MIRTAZAPINE TREATMENT OF ANXIETY IN CHILDREN AND ADOLESCENTS WITH PERVASIVE DEVELOPMENTAL DISORDERS

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A. SPECIFIC AIMS:

Autistic disorder (autism) and related pervasive developmental disorders (PDDs) are lifelong childhood neurobiological disorders that cause marked problems with social interaction and communication. Few biological treatments have been shown to be effective. This is especially tragic given that PDDs affect 0.6 % of children and are associated with a high degree of individual disability and great costs to the family and society. In addition to the core impairments in socialization and language, many persons with PDDs often experience high levels of psychiatric symptomatology. These psychiatric symptoms are treated with medication with varying success. Risperidone and aripiprazole are the only medications that are FDA-approved for use in children and adolescents with autism. There is less agreement about the efficacy of other medications to treat co-morbid psychiatric symptoms in autism.

One of the areas receiving surprisingly little attention in PDD treatment is that of anxiety. Anxiety is common in PDD, but has not yet been fully characterized. There continues to be an urgent need for additional studies aimed at the treatment of anxiety in persons with PDD. These symptoms are often treated with selective serotonin reuptake inhibitors (SSRIs) which are efficacious in some adults with autism (McDougle et al 1996a). In children with PDDs, however, SSRIs are not clearly efficacious and frequently cause activating side effects (Posey et al 2006a).

The **long-term goal** of this research is to advance the science behind the pharmacological treatment of PDDs by studying medications that reduce maladaptive symptoms and improve the quality of life of the individual and his/her family members. The **primary objective** of this application is to conduct a preliminary placebo-controlled trial of mirtazapine for the treatment of anxiety associated with PDDs. The principal investigator (PI) and colleagues have completed a retrospective, open-label study of mirtazapine in children with PDDs and believe this to be a promising new treatment for the target symptom of anxiety. Mirtazapine is novel in that it has both noradrenergic and serotonergic properties. Its unique mechanism of action is to block the presynaptic alpha-2-adrenergic receptor in addition to blocking serotonin 5-HT2 and 5-HT3 receptors.

The **central hypothesis** of this project is that mirtazapine will improve anxiety when administered to children and adolescents with PDD. We also hypothesize that mirtazapine will be safe and well tolerated. This study will provide much needed pilot data regarding efficacy and tolerability of mirtazapine for these symptoms in autism and is a necessary first step prior to conducting a larger, more definitive randomized controlled trial.

We plan to test the central hypothesis and accomplish the objectives of this application by pursuing the following **specific aims:**

1. **Determine whether mirtazapine shows evidence of efficacy, safety, and tolerability in children and adolescents with PDD.** This aim addresses our central hypothesis that mirtazapine will be efficacious and safe in the treatment of anxiety in children and adolescents with PDD.
2. **Determine the effects of placebo on anxiety symptoms in children with PDD.** This will be an important aim prior to conducting a more definitive trial since the magnitude of placebo effect for this specific symptom in PDD is unknown.

3. **Determine which semi-structured interviews and existing anxiety rating scales would be most useful for characterizing participants and assessing anxiety treatment effects.** A variety of outcome measures of anxiety will be used to better assess their applicability and feasibility in the PDD population.

B. IMPACT AND RELEVANCE:

PDDs are severe childhood-onset disorders which cause significant impairment in social interaction, communication, and behavior. The most common PDDs are autistic disorder, Asperger's disorder, and PDD not otherwise specified (NOS). These disorders affect 1 in 88 persons and lead to substantial impairment and disability.

Our group and others have been actively conducting clinical trials aimed at treating both core and associated symptoms of the disorder. Risperidone was approved by the FDA for the treatment of irritability in children and adolescents with autistic disorder. This approval was based in large part on studies conducted by the National Institute of Mental Health (NIMH)-funded Research Units of Pediatric Psychopharmacology Autism Network which included our center and four other academic sites (RUPP Autism Network 2002). Our group has also been involved with clinical trials aimed at finding better approaches to the treatment of hyperactivity co-morbid with PDD (Posey et al 2006b; Posey et al 2007; RUPP Autism Network 2005).

Interfering repetitive behavior and other symptoms that resemble obsessive-compulsive disorder (OCD) have also been studied in PDD. These symptoms are often treated with selective serotonin reuptake inhibitors (SSRIs) which work relatively well in adults with autism (McDougle et al 1996a). In children, however, their efficacy has been less robust and the frequent occurrence of activating side-effects is problematic (Posey et al 2006a).

This potential for activating side-effects with SSRIs was recently confirmed by a large multi-site clinical trial sponsored by the National Institutes of Health. In this trial from the Studies to Advance Autism Research and Treatment (STAART) network, 149 children and adolescents (ages 5-17 years) were randomized to flexible dose treatment with either citalopram (dose range 2.5-40 mg; mean dose 16.5 mg) or placebo (King et al 2009). Citalopram was no better than placebo on measures of global outcome and repetitive behavior, and was associated with adverse effects including hyperactivity, increased energy level, impulsivity, and gastrointestinal symptoms.

