STUDY TITLE: "PHASE 2 STUDY: INTRANASAL OXYTOCIN VS. PLACEBO FOR THE TREATMENT OF HYPERPHAGIA IN PRADER-WILLI SYNDROME"

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SPECIFIC AIMS

OBJECTIVE

The primary objective of this Phase 2 trial is to compare the change from baseline to week 8 of the peptide intranasal oxytocin (IN-OXT) on changes in hyperphagia using the Hyperphagia Questionnaire-Clinical Trials (HQ-CT) in children and adolescents with PWS. We have obtained an IND for the use of IN-OXT for PWS (IND 121109) and have acquired both the IN-OXT and matching placebo (Manufactured by Novartis as Syntocinon - see letter and COA of Batch in appendix). We will also compare the effects of IN-OXT from baseline to week-8 on repetitive behaviors (RBS-R), weight, BMI and body composition, quality of life (WHOQOL, Caregiver Strain Questionnaire), salivary oxytocin concentration, and safety measures. Safety will be assessed with routine laboratory monitoring, physical exam, urine pregnancy test for menstruating females, and collection of vital signs. The Aberrant Behavior Checklist (ABC) will also be used as a safety measure for both suicidality (per FDA guidelines) and for adverse events (aggression/temper tantrums). This Phase 2 study is designed to generate preliminary data for future Phase 3 trials, and targets symptoms in this population that currently have no effective treatments. By treating these symptoms earlier in life in a pediatric PWS population we may maximize the treatment impact. This study supports the Orphan Product Divisions goal of identifying and promoting the development of treatments indicated for rare diseases or conditions. Our proposed treatment will target hyperphagia in subjects with PWS, a symptom for which there is currently no effective treatment.
This study will provide essential data that is needed for drug development and eventually drug approval.

We propose a three-year randomized, double-blind, placebo-controlled 8-week treatment trial of IN-OXT 16 IU/day in 50 subjects aged 5 to 17 years with PWS. To allow for dose optimization, we allow one downward titration to 12 IU if adverse events occur, and one upward titration to 24 IU at week 2 for lack of response on hyperphagia.

Primary: To compare IN-OXT vs. placebo on changes on the Hyperphagia Questionnaire-Clinical Trials (HQ-CT) from baseline to week 8 in children with PWS. We hypothesize that IN-OXT will be significantly superior to placebo in improving hyperphagia.

Secondary: To compare the change from baseline to week-8 of IN-OXT vs. placebo on:

1. Repetitive Behavior Scale-Revised (RBS-R).
   - We hypothesize that IN-OXT will significantly reduce repetitive behaviors from baseline to endpoint as measured by a reduction of the score on the RBS-R when compared to placebo.
   - We hypothesize that IN-OXT will significantly reduce rigid behaviors from baseline to endpoint as measured by a reduction of the score on the MERS-PWS when compared to placebo.
2. Weight, BMI (z-score) and Body composition via bioelectrical impedance analysis
   - We hypothesize that IN-OXT will significantly reduce weight and BMI compared to placebo.
3. We hypothesize that IN-OXT will significantly reduce body fat compared to placebo, as measured by body composition via bioelectrical impedance analysis
4. Quality of Life Measures
   – World Health Organization Quality of Life Questionnaire (WHOQOL)
     • We hypothesize that IN-OXT will improve quality of life measured by an increase in overall score on the WHOQOL from baseline to endpoint when compared to placebo.

- Caregiver Strain Questionnaire (CSQ)
  - We hypothesize that IN-OXT will improve quality of life measured by a reduction in overall score on the CSQ from baseline to endpoint when compared to placebo.

5. Salivary Oxytocin Concentration
   - We hypothesize that higher concentrations of salivary oxytocin will be correlated with positive changes on each outcome measure, indicating improvement in symptoms.
6. Safety Analyses
   - We hypothesize that IN-OXT will not increase side-effect burden from baseline to endpoint compared to placebo.

Exploratory:

1. To compare IN-OXT vs. placebo on changes in dietary intake using the ASA 24: Automated, Self- Administered, 24 hour Recall diet diary system, as provided by the National Cancer Institute (NCI).
2. To examine the impact of medical co-morbidities on treatment outcome.
3. To examine the relationship between weight-based dosing and treatment response on hyperphagia.
4. To compare IN-OXT vs. placebo on changes in hormone levels at baseline and endpoint. Measured hormones will include ghrelin, pancreatic polypeptide, peptide YY, GLP-1, insulin, glucagon, testosterone and estrogen. We will also look at changes in HbA1C.

Phase 2 Study: Intranasal Oxytocin vs. Placebo for the Treatment of Hyperphagia in Children and Adolescents with Prader-Willi Syndrome

[IND 121109 acknowledged 8/27/14]

BACKGROUND AND SIGNIFICANCE

Prader-Willi Syndrome (PWS) has been designated as a rare disease by the National Institutes of Health and is categorized as such with the NIH Office of Rare Diseases Research. [1]. Inclusion on this list requires a prevalence of less than 200,000 people in the United States. According to the Prader-Willi Syndrome Association USA and a review of the literature, current estimates of the prevalence of PWS range from 1: 8,000 – 1: 30,000 with the most likely prevalence falling around 1 : 15,000 individuals [2-8]. Based on current U.S. Census data there are 317,434,622 people living in the U.S. Using the above prevalence estimates, the prevalence of PWS ranges from 10,581 to 39,679, and the most likely prevalence is approximately 21,000 Americans [9]. Using the most current available data from the U.S. Census (2010), there are 74,181,467 people in the pediatric population in the US (age < 18 years) [10]. Assuming that the prevalence of PWS falls between 1: 8,000 and 1: 30,000, there are currently between 2,473 and 9,273 people under age 18 with PWS. PWS is a rare neurodevelopmental disorder caused by lack of expression of paternally derived imprinted material on chromosome 15q11-q13. Maternal duplications and triplications of the 15q11-q13 region also account for the most frequently observed autosomal abnormalities in idiopathic ASD [11 – 19]. PWS is characterized by mild to moderate intellectual disabilities, repetitive/compulsive behaviors and rigidity, social cognition deficits and severe hypotonia at birth, followed by the onset of hyperphagia later in life.

This study supports the Orphan Product Divisions goal of identifying and promoting the development of treatments indicated for a rare disease or condition. The proposed product is intranasal oxytocin (IN-OXT) (Syntocinon, manufactured by Novartis). The batch certificate of analysis for the product lot manufactured on 9/13/2014 and having an expiration date of 2017 has been submitted to the IND; see letter in appendix). This proposal aims to target symptoms of PWS for which treatment options do not currently exist, and will provide essential data that is needed for product development and eventually product approval. In this Phase 2 trial of IN-OXT we will evaluate the effectiveness of the product for individuals with PWS and determine the common short-term side effects and risks associated with the treatment. We have submitted this protocol to FDA and have received IND 121109. All required documents are on file with the FDA and are included in the appendix of this proposal, including the Certificates of Analysis for both drug and placebo, the product insert and the Container Label Statement.

We propose a three-year randomized, double-blind, placebo-controlled 8-week treatment trial of IN-OXT 16 IU/day in 50 subjects aged 5 to 17 years with PWS. A recent 1 week crossover trial by Jennifer Miller (a consultant on this proposal) using this dose of IN-OXT in PWS demonstrated improvement in hyperphagia, and we discuss her results, as well as our pilot data with this dose below. To allow for optimization, we allow one downward titration to 12 IU if adverse events occur, and one upward titration to 24 IU at week 2 for lack of response on hyperphagia.

We will determine if IN-OXT is effective in improving hyperphagia in PWS as measured by changes on the Hyperphagia Questionnaire-Clinical Trials (HQ-CT). We will also examine secondary outcomes of changes in repetitive behaviors (i.e. skin picking and nail-biting that co-occur with hyperphagia) using the reliable and valid Repetitive Behavior Scale-Revised (RBS-R). We will also measure weight, BMI (z-score) and body composition (via bioelectrical

impedance analysis), quality of life, salivary oxytocin concentration, and safety measures in patients with PWS. PWS has well-known genetic targets and documented structural and functional lesions of the OXT neurons of the PVN. PWS is a unique and relatively genetically homogenous syndrome. Furthermore, IN-OXT has a favorable side effect profile and will target core symptoms for which there are no effective available treatments. Previous studies of IN-OXT treatment for PWS had several limitations in study design which our group has improved upon, as noted above. Based on Jennifer Miller's positive short-term trial of IN-OXT for the treatment of hyperphagia in PWS, we have opted to use a dose of 16 IU/day. Her study yielded weight based dosing of 0.2 IU/lb – 0.4 IU/lb. One patient who was underweight (32 lb) had a 0.5 IU/lb weight based dose and experienced temper tantrums. Thus we will not enroll subjects who weigh less than 40lb in order to maintain weight based dosing below 0.4 lb/IU. It is hypothesized that lower doses of IN-OXT may reduce binding to vasopressin receptors, and decrease the risk of the temper tantrum side effect observed in other trials. This is consistent with a recent single-dose crossover trial of IN-OXT in healthy controls with a modified nasal delivery device demonstrated that plasma OXT levels were similar in low-dose and high-dose IN OXT groups and both were greater than in the placebo group, but that the low dose IN-OXT was superior to the high dose IN OXT and to placebo in reducing anger ratings in response to emotionally ambiguous faces [101]. As the two existing trials of IN-OXT in PWS have enrolled both males and females, and the hyperphagia symptoms of PWS are not sexually dimorphic, we intend to enroll both sexes in equal numbers. IN-OXT has been used safely in multiple human studies involving men and women and is well tolerated in both adult and pediatric PWS populations and we expect a similar safety profile in our proposed study.

In selecting an orphan population, PWS, with a known specific genetic locus, and in selecting a treatment, IN- OXT, that specifically matches the underlying deficit in the structure and function of OXT neurons in PWS, we expect that this study will have substantially greater power to detect significant drug vs placebo differences.

Hyperphagia and Obesity in Prader-Willi Syndrome: Behavioral and fMRI studies have demonstrated that compulsive eating in PWS is due to decreased satiety rather than increased hunger. The characteristic behavioral pattern of PWS begins around age four in tandem with the onset of hyperphagia and is characterized by obsession with food, temper tantrums, stubbornness, controlling and manipulating behavior, compulsive behaviors and difficulty with changes in routine [4, 22].These behavioral problems are the most challenging problems in PWS and greatly impact the quality of life of individuals with PWS and their families [23 – 25].

Several studies have determined that the eating behaviors observed in PWS are due to decreased satiety rather than increased hunger [33, 34]. Functional magnetic resonance imaging data has revealed that after eating a meal, PWS subjects display hyperfunctionality in the limbic and paralimbic reward regions (amygdala, NAc) that drive eating behavior, and in regions that suppress food intake such as the medial prefrontal cortex. They also had lower activity in the hypothalamus and hippocampus in response to food [38, 39]. These results further implicate the failure of satiety that is observed in PWS and suggest an abnormal food motivation phenotype that involves hyperactivation of the subcortical reward circuitry and hypoactivation in cortical inhibitory regions resulting in compulsive eating. Obesity secondary to the disordered eating behaviors that characterize the PWS phenotype also contributes to the increased morbidity and mortality of the syndrome.

Obesity-related cardiovascular complications and sleep apnea are among the most common causes of death in adults with PWS. Additionally, diabetes mellitus secondary to disordered eating and obesity is common [27]. The mortality rate in PWS is up to six times higher than in

IQ matched controls, with obesity being the main factor [28].

There is conflicting evidence relating to the levels of hormones responsible for feelings of satiety and hunger in PWS. Comparisons of PWS patients to BMI and adiposity-matched obese patients demonstrated similar responses of gut hormones fasting and post-prandial, aside from overall increased levels of ghrelin [36]. It is believed that the increased levels of ghrelin could be responsible for increased hunger, despite high levels of postprandial satiety in PWS patients. Other studies have demonstrated increased leptin levels, and decreased fasting pancreatic polypeptide levels in PWS patients compared to BMI matched patients [37]. More recently, ghrelin concentrations were measured across nutritional phases in a 12-year longitudinal study of individuals with PWS (5 weeks old to 21 years old), individuals with early onset obesity of unknown origin and non-obese sibling controls. They concluded that ghrelin concentrations are significantly higher in early nutritional phases of PWS long before the onset of hyperphagia and especially during phases of poor appetite and feeding. Thus it is possible that high ghrelin concentrations do not contribute to the hyperphagic nutritional switch seen in PWS [102].

