PROTOCOL TITLE: Long-term Effects and Safety of DHA+AA Supplementation in Toddlerhood for Children born Preterm

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REVISION HISTORY

Revision #	Version Date	Summary of Changes	Consent Change?
1	7/2/2021	We modified our target age window, made edits to caregiver and child protocols (ie: added new activities, modified existing activities), made plans to provide families a generalized results letter, and edited details in our statistical analyses. We've also omitted references to any social media group.	Yes
2	9/14/2021	We are adding an exclusion criterion and designating another instrument as a secondary outcome measure.	No
3	3/29/2022	We included a way we will compensate schools if a teacher cannot personally accept compensation for completing teacher surveys. We are also clarifying the consent process for instances where families are "surveys only."	Yes
4	6/20/2023	We modified how we collect medical information. Parents will be asked for contact information for their doctor(s) and may be asked to complete a medical release form if required by their doctor's office. Modified consent form to reflect this change.	Yes
5	3/18/2024	We are modifying our recruitment methods to allow us to approach families in clinic if they	No

		have an appointment scheduled at NCH. We are also adding additional recruitment efforts to families who have not enrolled or refused as of 3/18/2024. We modified the amount of time that we will store PHI to align with current guidelines.	

1.0 Study Summary

Study Title	Long-term Effects and Safety of DHA Supplementation in Toddlerhood for Children born Preterm
Study Design	The present follow up study involves a prospective cohort of toddlers born preterm from our previous randomized, double-blind, placebo-controlled Omega Tots trial (ClinicalTrials.gov Identifier: NCT01576783, IRB11-00343, IRB14-00342).
Primary Objective	The primary objective for this study is to determine the long-term effect of daily dietary supplementation at age 1 year with omega-3 docosahexaenoic acid (DHA) and omega-6 arachidonic acid for children born preterm by comparing general cognitive ability, language, and multiple facets of executive function between the intervention and placebo arms at ages 8½ -10 ½ years.
Secondary Objective(s)	The secondary objective of this study is to determine the role of variability in fatty acid metabolism genetics en masse on the effect of supplementation on short and long-term outcomes, and further focus on 2 previously published FADS2 genetic variants.
Research Intervention(s)/ Investigational Agent(s)	Children from the original Omega Tots trial (NCT01576783) were previously randomized to 180 days of DHA+AA (200mg DHA+200mg AA daily) or placebo (400mg corn oil), dissolving powders in blinded foil packets. At this time, participants will not receive any further intervention or investigational agents. Long-term outcomes will be observed and recorded accordingly across intervention groups
IND/IDE #	*112885*
Study Population	Participants will be the 377 parent-child dyads from the original Omega Tots trial. They were born at <35 weeks' gestation, were 10-16 months of age (age corrected for prematurity) at trial enrollment, and had been patients at Nationwide Children's Hospital NICU or Follow-up clinic.
Sample Size	All 377 randomized children from the original Omega Tots trial (NCT01576783) will be eligible for in-person follow-up at age 8½ - 10 ½years.
Study Duration for individual participants	Participants will partake in one comprehensive study visit when children are sometime between 8 years, 180 days and 10 years, 180days of age. It is expected that participant enrollment will begin by September 1, 2020, and that human subjects' activity will be completed by February 1, 2025.
Study Specific Abbreviations/ Definitions	Nationwide Children's Hospital (NCH); Randomized Controlled Trial (RCT); docosahexaenoic acid (DHA); arachidonic acid (AA); Adverse Event/Adverse Experience (AE); Serious Adverse Event/Experience (SAE)

Statement of Compliance

The study will be carried out in accordance with Good Clinical Practice (GCP) as required by the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46; 21 CFR Part 50, 21 CFR Part 56, and 21 CFR Part 312)
- ICH E6; 62 Federal Register 25691 (1997)
- NIH Clinical Terms of Award

All key personnel (all individuals responsible for the design and conduct of this study) have completed Human Subjects Protection Training.

2.0 Objectives

Our long-term goal is to ensure that interventions to help children born preterm succeed in school are safe and effective.

Our overall objective with the present study is to determine the long-term effects of docosahexaenoic acid (DHA) plus arachidonic acid (AA) supplementation (hereafter called "DHA+AA") on general cognitive ability, language, and executive function, and to examine genetic explanations for treatment effects, through one comprehensive study visit with children and parents from our Omega Tots trial cohort (NCT01576783).