One target symptom that has received little attention in PDD treatment trials is that of anxiety unrelated to obsessive or compulsive behavior. Anxiety is common in PDD, but has only recently received research attention. Simonoff et al (2008) reported that social anxiety disorder was the most common psychiatric disorder occurring in 29.2% of a population-derived cohort of 112 children (ages 10-14 years) with autism spectrum disorders. Overall, any anxiety disorder was present in 41.9% of the population. The prevalence of generalized anxiety disorder, panic disorder, simple phobia, obsessive-compulsive disorder, agoraphobia, and separation anxiety disorder in this narrow age group was 13.4%, 10.1%, 8.5%, 8.2%, 7.9%, and 0.5%, respectively. Part of the challenge in the reliable characterization of these symptoms in patients is that individuals with PDDs often have poor expressive language skills. However, there continues to be a need for additional studies aimed at the treatment of anxiety and anxiety disorders in persons with PDD.

Mirtazapine is a novel drug that has both noradrenergic and serotonergic properties. Its primary mechanism of action is to block the presynaptic alpha-2-adrenergic receptor in addition to blocking serotonin 5-HT2 and 5-HT3 receptors. Antagonism at presynaptic alpha-2-adrenergic receptors (autoreceptors) increases norepinephrine (NE) release. Increased NE facilitates 5-HT release via alpha-1-adrenergic receptors on 5-HT neurons. This action is augmented by mirtazapine's alpha-2-adrenergic antagonism at the presynaptic alpha-2-adrenoreceptor on 5-HT neurons (heteroceptors) and resultant disinhibition of 5-HT release.

Mirtazapine's potent antagonism at the 5-HT2 and 5-HT3 receptors underlies its anxiolytic and hypnotic properties, as well as its low incidence of activating (5-HT2) and gastrointestinal (5-HT3) side effects (de Boer 1996). Antagonism at 5-HT2 receptors may be especially important in the treatment of PDD given the marked efficacy of risperidone, an atypical antipsychotic that also exhibits potent blockade of 5-HT2A receptors (Marek et al 2003).

Mirtazapine has a half-life of 20–40 hours and minimal potential for pharmacokinetic drug–drug interactions (Preskorn 1997). No specific laboratory monitoring is recommended with mirtazapine treatment, but there have been isolated reports of elevations in liver enzymes and exceedingly rare reports of agranulocytosis (Stimmel et al. 1997).

Mirtazapine is FDA-approved for the treatment of depression in adults and is currently available as a generic. Studies conducted since its approval have included children and targeted anxiety disorders. Mirtazapine was shown to be efficacious in a randomized, double-blind, placebo-controlled study of 66 women with social phobia (Muehlbacher et al 2005). Mirtazapine was also shown to be comparable to fluoxetine in a small (n=27) randomized, double-blind study of panic disorder (Ribeiro et al 2001). Mirtazapine has been beneficial in open-label trials involving children with depression (Haapasalo-Pesu et al 2004) and social phobia (Mrakotsky et al 2008), as well as adults with generalized anxiety disorder (Gambi et al 2005; Goodnick et al 1999). Mirtazapine has been well tolerated in children and adolescents. Adverse events have been comparable to those found in adults and include most commonly sedation and increased appetite. Additional potential side-effects reported have included sleep disturbance, dry mouth, nausea/vomiting, constipation, dyspnea, urinary frequency, back pain, edema, myalgia, dizziness, weakness, tremor, irritability, abnormal dreams, abnormal thinking, and confusion.

Mirtazapine has been proposed as a good option for patients who cannot tolerate SSRIs (Fava et al 2001). It improves sleep continuity without affecting sleep architecture (Winokur et al 2000). In schizophrenia, there is evidence that the addition of mirtazapine to antipsychotics improves both positive and negative symptoms, as well as cognitive dysfunction (Berk et al 2001; Delle Chiaie et al 2007; Joffe et al 2009; Zoccali et al 2004). Negative symptoms and cognitive dysfunction in schizophrenia may be relevant to autism given some overlap with the social and cognitive impairments found in PDDs.

In addition to evidence for the efficacy of mirtazapine in treating anxiety and other symptoms relevant to PDD, its effects on the serotonin system may also be important. Serotonergic abnormalities are well-known in autism and include elevations of whole blood serotonin (Schain & Freedman 1961; Anderson et al 1987), abnormal neuroendocrine responses to pharmacological probes of the 5-HT system (Hoshino et al. 1984; McBride et al. 1989), and worsening of behavioral symptoms in autistic adults after acute dietary depletion of tryptophan (McDougle et al. 1996b).

