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TITLE: Developing Treatment, Treatment Validation & Treatment Scope in the Setting of an Autism Clinical Trial

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14. ABSTRACT We submitted and received approval for our latest Continuing Review (CR). In addition a restructuring of this project was done along with related changes to the protocol to help increase the rate of subject recruitment. This includes changing of responsibilities and effort for some of the project member. These changes were in conjunctiwith the no-cost extension and were also approved by our IRB. Several amendments to our protocol were made increase recruitment and reduce burden on subjects including changes to recruitment tools, addition of new recruitment tools and addition of recruitment sources. We removed two blood tests that were no longer useful to the project and were therefore an excess burden to the subjects. Our new recruitment model contains the additioof other autism sites such as Children's Specialized Hospital and others. We have now begun tasks 3-7 and we wcontinue to refine and increase our recruitment tools and sources to achieve our goal. Please see initiating project W81XWH-08-1-0728 and partnering project W81XWH-08-1-0729.					
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Introduction:

This project is to test to see if DHA treatment can beneficially affect excretion of urinary biomarkers of oxidative stress and the autism clinical phenotype. In addition polymorphic variants of genes of certain enzymes that synthesize and metabolize docosahexaenoic acid (DHA) may contribute to the phenotype of some autism cases. We will test to see if any of these genes are risk factors for autism. We will also measure changes in excretion of the polyunsaturated fatty acid (PUFA) derived biomarkers of oxidative stress (isoprostanes and neuroprostanes) together with the changes in production of anti-inflammatory lipid mediators. We will test these biomarkers to see if we can monitor and validate effectiveness of DHA therapy. We will also test the genotypes of key DHA-metabolizing enzymes can predict which patients will respond to therapy.

Please see initiating project W81XWH-08-1-0728 and partnering project W81XWH-08-1-0729.

Body:

Project 1: PI Sherie Novotny, MD, Partnering PI, W81XWH-08-1-0730

Please see initiating project W81XWH-08-1-0728 and partnering project W81XWH-08-1-0729.

Task 1 Full board review with pending IRB approval prior to beginning (4-6 months, S. Novotny).

Our latest Continuing Review (CR) was approved on September 29th 2011. In addition a restructuring of this project was done along with related changes to the protocol to help increase the rate of subject recruitment. These changes were also approved by our IRB on September 29th 2011. For a more detailed description of these changes see below in Task #2. The approved CR was sent to the HRPO on October 1st 2011. Please also see partnering project W81XWH-08-1-0728 Tasks #1 and 2.

Additional amendments in the past year:

We submitted amendments to our IRB to add additional sources of subjects.

First, a flier, a webpage and a weblink were generated and submitted to our IRB for approval. We received approval on December 9th 2010.

We submitted another amendment to our IRB to add additional sources of subjects. Recruitment letters to be sent to families with children at the DDDC and to subjects of previous research studies of Dr's Ming, Johnson and Lambert that expressed interested in being part of future research projects (already allowed by the protocol) were sent to our IRB for review. The amendment was submitted December 10th. We received IRB approval to distribute the recruitment letters on February 4th 2011.

We submitted an amendment to our IRB containing a number of issues including modifying recruitment tools and two changes to the protocol removing two blood tests on May 9, 2011. We re-wrote our flier and made additional fliers targeted to different audiences (doctors of potential autism subjects, families etc). Two blood tests were removed from the protocol. In brief, to decrease the burden on the subjects we removed the PT/PPT bleeding profile. It is known that there is a risk of reduced platelet activity and increased bleeding times when very large doses of omega-3 fatty acids are given. We learned that decreased platelet function and increased bleeding times due to high doses of fish oil are due to the action of eicosapentaenoic acid's

(EPA's) down regulation of *PGE2* and *TXA2*¹. EPA is a different component of fish oil and the tablets we are using contain no EPA. In addition Nelson, GL et al 1997² previously tested DHA to see if there was any effect of DHA on bleeding time. They gave 15 grams (375 times the dosage we are using) of DHA per day for 90 days and saw no changes in platelet aggregation or prothrombin bleeding time. We therefore feel that the increased burden on the subjects of requiring additional blood drawing is not productive and does not increase the safety of this trial.

In addition, in our original study design when the grant was written in 2006-2007 we had planned on testing certain environmental toxins. As results from other studies became available including Dr. Lambert's study we decided that the tests would not be scientifically productive and we decided it would not be proper to require the subjects to give additional blood for these tests. We therefore removed them from the study and the protocol. A clerical error on our part reintroduced the text back into the protocol and this text was in the final IRB approved version. This amendment was approved on August 2nd 2011

Four subjects were enrolled during the review process and we did not draw blood for these two tests which represents a protocol deviation. We informed our IRB and the DOD of this. We received a letter accepting our report and determined that the subjects were not exposed to any additional risks from our IRB on August 2nd 2011. A copy was sent to the DOD HSROP on August 15th 2011.

Task 2 Volunteers recruited from local clinics, support groups and advocacy groups (6-30 months).Forty four child or adolescent outpatients per year with age ranges from 5-17, for three years totaling 132 patients, will be randomized into the 12-week double-blind, placebo-controlled parallel treatment study.