2.1 Hypotheses & Aims

Our central hypothesis is that preterm children who were randomized to 180 days of DHA+AA at age 1 will exhibit poorer general cognitive ability, greater language deficits, and more impaired executive function at age 8½ -10½ vs children randomized to placebo. Our hypothesis has been formulated primarily on the findings from Omega Tots and DINO, the largest DHA-supplementation trial of preterm infants. The rationale for this project is that a careful examination of the long-term effects of DHA+AA supplementation will offer much-needed clarification about the appropriateness of DHA+AA as an intervention to promote neurodevelopment among children born preterm. We plan to pursue our central hypothesis and accomplish our objective by pursuing the following specific aims:

***Aim 1*: Determine the long-term effect of DHA+AA supplementation at age 1 year by comparing general cognitive ability, language, and multiple facets of executive function between the DHA+AA and placebo arms at age 8-9.**

- Based on our preliminary data, working hypothesis 1 is that children randomized to DHA+AA at age 1 will display at least a 0.3-SD (effect size) deficit on measures of general cognitive ability, language, executive function at age 8½ - 10½ vs placebo.
- Sub-aim 1: Explore sub-group differences by sex, birthweight, socioeconomics, and diet.

Aim 2: **Determine the role of variability in fatty acid metabolism genetics en masse on the effect of DHA+AA supplementation on short and long-term outcomes, and further focus on 2 previously published FADS2 genetic variants.**

- Based on prior observational studies, working hypothesis 2a is that common genetic variant-based heritability over long-chain fatty acid metabolism will be associated with poorer performance on our outcome assessments at ages 1, 2, and 8½ - 10½ years for children assigned to DHA+AA vs placebo. Genetic variation is based on measured SNPs in genes accounting for virtually all synthesis of very long-chain fatty acids. Further, we hypothesize (2b) that minor allele status for FADS2 rs1535 and rs174575, the most commonly replicated SNPs in the literature, will be associated with poorer outcomes for children assigned to DHA+AA, vs placebo.

Upon completion of the proposed research, our expected outcomes are to have determined the long-term effects of DHA+AA supplementation in toddlerhood for children born preterm and the role of genetics. These results are expected to have an important positive impact: we expect to either offer reassurance about the safety of DHA+AA-supplemented products for young children, or bring attention to negative long-term effects and, thereby, steer the field to seek alternative interventions and further examine mechanisms for adverse effects.

3.0 Background

One in 10 US children is born preterm. By school-age, children born very preterm have an average IQ deficit of 11 points and learning and behavior problems that pose triple the risk of being held back a grade by age 8. Early, sustained intervention can improve long-term outcomes, but Early Intervention programs for preterm children cost $611 million/year in the US, require substantial family involvement, and are not cures. Children with mild impairment remain at risk but rarely receive intervention and are the majority of those born preterm. Safe and effective, low burden interventions to promote neurodevelopment in this population remain a major need.

Omega-3 docosahexaenoic acid (DHA) dietary supplements have been touted to promote cognitive development in preterm children based on large RCTs showing short-term visual and cognitive benefits in infancy. DHA is added to virtually all infant formula products as a result. DHA is the dominant structural omega-3 fatty acid in the brain, where it rapidly increases in concentration to age 2 and is a key player in numerous neurologic processes. Some families continue supplementation beyond infancy in the hope of further benefit, despite no evidence for efficacy post-infancy. Our recent Omega Tots trial filled that gap by testing the effect of 180 days' supplementation with DHA plus omega-6 arachidonic acid (AA) vs placebo in 377 preterm toddlers. Interestingly, we observed adverse effects of DHA+AA supplementation on early effortful control and language development in 2 pre-specified sub-groups, and no developmental benefit. Six months' post-trial, we saw adverse effects for the entire DHA+AA group: a 0.3-SD deficit in global cognitive development scores. The Australian DINO trial supplemented preterm infants and reported negative effects on executive function 4-7 years later. However, all these adverse findings were based only on parent report. Biologic explanations for adverse effects remain entirely unexplored, but fatty acid desaturase genetic polymorphisms (SNPs) are top candidates because of their strong effects on the amount and balance of long-chain fatty acids available to the developing brain. Therefore, there is an urgent need to evaluate trial participants in more depth, long term, to ensure that DHA+AA supplementation is not causing lasting harm. Without such information, the estimated 3.3 million US children annually who consume supplemented formula and foods will continue to be exposed

without reassurance. This is particularly worrisome for children born preterm who are already at risk for cognitive deficits.

4.0 Study Endpoints

4.1 Describe the primary and secondary study endpoints.

Primary outcomes (child) - General cognitive ability, language, and executive function.