Our group was the first to report on the efficacy of mirtazapine in PDD (Posey et al 2001). We found it helpful for a variety of symptoms associated with PDD. One limitation of this study was that it was retrospective and so did not have uniform entry criteria, treatment or assessment. Furthermore, the subjects in the retrospective study had failed multiple other adequate medication trials (mean # previous trials=6). Thus, they were clearly treatment-resistant and not representative of the majority of persons with PDD. The response rate compared favorably to that of SSRIs that examined response rate retrospectively and over a longer period of time (Branford et al 1998). Since the publication of that first study, our group and others (Albertini et al 2006; Coskun & Mukaddes 2008, Nguyen & Murphy 2001) have continued to find it useful for a variety of symptoms including anxiety, insomnia, irritability, and hyperactivity dosing it both daily and twice daily.

In summary, anxiety frequently occurs in PDDs yet no treatment studies have been conducted which can guide treatment. SSRIs, which are frequently used to treat anxiety in children without PDD, cause an unacceptably high degree of activating side-effects.

Mirtazapine appears promising in the treatment of pediatric anxiety disorders and PDDs. A prospective, pilot study is urgently needed to address this large gap in treatment knowledge. This project is extremely relevant to advancing the knowledge about autism and its treatment. This proposal will fully characterize a cohort of children and adolescents with PDD and interfering anxiety. Each participant will then be randomized to either mirtazapine or placebo under double-blind trial conditions.

C. PRELIMINARY STUDIES: A RETROSPECTIVE ANALYSIS OF MIRTAZAPINE IN THE TREATMENT OF REFRACTORY SYMPTOMS IN PDD

Methods:

To date, our group's investigators at Indiana University and now at the Lurie Center/Massachusetts General Hospital has conducted the largest investigation of mirtazapine in persons with PDDs (Posey et al 2001). The first 26 subjects (5 females, 21 males) with PDDs treated with mirtazapine. Subjects' ages ranged from 3.8 to 23.5 years (mean age, 10.1 $\pm$ 4.8 years). All but one of the subjects had received previous trials of psychotropic medications. The majority of subjects were treatment-resistant; the mean number of previous unsuccessful medication trials was 5.5 $\pm$ 5.4. At the time of mirtazapine treatment, 17 subjects were taking concomitant psychotropic medications. Mirtazapine was prescribed for a variety of target symptoms including anxiety. Institutional review board approval was granted and written informed consent (and assent when appropriate) was obtained from the subjects' caregivers.

All subjects were diagnosed with PDDs by two board-certified child and adolescent psychiatrists using DSM-IV criteria. Twenty had autistic disorder, 4 had PDD NOS, and one each had Asperger's disorder and Rett's disorder. Twenty had co-morbid mental retardation (5-mild, 9-moderate, 6-severe). Based on a comprehensive clinical interview with the subjects and caregivers, five subjects had co-morbid Axis I disorders (2-intermittent explosive disorder, 1-bipolar disorder, 1-major depressive disorder, 1-oppositional defiant disorder). Three subjects had relevant co-morbid medical disorders that have been reported in association with autism (3-seizure disorder, 2-fragile X syndrome, 1-Sotos syndrome).

Once treatment with mirtazapine was begun, no additional changes in other psychotropic medications were made until after the assessment period. Mirtazapine was prescribed at a starting dose of 7.5 mg daily with dosage increases made in 7.5 mg increments up to a maximum of 45 mg daily in divided doses, depending on response of target symptoms and side-effects. Clinical response was monitored at each subject's regularly scheduled clinic appointments and/or via interim telephone calls that occurred at least monthly. In addition, height, weight, and vital signs were monitored on subjects at their clinic visits.

Baseline and endpoint ratings were conducted by two board-certified child and adolescent psychiatrists using the Clinical Global Impressions (CGI) scale (Guy 1976) during an interview with each subject's primary caregiver to determine overall response of target symptoms to mirtazapine. The CGI severity item (CGI-S) is rated on a scale from 1 to 7 (1 = normal, not at all ill; 2 = borderline ill; 3 = mildly ill; 4 = moderately ill; 5 = markedly ill; 6 = severely ill; 7 = among the most extremely ill patients). The CGI global improvement item (CGI-I) is also rated from 1 to 7 (1 = very much improved; 2 = much improved; 3 = minimally improved; 4 = no change; 5 = minimally worse; 6 = much worse; 7 = very much worse). Ratings of improvement were based on the global response of the subject's target symptoms to treatment with mirtazapine. Responders were defined by a rating of "much improved" or "very much improved" on the CGI-I item.

A structured side-effect checklist was used to query the subjects' caregivers about any new side-effects that occurred during treatment with mirtazapine. If a side-effect was reported, its severity was rated as transient, mild, moderate, or severe.