Clearly there are multiple neuropeptide and neurotransmitter mechanisms that can contribute to obesity and the onset of hyperphagia in PWS. In order to collect more information about these mechanisms we will measure ghrelin concentrations, as well as pancreatic polypeptide, peptide YY, GLP-1, insulin, glucagon, testosterone and estrogen, and HbA1C at baseline and endpoint to observe potential effects of IN-OXT treatment as it relates to both hyperphagia and the concentration of ghrelin and these other measures.

Proposed Mechanism of Oxytocin In Prader-Willi Syndrome, Hyperphagia And Obesity

Pathophysiology: Dysfunction of the oxytocin (OXT) system is implicated in the pathophysiology of PWS. Oxytocin (OXT) is a nonapeptide synthesized in the paraventricular (PVN) and supraoptic nuclei (SON) of the hypothalamus. It is proposed to play a role in social recognition, pair bonding, anxiety, maternal behaviors, metabolism, food motivation and hyperphagia. OXT receptors are expressed in numerous brain regions including the amygdala, ventromedial hypothalamus, and the brainstem. The number and size of OXT neurons in the PVN is significantly reduced in PWS [29;45].

Animal Models: Faulty OXT signaling is also associated with obesity, which is a large issue in the PWS population. OXT's role in obesity has been studied in mouse models. For example, OXT supplementation is demonstrated to reduce food intake and body weight in a mouse model of diet-induced obesity [26;46]. Mice with haploinsufficiency of SIM1, a gene essential for the formation of the PVN, are characterized by hyperphagic obesity, similar to patients with PWS, and reduced OXT and melanocortin-4 receptors [46]. When OXT was administered to these mice, their food intake decreased and they lost weight in excess of what could be explained by decreased food intake alone [46]. Thus, peripheral administration of OXT has been shown to treat obesity by reducing food intake and visceral fat mass in a mouse model [26]. In addition, neuronal c-Fos expression, a marker for cell proliferation, differentiation and survival, increases in the NTS and AP in response to peripheral OXT. The nucleus of the solitary tract (NTS) and area postrema (AP) are hindbrain areas heavily involved in the processing of satiety signals.

In early studies of hypothalamic function, lesions of oxytocin containing hypothalamic nuclei were shown to result in an increase in food intake and body weight [81-84]. In the 1990s, several studies reported on the anorexigenic effects of central oxytocin. Low doses of oxytocin given intracerebroventricular (ICV) dose- dependently inhibited food intake in rats, increased

the latency to begin feeding and reduced meal duration in both hungry and satiated animals. These actions could also be blocked by oxytocin receptor antagonists [85;86]. Longer term central infusions of oxytocin were also reported to reduce body weight gain in rats given a high-fat diet. However, in contrast to oxytocin's acute effects, chronic oxytocin infusions did not alter total food intake or meal patterning, but instead appeared to stimulate lipid metabolism in adipose tissue [88]. This suggests that similar to the effect of LH/FSH in inducing puberty, a pulsatile release of oxytocin may be preferable than a chronic infusion administration. Additionally, both male and female oxytocin-knockout mice show an elevation in body weight and fat stores in adulthood, but as with oxytocin receptor-deficient mice, this is not due to an increase in food intake and is solely related to the lack of oxytocin receptors [87]. While there have been no published reports of humans that completely lack either oxytocin or its receptor, most likely due to the fact that the absence of oxytocin or its receptor is incompatible with successful reproduction, a partial deficiency in central oxytocin production has been associated with the development of obesity in humans in two documented conditions.

Data from Studies in Humans: As shown above, oxytocin and its role in obesity and food motivation have been repeatedly investigated at the animal model level, but in humans it has only been studied limitedly, despite many models that demonstrate its role in these processes. Fortunately, studies are currently underway to study the effect of oxytocin on food motivation in humans [78:NCT02276677]. Additionally, a recent study of healthy volunteers [79] showed that IN-OXT reduced snack intake by 25%; attenuated basal and postprandial levels of adrenocorticotropic hormone and cortisol; and curbed post-meal glucose elevation. Although data on oxytocin as a treatment for hyperphagia in humans is limited, one study in human subjects with PWS demonstrated that 45% of those on IN-OXT vs. 10% of those on placebo had reduced hunger and decreased food intake (OR = 7.85) after a single dose of 24 IU IN-OXT [47]

Data from a Similar Disorder: Autism Spectrum Disorder (ASD): In addition to PWS, OXT has also been implicated in the pathophysiology of ASD, and multiple clinical trials of IN-OXT in this population have been completed as a result. While trials have differed in age range, outcome measures, dose of OXT and some studies have yielded mixed results, in general over different age ranges and with different outcome measures, most findings suggest some improvement of repetitive behavior (especially lower order repetitive behaviors) and social cognition with IN-OXT in ASD. Abnormalities in the neural pathways for OXT contribute to the behavioral manifestations of ASD [41]. Treatment with intranasal oxytocin (IN-OXT) vs. placebo in ASD populations has resulted in improvement in repetitive behaviors and social information processing deficits that are characteristic of PWS, in particular improvement was observed in the lower order self-stimulatory behaviors [42-44].

A recent double-blind, placebo-controlled, parallel design pilot study of 24 IU BID IN-OXT vs. placebo in 19 adults with ASD showed significant improvement on measures of repetitive behaviors, social cognition and quality of life with IN-OXT when compared to placebo [44]. Safety data showed no serious adverse effects, with mild to moderate irritability, nasal congestion/allergy symptoms, headache and increased energy reported when the patient was taking IN-OXT. The study included women (3/19) and after evaluating distributions to assess the need for data transformations, no statistical evidence of distributional concerns were found, therefore, no transformations were made [44]. Previous studies have shown a reduction in repetitive behaviors and an increased ability to assign emotion to speech intonation with titrated intravenous oxytocin in adults with ASD [42, 43], in addition to improved empathic accuracy in healthy volunteers with high scores on the Autism Quotient scale with 24 IU of IN-OXT [41].

The safety and efficacy of IN-OXT has also been demonstrated in children and adolescents with ASD. Adolescents with ASD treated with open label IN-OXT with 2 month stepwise dosage increases (8, 16, 24 IU) showed improvement on communication and social interaction domains of the Autism Diagnostic Observation Schedule (ADOS) and trends in improvement on the social withdrawal scale of the Aberrant Behavior Checklist (ABC) and the delinquent behavior scale of the Child Behavior Checklist (CBCL). As in previous studies there were no serious adverse effects or impairments in short-term memory. This is one of the few studies demonstrating the safety of long term administration (6 months) of IN-OXT [54]. In a similar study, males aged 12 to 19 years with ASD or Asperger's Disorder received a single challenge of IN-OXT (18 IU or 24 IU) one week apart. IN-OXT was noted to improve performance on the Reading the Mind in the Eyes Test (RMET), with a highly significant effect on the easy items of the assessment [55]. As seen in prior studies there was no difference across sessions with regard to drug identification or in the number of reported side effects. Side effects from IN-OXT included feeling tired and relaxed, and sweating, while those with placebo also reported feeling tired and relaxed, in addition to headaches and coughing. A recent negative trial of IN-OXT in male children aged 7 to 16 years looked at the effect of a 24 IU or 12 IU dose over the course of 5 days combined with Parent-Child Interaction Therapy (PCIT). Although there was no separation between placebo and IN- OXT, minimal side effects were observed, further demonstrating the safety of IN-OXT younger populations [56].

Previous Studies Of Intranasal Oxytocin In Prader-Willi Syndrome And Their Limitations: The first study showed that a single low dose of 24 IU IN-OXT vs. placebo in 24 adult patients with PWS (16 female and 8 male) had significant effects on reducing disruptive behaviors using a clinician-rated behavior grid (d = 0.72), and on increasing trust. IN-OXT was determined to be safe and effective. Additionally, although not statistically significant, 45% of those on IN-OXT vs. 10% of those on placebo had reduced hunger and decreased food intake (OR = 7.85). However, this study only involved a single administration of IN-OXT and did not employ any standardized measure to monitor food intake or behavioral measures [47].

The second study [72] enrolled 30 subjects aged 12 – 30 years (20 males and 10 females) in an 18-week, randomized, double-blind, placebo-controlled crossover trial. 11 subjects received 24 IU IN-OXT BID (16 years and older) or 18 IU BID (13 – 15 years). 18 subjects received 40 IU BID (16 years and older) or 32 IU BID (1315 years). The study found little impact on any of its outcome measures (Developmental Behavior Checklist Monitor, Parent, Teacher, and Adult; Yale-Brown Obsessive Compulsive Scale; The Dykens Hyperphagia questionnaire; Reading the Mind in the Eyes Test; Epworth Sleepiness Scale and the Movie Stills). However, there was an increase in the number of temper tantrums (p = 0.023) on the higher doses of IN-OXT (40 and 32 IU), which could have been due to increased binding of oxytocin to V1a receptors and have resulted in increased aggression. This could also be bias against finding improvement on other behavioral measures.

Jennifer Miller recently completed a double-blind, placebo-controlled crossover study of IN-OXT vs. Pbo in 24 children with PWS. Children were randomized to receive 5 days of either IN-OXT followed by placebo, or vice versa, with a minimum 4-week washout between the treatment periods. All children were in the Phase 2b or Phase 3 nutritional phase. Results revealed that all scales factor improvement from Day 3 to Day 6 favored oxytocin over placebo. In addition, after 5 days of treatment with IN-OXT, there was a 2.5 point decrease (i.e., improvement) on the Hyperphagia Questionnaire compared to a 1.6 point decrease on placebo. In addition, improvements were demonstrated on IN-OXT versus placebo on the ABC Factors of lethargy/social withdrawal, inappropriate speech, irritability, stereotypical behaviors and hyperactivity (107). Another recent multicenter trial using the oxytocin analogue carbetocin

conducted by Ferring Pharmaceuticals also demonstrated improvement in hyperphagia in PWS using the Revised Hyperphagia Scale [Personal Communication, July 2015].
Aside from Dr. Miller's study, these previous studies of IN-OXT treatment for PWS had several limitations which our group intends to improve upon. Firstly, the studies had small sample sizes. One was a pilot study and not powered to find statistically significant changes between treatment vs. placebo groups. Secondly, only a single dose of IN-OXT was administered and no standardized measures of hyperphagia or other outcomes were used. The second study found no separation between treatment with placebo vs. IN-OXT at high doses on the Dykens Hyperphagia Questionnaire or other outcome measures. However, this study was small (n = 30) and the dose of IN-OXT may have been too high and have worked against detecting a signal for the reasons noted above. Dr. Miller's study was also a small sample size and only dosed patients for 1-week with IN-OXT. Both she, and we, agree that further studies are necessary to look at long term effects of IN-OXT in PWS and to replicate findings at this dose of OXT.

In our currently blinded and ongoing pilot study of 16IU/day intranasal oxytocin vs. placebo, sponsored by the Foundation for Prader-Willi Research, we have enrolled 12 and randomized 9 patients with PWS over the last 3 months. Our recruitment goal is 24 patients. Thus far we have observed minimal adverse events, with only one, polyuria, possibly related to study drug. There have been no serious adverse events. We have also noted positive reports of hyperphagia, compulsivity and other behaviors (below).

Oxytocin and Vasopressin Effects and Evidence Supporting Efficacy of Low Dose Oxytocin: Vasopressin and oxytocin are molecularly similar and there is some overlap in receptor occupancy. As vasopressin (V1a) acts to increase behavioral activation and can lead to aggression and irritability, occupation and thus activation of vasopressin receptors by oxytocin, could theoretically lead to increased aggression and irritability. Increased aggression and irritability could be exacerbated in individuals with PWS who are known to have genetic lesions of the oxytocin system. This also raises issues over ligand selectivity [90]. Oxytocin and vasopressin are structurally related analogs that differ by only two amino acids and share an evolutionary history. They also lack absolute receptor subtype selectivity and their receptor subtype selectivity is always relative and concentration dependent. Selectivity may be defined on the basis of the binding affinity of the ligand. To be ''selective'' on one receptor subtype, a ligand's affinity constant on the other receptor subtypes should be at least two orders of magnitude higher. Thus, we posit that the increase in the number of temper outbursts observed in the Einfeld study [72] could have been caused by ligand promiscuity. Higher concentrations of oxytocin may have overwhelmed the OXT-R subtype and spilled over to bind with vasopressin receptors, an event that could have led to the observed increased aggression and irritability. This is particularly likely in the PWS population, a population known to have abnormal oxytocin production. Using the lower dose of 16 IU qd in our study replicates the dose that yielded positive findings in children with PWS by Jennifer Miller and addresses this issue. Converging biological and behavioral evidence suggests that lower OXT doses may be more efficacious than higher doses. For instance, compared with higher doses, lower doses increased peripheral levels of OXT in saliva [65] attenuated cortisol stress responses [66] and increased eye gaze in patients with Fragile X syndrome [67]. In animals, a low dose of OT administered shortly after birth increased partner preference later in life, whereas higher doses did not [68]. Similarly, lower doses have been associated with stronger increases in social recognition compared with higher doses [69,70]. The dose–response data reported for reduction of anger in response to ambiguous faces in healthy controls in low dose vs high dose IN OXT also supports the selected dose of the study.