- General cognitive ability - We will use the *DAS-II General Conceptual Ability (GCA)* standard score as our primary measure for general cognitive ability.
- Language - We recognize that language is a major component of cognitive ability, but given the specific effects on language we observed in the original trial and the striking deficits in language caused by prematurity, we have elected to specifically focus on language as a separate primary outcome and examine it in more depth than the *DAS-II* can. Thus, we will use the *Clinical Evaluation of Language Fundamentals-5th ed (CELF-5)(core subtests)* as our measure for language. We will use the *Core Language standard score* as our primary measure of language.
- Executive function - Our primary EF measure will be an executive function factor derived from the following NIH Toolbox tasks: *Dimensional Change Card Sort, Flanker, and Pattern Comparison tests*.

Secondary outcomes and measures.

We propose a handful of secondary outcomes and measures to accompany our primary measures shown above. These serve 3 purposes. 1) Some are additional measures of the primary outcomes but rely on a different reporter. 2) Some were chosen because they compensate for small shortcomings in the Toolbox, and so they ensure we measure the 3 key aspects of EF (inhibition switching, working memory). These will be part of sensitivity analyses compared to the Toolbox. 3) Academic achievement will be measured because it will reveal how identified cognitive deficits manifest in academic skills particularly important in middle childhood.

***Secondary outcomes* - Child activities**
Grooved Pegboard Task
Wechsler Intelligence Scales for Children-V (digit span, picture span)
Kaufman Test of Educational Achievement-3 (Letter and Word Recognition, Math Computation, Object Naming Facility and Associative Fluency subtests)
Weight, height and BMI for age z-scores (WHO standards), percent body fat
NIH Toolbox Friendship (Ages 8-17) Fixed Form
***Secondary outcomes* - Caregiver report (teachers will complete instruments with *)**
Behavior Rating Inventory of Executive Function 2nd ed. *

Teacher Report Form 6-18 (Syndrome & DSM-Oriented subscales)*
Child Behavior Checklist 6-18
National Survey of Children's Health module (developmental and behavioral diagnoses)
Emotion Regulation Checklist
NIH Toolbox Positive Peer Interaction Parent Report Fixed Form (Ages 3-12)
Child Sleep Habits Questionnaire

4.2 Describe any primary or secondary safety endpoints.

N/A

5.0 Study Intervention/Investigational Agent

The current study will not include any new interventions or investigational agents. Instead, it is a follow-up study to our Omega Tots trial (n=377, 2012-17), which was the first to test the effect of supplementation (200 mg DHA + 200mg AA, orally) on developmental outcomes during the 2nd year of life—a period of rapid neurodevelopment and diminished dietary quality for preterm children.

Children in the DHA+AA supplementation group received 200mg algal-DHA plus 200mg algal-AA orally daily in the form of a microencapsulated powder contained in small foil packets for 180 days. AA was provided in addition to DHA because this combination of fatty acids mirrors the balance of what is found in infant formula and is the combination available in a powder form. The supplementation packets were provided by DHASCO® (life'sDHA^{TM}) and ARASCO®and are marketed in the U.S as over the counter supplements for individuals across the lifespan, including toddlerhood.

Children in the placebo group received 400mg of corn oil powder orally daily in the form of a microencapsulated powder contained in small foil packets. Parents were instructed to mix two packets of powder (either DHA+AA or placebo) per day into their child's drink or food. For an average 10 kg child this dose of DHA amounts to approximately 20 mg/kg/day and mirrors the intake recommendations of a 1994 report by the United Nations Food and Agriculture Organization. Previous studies that began supplementation just after birth vary widely in the duration of supplementation, from a few weeks to 12 months. This intervention lasted for 6 months. No previous studies began supplementation around 12 months of age. We chose 6 months for the duration of the intervention because we expected the rate of DHA+AA accretion to be potentially slower in this older age group and because we needed to allow sufficient time developmentally for differences to become apparent.

Although DHASCO and ARASCO are classified as Generally Recognized As Safe (GRAS) by the FDA and is the source of DHA and AA in most U.S. infant formula products, the FDA was consulted regarding the necessity of an IND for this study. Through these conversations it was determined that an IND was necessary for this product, and the application was filed on July 20, 2011. On October 4, 2011 the FDA approved the Omega Tots protocol. The IND Number for the Omega Tots study was 112885.

Because the current study is a follow up study to Omega Tots and will not include any new interventions or investigational agents, we have not included information on drug or device handling.