Comparisons of CGI-S ratings at baseline and after mirtazapine treatment were completed using paired *t*-tests. Comparisons of age and mirtazapine dose between responders and nonresponders were completed using student *t*-tests. Results are reported as the mean $\pm$ standard deviation with significance set at $p < 0.05$.

Results:

Twenty-five of 26 subjects completed at least 4 weeks of treatment with mirtazapine. The mean duration of treatment for the group was 150 $\pm$ 103 days (range, 11 to 368 days).

Nine of 26 subjects (34.6%) were judged responders ("much improved" or "very much improved" on the CGI-I), showing improvement in anxiety as well as irritability, hyperactivity, and depression. For the entire group of subjects, mirtazapine treatment led to a statistically significant improvement in CGI severity ratings (baseline = 5.00 $\pm$ 0.89; endpoint = 4.65 $\pm$ 0.94; paired $t = 11.29$; $df = 25$; $p < 0.04$).

Eight of 26 subjects (30.8%) were judged responders on the modified CGI improvement item assessing sleep quality. Statistically significant improvement was seen on the CGI severity ratings of sleep (baseline = 4.00 $\pm$ 1.94; endpoint = 2.80 $\pm$ 1.80; paired $t = 18.54$; $df = 24$; $p = 0.002$).

Side-effects were usually transient or mild in severity and included increased appetite, irritability, and sedation. Three subjects experienced significant weight gain (greater than 7 % over baseline) ranging from 7 to 10 lbs.

Summary:

In this naturalistic, retrospective study, mirtazapine was effective for the treatment of interfering behavioral symptoms in approximately 35% of treatment-refractory patients with PDDs. This modest rate of response is misleading since it does not take into account the fact that the subjects were markedly ill (mean CGI severity rating of 5) and had already failed to respond to a numerous previous medication trials before mirtazapine. Persons with PDD that are not refractory to treatment will likely show a much better response. In addition, outcome was measured after a mean five months of treatment (range 11-368 days) and thus reflects a longer period of follow-up compared to the majority of published short-term efficacy trials which are usually 2 months in duration. Finally, the study design was retrospective which leads to lack of precision in terms of some of the assessments.

D. INNOVATION

Studying the treatment of anxiety in PDDs is highly novel. Anxiety is a common co-morbidity in PDD though hasn't received attention in terms of characterization or treatment. This trial when completed will be the first study of a medication in the treatment of anxiety in PDD and the first prospective and placebo-controlled trial of mirtazapine in PDD. Mirtazapine itself has a unique mechanism of action and appears promising in treating PDD, but also in the treatment of symptoms (e.g., anxiety, social impairment, cognitive dysfunction) associated with other neuropsychiatric disorders with relevance to PDD.

Our group has pilot data with this drug in children and adolescents with PDD. We continue to use it clinically at our autism center, often as our first-line pharmacotherapy for interfering anxiety in PDDs. Over the years, we have found mirtazapine to be particularly effective for the treatment of disabling anxiety when other drugs such as SSRIs proved ineffective. This project represents a novel avenue of study and we expect that it will advance knowledge on how best to treat anxiety in autism. This data could also be used to obtain future funding for a more definitive large-scale clinical trial.

E. RESEARCH STRATEGY

Study Design:

A 10-week, flexibly-dosed, double-blind trial with 2:1 randomization to either mirtazapine or placebo was chosen as the optimal way of gathering quality preliminary data. Participants will be seen at screen, baseline (randomization), and following 2, 4, 6, and 10 weeks of treatment. The duration of the trial will allow us to adjust the dose of study drug over the first 6 weeks and allow for stable dosing during the final 4 weeks of the trial. Unbalanced randomization was chosen so that the majority of subjects will receive mirtazapine which will allow us to gather more information about the efficacy of mirtazapine in a broader range of subjects. A placebo control group will help us determine placebo effects in the treatment of anxiety in PDD.

Participants:

Thirty children and adolescents, ages 5-17 years, with a DSM-IV diagnosis of autistic disorder, Asperger's disorder, or PDD NOS exhibiting clinically significant anxiety on standardized rating scales will be recruited for this trial. All caregivers calling for an appointment at our Center are asked if they have any interest in participating in research. The Lurie Center's IRB-approved research registry will also be searched to identify participants eligible for this project. Recruitment goals will be established at the beginning for all study staff. Recruitment is reviewed weekly by all research staff, and any shortfalls lead to detailed discussion and problem solving around recruitment difficulties. One staff person will be primarily responsible for subject recruitment and report directly to the PI. Given the size of the Lurie Center, we do not expect significant difficulty in recruiting 30 subjects over the 3 years of the project.