We have continually updated and improved our recruitment techniques and tools (please see Task #1 for specific protocol changes). With the goal of increasing the recruitment rate we started to review and analyze our protocol and recruitment strategies in March of 2011. We spoke with others that have completed studies and are moving forward with some of the suggestions. We decided that our protocol and recruitment model were adequate for recruiting given the full three years of the project but are not with the limited time we currently have. We decided we needed to make changes in the protocol and recruitment model to help Dr. Novotny and the study coordinator Dr. Wasiulla meet the recruitment goals. We found two areas that we thought could make a large difference in recruitment. First, in our protocol, recruiting, initial evaluation and consenting are all in the hands of either Dr. Novotny or Dr. Wasiulla. Second, our recruitment model limits us by having our recruitment sources only refer subjects to the project. We therefore initiated the following changes (these changes are interrelated to the no-cost extension and therefore made together).

To facilitate these changes our first step was to assess our current status. Dr.'s Novotny and Ming began a review of who has responded to our recruitment tools and who has not as well as a review of those that have contacted us and expressed an interest in the study but have not as of yet decided to enter the study. We found several things. First is that travel is an issue for many of the families. Second, some parents are already giving fish oil to their children and do not want their children to stop taking it if there is a chance that they may receive placebo. They are asking us to guarantee that their child will receive DHA and not placebo. Since this is impossible we are trying to determine if after the end of the study (once the study is over and decoded) we can give all subjects that received placebo DHA as an incentive. Third, is that while the letters we sent to subjects of previous studies were effective in letting people know

about this study they are not effective in recruiting without a follow up phone conversation from the PI of the previous project. Trust and having an established relationship with these families is important. In addition these families have very significant time constraints and being interested in being part of the project does not translate to recruitment without further follow up and explanation of the benefits of the trial. Dr. Ming has just begun this process and 6 of her previous subjects have contacted us to enter the study. She will continue this process. Dr's Lambert and Johnson have begun this process.

The second step is to amend the protocol to allow other study members to do preliminary testing and consent the subjects. Along with this we are increasing the amount of effort that Dr. Ming will spend on this project. Now that this amendment has been approved Dr. Ming is able to help Dr. Novotny by screening and consenting subjects. This amendment was approved on September 29th 2011 (please see Task #1). Also, please see Task #2 of W81XWH-08-1-0728 (Dr. Johnson).

In concert with the second step our third step was to restructure our recruitment model. In this new model we divided recruitment into two areas. Dr. Novotny is responsible for recruitment in the New Brunswick/Piscataway area (central NJ) and Dr. Ming is responsible for recruiting in the Newark area (northern central NJ). Our study coordinator Ms. Wasiulla is splitting her time between the New Brunswick / Piscataway area and Newark to complete psychological testing for diagnostic confirmation and other baseline behavioral testing. This way all parts of the trial will be done where the subject is recruited with the exception of the randomization/initial sample collection and the final sample collection. This should minimize burden on the subjects and their families and should reduce travel time greatly. Mr. Stenroos will now start to assist Dr's Novotny and Ming and Dr. Wasiulla in recruitment and follow up. This restructuring was completed with the amendment approved on September 29th 2011.

In addition each area is utilizing additional sites to maximize recruitment.

We have also contacted the director of Children's Specialized Hospital which has several branches in NJ. These hospitals cater to a large section of children and adolescents with mental health issues in NJ. According to the director they have an overload of children with Autism Spectrum Disorders who are in the waiting list for testing and treatment services from the hospital. The director, Dr. Frank Castello believes this study is of interest to his patients and families and that those that decide to enter the study will receive the testing that they have been waiting for more quickly.

Dr. Ming has contacts at the Children's Specialized Hospital at Mountainside. Dr. Evelyn Okouneva is a graduate of Dr. Ming's Pediatric Neurology program who is working at Children's Specialized Hospital at Mountainside, NJ. She sees patients five days a week, one third of her patients are autistic. She inherited many patients from Dr. Brenda Torres who was the developmental pediatrician at the Children's Specialized Hospital. Dr. Okouneva is enthusiastic about helping us recruit. We think it is best to meet the subjects at the Children's Specialized Hospital at Mountainside and do the pre-screening and consenting there by Ms. Wasiulla and Dr. Ming.

St. Peter's Hospital. Dr. Barbie Zimmerman-Bier runs an autism center at St. Peter's hospital and sees patients 2 days a week. She is enthusiastic about helping us recruit for this project.

Dr. Ming will begin recruiting from her autism clinical population. Dr. Ming has had great success in her past research projects recruiting this way.

Dr. Ming will meet with Susan Adubato to try to find more contacts.

Sr. Adubato's effort has been changed to allow a higher throughput of testing.

Mr. Stenroos has been given the responsibility of all IRB issues. He will also assist Dr.s Novotny Wasiulla and Ming in subject recruitment consenting etc. He will be responsible for managing recruitment sources.

We are in the process of evaluating if dividing up the areas further is a benefit. If this is the case we may need to add additional study personnel.