6.0 Procedures Involved*

6.1 Study Design

This is a prospective cohort, built from our randomized, blind, placebo-controlled Omega Tots trial of DHA+AA supplementation for 180 days involving children 10-16 months of age (adjusted for prematurity) at enrollment. Participants are Nationwide Children's Hospital (NCH; Columbus, OH) former NICU patients born at <35 weeks' gestation (n=377). All 377 randomized children will be eligible for in-person follow-up at age 8½ - 10½, even those with missing data from the trial, per intent-to-treat. All investigators (except the Principal Investigator and biostatistician), study staff, clinic staff, parents, and children will remain blind to the child's treatment assignment.

The cohort study will be conducted at NCH. There are no collaborating sites where enrollment or data collection will be performed. To recruit families, an attractive "mailer box" will be sent to all eligible families. A Recruitment and Retention (R&R) Specialist (part of the study team at NCH) will call families approximately 1week post-mailer to further describe the study and gauge child and caregiver interest. Non-responsive or "hard to reach" families will be contacted with further, well-honed attempts by the R&R Specialist. Participation or refusal to participate in the study will not affect entitlement to clinical care.

Children and a parent/guardian will come to NCH when the children are between 8 years, 180 days and 10 years, 180 days, and provide written consent/assent. It is expected that participant enrollment will begin by August 2021, and that human subjects' activity will be completed by February 2025.

Visits will occur in the Center for Biobehavioral Health, child-friendly observation rooms (x8) with video recording equipment built for assessment, observation, and interviews. Trained, experienced staff will carry out the visits 7 days/week including evenings for family convenience. Proposed visit length is approximately 161 minutes.

The measures administered during this single visit involve combination of computerized and manual tests described in section 6.2. Testing will occur in a fixed order with 2 scheduled breaks unless children lose attention or express the need for a break at other times. The parent will be in an adjacent room completing questionnaires described in detail in section 6.3. Saliva collection will occur using procedures detailed later (see sections 6.2 – 6.5). We will offer the parent a $100 incentive, parking pass, a small gift or snack (value ~$5), and a letter summarizing the child's general standardized test results (e.g. average, below average, above average). We will offer the child a $10 incentive, an age appropriate book, and a small gift or snack (value ~$5) upon completion of all study components. All study activities and experiences will be documented on appropriate Clinical Research Forms (CRFs), according to NCH IRB standards. If families are unable to complete a study visit at NCH, staff will offer the family the opportunity to carry out the visit in a convenient community location near their home (e.g. library, NCH Close to Home site) or the participants home if absolutely necessary. If none of these is possible the family can become a "survey only" family, where they will not complete an "in person visit," but may still complete surveys either online, via phone or via postal mailed responses, or telemedicine. In these cases, staff will mail out

a saliva kit including instructions how to give a sample and mail back in provided packaging. Participants will be informed that this "survey only" option is accompanied by partial compensation, but it is again completely voluntary. Staff will emphasize that participation is valued, but if they choose to stop participating there will not be a penalty or loss of benefits to which they are otherwise entitled (e.g. clinical care at NCH).

If "in person" study visits are no longer permitted due to the COVID-19 pandemic or other extenuating circumstances, staff will transition to collecting as much data as possible via the remote means mentioned above for "surveys only" family cases (e.g. phone, online, mail, telemedicine). Staff would also mail out a saliva kit including instructions how to give a sample and mail back in provided packaging.

6.2 Study measures: Direct assessment of child

Child testing will include the following measures. All assessments will be administered or coordinated by trained staff. The order that measures are administered may change slightly based on child's engagement, time constraints, or other factors.

a) *Differential Ability Scales-II General Conceptual Ability assessment*. This instrument can assess and identify children's cognitive strengths and weaknesses that are important to learning by utilizing various stimulus books and manipulatives (i.e.: blocks). Assessment time is approximately 50 minutes.

b) *Clinical evaluation of Language Fundamentals, 5th edition core tests*. This is a comprehensive instrument has a total of 16 subtests that can assess children's written and oral language skills, as well as their reading comprehension. Assessment time is approximately 30 minutes.

c) *NIH Toolbox Cognition Battery*. Total assessment time is approximately 20 minutes. The NIH Toolbox is a multidimensional set of brief measures assessing cognitive function for participants aged 3 – 85 years. *The Flanker, Dimensional Card Sort, and Pattern Comparison subtests* are part of the child Cognition Battery that assesses executive function, cognitive flexibility, attention, episodic memory, processing speed, and working memory.

d) *Kaufman Test of Educational Achievement-3 (Math Computation, Letter and Word Recognition, Object Naming Facility and Associative Fluency)*. This brief assessment can produce composite scores that measure children's reading, math, and written language skills. Assessment time is approximately 20 minutes.