Subjects and their legal guardians interested in participating in the study will have a face-to-face interview with the PI and/or the research coordinator/assistant where the nature of the project, the risks, the benefits, and the alternatives to participation in the project are discussed. Prior to engaging in research, formal written consent will be obtained from the parent(s)/legal guardian(s) on an IRB-approved consent form. An IRB-approved assent form will be also be used for the subjects since they are under the age of 18. The only exception will be for younger children with lower developmental levels who are assessed as incapable of participating in even a simplified discussion of benefits, risks, and alternatives.

Inclusion Criteria:

1. **Age 5-17 years**.
2. **Diagnosis of autistic disorder, Asperger's disorder, or PDD NOS based on DSM-IV-TR criteria.** A final diagnostic determination will be made by a board-certified child and adolescent psychiatrist with experience in the diagnosis of PDDs based on clinical history, review of records, mental status exam, and a thorough review of the information obtained from the Autism Diagnostic Interview-Revised (Lord et al 1994) and other administered rating scales.
3. **Clinically significant anxiety as evidenced by a Pediatric Anxiety Rating Scale (PARS) score of 10 or greater (5-item scale).** The PARS (Research Units on Pediatric Psychopharmacology Anxiety Study Group 2002) was chosen as an inclusion criteria (and outcome measure) since it assesses severity across common anxiety disorders in children including generalized anxiety, social anxiety, separation anxiety, and transition-associated anxiety. In addition, it is an instrument that allows the clinician to incorporate both child and parent report into a final clinician-rated score for each item.
4. **Abbreviated IQ greater than 50 on the Stanford Binet 5th Edition.**

Exclusion Criteria:

1. **Diagnosis of Rett's disorder or childhood integrative disorder**. Subjects with these other PDDs will not be enrolled since these disorders have a different etiology, course, and treatment response.
2. **Diagnosis of OCD, post-traumatic stress disorder, major mood disorder, psychotic disorder, or substance use disorder**. These disorders are exclusionary since the primary treatment of these disorders may require acute psychosocial treatments or other medications that would confound the assessments.
5. **Presence of any past or present medical conditions that would make treatment with mirtazapine unsafe**. This includes allergy to mirtazapine; agranulocytosis/bone marrow suppression; heart, kidney, or liver disease; unstable seizure disorder; and pregnancy (or being sexually active without using acceptable methods to prevent pregnancy).
3. **Use of other antidepressants or benzodiazepines.** Subjects will need to be off medications from these classes for at least 5 elimination half-lives prior to randomization.
6. **Use of other psychotropic medications which are ineffective, poorly tolerated, or sub-optimal in terms of dose**. A board certified child and adolescent psychiatrist will assess any other psychotropic medications being used and determine whether they are effective, tolerated, and optimal in terms of dose. Concurrent use of a psychotropic medication (other than antidepressants or benzodiazepines) will be allowed if the dose has been stable for 60 days and if they meet the above criteria of effectiveness, tolerability, and dose. Medications for concomitant attention-deficit/hyperactivity disorder symptoms (e.g., stimulants), insomnia (e.g., melatonin), and irritability (e.g., atypical antipsychotics) will be allowed if they meet the above criteria. Any medication that is assessed as possibly contributing to the subject's anxiety symptoms will not be allowed.
7. **Use of linezolid (Zyvox).** Subjects will need to refrain from taking linezolid during the study to prevent a potential drug interaction related to the serotonin syndrome. Linezolid taken in conjunction with mirtazapine could increase the risk of increasing serotonin activity to toxic levels, causing what is known as serotonin syndrome. This syndrome is characterized by at least 3 of the following symptoms: agitation, diaphoresis, diarrhea, fever, hyper-reflexia, incoordination, myoclonus, shivering, or tremor.
8. **Previous adequate trial of mirtazapine**. An adequate trial will be defined as a dose of 15 mg for at least 4 weeks. In addition, subjects who developed significant adverse effects during a trial of mirtazapine at any dose or duration will be excluded.

Baseline Characterization:

All subjects will have a basic psychiatric diagnostic evaluation done to gather a thorough developmental, medical, and psychiatric history, as well as complete a physical and mental status exam. The medical history and exam will be aimed at ruling out other medical conditions that could exacerbate anxiety or behavioral symptoms (e.g., chronic gastrointestinal disease, obstructive sleep apnea, thyroid disease). The following standardized scales and testing will also be conducted.

1. **Autism Diagnostic Interview-Revised (ADI-R)**.
2. **Anxiety Disorders Interview Schedule (ADIS-IV)**. The ADIS-IV (Silverman & Albano 2004) consists of semi-structured interviews for children and parents organized diagnostically to permit differential diagnoses among the DSM-IV anxiety disorders. It also includes questions on mood and externalizing disorders. We expect that not all child questions will be answerable depending on the communication abilities of the child. However, this will still provide information about the usefulness of this instrument in future trials involving children with PDD.
3. **Stanford-Binet Intelligence Scales, Fifth Edition (SB5)**. The SB5 (Roid 2003) will be used to calculate an abbreviated battery IQ. The abbreviated IQ was chosen in order to

limit subject burden given that a number of other measures will be conducted at screening.