Gender Effects of Oxytocin: Justification for Inclusion of Both Genders: While IN-OXT

has been shown to have a sexually dimorphic effect on social cognition measures, we do not believe this will be the case for the primary outcome of changes in hyperphagia on the Dykens Hyperphagia Questionnaire. PWS is not a sexually dimorphic disorder and all patients are equally affected by hyperphagia symptoms and weight gain regardless of sex. Including both genders in this study is a strength of our proposal. Additionally, data on the use of IN- OXT in females is needed. Families are already trying this treatment off-label and the evaluation of the use of IN-OXT in females, in any disorder, is lacking. Although it is not our primary outcome measure, it is also important to assess for gender effects on social cognition, which is deficient in individuals with PWS. Animal models have shown that low doses of OXT seem to enhance social recognition, [91] in both female and male rats. Although, previous studies have shown sexually dimorphic effects on social cognition tasks with IN-OXT administration [77], and further data is needed regarding this gender split in syndromes such as PWS. Nevertheless, it is unlikely that the same gender effects will be observed in our primary outcome measuring hyperphagia.

Summary: Given the limited number of trials, the lack of sufficient information about IN-OXT's effects on hyperphagia, and the conflicting results with higher doses, more information is needed to determine whether IN-OXT is a safe and effective treatment for hyperphagia in PWS. Administering IN-OXT in childhood could slow or prevent the onset of obesity and reduce its long-term sequela. In this study, we will determine if IN- OXT reduces hyperphagia in children with PWS as assessed by changes on the PWS specific Revised Dykens Hyperphagia Questionnaire. Secondary outcomes of changes in repetitive behaviors, weight and body composition via bioelectrical impedance analysis, quality of life measures, salivary oxytocin concentration, and safety measures will also be assessed. IN-OXT is a promising treatment for hyperphagia and other symptom domains of PWS, such as repetitive behaviors.

INNOVATION

This proposal shifts current drug development paradigms by selecting an orphan population, PWS, characterized by a known genetic and neuropathological mechanism (disruption of chromosome 15q11-q13 and a decreased of number and size of OXT-producing neurons in the PVN nuclei of the hypothalamus), and matching an available well tolerated treatment, IN-OXT, with a mechanism that targets the specific pathophysiology of the condition (faulty OXT signaling) to a specific homogeneous disorder. This novel drug development approach provides a substantially greater chance of detecting a drug vs. placebo signal in a more targeted and homogeneous population. This approach also targets the core deficits of PWS, disordered eating behaviors, which currently lack effective therapeutic interventions and contribute to disease burden and caregiver strain.

Our study will improve upon the design from the Einfeld study of IN-OXT [72] in six key parameters.

1. We have lowered the dose in response to the temper tantrums side effect observed, which we believe is a result of excess oxytocin binding to vasopressin receptors. Additionally, a lower dose may have a better impact on hyperphagia issues, as discussed above.
2. The Einfeld study [72] was a crossover design which raises issues regarding carryover and phase order effects, whereas our proposed study is a parallel design study.
3. The Einfeld study [72] sample size was much smaller (n=30). Our sample size is 50 subjects (25 vs 25).
4. Their population was older (ranging from 12 – 30 years), whereas in our proposed population (5 - 17), we have the ability to intervene at an earlier stage of the illness.
5. We only include subjects in PWS nutritional phase 2b or 3, in order to maximize severity of hyperphagia at baseline in our subjects. This will enhance our ability to detect a significant

reduction in hyperphagia for our active treatment vs placebo.

6. We will replicate the dose from the successful trial of Jennifer Miller in childhood PWS, and from our current pilot trial of IN-OXT in patients with PWS.

PRELIMINARY DATA

Our group has extensive experience with IN-OXT for the treatment of ASD in clinical trials. The following study synopses present our findings that IN-OXT is safe, well-tolerated and effective in managing repetitive behaviors and social cognition deficits and improving quality of life for patients and caregivers.

Preliminary Study 1: Feasibility Treatment Study of Intranasal Oxytocin in Adults with ASD:

In a 6-week pilot study, we investigated the effects of IN-OXT on core ASD symptoms [44]. Nineteen high functioning patients meeting criteria for an ASD based on DSM-IV diagnoses and Autism Diagnostic Interview were randomized into IN-OXT 24 IU IN BID (i.e. 3 puffs/nostril twice daily, morning and noon) or placebo. Side effects were minimal. One patient randomized to IN-OXT reported side effects of fatigue and sneezing, which were mild in nature. No additional side effects were recorded for either IN-OXT or placebo subjects. **On primary outcome measures, there were significant differences in performance on the repetitive behavior measure Repetitive Behavior Scale-Revised (RBS-R) lower-order behaviors (t = -2.17, df = 16.78, p = 0.045, Cohen's d effect size = 0.64)** but not on the Diagnostic Analysis of Nonverbal Accuracy (DANVA) or Clinical Global Impressions Severity and Improvement Scales (CGI-S/CGI-I). Although baseline differences were not statistically significant, they were somewhat large and as such the analysis was repeated with baseline added as a covariate. The difference in the RBS-R lower-order behaviors then showed a trend towards significance (t = -1.971, df = 17.18, p = 0.065). For the rest of the measures, co-varying for baseline did not change the conclusions of the analyses. Thirty percent (3/10) of participants in the IN-OXT group were rated as improved on the CGI-I, whereas 11% (1/9) of participants in the placebo group were rated as improved. On secondary measures, significant improvements were noted on the Reading the Mind in the Eyes Test-Revised (RMET-Revised), a social cognition measure, (t = 3.91, df = 9.01, p = 0.004, Cohen's d effect size = 1.2) and the World Health Organization Quality of Life (WHOQOL) emotional subscale (t = 2.42, df = 10.89, p=0.034, Cohen's d effect size = 0.84 (**Table 1**). The results remained significant for the RMET (t = 4.045, df = 10, p = 0.002) and the WHOQOL-emotional (t = 2.43, df = 12.37, p = 0.031) with baseline covariate. Differences did not reach significance on the Social Responsiveness Scale (SRS), Yale-Brown Obsessive Compulsive Scale (YBOCS), and RBS-R higher-order behaviors; however this pilot study in idiopathic ASD was powered to detect only very large effect sizes.

Table 1: Parameter Estimates and p-values From Full-Information Maximum-Likelihood Mixed-Effects Regression Analyses

	Baseline	6-weeks from baseline	p-value	Mean (SD) Week 0	Mean (SD) Week 6	d-value
Social Cognition						
RMET	-	22%				1.2
Oxytocin			0.004	48% (20%)	61% (24%)	
Placebo			0.002*	74% (14%)	63% (12%)	
Repetitive Behaviors						
Primary						
RBS-R lower order	-	-2.25				0.64
Oxytocin			0.045	5.8% (4.6%)	2.4% (2.3%)	
Placebo			0.065*	4.9% (3.7%)	3.7% (2.6%)	
Quality of Life						
WHOQOL– emotional	-	9.5%				0.84
Oxytocin			0.034*	47.8% (16.3%)	59.5% (16.0%)	
Placebo			0.031*	65.2% (12.3%)	63.2% (12.3%)	

*p-values if the baseline is used as a covariant

Preliminary Study 2: Intravenous Oxytocin and Repetitive Behaviors in Adults with ASD: We obtained preliminary support for the therapeutic effects of OXT on repetitive behaviors in ASD in a randomized, double-blind, placebo- controlled crossover challenge in which 15 adults with ASD completed both OXT and placebo challenges on separate days. Participants reported to the General Clinical Research Center where they spent the night before the challenge session; all participants fasted overnight. At 8:00 am participants were awoken and an indwelling intravenous catheter was inserted; vital signs were taken at 8:30 am. At 9:00 am, 6 cc^3 of blood was drawn and participants were randomized in a double-blind fashion to receive synthetic OXT (Pitocin) or placebo via intravenous infusion. The initial vial of Pitocin (10 u/ml), combined aseptically with a 1.0 l bag of normal saline, was first given at a rate of 10 ml/h to minimize potential side effects, and was gradually titrated up to 700 ml/h during the fourth hour. Participants reported the frequency of characteristic repetitive behaviors such as need to know, repetitive language , ordering, need to tell/ask, self-injury, and touching at baseline (0), 60, 120, 180, and 240 minutes. **Results from repeated measures analyses of variance comparing the two infusions (OXT vs. placebo) over the five time points revealed a significant Treatment x Time interaction, *F* = 3.487, *df* = 4, 52, *p* = .027. Frequency of repetitive behaviors was reduced over time following OXT compared to placebo infusion (Figure 1).** Moreover, OXT decreased the total number of different repetitive behaviors. Side effects from OXT were mild and included drowsiness, anxiety, depression, headache, tingling, backache, trembling, restlessness, stomach cramps, and enuresis.

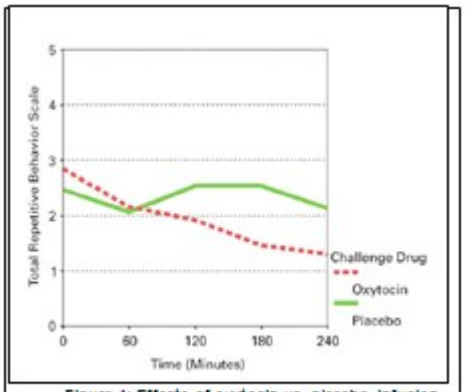

Figure 1: Effects of oxytocin vs. placebo infusion on repetitive behaviors over time.

Preliminary Study 3: Intravenous Oxytocin and Social Cognition in Adults with ASD: We also have preliminary data on the effects of intravenous OXT on aspects of social cognition in adults with ASD [43]. Patients with autism or Asperger's Disorder received an intravenous infusion of OXT or placebo over a 4-hour period and ability to assign affective significance to speech was assessed using the ASR (see above for challenge methods) at baseline, just prior to the infusion, and at 30, 60, 120, 180, and 240 minutes over the course of the infusion. In this task, participants were presented with four pre-recorded sentences of neutral content (e.g., "The boy went to the store"); each sentence was presented with one of four emotional intonations (*happy*, *indifferent*, *angry*, and *sad*), with the pairing of emotional expression and sentences in 1 of 6 counterbalanced orders. Participants were instructed to identify the emotional mood of the speaker. This task turned out to be relatively easy for the adult participants in this study and the findings were negatively skewed. To reduce the negative skew and better balance the difficulty of the task, the outcome measure was scored dichotomously as 1 (*all items correct*) and 0 (*not all items correct*). Results from the mixed regression analysis revealed a significant three-way interaction of Time x Treatment x Order for the dichotomized comprehension of affective speech score (*z* = -2.134, *p* = 0.033, estimate = -0.170). As depicted in **Figure 2,** Subjects

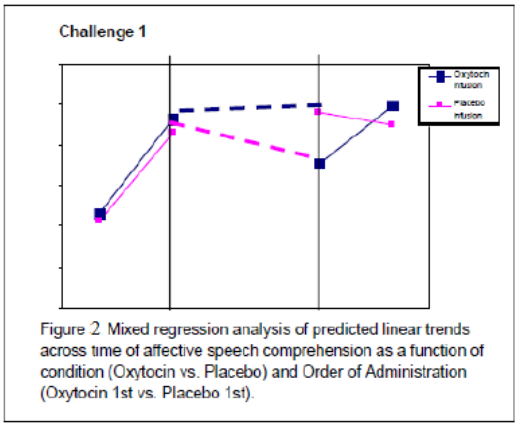

Figure 2 Mixed regression analysis of predicted linear trends across time of affective speech comprehension as a function of condition (Oxytocin vs. Placebo) and Order of Administration (Oxytocin 1st vs. Placebo 1st).

showed pretest to posttest improvement for 3 of the 4 Treatment x Order conditions (i.e. OXT 1st, Placebo 1st, OXT 2nd), whereas for the Placebo 2nd condition, there was a slight drop in comprehension of affective speech from pretest to posttest (0.958 to 0.898). Thus, subjects who received OXT 1st showed increased levels of retention on the task, and did not show a tendency to revert to baseline when retested after a delay, whereas subjects who received placebo 1st tended to revert to baseline after the delay. The difference between predicted pretest scores for subjects who received placebo 2nd (0.958) and placebo 1st (0.712) is 0.246, which corresponds to a medium-large effect size d = 0.66.