We will be attending the annual conference of Autism NJ. This conference is the largest of its kind in the state. It is a large and valuable tool and is attended by families of individuals with autism, doctors, representatives of autism schools and autism centers/departments of hospitals as well as researchers. We have attended before and it has been a valuable source in the past. Unfortunately we were unable to utilize fully last year since we did not have full approval at that time.

Dr. Novotny will be making a presentation titled "Alternative Treatments in Autism" at the conference. In her talk she will be speaking of the use of Omega-3 fatty acids in autism. Partnering PI, Dr. Stein (project W81XWH-08-1-0729) will be presenting a poster titled "Polyunsaturated fatty acid metabolism in Autism".

We will have a booth to distribute our recruitment materials and Dr's. Ming and Wasiulla and Mr. Stenroos will attend the conference with the goal of informing autism families, doctors, representatives of autism schools and autism centers/departments of hospitals as well as researchers of our study and making contact with potential recruitment sources.

The annual Data and Safety Monitoring Board (DSMB) meeting is being set up and will be held in the beginning of November. The board consists of Wei-Ting Hwang of UPenn, Kapila Seshadri of UMDNJ-RWJ and Bart Kamen of UMDNJ-RWJMS.

Task 3 Informed consent/assent obtained (6-30 months).

This task has begun and will continue until recruitment is closed. To date we have consented 13 subjects and assented 2.

Task 4 Full diagnostic assessment with Autism Diagnostic Interview-revised (ADI-R), Autism Diagnostic Observation Scale (ADOS), Vineland Adaptive behavior scale and Leiter Intelligence Scale (E Roberts); DSM IV criteria (S. Novotny) for eligibility & diagnosis. Parents will complete baseline Aberrant Behavior Checklist; study psychiatrist will complete Clinical Global Improvement, baseline Severity Scale (6-30 months, 21-42 months in the current SOW).

This task has begun and will continue until recruitment is closed. To date 11 subjects have undergone ADOS, ADI, Vineland and Leiter,

Task 5 Cases undergo full medical evaluation to determine health; at this visit will have phlebotomy including 10 mls for blood chemistry, PT/PTT, hematology, 10 ml for

genotyping (Project III), urine for pregnancy test, drug screen as indicated, routine urinalysis; urine collected for Project II.

This task has begun and will continue until recruitment is closed. To date 9 subjects have undergone full medical evaluation and had phlebotomy..

Task 6 Cases randomized to receive either DHA, 200mg daily, or placebo. Cases given DHA after physical exam and routine lab-work completed.

This task has begun and will continue until recruitment is closed. To date 9 subjects have been randomized and received either DHA, 200mg daily, or placebo.

Task 7 Cases seen weekly for four weeks and biweekly for the remaining 8 weeks. Aberrant Behavior Checklist done every 4 weeks and at termination and Clinical Global Improvement Scale done every 2 weeks and at termination.

This task has begun and will continue until recruitment is closed. To date, only 4 subjects have completed the study, so ABC's and CGI's were only completed by those 4. Others who are in the study are being administer these tests bi weekly.

Task 8 Cases complete the study with repeat ADOS, Vineland Adaptive Behavior Scale (E Roberts) and Aberrant Behavior checklist (parent) and Clinical Global Improvement Scale (S Novotny). Blood work for safety measures; urine will be collected for Project II during last week of DHA or placebo.

This task has begun and will continue until recruitment is closed. To date, only 4 subjects have completed the study, so ABC's and CGI's were only completed by those 4. Others who are in the study are being administer these tests bi weekly.

Task 9 Data will be collected and analyzed (6-36 months, 04 year as per the current SOW S Buyske).

This task has not begun yet. This will start once when analysis of samples is completed.

Task 10 Manuscripts prepared and submitted for publication (03 year, 04 year as per the current SOW all investigators)

This task is to be done when the analysis of the data is completed.

Key Research Accomplishments

There are no Key Research Accomplishments yet.

Reportable Outcomes:

There are no reportable outcomes for any of the three projects as of yet.

Conclusion:

A large amount of time was spent on getting IRB approval for this project. We are now working on making up the lost time. We have now begun tasks 3-7. In addition we have restructured this project to increase our recruitment ability and rate as well as structured a no-cost extension. We anticipate a significant increase in subject recruitment and no other potential problems that would impede progress in continuing to recruit subjects. We expect that we can move forward rather quickly. We are and will continue to add new subjects including those that have already been tested for ADOS and ADI. We are seeking out new recruitment sources and will continue to so. We will continue to refine and increase our recruitment tools and sources to achieve our goal.

References:

1. Bernardini R, Chiarenza A, Calogero AE, Gold PW, Chrousos GP. Arachidonic acid metabolites modulate rat hypothalamic corticotropin-releasing hormone secretion in vitro. *Neuroendocrinology* 1989;50:708-15.
2. Nelson GJ, Schmidt PS, Bartolini GL, Kelley DS, Kyle D. The effect of dietary docosahexaenoic acid on platelet function, platelet fatty acid composition, and blood coagulation in humans. *Lipids* 1997;32:1129-36.

Appendices:

There are no appendices.