4. **Peabody Picture Vocabulary Test, Fourth Edition (PPVT-4)**. The PPVT-4 (Dunn & Dunn 2007) will be done to assess receptive language skills.
5. **Vineland Adaptive Behavior Scales, Second Edition (Vineland II)**. The Vineland-II (Sparrow et al 2005) will be used to assess adaptive functioning in four domains: Communication, Daily Living Skills, Socialization, and Motor Skills. This is a well-standardized open-ended interview used to assess the overall functioning of children and adults. This measure is especially important for subjects with PDDs given that their intellectual level is not always comparable to their adaptive functioning (Volkmar et al., 1993).
6. **Laboratory evaluation**. This will consist of a complete blood cell count with platelets and differential, a comprehensive metabolic panel (including electrolytes, renal function tests, liver function tests, glucose), and thyroid function tests. These tests will be drawn to rule-out any occult medical problems not detected by history and physical exam alone. A urine pregnancy test will be obtained from females of childbearing potential.

Mirtazapine/Placebo Treatment:

Subjects will receive 7.5 mg of mirtazapine nightly at the start of the trial. The dose will be increased by 7.5 mg per week for subjects with weights less than 50 kg and by 7.5 or 15 mg per week for with subjects weight greater than 50 kg depending on efficacy and tolerability. During the first 6 weeks, patients will be seen bi-weekly with additional telephone visits conducted in between clinic visits for additional monitoring of adverse effects and for dosing adjustment as necessary. The optimal dose will be reached by week 6 of treatment. A final visit will be conducted at week 10 with a follow up phone call at week 8. The minimum starting dose will be 7.5 mg and the maximum total daily dose will be 45 mg (based on our preliminary study and clinical use). Medication will be dosed once or twice daily depending on response and tolerability. Our previous experience has found the addition of a morning dose to be well-tolerated and effective in a clinical sample (Posey et al 2001). Once daily dosing at night only will be allowed if children are unable to tolerate a morning dose due to sedation.

Matching placebo prepared by a compounding pharmacy will be identical to mirtzapine in appearance and taste.

Outcome Measures:

1. **PARS**. The PARS will be the primary outcome measure. It will be conducted at screening, baseline, and each follow-up visit.
2. **Screen for Childhood Anxiety Related Emotional Disorders (SCARED)**. The SCARED (Birmaher et al 1999) is a 41-item scale that includes both a child/self-report and parent-report form. It assesses symptoms of panic disorder, separation anxiety disorder, social phobia, and generalized anxiety disorder, as well as school avoidance. It will be conducted at baseline and each follow-up visit.
3. **Child and Adolescent Symptom Inventory (CASI) Anxiety Items**. These include 20 parent-rated items from the Child and Adolescent Symptom Inventory that has been administered to children with PDD in previous trials (Sukhodolsky et al 2008). It will be conducted at baseline and each follow-up visit
4. **Aberrant Behavior Checklist (ABC)**. The ABC is a 58-item questionnaire with 5 subscales derived by factor analysis: Irritability, Social Withdrawal, Stereotypy, Hyperactivity, and Inappropriate Speech (Aman et al., 1985). It has been extensively used in psychopharmacological studies of autism and assesses many symptoms that are either central to autism (Social Withdrawal, Stereotypy, Inappropriate Speech) or

frequently a target of treatment (Irritability). It will be conducted at baseline and each follow-up visit.

5. **Children's Sleep Habit Questionnaire (CSHQ)**. The CSHQ (Owens et al 2000) is a 46-item parent-report questionnaire that assesses bedtime resistance, sleep onset delay, sleep duration, sleep anxiety, night waking, parasomnia, sleep disordered breathing, and daytime sleepiness that has been used in children with PDD (Honomichl et al 2002). It will be conducted at baseline and each follow-up visit.
6. **Clinical Global Impressions (CGI)**. A trained clinician blind to treatment assignment and detailed side-effect information will perform the CGI. The CGI is designed to take into account all factors to arrive at an assessment of severity and response to treatment, including parent report, parent-rated measures, teacher-rated measures, and clinician-rated measures (as described below). The CGI Severity (CGI-S) item is rated on a scale from 1 to 7 (1 = normal, not at all ill; 2 = borderline ill; 3 = mildly ill; 4 = moderately ill; 5 = markedly ill; 6 = severely ill; 7 = among the most extremely ill patients). The CGI Global Improvement (CGI-I) item is also rated from 1 to 7 (1 = very much improved; 2 = much improved; 3 = minimally improved; 4 = no change; 5 = minimally worse; 6 = much worse; 7 = very much worse). Subjects receiving a CGI-I rating of 1 or 2 will be classified as **responders** to treatment. The severity ratings will be conducted at baseline and the last follow-up visit. The improvement ratings will be conducted at each follow-up visit.
7. **Developmental Disability-Child Global Assessment Scale (DD-CGAS)**. The DD-CGAS (Wagner et al 2007) is a modified version of the CGAS for use in PDD treatment trials. It will be conducted at baseline and at the last follow-up visit.