Preliminary Study 4: Ongoing Pilot Study of IN-OXT/Placebo in Childhood PWS:
Our current pilot study of an 8-week randomized placebo-controlled double-blind trial of 16 IU/day of IN-OXT in children and adolescents with PWS is ongoing and blinded. To date, over a 3 month time period, we have enrolled 12 pediatric PWS subjects, and randomized 9 subjects. We aim to randomize 24 subjects. Although the data remain blinded at this time, we briefly describe reports of efficacy and side effects observed in the study thus far. The only AE noted in 1 blinded subject was polyuria/nocturia, of mild severity, and possibly related to study drug. Subjects on the blinded 16 IU/day of IN-OXT/placebo dose were noted to have the following responses by parent report to study clinician: 1. improvements in hyperphagia- “for the first time left food on the plate”, “best Thanksgiving ever”, “less asking for food”, “less freak-outs around food”; 2. improvements in compulsivity- “better able to transition and switch to another topic other than food”, “less compulsive questioning”, “less skin picking”, “less need for control”, “less restricted interests in stuffed animals”; and 3. improvements in other behaviors- “less anxiety”, “less tantrums”, “more verbal utterances”,” longer string of words”, and “more back and forth banter”. Of note, the study has not been complicated by unmasking of medical co-morbidities. There have been no tolerability or safety issues to date. Subjects have not required downward titration or had worsening of laboratory parameters to date.

APPROACH
Objective: The overall objective of this Phase 2 trial is to compare the change from baseline to week 8 of IN- OXT on hyperphagia in children with PWS. We have obtained an IND for the use of IN-OXT for PWS (IND 121109) and will utilize IN-OXT and matching placebo (Manufactured by Novartis as Syntocinon - see letter in appendix regarding batch number and certificate of analysis).

We will also compare the effects of IN-OXT from baseline to week-8 on repetitive behaviors (Repetitive Behavior Scale-Revised - RBS-R), weight, BMI (z-score), and body composition via bioelectrical impedance analysis, quality of life (World Health Organization Quality of Life scale – WHOQOL and Caregiver Strain Questionnaire -CSQ), salivary oxytocin concentration, and safety measures. Safety will be assessed with monitoring of routine laboratory tests and levels of testosterone and estrogen, physical exam, urine pregnancy test for menstruating females, and collection of vital signs. The Aberrant Behavior Checklist (ABC) will also be used as a safety measure for both suicidality (per FDA guidelines) and for adverse events (aggression/temper tantrums). In addition, we will monitor measures of hypogonadism and sleep apnea throughout the study. This Phase 2 study is designed to generate preliminary data for future Phase 3 trials, and targets symptoms in this population that currently have no effective treatments. By treating these symptoms earlier in life in a pediatric PWS population we may maximize the treatment impact.

This study supports the Orphan Product Divisions goal of identifying and promoting the development of treatments indicated for rare diseases or conditions. Our proposed treatment

will target hyperphagia in subjects with PWS, a symptom for which there is currently no effective treatment. This study will provide essential data that is needed for drug development and eventually drug approval.

SPECIFIC AIMS

Primary: To compare IN-OXT vs. placebo on changes on the Hyperphagia Questionnaire-Clinical Trials (HQ-CT) from baseline to week 8 in children with PWS. We hypothesize that IN-OXT will be significantly superior to placebo in improving hyperphagia.

Secondary: To compare the change from baseline to week-8 of IN-OXT vs. placebo on:

1. Repetitive Behavior Scale-Revised (RBS-R).
   - We hypothesize that IN-OXT will significantly reduce repetitive behaviors from baseline to endpoint as measured by a reduction of the score on the RBS-R when compared to placebo.
   - We hypothesize that IN-OXT will significantly reduce rigid behaviors from baseline to endpoint as measured by a reduction of the score on the MERS-PWS when compared to placebo.
2. Weight, BMI (z-score) and Body composition via bioelectrical impedance analysis
   - We hypothesize that IN-OXT will significantly reduce weight and BMI compared to placebo.
   - We hypothesize that IN-OXT will significantly reduce body fat compared to placebo, as measured by body composition via bioelectrical impedance analysis.
3. Quality of Life Measures
   - We hypothesize that IN-OXT will improve quality of life measured by an increase in overall score on the WHOQOL from baseline to endpoint when compared to placebo.
   - We hypothesize that IN-OXT will improve quality of life measured by a reduction in overall score on the CSQ from baseline to endpoint when compared to placebo.
4. Salivary Oxytocin Concentration
   - We hypothesize that higher concentrations of salivary oxytocin will be correlated with positive changes on each outcome measure, indicating improvement in symptoms.
5. Safety Analyses
   - We hypothesize that IN-OXT will not increase side-effect burden from baseline to endpoint compared to placebo.

Exploratory:

1. To compare IN-OXT vs. placebo on changes in dietary intake using the ASA 24: Automated, Self- Administered, 24 hour Recall diet diary system, as provided by the National Cancer Institute (NCI).
2. To examine the impact of medical co-morbidities on treatment outcome.
3. To examine the relationship between weight-based dosing and treatment response on hyperphagia.
4. To compare IN-OXT vs. placebo on changes in hormone levels at baseline and endpoint. Measured hormones will include ghrelin, pancreatic polypeptide, peptide YY, GLP-1, insulin, glucagon, testosterone and estrogen. We will also look at changes in HbA1C.

Research Strategy And Feasibility: We propose a three year, phase 2, randomized double-blinded 8-week treatment trial of IN-OXT vs. placebo in 50 child and adolescent subjects (25 male and 25 female) aged 5 to 17 years with a diagnosis of PWS. PWS diagnosis will be confirmed by study team via patient interview and confirmation by patient's past medical

records and genetic testing results. At the screening visit, patient will have the opportunity to learn about the study and seek clarification on any outstanding questions. If patient voluntarily agree to participate, study team will work with the patients to complete the informed consent and if applicable, assent. In addition, a psychiatric interview and medical history review will be conducted to screen the patient for eligibility. The study team will also review patient's concomitant medications, assess for patient condition with CGI-S and hyperphagia using the Hyperphagia Questionnaire-Clinical Trials (HQ-CT). If patient meets selection criteria, the study team will arrange on behalf of the patient for an out-patient Quest visit, at which time patient will be asked for a screening blood draw to provide information on CBC, BMP, LFT, IGF-1, thyroid panel, lipid panel, testosterone, estrogen, hemoglobin A1C, and for applicable patient, a urine pregnancy test. Once the screening blood work results are available and all results are within acceptable range, and patient is otherwise eligible, patient will be invited for an in person visit to clinic where additional assessments including a complete physical and neurological exam, intelligence test, interval health or medication change, and if applicable, repeat urine pregnancy test will be conducted. If patient continues to meet all study inclusion and exclusion criteria and agrees to continue with study participation, the study team will formally submit the patient for randomization. The randomization is done in a 1:1 placebo to oxytocin ratio and stratified for gender. The result is blinded to the study team and the process is performed by the study pharmacist at the Clinical Research Pharmacy using a computer generated randomization table. Patients will receive a supply of oxytocin spray bottle with each spray dispensing 4 IU, or a matching placebo. The participant will start with an initial dose of 2 sprays per nostril (4 sprays per day), or 16 IU/day IN-OXT or placebo. To allow for optimization for adverse events or lack of response, we allow for one downward titration to 12 IU (3 sprays per day), and one upward titration to 24 IU (6 sprays per day) at week 2. This will also allow us to examine the relationship between weight based dosing and treatment response. Syntocinon (synthetic intranasal oxytocin) and matched placebo made by Novartis will be used in this protocol. We have an IND for the use of IN-OXT in PWS (IND 121109).

Study Intervention: OXT and placebo will be administered intranasally. There have been multiple trials of IN- OXT in children, adolescents and adults with ASD, and related genetic disorders with ASD features, such as PWS. To date, no trials of IN-OXT in these populations have recorded any serious adverse effects and the treatment has been well tolerated. We are choosing an initial dose of 16 IU/day, as used in Dr. Jennifer Miller's recent successful trial and our current ongoing pilot trial of IN-OXT vs. placebo in children with PWS.

Inclusion Criteria:

1. Male or female pediatric outpatients aged 5 to 17 years.
2. Must be in PWS nutritional phase 2b or 3 as determined by PI.
3. Must be on growth hormone treatment and have been receiving stable dose of growth hormone treatment for at least three months prior to screening date. Treatment cannot have been interrupted for more than one week within 3 months of screening.
4. Diagnosis of PWS confirmed by exam, genetic testing and patient medical records.
5. A score of at least moderate severity on the Hyperphagia Questionnaire-CT at both screening and baseline visits
6. Stable dosages of hormone treatments (including testosterone and estrogen supplements) for 4 weeks prior to randomization and for the duration of the study.
7. Stable dosages of metabolic treatments that could affect appetite (including metformin)

for 4 weeks prior to randomization and for the duration of the study.
8. Stable pharmacologic, educational, behavioral and/or dietary interventions for 4 weeks prior to randomization and for the duration of the study.
9. Physical exam and laboratory results that are within the normal range for individuals with PWS.
10. Presence of a parent/caregiver/guardian that is able to consent for their participation and complete assessments regarding the child's development and behavioral change throughout the study.

Exclusion Criteria:
1. Exposure to any investigational agent in the 30 days prior to randomization
2. Child not receiving growth hormone treatment.
3. Children weighing less than 40 lbs.
4. Children with unstable Type 2 Diabetes confirmed by Hemoglobin A1C level >=6.5% at screening.
5. Children with unstable medical co-morbidities at baseline.
6. Children with active upper respiratory infections at screening.
7. A primary psychiatric diagnosis other than ASD, including bipolar disorder, psychosis, schizophrenia, PTSD or MDD. These patients will be excluded due to potential confounding results.
8. Pregnant or lactating patients or patients who will not agree to use a double barrier method of contraception. IN-OXT has not been studied in pregnant or lactating women.
9. Females using an estrogen-based contraceptive. As an alternative to an estrogen based contraceptive, subjects will be counseled to use progesterone-based contraceptives; cervical caps; cervical sponges; or spermicidal foam in combination with a condom. Subjects will need to use a double barrier method to be in the study.
10. A medical condition that might interfere with the conduct of the study, confound interpretation of study results or endanger the subject's well-being.
11. A known diagnosis of Rett's Syndrome or Childhood Disintegrative Disorder or marked sensory impairment such as deafness or blindness.
12. Subjects who have had changes in allied health therapies, behavioral or educational interventions within four weeks prior to randomization other than those associated with school holidays.
13. Subjects who have had changes in medications or medication doses of risperidone, aripiprazole, other antipsychotic medications, clonidine, guanfacine, stimulants or anti-convulsants within four weeks of randomization.

Titration Plan and Schedule: If side effects occur, the medical monitor, PI and co-I's will determine if and when to titrate based on severity of the adverse event and relation to the study drug. If there is a significant exacerbation of known PWS co-morbidities, including Type 2 diabetes, sleep apnea or hypogonadism, or any other adverse event rated by the study physician as severe, a clinical determination will be made by the medical monitor, PI and co-I's, as to the patient's eligibility to continue in the study, or to follow the titration schedule, as described below. Both co-investigators, Dr. Rubina Heptulla and Dr. Judith Wylie-Rosett, have extensive expertise in co-morbidities associated with PWS, as does consultant Dr. Moris Angulo.

Titration Schedule:

Adverse events: Study physician will review with study participants regarding the nature,

duration and severity of noted adverse events. The study physician will make clinical judgements on the relationship between reported adverse events with study medication. If there is a high likelihood of adverse events being related drug effects, the subject will titrate down from 16 IU/day (4 sprays per day) to 12 IU/day (3 sprays per day) and will be reassessed in one week). At the next assessment the side effects and tolerability will again be reviewed by the PI and medical monitor. If the side effects have resolved, the subject will be titrated back up to 16 IU/day. If the side effects persist, the subject may continue on the 12 IU/day for another week; or will be discontinued from the study at the judgement of the PI. Subjects who do not receive the full dose by week four will be discontinued. Any subject who is discontinued from the study will be referred for continued medical follow up.