e) *A Grooved Pegboard Task* and *Edinburgh Handedness Inventory-Short From.* This brief task requires children inserting pegs inside a board. The handedness form is a 4-item measure that assesses the child's handedness. Child are verbally asked which hand they prefer to use for 4 tasks. It takes approximately 5 minutes to complete these activities.

f) The *Wechsler Intelligence Scales for Children-V digit span*, and the *Wechsler Intelligence Scales for Children-V picture span* are brief cognitive assessment that measure working memory. Assessment time is approximately 15 minutes.

g) Peer Relationships are assessed using the *NIH Toolbox Friendship (Ages 8-17) Fixed Form.* This 5-item measure assesses quality of relationships with friends and other

acquaintances over the past month. Children are verbally asked each question and asked to answer on a 5-point scale ranging from *Never* to *Always.* It takes 1 minute to answer these questions.

h) Physical Activity-Children will be asked an adapted version of the *Child Heart And Health Study in England (CHASE)* questionnaire that asks about their physical activity inside and outside of school. Children are verbally asked each question and asked to answer with one of the multiple choices provided. It takes about 5 minutes to answer these questions.

i) Genetic sample (saliva) – Child saliva will be collected via saliva tube. Collection will only be done with children that do not have a stored blood white blood cell sample from their previous participation in Omega Tots. At least 1 hour after eating, children will rinse their mouth with water, wait 10 min while doing other activities, and then will be assisted in drooling a 1.5-2.0 mL sample into the collection device (a sponge alternative will be available for children who have difficulty). This procedure is routinely successful with children as young as 5 years, and the Keim Lab staff will be trained by the genetics team (under Dr. Chris Bartlett), as the Bartlett Lab staff are leading experts in genetic material collection techniques. Colorful, scientific drawings may be used to explain the procedure to the child. Collectors will pantomime the procedure to ensure comprehension and to reduce the potential for child discomfort. The parent may also be present to support the child during saliva collection.

j) Anthropometrics: To assess body composition, height and weight of the child are measured. Three measurements for each type of body measurement are taken. To assess adiposity, a bioelectric impedance scale will be used to calculate the child's body fat percent and BMI z-score. This takes approximately 5 minutes

6.2 Study measures: Parent report

Parent surveys and testing will include the following constructs & measures. All assessments will be administered or coordinated by trained staff.

a) Child executive function/behavior– Child executive function will be collected via parent report, using the following measures: the *Behavior Rating Inventory of Executive Function, 2nd edition (BRIEF-2)*; the *Child Behavior Checklist 6-18*; and the *Emotion Regulation Checklist*. Assessment time is approximately 30 minutes.

   i. *BRIEF-2 (ages 5 to 18 years)* measures a range of executive functioning capabilities in children. Caregivers are asked to record if the described behavior has *never, sometimes,* or *often* been a problem for their child in the past 6 months. This measure takes approximately 10 minutes to complete.
   ii. *CBCL (6-18 form)* is a 113 item questionnaire completed by caregivers to reflect their child's behavior in the previous six months. Caregivers rate the items on a 3-point scale: 0-Not True, 1-Somewhat or Sometimes True, 2-Very True or Often True. It takes approximately 15 minutes to complete the full *CBCL*.
   iii. *Emotion Regulation Checklist* is a 24 item questionnaire completed by caregivers to reflect on their child's emotions and select if items are *Never, Sometimes, Often,*

or *Almost Always* applicable to their child. It takes approximately 5 minutes to complete.

b) Caregiver executive function – Caregiver executive function will be collected using the *NIH Toolbox Adult Cognition Battery*. Assessment time is about 45 minutes.

   i. The adult Cognition Battery assesses executive function, cognitive flexibility, attention, episodic memory, language, processing speed, and working memory. The following subtests will be administered to obtain a Total Composite Score, Crystallized Composite Score, and Fluid Cognition Composite Score: *Flanker Inhibitory Control and Attention Test, Dimensional Change Card Sort Test, Picture Sequence Memory Test, Picture Vocabulary Test, Oral Reading Recognition Test, Pattern Comparison Processing Speed Test,* and the *List Sorting Working Memory Test.*

c) Child developmental/behavioral diagnoses – Child developmental/behavioral diagnoses and other major disease diagnoses will be collected via parent report using the *National Survey of Children's Health module.* Caregivers are asked if a doctor or health professional has ever told them that the child had a specific health condition presented from a list of specific health conditions, behavioral problems, or developmental delays. Assessment time is approximately 2 minutes. Caregivers will also be asked to provide contact information for their child's doctor so that the study team can access medical records to allow study staff to document and consider major diagnoses since birth (developmental and behavioral and mental health, respiratory, allergy) and any associated treatments the child has experienced, and growth indicators (height/length and weight since birth) based on NCH medical records and records from their other doctor(s) if not NCH. Caregivers may also be asked to sign a Medical Release Form if required by their child's doctor's office.