Safety Monitoring:

Adverse events, vital signs, height, and weight will be collected at each clinic visit. A Comprehensive Metabolic Profile and CBC will be collected at baseline and endpoint. Standard methods and precautions will be used to protect the venipuncture site from bleeding and infection. In addition, a trained pediatric phlebotomist will perform all venipunctures that are required. A research assistant, who will be familiar with the subjects, will accompany the subject and their parents for the venipuncture. Parents are encouraged to remain with the subject at all times. To minimize the subject's anxiety and phobic reactions, we "talk through" the procedure with the subject so that they might know, to some extent, what to expect. For subjects who are minimally verbal, we use visual supports in order to prepare the participants for the blood draw. Topical anesthetics (e.g., EMLA or lidocaine cream) are sometimes used as well at the discretion of the nurse or investigator.

Adverse events will be collected via a structured side effect rating scale completed with the participant and their primary caregiver. This will include a list of side-effects that have been reported with mirtazapine at a rate greater than 1% including sedation, sleep disturbance, dry mouth, increased appetite, nausea/vomiting, constipation, dyspnea, urinary frequency, back pain, edema, myalgia, dizziness, weakness, tremor, irritability, abnormal dreams, abnormal thinking, and confusion. Weight gain is a potential side effect especially in those subjects that have been on atypical antipsychotic medications which can also cause weight gain. Parents of the subject will be informed of this risk in the informed consent prior to participation in the study. Each of these side-effects plus any additional complaints will be rated at baseline on a 4-point scale by the caregiver as follows: 0 = none; 1 = mild; 2 = moderate; 3 = severe. This scale is similar to that used by the RUPP Autism Network (Aman et al., 2005). Suicidality will be assessed at each visit by directly asking the subject (when verbal skills are sufficient) and caregiver about any thoughts or behaviors that directly or indirectly might indicate suicidality (e.g., morbid thoughts, self-injury, statements about life not being worth living), when indicated by positive responses. At baseline, the Columbia-Suicide Severity Rating Scale (C-SSRS) will be administered per the instructions at www.cssrs.columbia.edu/clinical_trials.html. The C-

SSRS will also be administered at each subsequent visit following the first report of suicidality. Subjects and caregivers will also have information on how to contact the on-call physician from our practice group who provides coverage 24 hours/day and 365 days/year. Physicians taking call are familiar with research protocols and can contact the principal investigator at any time.

All adverse events picked up on the rating scale as new will be recorded by the physician. The physician will also ask about any visits to the doctor, new medication use (e.g., OTC cold medicine), or any other complaints, in order to be confident that most AEs are uncovered. The physician will keep a running log of adverse events that will record the date of onset, date of resolution, seriousness, severity, and relationship to study intervention (e.g., definite, probable, possible, remote, or none) as well as whether the adverse event led to a change in study intervention or other treatment.

A physician will review vital signs and laboratory data, as they become available. All significant AEs as well as the progress of the study will be reviewed and discussed in detail at the biannual meetings of the **Lurie Center Data and Safety Monitoring Board (DSMB).** Special attention will be made by the treating physician to note all weight gain trends and bring any concerns to the DSMB. The DSMB is currently being formulated for the Lurie Center and will include rotating members of the Lurie Center including pediatricians, pediatric and adult neurologists, child and adolescent psychiatrists and independent members outside the Lurie Center.

Statistical Analysis Plan:

Since this is a preliminary trial that is not powered to show that mirtazapine is better than placebo in the treatment of anxiety in children and adolescents with PDD, we will analyze the data to determine whether the following pre-specified positive indicators of efficacy are present.

1. Significant improvement in PARS score with mirtazapine.
2. Proportion of subjects receiving mirtazapine rated as responders is 50% or greater.
3. Change in PARS score is numerically greater with mirtazapine than placebo.
4. Proportion of subjects rated as responders to mirtazapine is numerically greater than placebo.

Outcome measures will also be analyzed to determine whether there are statistically significant differences between mirtazapine and placebo. However, these will be considered exploratory since they may be underpowered.

The primary outcome measure, PARS score, and other continuous measures will be analyzed using a mixed effects linear model using PROC MIXED in SAS with a REPEATED statement. Prior to running analyses, each dependent variable will be checked for normality and transformed if necessary. In accordance with intention-to-treat principles, data from all subjects having at least one visit following baseline will be analyzed. Significant main effects will need to be significant at an alpha level of 0.05.

