12 change scores. Our Type I error rate (α) was set at .05 and we have adequate power to detect large effects in the within-subject analysis (1). A large effect size is deemed feasible based on the potentially disease modifying effects of IGF-1 and evidence of large effects with risperidone (RUPP, 2002) and aripiprazole (Owen et al., 2009) using the ABC as the primary outcome measure. However, our primary aim is to evaluate feasibility and detect signal of improvement.

Effect Size	Full Within Subject	Within Subject x Order	Phase 1 Between Group
.80	.89	.56	.36
1.00	.98	.75	.51
1.20	.998	.88	.67

Expected outcomes. We expect to provide evidence for the safety and feasibility of IGF-1 in ameliorating social withdrawal in children with Autistic Disorder. Further, we expect to demonstrate that IGF-1 is associated with improvement on secondary outcomes of social impairment, language delay, and repetitive behavior, as well as on functional outcomes of global severity and caregiver strain. We further hypothesize that treatment with IGF-1 will result in improved expressive speech, reflected by an increase in the frequency of vocalizations, increased well-formed babbles, increase in the proportion of speech sounds to non-speech vocalizations and increases in words and turn-taking as measured by LENA. We also predict language increases will be evident across assessment settings, the clinic and home. Functional improvements in language are not expected in the short-term treatment, but will be evaluated for indication of a signal on this clinically meaningful outcome. Significant improvements on measures of life skills (e.g. Vineland) are likewise not expected in the short-term, but will be evaluated at major study time points to provide indications of potential impact on functional skills domains. *If positive, results from this clinical trial will demonstrate potentially disease-modifying effects on core and associated symptoms of ASD and may inform future trials.*

Timeline and Milestones. We will require six months for study preparation and 18 months for data collection at a randomization rate of one subject per month. The last subject will be randomized in July, 2015 to allow for completion of the trial and leave five months for data cleaning, analysis, and manuscript preparation

Potential problems and alternative strategies. Crossover trial designs carry risks of treatment order effects. We have provided a four-week washout period to minimize this risk and will use a between subjects Order variable to evaluate the differential effect of treatment over time in the context of order. We believe this design is important because *SHANK3* deficiency is a rare disease without available treatments and all patients will be afforded the opportunity to receive active medication; the cost of IGF-1 does not make an open label extension trial feasible at this time. Second, the burden of frequent monitoring visits and subcutaneous injections risks subject withdrawal; we will use the intent to treat principle so all randomized patients will be included in the data analysis. Third, concerns about recruitment feasibility must be raised with rare diseases. We have established an excellent working relationship with the national Foundation for affected families (see Letter of Support) and have already evaluated 32 children. There has never been a treatment trial in this devastating disorder and 52 patients have thus far expressed interest in participating. Finally, the possibility that our hypotheses are not supported must be considered because the preliminary evidence is drawn from a preclinical mouse model and we do not have clear evidence for our phenotypic targets. Yet, we have selected a measurement tool (ABC) validated in developmentally delayed populations, sensitive to change with treatment, and reflective of the phenotype in our preliminary studies. Several secondary and exploratory outcomes will also be available for analysis and will provide meaningful data for future research should signal be detected elsewhere and not on the primary outcome. Standardized measures of receptive and expressive language (e.g., Vineland) that we use as part of our studies may not be sensitive change in this intervention trial as standard error of measurement is approximately three months. For this reason, we have chosen an additional focus on measures of expressive language using natural language samples and objective measurement of language production in semi-structured and naturalistic settings.

Future directions. Future studies will refine and characterize the phenotype using functional neuroimaging and prospectively studying the natural history of the disorder. If results are promising with IGF-1, a future clinical trial will include the identification of minimal effective dose and potential biomarkers to predict treatment response. Pilot studies to explore the use of IGF-1 in ASD *not* associated with *SHANK3* deficiency are also being proposed (IND #113450).