Lack of response: One upward titration will be allowed at week 2 to 24 IU/day (4 sprays per day) if study clinician, with consultation of the PI, determines that study subject experiences no response on hyperphagia via a categorical yes/no determination, and has no adverse events. In our current ongoing pilot study, and in Jennifer Miller's study, the initial hyperphagia response was observed at the week 1 and week 2 ratings.

Instructions for Dosing: Once the cap has been removed from the spray, the device should be held upright to the nostril and the actuator depressed. The patient should be in the sitting position and should be instructed to inhale gently through the nose while the actuator is being depressed. The head should be tilted back once the actuator is depressed to ensure that no fluid runs out of the nose. Parents may assist the child with dosing.

Outcome Measures: Project outcomes will be determined by changes in scores of parent and clinician rated measures from baseline to week 8 and comparisons of week 8 measures between active treatment and placebo groups. Assessments will be administered according to the schedule of events in **Table 2.**

Safety monitoring will be completed at each visit in both remote and in-clinic settings. Participant will complete concomitant medication review, adverse event (AE) monitoring, ABC rating, and be monitored for hypogonadism, sleep apnea and lethargy, In addition, a complete physical and neurological exam and full panels of clinical labs (CBC, BMP, LFT, IGF-1, thyroid panel, lipid panel, HbA1C, testosterone, estrogen, growth hormone, ghrelin, pancreatic polypeptide, peptide YY, leptin, GLP-1, insulin and glucagon) will be completed at baseline and week 8 in person visits.

For participants who are able to provide salivary samples and co-operate with the collection process, the study team will attempt to obtain pre- and 1 hour post- study IP administration saliva at baseline, week4, and week 8 using the Oragene-Discover collection kit (ORG-500). The salivary samples will be used to measure patient oxytocin levels.

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As required by the Division of Psychiatry Products and suggested by FDA review, we have also included the ABC-I subscale at every visit as a prospective assessment for suicidal ideation.The ABC will also be used to assess for adverse events, including increased aggression or temper tantrums. Urine pregnancy tests will be completed at every visit to ensure subject safety. If a subject becomes pregnant during the study, she will immediately be discontinued from the medication. All subjects must use abstinence or a valid method of non-estrogen based double barrier contraception to participate in the study.

While some of these secondary measures have not been studied in PWS, we believe that they

are appropriate for this population as a valid measure of treatment efficacy and focus on important target issues of PWS – including repetitive behaviors (Repetitive Behavior Scale-Revised). The WHOQOL is a validated measure used to assess quality of life and has been used in a vast array of clinical trials in varied disorders (muscular dystrophy, hypertension, tuberculosis, etc.). The Caregiver Strain questionnaire (CSQ) is a commonly used and well-validated self-report questionnaire developed to assess caregiver strain for families with a child living with an emotional or behavioral disorder. The ABC has been used to assess disruptive behaviors in other disorders such as Down Syndrome and other intellectual disabilities

Study Time Commitment and Reduction of Burden: Every effort to reduce the time burden on patients and caregivers has been made. In the era of COVID-19 pandemic and travel restriction, we are implementing telemedicine for remote visits in some of the study visits to reduce patient's potential exposure and travel, while ensure adequate study monitoring are in place.

The HQ-CT, ABC, WHOQOL, CSQ, MERS-PWS and RBS-R assessments are completed with the parent and not the patient, as specified in the schedule of events. As is the case in most clinical trials, the most significant burden to the parent and child is the screening visit, which is done remotely and avoided participant's need to travel. Each parent questionnaire takes between 5 and 25 minutes to complete and child assessments are kept to a minimum, with optional breaks to maximize patient comfort. Screening lab work will be done locally at participants nearest Quest lab. This is particularly beneficial for out of state participants. Clinical lab works are collected only at in person visits at baseline and week 8 during in person clinic visits. We have eliminated the eye- tracking measures as they are not central to our hyperphagia and repetitive behavior aims and to reduce burden. This ensures minimal burden to the family and decreases the time commitment while reducing testing bias on measures. As parent rated measures are questionnaires, no repeat testing bias is expected.

The time commitment and study burden is similar to other current studies in this population and the ASD population. If needed, visits can be split into two visits. Parents may prefer to attend a visit by themselves without the child to complete their measures. Visits at screening, weeks 2, 4, 6, and 10 (follow-up) are remote visits done via tele-medicine. The study clinicians will complete the video conference call with the parent. This will further reduce the family's burden and decrease the amount of times they need to travel to the study site. As much flexibility in scheduling as necessary will be given to the patients and families. Reimbursement for time and travel is provided at each visit ($150 – in-person; $30 – phone visit). Additional reimbursement for travel will be provided as needed for families traveling long distances. The average time needed for parents, child and clinician to complete the assessments is detailed below. Details about which measures are included in each visit can be seen in **Table 2: Schedule of Events.** Some assessments require the participation of both the parent and child and times reflect how long each individual is needed independent of combined measures. Families may differ in how long it takes them to complete certain measures and the times do not include any breaks for meals or walking between sites at the clinic.

Screening Visit (remote): 3-4 hours
Baseline Visit (in-person): 4-5 hours
Week 2 and Week 6 (remote): 30 minutes
Week 4 (remote): 2-3 hours
Week 8 (in-person): 4-5 hours
Week 10 Follow Up (remote): 2-3 hours

PRIMARY OUTCOME:

Hyperphagia Questionnaire-Clinical Trials (HQ-CT): The Hyperphagia Questionnaire-Clinical Trials (HQ-CT) is the standard for assessing how potential therapies may impact hyperphagia in PWS clinical trials. It is a caregiver-rated assessment comprised of 9-items that measure and track the food seeking behaviors and drive for food that is associated with PWS. The HQ-CT is a modified version of the Dykens-Hyperphagia Questionnaire (a well-established questionnaire), with changes based on industry and regulatory standards (e.g., Food and Drug Administration, 2009). These modifications included a reduced recall period, optimization of response scales, and limiting item content to observable behaviors that have the potential to change during the course of a clinical trial. Psychometric evaluation demonstrated the HQ-CT's reliability and validity, and the HQ-CT total score has demonstrated improvements in hyperphagia-related behavior between treatment groups in children with PWS. The HQ-CT will be administered at screening, baseline, and at all subsequent visits. In order to be eligible to participate in the trial, scores on the HQ-CT will need to be at least of moderate severity at both screening and baseline visits. It will take about 15 minutes to complete.

SECONDARY OUTCOME MEASURES:

Repetitive Behavior Scale-Revised (RBS-R): The RBS-R is a 44-item self-report questionnaire that is used to measure the breadth or repetitive behavior in children, adolescents and adults with PWS. The RBS-R provides a quantitative, continuous measure of the full spectrum of repetitive behaviors. The RBS-R consists of six subscales including: Stereotyped Behavior, Self-injurious Behavior, Compulsive Behavior, Routine Behavior, Sameness Behavior, and Restricted Behavior that have no overlap of item content. This permits differential identification and scoring of discrete varieties of repetitive behaviors. Participants are asked to read a list of behaviors and choose a score that best describes how much of a problem the behavior has been of the last month. Behaviors are rated on a 4-point scale: 0 - behavior does not occur, 1 - behavior occurs and is a mild problem, 2-behavior occurs and is a moderate problem, 3 - behavior occurs and is a severe problem. On the last question, participants are asked to "lump together" all of the behaviors described in the questionnaire and provide a rating for how much of a problem these repetitive behaviors are overall on a scale from 1 – 100. 1-Not a problem at all to 100-as bad as you can imagine. The RBS-R measures repetitive behaviors that are specific to PWS symptomatology. It has been used in previous trials of oxytocin in adults with success, as seen in our preliminary data. It is completed by the parents at baseline and weeks 4 and 8 and takes approximately 25 minutes to complete.

Montefiore-Einstein Rigidity Scale-Revised PWS (MERS-PWS): The Montefiore-Einstein Rigidity Scale-Revised (MERS-PWS) is designed to assess three domains of rigid behavior in children and adults with PWS: 1. Behavioral Rigidity (e.g., insistence on sameness, things must be done in his/her way, etc.) 2. Cognitive Rigidity (e.g., special interests, inflexible adherence to rules, etc.) 3. Protest (in response to deviation from rigidity; e.g., verbal objection, tantrum, physical aggression) The MERS-PWS is a clinician-rated scale in that will be completed at baseline, week 4, week 8 and follow up and takes about 20 minutes to complete.

Weight, BMI (z-score) and Body Composition via Bioelectrical Impedance Analysis: Weight and BMI (z-score) will be measured at each visit using a regularly calibrated scale. Body composition, in particular fat mass, will also be measured at each visit using bioelectrical impedance analysis. The Einstein-Mount Sinai Diabetes Research Center regularly completes and advises on the administration of bioelectrical impedance analysis and will provide the necessary equipment and services for this proposal. Both subject weight and body composition

will serve as markers of treatment efficacy as weight and fat loss are expected outcomes of treatment with IN-OXT. Although it is possible oxytocin could modulate energy expenditure, weight and fat loss observed during the 8 week period would be a strong indicator of a decrease in hyperphagia. This should take 15 minutes.

World Health Organization Quality of Life Questionnaire (WHOQOL): The WHOQOL is a quality of life assessment developed by the WHOQOL group with fifteen international field centers in an attempt to develop a quality of life assessment that would be applicable cross-culturally. The full-scale assessment WHOQOL-100 comprises 100 questions while the WHOQOL-BREF is a shorter version comprised of 26 items which measure the following broad domains: physical health, psychological health, social relationships and environment which is more convenient for use in large research studies or clinical trials. As seen in our preliminary data, it has proven to have significant change in trials with OXT and will be used to measure quality of life and caregiver burden in this study. PWS has a substantial impact on caregiver burden and quality of life. Thus, it is important to demonstrate that symptom reduction is also clinically meaningful. This scale, completed by parents on a bi- weekly basis, has been validated in other studies, including in PWS. However, WHOQOL is not a PWS- specific assessment. It has been used in multiple disorders including muscular dystrophy, tuberculosis and hypertension. WHOQOL is a parent questionnaire that will be completed at baseline, week 4, week 8 and follow up and takes about 20 minutes to complete. This scale is completed by the parent and is meant to show the burden to the caregiver and the family by the disorder being studied, in this case, PWS.

Caregiver Strain Questionnaire (CSQ):
The Caregiver Strain Questionnaire is a well-validated, reliable 21-item measure of self-reported strain experienced by caregivers and families of youth with emotional problems, with responses on a 5-point Likert scale (0 = Not at all, 4 = very much).

Clinical Global Impression Scale – Improvement (CGI-I): The CGI-I will be used as a measure of improvement and contains a 7 point scale as follows: 1 = very much improved, 2 = much improved, 3 = minimally improved, 4 = no change, 5 = minimally worse, 6 = much worse, 7 = very much worse. The CGI-I has been used as a measure in previous clinical psychopharmacology trials and will be completed by the study physician and clinician at each visit based upon clinical observations. It is a clinician rated global measure of improvement that will be completed at baseline, weeks 2 through 8 and at follow-up. It takes about 5 minutes.