For the CGI-I rating, subjects classified as “much” or “very much improved” will be defined as **responders** and all other classifications will be regarded as non-responders. We will be using this classification of the subjects throughout the trial as the dependent variable in these analyses. Hence, the CGI-I dichotomous variable will be examined over the study using a model with time treated as a repeated factor and treatment group as a fixed effect.

The occurrence of particular adverse effects during the 10-week treatment period will be compared between groups using Chi-square tests. In addition to absolute measures, height, weight and body mass index will be transformed to standardized z scores using anthropometric indices based on the 2000 Centers for Disease Control and Prevention age- and gender-normed growth charts (http://www.cdc.gov/growthcharts/). These z scores will then be analyzed in a similar manner to those used for other continuous measures described above.

Sample Size Consideration and Power Analysis:

The baseline to endpoint comparisons of mirtazapine (n=20) have 80% power to detect an effect size of 0.7 (two-tailed significance of 0.05). The mirtazapine (n=20) vs. placebo (n=10) analyses of the secondary comparisons have 80% power to detect an effect size of 1.1.

Training and Reliability on Instruments:

Training on clinician-rated instruments consists of observing videotaped interviews conducted by trained interviewers, then interviewing under direct supervision. CGI Reliability will be conducted in a manner similar to that used by our site in the RUPP Autism Network. Our site is fully certified for using the ADI-R for research purposes.

Data Management:

When a participant is enrolled in the study, he or she will be assigned a unique identification number that is used to identify all data associated with that person, including hard copy, biological specimens, and computerized data. Data will be collected on hard-copy forms and then verified by data entry personnel. All of the hard copy research data is kept in locked file cabinets at the Lurie Center. Only the PI and primary research assistants will have access to these files, ensuring security of the hard copy records.

Once data is obtained, the clinician will review the form to make sure that all required items are completed and to clarify any ambiguous notations before giving it to the research assistant responsible for data entry. Several quality control measures are built into our computerized data management. Data entry forms have been designed that correspond to the measures used in this study. These forms are configured so that out-of-range values cannot be entered; data entry prompts appear in the correct order, including skipping questions when appropriate; and entered values cannot be inadvertently overwritten. Immediately after data entry, a series of logic check programs are run automatically, indicating any entered values that appear incorrect and the reason why. Logic check reports will be reconciled with the hardcopy and, if necessary, the clinician. Changes will then be entered, and the logic check programs executed again in order to detect any new errors resulting from the changed values. The process will continue until no further errors are detected by the logic check programs.

Project Milestones:

Year 1: The trial will be initiated after obtaining IRB approval, training of all personnel on study procedures, and achieving reliability on screening and outcome measures. Nine subjects will enroll into the protocol.

Year 2: Trial enrollment continues. An additional 12 subjects will be enrolled into the protocol.

Year 3: Trial enrollment continues. The final 9 subjects will be enrolled into protocol. Data will be analyzed.

End of Trial Alternatives:

At the completion of the 10-week trial, the prescribing physician will break the blind in order to decide on an appropriate next step for the participant. Placebo treated subjects still exhibiting clinically significant anxiety will be entered into a 10-week open-label trial of mirtazapine of similar design to the double-blind trial with the exception that the outcome measures will be more limited in scope and conducted by the physician. This will help to protect the blinded rater from gaining any knowledge that might influence their ratings during the course of the study. This mechanism has been used successfully in studies conducted by the RUPP Autism Network.

Schedule of Measures:

Measure	SC	BL	W2	W4	W6	W10
Diagnostic Exam including history and mental status exam	X					
Physical exam, labs	X					X
Urine Pregnancy Test (females of child-bearing potential only)	X					
Vital signs, height, weight, AEs, Con Meds	X	X	X	X	X	X
ADI-R, ADIS-IV, PPVT-4, SB5, Vineland-II	X					
PARS	X	X	X	X	X	X
SCARED, CASI Anxiety, ABC, CSHQ		X	X	X	X	X
CGI-S, DD-CGAS		X				X
CGI-I			X	X	X	X
C-SSRS	X*	X*	X*	X*	X*	X*

* - C-SSRS will be administered to child and/or parent if any positive suicidal ideation is reported during clinical assessment. Each visit following the first report of suicidal ideation will include the administration of the C-SSRS.

G. ENVIRONMENT

The performance site is the Lurie Center in Lexington, Massachusetts. It serves as the major referral site for children, adolescents, and adults with autism and serious developmental disabilities from throughout the State of Massachusetts, as well as neighboring states. Research is a major focus of the Center's mission. Approximately 450 patients with autism or another PDD are seen annually for an initial evaluation thus ensuring an adequate sample from which to recruit.

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