d) Family demographics – A basic demographic profile will be collected via parent self-report. Assessment time is approximately 3 minutes.

e) Diet – Child & family diet will be collected via parent report, using the *Fatty Acid Food Frequency Questionnaire.* This assessment will be conducted interview style, where *s*taff will read the question items and provide answer choices. This also allows caregivers the opportunity to ask questions and receive clarifications when needed. Assessment time is approximately 3 minutes.

f) Home dynamic and environment – Elements of the child home environment will be collected via parent report, using the *Middle Childhood- Home Observation for Measure of the Environment (MC-HOME),* and the *Family Assessment Device-General Functioning subscale (FAD-GF).*The MC-HOME questions pertain to opportunities for variation in daily stimulation (i.e.: playing outside, visiting relatives, etc.) and support available to the child at home. The FAD-GF is a 12-item scale that measures general characteristic of families. Assessment time is approximately 15 minutes.

g) Parent mental health – Parent mental health will be assessed using the *Adult Self-Report (ASR),* the *Parenting Stress Index, 4th edition-Short Form (PSI-4), and the Patient Health Questionnaire-4 (PHQ-4)*. The ASR is a self-report on adaptive functioning, problems, and substance use. Caregivers will rate 126 items on a 3-point scale: 0-Not True, 1-Somewhat

or Sometimes True, 2-Very True or Often True. Assessment time is approximately 20 minutes. The PSI short form contains 36 items of parenting stress. Assessment time is approximately 5 minutes. The PHQ-4 is a very brief, 4 item questionnaire used to detect depressive and anxiety disorders. Assessment time is approximately 1 minute.

h) Child medication use-Caregivers will be asked to report the child's use of any dietary supplement, vitamin, prescription, and nonprescription medication within the last 30 days. Assessment time is approximately 5 minutes.

i) Child sleeping habits-Child's sleep patterns and behaviors will be collected via parent report using the *Children's Sleep Habit Questionnaire (CSHQ).* The CSHQ is a 33 item questionnaire that asks parents to report on their child's bedtime behavior, sleep duration, behavior during sleep, etc. Items will be rated on a 3 point scale of Usually, Sometimes, or Rarely. Assessment time is approximately 10 minutes.

j) Food security-Caregivers will be asked 2 brief questions from the *Household Food Security Survey (HFSS)* to assess the household's food security. This takes approximately 1 minute.

k) Child peer relationships-This will be assessed using the *NIH Toolbox Positive Peer Interaction Parent Report Fixed Form (Ages 3-12).* This 4-item measure asks the caregiver to assess their child's quality of relationships with friends and other acquaintances over the past month. Caregivers rate the items on a 5-point scale from Never to Always. This takes approximately 1 minute.

l) Genetic sample (saliva)- Saliva will be collected via saliva tube from the child's biological parent. This procedure will not be done if child's legal guardian is not their biological parent. At least 1 hour after eating, the adult will rinse their mouth with water, wait 10 min while they complete other activities, and then will be assisted in drooling a 1.5-2.0 mL sample into the collection device. Keim Lab staff will be trained by the genetics team (under Dr. Chris Bartlett), as the Bartlett Lab staff are leading experts in genetic material collection techniques.

6.3 Study measures: Teacher report

Teacher questionnaires will include the following constructs & measures. While online completion is preferred, teachers may complete surveys via mail, phone, or email, whichever is feasible for the teacher. All assessments will be administered or coordinated by trained staff. Teachers will receive a $25 incentive for completion. If the teacher is unable to accept the incentive, per their school's policy, study staff will send a gift card to the school's office to use at their discretion.

a) Child executive function – Child executive function will be collected via teacher report, using the *Behavior Rating Inventory of Executive Function, 2nd edition (BRIEF-2)* as well as select scales in the *Teacher Report form (TRF 6-18)*. The BRIEF-2, ages 6 to 18 years, measures a range of executive functioning capabilities/behaviors in children in the home and school environments. Respondents are asked to record if the described behavior has never, sometimes, or often been a problem for their child in the past 6 months. The selected scales in the TRF (6-18) include: Syndrome Scales *(Anxious/Depressed, Withdrawn/Depressed, Somatic Complaints, Social Problems, Thought Problems, Attention Problems, Rule-Breaking Behavior, Aggressive Behavior)* and DSM-Oriented Scales *(Affective Problems, Anxiety Problems, Somatic Problems, ADHD Problems,*

Oppositional Defiant Problems, and Conduct Problems). These ask about the child's behavior in the previous six months. Teachers rate the items on a 3-point scale: 0-Not True, 1-Somewhat or Sometimes True, 2-Very True or Often True Assessment time is approximately 15 minutes.