Salivary Oxytocin Levels: Saliva will be collected by means of an Oragene-Discover collection kit (ORG-500). If able, subjects will spit saliva directly into the collection tube. If the child is unable to spit into the tube, we will use Oragene's OG-AC1 assisted collection swabs to collect saliva from the inside subject's cheeks. Samples will be collected pre-dose and one-hour post-dose at baseline, week 4 and week 8. A salivary sample will also be taken at the follow up visit. It takes approximately 10 minutes to complete.
Justification for Measurement of Salivary Levels: We have selected salivary measures of OXT, since Gordon et al [73] demonstrated that salivary OXT levels are directly correlated with brain activity and enhancements in brain function. After administration of IN-OXT, levels of salivary OXT increase markedly and have been shown to remain elevated for up to 7 hours after administration vs. placebo. In fact, low doses (16 IU OXT) did not show weaker effects and tended to elevate the initial salivary OXT compared to higher doses (24 IU). Salivary OXT levels on placebo were highly correlated over time, indicating individual stability of OXT levels. Another study [100], utilized a double-blind placebo-control within-subject design. Ten subjects

were administered IN- OXT or placebo and salivary OXT was measured ten times over four consecutive hours. IN-OXT increased salivary OXT across the entire period. OXT rose dramatically 15 min after administration (from 6.9 pg/ml at baseline to 1265.4 pg/ml), reached plateau at 45—120 min (range = 131.6 and 105.3 pg/ml), and did not return to baseline by 4 h. The findings from these studies support target engagement in the CNS and refute the idea that salivary elevation results solely from movement of mucus from the nasal cavities back into the mouth.
We believe that plasma OXT levels are of less importance, as central and clinical effects do not reflect plasma PK levels, but that salivary OXT levels may be more closely correlated with CNS effects. We do not expect our one hour post-dose measurements to be affected by IN-OXT residue in the nasal cavity. As stated above, Gordon et al. [73] demonstrated salivary levels of OXT 30 minutes post-dose were strongly correlated with changes in brain activity and van IJzendoorn et al [99] demonstrated that salivary OXT levels peak 1 hour post- dose and a dose of 16 IU was correlated with higher salivary OXT levels than a 24 IU dose. Salivary concentrations are being measured at weeks 4 and 8 to determine if salivary OXT concentrations accumulate over time with chronic treatment of IN-OXT and to determine if the levels correlate with changes in clinical symptoms. We will measure both group and individual differences in salivary OXT concentration in addition to correlating individual salivary OXT concentrations at day 1 and weeks 4 and 8 with changes on the Dykens Hyperphagia Questionnaire. We also believe that salivary OXT concentration measurements will induce less discomfort than plasma measurement for the children enrolled in the study. In sum, while levels of salivary oxytocin remain elevated for up to 7 hours, the standard and maximum levels observed in prior studies was observed at the 1 hour post-treatment measurement.

IQ Test: Stanford-Binet Intelligence Scales, Fifth Edition: The Stanford-Binet will be administered to obtain data on adaptive behavior and intellectual functioning for correlation during analyses. It is a gold standard measure of intelligence used in clinical and research settings in those aged 2 to 85 years. The fifth edition has a 50/50 balance between verbal and nonverbal subtests, and the Stanford-Binet is considered the best assessment of intelligence in those with intellectual disability and mental retardation 12. Administration time is approximately 5 minutes per subtest.

Dietary Assessment: Dietary intake will be assessed using the National Cancer Institute (NCI) 24-hour automated recall method, which will be completed by the primary caregiver in 15 to 20 minutes. Three dietary recalls (randomly assigned days to include two weekdays and one weekend day) will be obtained to assess pre-treatment dietary habits at baseline and repeated at endpoint. The NCI nutritional program output will allow for secondary analyses of impact of treatment on both dietary macronutrient ratio (percent carbohydrates, proteins and fats) and caloric intake.

Safety Measures:
Aberrant Behavior Checklist – Irritability (ABC-I): The ABC is an informant rating instrument that was empirically derived by principal component analysis to measure behavior in those with developmental disability and ASD. It contains 58 items that resolve into 5 subscales. The subscales and the respective number of items are as follows: (a) irritability – 15 items, (b) lethargy/social withdrawal – 16 items, (c) stereotypic behavior – 7 items, (d) hyperactivity/noncompliance – 16 items, and (e) inappropriate speech items. The ABC was designed to be completed by any adult who knows the patient well, such as a parent/caregiver or teacher. This instrument measures behavior on a four point severity scale where 0 = no problem at all, 1 = behavior is a problem but in a slight degree, 2 = problem is moderately serious, and 3 = problem is severe in degree. As suggested by the FDA, we have included the

ABC-I subscale as a prospective assessment for suicidal ideation and behavior at every visit. Given the concern over the possibility of increased temper tantrums raised by a previous study of high dose IN-OXT in the PWS population, the ABC-I can also inform investigators if there are any trends towards increased irritability and disruptive behavior. The Division of Psychiatry Products (DPP) has developed a policy that all clinical protocols for products developed in DPP, whatever the indication, include a prospective assessment for suicidal ideation and behavior. Per the DPP, these assessments need to be included in every clinical protocol at every planned visit and in every phase of development. Collecting this data ensures that patients in clinical trials who are experiencing suicidal ideation or behavior are detected and adequately managed. This is important whether or not a particular drug is associated with treatment-emergent suicidal behavior. As per the recommendation of the FDA, in studies of subjects with ASD, investigators frequently use the ABC-I subscale to fulfill this requirement. The ABC may help identify any increased risk to self through observation rather than self-report. In particular, the ABC-I can be useful for obtaining information about treatment-emergent irritability, self-injurious behavior or worsening of aggressive behavior. The ABC will be completed at every visit and requires approximately 25 minutes.
Monitoring for Co-morbidities- The study doctor will monitor hypogonadism, sleep apnea and lethargy throughout the course of the study.
Hormone Levels: Ghrelin, Pancreatic Polypeptide, Peptide Y, Leptin, insulin, glucagon, GLP-1, Growth Hormone, Testosterone and Estrogen will be measured using blood plasma at baseline and endpoint.

COVID-19 Related Assessments:
COVID Stressors questionnaire: This questionnaire will be used to assess any changes in eating patterns or weight changes caused due to COVID-19 stressors.

Adverse Event Reporting and Measures of Compliance:
Drug accountability will be completed by the Pharmacist at the Clinical Research Pharmacy and will serve as a measure of study compliance. IN-OXT and Placebo nasal spray bottles will be weighed pre-and post- dispensing to ensure the correct amount of drug/placebo was administered to the patient. The pharmacist will notify the study team if a subject is non-compliant with the study drug/placebo, and the study team will address this with the subject and his/her parent.
Adverse events will be reported on logs kept in the subject binder and completed by the study physician at each visit. Reporting requirements for the FDA (within 7 days) and the IRB (within 5 days) will be followed for any suspected unexpected related adverse event. For any SAE the IRB will be notified within 24 hours, and the FDA within 48 hours.

Special Circumstances

During special circumstances (e.g. COVID-19 pandemic), the specific guidance from local public health and other competent authorities regarding the protection of individuals' welfare must be applied. In cases where participants are not able to perform all protocol-defined assessments due to special circumstances, the investigator must discuss with the medical monitor potential mitigation approaches.

For the duration of such special circumstances, the following measures may be implemented for enrolled subjects:

A. Safety follow-up may be done by a telephone call, other means of virtual contact or home visit, if appropriate.

B. Patient and/or clinician-rated outcomes assessments may be done by video-conference, telephone call, other means of virtual contact, if possible.
C. An alternative approach for IMP dispensing, secure delivery and collection may be sought
D. Visits may take place in a different location than defined in the protocol. If this is not feasible, then the visit may take place virtually with documentation of the means of communication (e.g., phone call or videoconference).
E. Biological samples may be collected and analysed at a different location than defined in the protocol. Biological samples should not be collected if they cannot be processed in a timely manner or appropriately stored until shipping/processing.
F. If despite best efforts it is not possible to collect the biological samples or safety assessments (e.g. ECG, vital signs), the investigator must review the benefit-risk for patient continuation in the study and record this in the medical records

The rationale (e.g., the specific limitation imposed by the special circumstances that led to the inability to perform the protocol-specified assessment) and outcome of the discussion with the medical monitor will be documented in the medical record. Information on how each visit was performed will be recorded in the eCRF.

Procedure	Time	Completed By	Screening (remote)	Baseline (in-person)	Week 2 (remote)	Week 4 (remote)	Week 6 (remote)	Week 8 (in-person)	Week 10 Follow Up (remote)
Informed Consent	60 min	Parent/Child/Clinician	X						
I/E Criteria	10 min	Clinician/ Parent	X	X					
Medical Hx/Demographics	30 min	Parent/Child/Clinician	X						
Hypogonadism/sleep apnea Hx	10 min	Parent/Child/Clinician	X						
Physical/Neuro Exam	20 min	Clinician/ Child		X				X	
Psychiatric Interview	30 min	Parent/Child/Clinician	X						
Intelligence Test	60 min	Clinician/ Child		X					
Randomization	0 min			X					
Concomitant Medications	5 min	Parent/Child/Clinician	X	X	X	X	X	X	X
Adverse Events/Monitoring for hypogonadism, sleep apnea, lethargy	10 min	Parent/Child/Clinician	X	X	X	X	X	X	X
Standard Laboratory Tests*	10 min	Clinician/ Child	X***	X				X	
Hormone and Metabolic Laboratory Tests**	10 min	Clinician/ Child		X				X	
Treatment Given	10 min	Parent/Child/Clinician		X		X		X	
Urine Pregnancy Test (if appropriate)	5 min	Child/Clinician	X	X				X	
Vital signs	15 min	Clinician/ Child		X				X	
Eating Behavior Measures									
Hyperphagia Questionnaire-Clinical Trials (HQ-CT)	15 min	Parent/Child/Clinician	X	X	X	X	X	X	X
Weight, Body Composition and BMI	5 min	Parent/Child/Clinician		X				X	
Dietary Diary ASA24 Automated Self-	15 min	Parent		X				X	

Administered 24 Hour Recall									
Repetitive Behavior Measures									
Montefiore-Einstein Rigidity Scale –Revised (MERS-PWS)	15 min	Clinician		X		X		X	X
RBS-R	15 min	Parent		X [pre-dose]		X		X	X
Oxytocin Measures									
Salivary Oxytocin Levels	10 min	Clinician/Child		X [pre- and 1 hour post dose]		X [pre- and 1 hour post dose]		X [pre- and 1 hour post dose]	X
Global Clinical Improvement and Quality of Life Measures									
ABC-I (Full ABC will be completed to measure this subscale)	25 min	Parent		X [pre-dose]	X	X	X	X	X
CGI-I	5 min	Clinician			X	X	X	X	X
CGI-S	5 min	Clinician	X	X					
WHOQOL	20 min	Parent/Child/ Clinician		X		X		X	X
Caregiver Strain Questionnaire	10 min	Parent		X		X		X	X

* Standard Laboratory Tests: CBC, CMP including sodium, IGF-1, thyroid panel, lipid panel, hemoglobin A1C, and urine pregnancy test
** Hormone and Metabolic Laboratory Tests: testosterone, estrogen, growth hormone, ghrelin, pancreatic polypeptide, peptide YY, leptin, GLP-1, insulin, glucagon
***** To be done at local Quest**

Table 2: Intranasal Oxytocin vs. Placebo for the Treatment of Hyperphagia in Prader-Willi Syndrome SCHEDULE OF EVENTS

RECRUITMENT

We have received letters of support from Theresa Strong from the Foundation for Prader-Willi Research, Jennifer Miller, MD, Elisabeth Dykens, PhD and Moris Angulo, MD. We are in communication with the local chapter of the Prader-Willi Syndrome Association USA. We have also collected over 40 patients who are interested in the study and meet with inclusion/exclusion criteria. These patients contacted us after Dr. Eric Hollander presented at the Foundation for Prader-Willi Research Meeting (November 2014), Prader-Willi Syndrome Mental Health Strategy Workshop (March 2015) and the "Overcoming Bottlenecks in Clinical Trials of Investigational Medicinal Products for Hyperphagia in Prader-Willi Syndrome" FPWR Meeting (July 2015), all which were geared toward the development of clinical trials for this population. Additionally, Dr. Hollander's team presented at the recent Foundation for Prader-Willi Research Meeting held in Austin, Texas. These families are very enthusiastic about participating in research trials.

Theresa Strong PhD, the Chair of the Scientific Advisory Board for the Foundation of Prader-Willi Research (FPWR), is a co-investigator on this proposal, brings a wealth of research and clinical PWS expertise to the proposal, and is committed to aid us in recruitment. The mission of the Foundation for Prader-Willi Research (FPWR) is to eliminate the challenges of Prader-Willi syndrome through the advancement of research. FPWR has over 1,000 members, including families in the USA, Canada and around the world with a social media reach of >7,500 individuals, and continues to grow rapidly. Their largest concentration of members is in the northeast, within quite reasonable travel distance to our institution. Their community is highly engaged and enthusiastic in its support of research; in fact, a recent online family survey indicated that more than 95% ~ 750 respondents would consider enrolling in a clinical trial. To ensure the successful completion of our clinical study, FPWR is committed to assisting us in the recruitment of participants by posting information on the FPWR website and social networks. They will also feature our study in their "Clinical Trials Alert" e-newsletter, which reaches approximately 700 families, and in their "Clinical Trials" webinar series, which is also well attended.