6.4 Source records

Data for this study will include medical record reviews, questionnaires, direct testing/observation of cognitive abilities and behavior, and biospecimens (saliva, blood).

Please see supplemental materials for all applicable source records.

All of the research procedures and questionnaires are designed to meet the definition of "minimal risk" in §45 CFR 46.102(i) and to be reviewed by the IRB under §45 CFR 46.404 "Research not involving greater than minimal risk."

The Principal Investigator will ensure that this study is conducted in full conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research and codified in 45 CFR Part 46 and/or the ICH E6; 62 Federal Regulations 25691 (1997). Study staff are also committed to minimizing risks even though they are minimal.

7.0 Data and Specimen Banking

Following collection procedures outlined above (see section 6.2, i and 6.3, l), saliva samples will be stored in a dry, dark secure drawer per Dr. Bartlett's instruction, until they are transferred to his team for analysis purposes. All samples will be labeled using de-identified participant identification methods (i.e. assigned, numerical participant study IDs), and will never include participant PHI.

Saliva samples will be transferred to Dr. Bartlett's team for analysis. Dr. Bartlett's molecular genetics laboratory is experienced in DNA extraction, using protocols his lab developed expressly to maximize the yield of high-quality DNA suitable for genetics assays. DNA from saliva will be maintained after the study is complete and will be stored indefinitely for future analysis and use, per the participant consent document. Specimens may be analyzed for future studies of nutritional and genetic factors in relation to child growth and development. Specimens will be maintained in a secure location in the NCH Center for Biobehavioral Health or Battelle Center for Mathematical Medicine and will be labeled with a numeric code and no identifying information. The numeric code will be linked to the other deidentified study data. Only the study investigators and their staff IRB-approved to work on this research and internal and external human subjects' protection officials and auditors (IRB, FDA, NIH) will have access to the specimens. De-identified study data (but not biospecimens) will be available to scientists external to the study team upon securing appropriate Data Use Agreements and per the informed consent form.

Blood was collected and stored during the original trial from about half of the participants, and these samples have been stored in the Keim Lab - 80°C freezer. The consent form from the original trial included consent for future use of leftover blood. Stored blood from the original trial will be used in lieu of saliva collection if that family provided permission.

8.0 Sharing of Results with Subjects

Upon completion of the study visit, staff will send a letter to the family that includes the child's generalized results from the standardized testing. This letter will not include specific scores, but will include the range the child scored in (e.g. average, below average, above average). Staff will also explain to families that the testing was completed for research purposes only. Families can contact the study with any questions. Dr Taylor is available to assist families in interpreting their letter.

9.0 Study Timelines

9.1 Describe:

Anticipated study enrollment will occur from August 2021 through February 2025. During this time, participants will be invited to participate in a single study visit that lasts 3 hours along with any necessary follow up appointments to address missing or poor quality data. Estimated time for study investigations to complete the study and primary analysis is June 2025.

The duration of an individual subject's participation in the study consists of a single study visit (when child is between 8 years, 180 days and 10 years, 180 days) and any necessary follow-up to address missing or poor quality data.

10.0 Inclusion and Exclusion Criteria

For participation in the original Omega Tots trial, participants met the following inclusion criteria: age 10-16 months (corrected age) at baseline, gestational age <35 completed weeks, discontinued breastfeeding and formula feeding, English language ability adequate to understand the study and its risks. Exclusions: Feeding problem or malformation; metabolic or digestive disorder precluding participation or absorption of the supplement; weight <5th or >95th percentile for corrected age (WHO growth charts); consume a DHA supplement, Pediasure, or fatty fish more than twice per week; plans to leave the area; corn, soy or fish allergy.

The only criteria for the present study are previous participation in Omega Tots, and a current age of 8 years, 180 days to 10 years, 180 days. Our study will include children, their parent or legal guardian, and the child's primary teacher. Any child that is in custody of children's services for their window of eligibility will be excluded. Our study will not include adults unable to consent or prisoners.