Jennifer Miller, MD is an Associate Professor in the division of pediatric endocrinology at the University of Florida. She graduated with her M.D. from the University of Florida in 1998, and her M.S. in Clinical Investigation from the University of Florida in 2005. Her research focus includes: investigating the effects of growth hormone treatment on brain development, sleep, and appetite in individuals with PWS; investigating the effects of early-onset weight gain on brain development; studying the sleep abnormalities in individuals with PWS; and finding treatments for the hyperphagia and obesity in individuals with PWS. Dr. Miller has followed hundreds of patients with PWS and provides ongoing advice to patients on the most effective and newest management strategies and treatments for handling PWS. To date, Dr. Miller has been at the helm of several research projects with a focus on evaluating the effects of early-onset obesity (i.e. obesity occurring before age
5) on the developing brain. She currently sits on the Scientific Advisory Board for the Foundation for Prader- Willi Research, and recently completed a clinical trial at the University of Florida on the effects of Intranasal Oxytocin on individuals with PWS. She follows over 450 patients per year with PWS at the University of Florida, the largest PWS program in the country. Dr. Miller will serve as a consultant on this proposal and will be an integral part of the protocol design and study recruitment.

Elizabeth Dykens, PhD is a Professor of Psychology and Director of the Vanderbilt Kennedy Center for Research on Human Development, and Co-Director for the University Center for

Excellence in Developmental Disabilities. Her research examines psychopathology and areas of strength in persons with intellectual and developmental disabilities, especially those with genetic syndromes. Her studies focus on the development and correlates of psychopathology and behavioral problems in Prader-Willi syndrome, including marked obsessive- compulsive behaviors in Prader-Willi syndrome. Dykens also examines profiles of neurocognitive and adaptive strengths and weaknesses in PWS, and how these unusual profiles refine treatment and shed light on normal development. Some of her current studies include looking at the physiological and neurological mechanisms of compulsive behavior in persons with Prader-Willi syndrome and the visual-spatial strengths in persons with Prader-Willi syndrome. Dr. Dykens is also the creator of the Dykens Hyperphagia Scale, a validated measure that has been used to measure hyperphagia severity in children and adults with PWS in multiple clinical trials. Dr. Dykens will serve as a consultant on this proposal.

Moris Angulo, MD is a pediatric endocrinologist and genetic specialist who established one of the largest PWS centers in the country at Winthrop University Hospital in Mineola, New York. Winthrop's Division of Pediatric Endocrinology and Genetics has more than 300 patients with Prader-Willi Syndrome in its care and has helped to establish three local PWS group homes with trained personnel for the care of adult individuals. Winthrop also has parental and familial support groups that are run by a genetic counselor and play a key role in the management of the syndrome and the emotional issues in those caring for PWS children and adults. Dr. Angulo will serve as a consultant on this proposal.

The Prader-Willi Syndrome Association USA (PWSA): PWSA is an organization of families and professionals working together to raise awareness, offer support, provide education and advocacy, and promote and fund research to enhance the quality of life of those affected by Prader-Willi Syndrome.

Global PWS Registry: The Global Prader-Willi Syndrome Registry is sponsored by the Foundation for Prader-Willi Research (FPWR) and hosted by the National Organization of Rare Diseases (NORD). The primary objective of the Registry is to develop a comprehensive database of individuals with PWS to better understand the full spectrum of the PWS phenotype. Secondary objectives include accelerating enrollment in clinical trials by locating potential research participants quickly and efficiently. The Registry question set was developed in collaboration with leading clinicians in the field of PWS, including Drs. Jennifer Miller (University of Florida, Endocrinology); Merlin Butler (Kansas University Medical Center, Genetics); Harold van Bosse (Shriner's Hospital, Orthopedic Surgery), and Tony Holland (Cambridge University, Psychiatry). The registry will collect data from parents/caregivers (or the person with PWS, if s/he is able) with respect to Demographics (including weight, height and age), Diagnosis, Development, Medical History (eg, endocrine, neurological, orthopedic), Medications, Behavior and Appetite, Mental Health and Quality of Life.

The design and implementation of the Registry was guided by current best practices in advocacy group- sponsored rare disease registries. To this end, Dr. Strong participated in the Genetic Alliance "Registry and Biobank Boot Camp" in Washington DC (2012), while Dr. Bohonowych attended registry-focused sessions at the annual meeting of the National Organization of Rare Disorders (2013) and at the annual DIA conference (2014), where she was a DIA Patient Advocate Fellow. Drs. Strong and Bohonowych have also consulted with other advocacy groups who have launched successful registries and Dr. Yaffa Rubenstein, Director of Patient Resources for Clinical and Translational Research, Office of Rare Disease Research, NCATS, NIH. The Registry protocol and informed consent have been reviewed and

approved by an Institutional Review Board (Chesapeake). A governing body consisting of the Registry Investigators (Drs. Strong, Bohonowych, Miller and Butler), parent advocates (2), has been established.

Predicted Enrollment: We expect that the Foundation of Prader-Willi Research (FPWR) and their Global PWS Registry to be the greatest source of recruitment for our study. There are over **700** patients interested in participating in research that we will have access to with their Clinical Trials Alert newsletter. Additionally, the Global PWS Registry will collect data that will allow us to pre-screen patients based on our inclusion/exclusion criteria. Through Dr. Miller's center we will have access to over **450** patients. Dr. Angulo's practice has over **300** patients with PWS. We have budgeted significant funding to provide travel reimbursement to patients that will need to travel long distances to our center and do not expect that to play an issue in the recruitment and enrollment for our study due to the enthusiasm of this population for clinical research. In total we will thus have access to, at minimum, **1450** patients and families affected by Prader-Willi Syndrome. Even if we use an elevated pre-screening/screen failure rate of 80%, to account for patients that are not interested, do not meet I/E criteria, or who do not wish to travel to our site, we would still have access to approximately **300** patients that would be study eligible.

As mentioned above, we are already in contact with 40 families/patients that are interested in research and meet the criteria for this study. Study subjects will be drawn from the resources above and referrals, as well as from the diverse and large clinical practice of the PI, Dr. Eric Hollander. Additional recruitment methods include advertisements in local media, referrals from patients' personal physicians and the clinical affiliations of the PI and collaborators. Pre-screening/screening procedures are detailed below. Study visits have been kept to the safest minimum to reduce caregiver burden and travel time. Reimbursement of $150 for each in-person visit (2 visits) and $30 for each remote visit (5 visits) will be provided, for a total of $450 over the course of the study. This reimbursement covers compensation for time and reimbursement for travel expenses to the site. Additional funds have been allocated to provide for long-distance travel for patients outside of the tri-state area.

Timeline: We anticipate completing this study in 3 years, with 10 subjects enrolled in year 1, 20 subjects enrolled in year 2, and 20 subjects enrolled in year 3. A 6 month start-up period will allow for IRB approval and an update to the IND; followed by 24 months of recruitment/enrollment and a 6 month period to complete data analysis and prepare for publication. Data will be entered into the database prospectively.

Assessment of Safety:
The safety and tolerability of the intranasal oxytocin will be determined by clinical laboratory results, physical examination and adverse events monitoring. Adverse Event (AE) collection will begin when subjects are enrolled and randomized at the baseline visit, and will occur throughout the study, or last study visit if the patient withdraws or is discontinued from the study. AEs will be recorded as volunteered by the patient or solicited through indirect questioning. An AE will be

Table 3: Adverse Event Relationship to Study Drug

Possibly Related	There is "a reasonable possibility" that the event may have been caused by the product under investigation (i.e., there are facts, evidence, or arguments to suggest possible causality). Individual AE reports will be considered "related" to the use of the product if the "not related" criteria are not met.
Not Related	There is an unreasonable temporal relationship between administration of the product and the onset of the AE (i.e., the event occurred either prior to randomization, or too long after administration of the product for it to be considered product-related); The causal relationship between the product and the AE is biologically implausible (i.e., death of a passenger in an automobile accident); There is a clearly more likely alternative explanation for the AE (i.e., typical adverse reaction to a concomitant drug and/or typical disease-related event).

considered “related” or “not related” to the use of the product based on the criteria listed in **Table 3**. Assessment of the causal relationship between any Serious Adverse Event (SAE) and study drug administration will be performed by both the Investigator and the Sponsor. If at least one of the parties assesses the event as related, it will be reported expeditiously as related to the appropriate parties (i.e. FDA and IRB). The severity of each AE should be characterized and then classified into one of three clearly defined categories as follows:

Mild: The AE does not interfere in a significant manner with the subject’s normal functioning level. It may be an annoyance.
Moderate: The AE produces some impairment of functioning, but is not hazardous to health. It is uncomfortable or an embarrassment.
Severe: The AE produces significant impairment of functioning or incapacitation and is a definite hazard to the subject’s health.

These 3 categories are based on the Site Investigator’s clinical judgment, which in turn depends on consideration of various factors such as the subject’s reports, the physician’s observations and the physician’s prior experience. The severity of the AE should be recorded in the source documentation. The evaluation of severity is distinguished from the evaluation of “seriousness.” A severe event might not meet the criteria for seriousness and a serious event might be evaluated as mild.

Safety Analyses: Safety will be evaluated from concomitant medications, study drug exposure, treatment emergent adverse events, serious adverse events, death, suicidal ideation, physical examinations, clinical laboratory test results and vital signs. All summaries of adverse events and suicidal evaluation will be presented. Serious adverse events, deaths (if any), abnormal laboratory results and abnormal vital signs will be listed in patient listings. All AEs will be coded by the system organ class (SOC) and preferred term (PT) using the Medical Dictionary for Regulatory Activities (MedDRA).

Non-treatment emergent AEs are defined as
- AEs that started and resolved prior to the first study drug dosing
- AEs that started prior to the first study drug dosing, resolved after first study drug dosing and the grade remained the same or reduced.

Treatment emergent adverse events (TEAEs) are defined as all AEs that begin or worsen after the patient receives the first dose of the study drug until follow –up. All reported AEs (including non-treatment-emergent adverse events) will be listed. Overall summary of TEAEs, such as total number of reported TEAEs, total number of patients reporting at least one TEAE, severity of TEAEs, relation to study drug, serious AEs, any deaths, TEAEs leading to study drug discontinuation, will be summarized by treatment group. In addition, TEAEs will be tabulated by SOC and PT. A table will contain the number of TEAEs (frequency of occurrence, number and percentage of patients) by treatment group will be created.

For summaries of TEAEs by maximum severity, body system, and preferred term, the rule will be as follows: if a patient experiences more than one episode of a particular coded adverse event, the patient will be counted only once by the maximum severity of these episodes (preferred term). Similarly, if a patient has more than one adverse event within a body system, the patient will be counted only once by the maximum severity among adverse events in that body system. For the purpose of summary of adverse events, missing severity events will be included in the Grade 3 (Severe) event category. Summary of TEAEs by SOC and PT will also

be presented fort the following categories, when data are available.

1. Grade 3 TEAEs
2. Related and Grade 3 TEAEs
3. TEAES leading to study drug discontinuation
4. Serious events
5. Serious and related events
6. Events resulting in death

Additional Safety Monitoring and Adverse Event Reporting Adverse events that are serious and unexpected will be immediately reported to the IRB, and FDA. Non-serious unanticipated problems will be described in annual reports to the IRB and FDA. Additionally, a pediatrician not affiliated with this study will serve as a medical safety monitor for this study. The safety monitor will review subjects' laboratory values and adverse events on at least a monthly basis. This physician will be available to immediately address the clinical circumstances for any acute problem and will promptly notify appropriate regulatory bodies.

Independent Data Safety Monitoring Committee
An Independent Data Safety Monitoring Committee (DSMC) will review safety and clinical outcome data including data on adverse events (AEs) and serious adverse events (SAEs) at regular intervals. They will meet biannually to review study reports. The DSMC will be independent of the investigator and will be empowered to recommend stopping the study due to safety concerns, but not for efficacy or futility. The DSMC may review blinded, unblinded, or partially unblinded data, but the investigator will remain blinded until the official unblinding of the database.

Statistical Analyses: Analyses will be based on the intent-to-treat approach in which subjects are analyzed in the group to which they were originally assigned regardless of treatment compliance. All variables will be initially screened for inconsistent or abnormal values, and continuous measures will be assessed for skew and outliers. Transformations to improve normality will be applied when necessary. The demographic characteristics of all randomized patients will first be summarized using standard descriptive statistics (means, medians, standard deviations, and ranges for continuous variables and frequencies for categorical variables). Although subjects will be randomized 1:1 to the two treatment groups, imbalances in patient characteristics may still occur by chance given the limited sample size of the Phase 2 trial. Between group differences in the baseline demographic and other prognostic variables will be assessed using the chi- square test for discrete variables and the T-test or Wilcoxon rank sum test for continuous variables. Baseline characteristics which are found to be significantly different will be considered for inclusions as potential confounders in secondary analyses of treatment effects.

Summary of Analytic Approach: The primary outcome measure is change on the continuous measure of the Hyperphagia Questionnaire-Clinical Trials, a valid and reliable measure of the food seeking pathology in PWS. Secondary outcome measures include a measure of repetitive behavior (RBS-R and MERS-PWS), weight, measures of Quality of Life and Global Functioning (WHOQOL, CGI), and measures of salivary Oxytocin concentration. Given that these outcomes are all continuous variables, they will be analyzed using the same general statistical strategy as outlined below.

Measurements obtained at a specific time point (e.g. 8 weeks) will be compared between the IN-OXT and placebo groups with the two sample t-test or Wilcoxon rank sum test. In addition, the change in outcome measures between baseline and 8 weeks will be compared between

treatment groups. Exploratory analyses will first be performed to evaluate whether the magnitude of changes in these variables between the two time points depends on the baseline value. If there is no evidence that change is dependent on baseline, then two sample tests will be used to compare the mean change in each variable between treatment groups; otherwise, analysis of covariance models will be fit to the data where the outcome is the level measured at 8 weeks, and the main effects are treatment group and baseline value [15;37]. Adjustment for any patient characteristics which appear to be imbalanced across treatment groups will be accomplished by including the relevant terms as additional covariates into the model. To compare longitudinal trends in outcomes between treatment groups, linear mixed effects models will be fit to all repeated measures obtained during follow-up, with main effects for time and treatment group and a time x treatment interaction term; this is a widely used statistical approach in longitudinal studies of psychiatric outcomes. Appropriate data transformations, such as the log transform, will be applied if the data deviate from the normal distribution. Although a number of different outcomes will be analyzed, we will not adjust for multiple testing given the exploratory nature of this study.
Additional subgroup analyses will also be performed to evaluate and compare treatment effects in males and females. Given the limited sample size of this Phase 2 trial, however, any subgroup analyses will be viewed as exploratory.

Detailed Description of Analyses

Primary Analyses: Analyses for Specific Aim (1): *To compare IN-OXT vs. placebo with respect to improvement in the hyperphagia severity in children with PWS via improvement in scores on the* Hyperphagia Questionnaire-Clinical Trials.

The primary analysis will evaluate the hypothesis that IN-OXT will decrease hyperphagia vs. placebo.

1. Response to treatment will be assessed in two ways:
    a. The effects of IN-OXT on hyperphagia will be analyzed using an intent-to-treat approach with the two-sample T-test or Wilcoxon rank sum tests to compare treatment groups at specific visits as well as linear mixed effects models to analyze the repeated measures and changes over time.
    b. The proportion of responders in each treatment group will also be compared. Response will be defined as a 30% or greater decrease in food-seeking and eating behaviors from baseline on the Hyperphagia Questionnaire-Clinical Trials. Dropouts for clinical reasons will be coded as non- responders. Differences in the proportion of responders in each treatment group will be evaluated using the chi-square test and time to response will be assessed using survival analytic techniques.
    c. All tests will be two-tailed with an alpha level of 0.05.

***Secondary Analyses*:** Analyses for Specific Aims (2) through (8): *To compare IN-OXT vs. placebo in children with PWS on change in:(2) Repetitive behaviors via scores on the Repetitive Behavior Scale –Revised (RBS-R) and Montefiore-Einstein Rigidity Scale-Revised (MERS-PWS)(3) Weight and body composition via impedance analysis. (4) Quality of Life Measures on the World Health Organization Quality of Life Scale (WHOQOL)(5) Clinical Global Impression Scale (CGI-I) (6) Salivary Oxytocin Concentration (7) Safety Analyses*

IN-OXT and placebo groups will be compared to the above secondary measures will be analyzed using methods similar to those outlined above. Associations between outcome measures at a specific time point will be analyzed with the Spearman rank or Pearson

correlation coefficient.

1. Secondary Aim (1) Repetitive Behaviors
    a. The effects of treatment on frequency and severity of repetitive behaviors will be assessed by fitting linear mixed effects models to the data on the RBS-R scores. We hypothesize that IN- OXT will significantly reduce repetitive behaviors from baseline to endpoint compared to Placebo.
    b. The effects of treatment on frequency and severity of repetitive behaviors will be assessed by fitting linear mixed effects models to the data on the MERS-PWS scores. We hypothesize that IN- OXT will significantly reduce repetitive behaviors from baseline to endpoint compared to Placebo.
2. Secondary Aim (2) Weight, BMI (z-score) and body composition via bioelectrical impedance analysis
    a. Weight and BMI (z-score) will be measured at each visit. It will be utilized to evaluate whether IN-OXT significantly modulates energy metabolism and reduces weight compared to placebo. Body composition via impedance analysis will be measured at baseline and endpoint.
3. Secondary Aim (3) Quality of Life Measures
    a. The relationship between quality of life and treatment will be assessed using scores on the WHOQOL. We hypothesize that IN-OXT will improve quality of life from baseline to endpoint as measured by an increase in overall score on the WHOQOL from when compared to placebo.
    b. The relationship between caregiver strain and treatment will be assessed using scores on the CSQ. We hypothesize that IN-OXT will caregiver strain from baseline to endpoint as measured by an reduction in overall score on the WHOQOL from when compared to placebo.
4. Secondary Aim (4) Salivary Oxytocin Levels
    a. Both group and individual differences in salivary OXT concentration will be measured. Correlations between individual salivary OXT concentrations at day 1 and weeks 4 and 8 as well as correlations between OXT and changes in our primary outcome measure, hyperphagia assessed by the Dykens Hyperphagia Questionnaire, will be evaluated using the Spearman rank or Pearson correlation. Additional correlation analysis will be performed on salivary oxytocin levels and secondary outcome measures: RBS-R, WHOQOL and CGI-I. We hypothesize that higher concentrations of salivary oxytocin will be correlated with positive changes on each outcome measure, indicating improvement in symptoms.
5. Secondary Aim (5) Safety Analyses
    a. Rates of specific side effects and other adverse events will be tabulated and compared between treatment groups using the chi-square test. Please see the section labeled “Safety Analyses” for further details on how adverse events will be monitored and rated.
    b. The relationship between frequency of suicidal behaviors will be assessed on the ABC-I to evaluate whether IN-OXT vs. placebo is associated with a change in the irritability subscale of the ABC-I which serves as a surrogate for suicidal ideation in this population.

Additional Analyses

1. Sample size permitting, we will explore the potential for ethnicity-by-treatment interactions. If effects of minority status are found that might be clinically meaningful, even if not statistically significant, we will report these to guide future research. Please see the Planned Enrollment Table for the breakdown of anticipated percentages of children of each ethnicity that will be enrolled.

2. We will test for gender by treatment interactions.
3. We will explore IQ by treatment interactions.
4. We will explore for co-morbidity (e.g. Type 2 Diabetes, Hypogonadism etc.) by treatment interactions.
5. We will explore for weight based dosing by treatment interactions.

Missing data: In any clinical trial, some subjects will be lost to follow up or will miss study visits but the expected rate is low given that this is only an 8 week trial. Primary analysis will be based on available data at each time point. The advantage of the linear mixed effects approach is that it can handle data which are missing at random and measurement times which are not evenly spaced. However, when the missingness mechanism depends on unobserved information, i.e., non-ignorable, parameter estimates and resulting tests on hypotheses will be biased without further adjustment. Different approaches for handling missing data will be performed in this study. Multiple imputation will be applied if missing data rates are observed to differ across observed covariates. Regardless of the technique, characteristics of patients who are lost to follow-up will be compared to those that remain in the study to assess the degree of any selection bias, and sensitivity analyses will be performed to evaluate robustness of conclusions to the different missing data approaches.

Power Analysis: Sample size justification: The target sample size for this Phase 2 trial is 25 patients per treatment group. The power for the study has been calculated to generate a detectable signal with the sample size of 50 subjects. With this sample size, the study will have 80% power to detect a minimum effect size of d= 0.8 standard deviations in the outcome measures between groups using a two-sample T-test and a two sided Type I error rate of 5%. The study is powered based on a conservative estimate from a heterogeneous population. We expect that in this homogeneous PWS population selected to match study drug to underlying mechanism, the true power will exceed the conservative estimate. This minimum detectable difference is smaller than the treatment effects observed in our previous studies of IN-OXT in adults with ASD, which demonstrated an effect size of d=1.2 on a social cognition measure (RMET) [3], and an effect size of d = 0.84 on a quality of life measure (WHOQOL) [3]. In addition, our previous study of a V1a antagonist in adults with ASD demonstrated an effect size of d=0.8 on a social cognition measure (ASR Lust) [80], and an effect size of d = 0.8 on an Eye Tracking measure (Biological Motion Preference) [90]. The above effect sizes observed in our pilot data were based on a heterogeneous sample of ASD patients characterized by over 100 known different genetic contributing factors. In the proposed trial, both males and females will be included, and any heterogeneity in outcomes due to gender may attenuate overall treatment differences. However, by selecting an orphan population (PWS) with a single unique genetic contribution, and selecting a treatment that matches the known molecular mechanism (IN-OXT), we expect that the treatment effects in this study will nevertheless be greater than the effects observed in earlier studies.

Data Management: The Biomedical Research Informatics Core (RIC) of the Institute for Clinical and Translational Research (ICTR) at Einstein and Montefiore will be used to create and manage the database for the trial. The RIC supports the clinical data pipeline for both Einstein and Montefiore including supporting EDC tools, customized databases and a solution to securely link patient specimens to clinical and pathological data. Database programming will begin at the study start date, and will be ready by the 6 month milestone mark, when study enrollment begins, so that data can be entered prospectively as subjects move through the study. Data will be encoded using unique patient identifiers, and not patient names. IDs will be assigned sequentially, in a manner unrelated to name or other easily identifiable information. Data will be entered for both screened and randomized subjects to allow analyses to assess

the degree to which randomized subjects may differ from other potential subjects. Periodic reports on recruitment, treatment, retention and follow up will be generated and reviewed by the study team. All subject files will be kept in a locked room at the study site. Another ICTR core, the Biostatistics and Study Design Core, directed by Mimi Kim, ScD, will be responsible for the statistical support of the study, including the final data analysis. The PI will meet regularly with the study team, including staff from the two ICTR cores, to review the status of the study and devise any strategies needed to meet the study goals.

Ethical Considerations: Parents/caregivers of potential participants that are referred or indicate interest in the study will be phone screened by trained staff to determine eligibility. If there are no clear exclusionary criteria, a consent form will be sent and an appointment will be set up to discuss the benefits and risks of study participation, duration of study, alternative treatment options and to answer any questions about the study.
When all questions are answered and the parent/caregiver and their child, when appropriate, indicate that they understand the study, written informed consent and/or assent will be obtained. Child assent/consent will be obtained as appropriate to age and capacity determined by the study physician/psychologist. Consent procedures will be completed by a trained member of the study staff and will be reviewed by the PI.

Risks: Studies have shown risks of IN-OXT to be minimal with no difference in AE burden between IN-OXT and placebo [46]. The most common categories of side-effects seen include:

- increased calmness/euphoria or more energy
- light headedness
- drowsiness/fatigue
- headache
- nasal irritation
- dry mouth/throat

As noted above, one study of IN-OXT in PWS noted increased levels of aggression, via temper tantrums, at higher doses of IN-OXT. As very few studies have reported on the extended use of IN-OXT there may be other unknown side effects, although our 6-week studies showed no difference in AEs. Other risks relate to venipuncture procedures for blood sampling taken at key points in the study as a safety measure. Blood draws and anthropometric measures will be completed at the CTSA sponsored Clinical Research Center (CRC), which has trained nursing and lab staff. In addition, patients may feel uncomfortable answering some of the questions presented during the assessments and breaks will be provided if needed. There is a risk of loss of confidentiality, although every step is being taken to keep patient files and data secure. All data is locked in staff offices or on staff computers and only authorized personnel or organizations indicated in the consent (IRB; FDA etc.) will have access. Pediatric neurologist Aleksandra Djukic, MD, PhD is the independent medical monitor and will review side effects after each study visit to ensure subject safety and to determine if a subject's participation should be terminated due to an AE.

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