11.0 Vulnerable Populations

This study intends to enroll children, a special population, and will accordingly adhere to additional protections specified under 45 CFR Part 46 Subpart D – Additional Protections for Children Involved as Subjects in Research (45 CFR Part 46.401-409). Follow-up at 8½ - 10 ½ years of age was selected because this is a critical developmental period for children born preterm.

Children (age 8½) may participate in this study under the written informed consent provided by a parent or legal guardian. If children are enrolled at an age ≥ 9, they will be assented in addition to acquiring caregiver informed consent. This study does not include any other vulnerable populations (i.e. adults unable to consent or prisoners). Parents/guardians who may be pregnant at the time of enrollment will be invited to participate as their pregnancy is in no way related to the study intervention. Children of a parent who is cognitively impaired or mentally ill are not eligible if the parent is not able to understand fully the research project and to grant informed consent.

12.0 Local Number of Subjects

We hope to enroll as many of the eligible 377 caregiver-child dyads as possible for the current study.

13.0 Recruitment Methods

13.1 Procedures

To recruit families, an attractive "mailer box" will be sent to all eligible families. Mailer boxes will be addressed to the child and their family, and include: child-friendly details such as a greeting card; a small, engaging gift or activity (e.g. sensory keychain or craft kit); an introductory letter (signed by the PI) that describes the child's previous participation and offers related details about the new study; and an attractive, professionally-designed study brochure that provides additional information about the research team.

Prior to the mailer box being sent, a Recruitment and Retention (R&R) Specialist will ensure that the child is not deceased or in the care of Children's Services by verifying this information in EPIC. R&R Specialists will reach out to families (via phone call/text/email). Approximately 1 week post-mailer, the R&R Specialist will reach out to families (via call/text/email) to further describe the study and gauge child and caregiver interest. Participation or refusal to participate in the study will not affect entitlement to clinical care. Study visits will be scheduled at convenient times and with additional services as needed. Families will be reminded of scheduled/upcoming appointments via phone, email and/or text (based on family preference). Attempts will be made via phone, email, text, and/or mail to contact families who do not show for their scheduled visits. Non-responsive or "hard to reach" families will be contacted with further, well-honed attempts by the R&R Specialist. Examples of this include using the child's electronic medical record (e.g. EPIC) to retrieve up-to-date contact information, connecting with families at an upcoming clinical care appointment, a web-based online search service (e.g., http://www.whitepages.com/) that gathers information through public records, similar to a Google search. The search service provides a fast and effective way to look-up or verify contact information about a person, phone number of address of interest. Additional methods to contact hard to reach families may include contacting additional contacts provided during the Omega Tots trial to aid in identifying updated contact information (postal mail, phone). If the additional contact listed for the caregiver also has outdated contact information, attempts will be made to reach them using the same web-based online search service (e.g., http://www.whitepages.com/). Before contact is made with each eligible family, life (or death) status of the caregiver and child will be affirmed using the child's electronic medical record (EPIC) as well as through vital records maintained by the Ohio Department of Health.

Families who have not enrolled or refused participation by March 18, 2024 will be sent an additional mailer box. Mailer boxes will be addressed to the child and their family and include: a small gift for the

child and their caregiver; a flyer that succinctly describes the child's previous participation, details about the new study, and options for participation; and a brochure that provides additional information about the study. Prior to the new mailer box being sent, a R&R Specialist will re-confirm that the child is not deceased or in the care of Children's Services by verifying this information in EPIC. Approximately 1 week post-mailer, R&R Specialists will reach out to the families in the same manner as the initial recruit attempts. Families who are still non-responsive after these attempts will receive a packet containing: a flyer that briefly describes participation options, QR codes to the study survey and refusal questionnaire, paper copies of the survey and refusal questionnaire, study information sheet, saliva collection kits and a pre-paid return envelope.

Children and a parent/guardian will come to NCH when the child is between 8 years, 180 days to 10 years, 180 days and provide written consent/assent. If the parent is interested in enrolling and the child is in the age range, the study staff will guide the parent and child through the process. Teacher recruitment will begin following family enrollment. The caregiver will fill out the "Release of Information Teacher Form," which permits study staff to contact the child's school and teacher, and staff may follow up with caregivers to affirm child's current teacher if visits occur during summer months.

Participants will be recruited from the original Omega Tots cohort (n=377). No additional sources or subject identification methods are necessary.

13.2 Describe materials that will be used to recruit subjects. (Attach copies of these documents with the application.

Please see supplemental materials for recruitment materials.

13.3 Describe the amount and timing of any payments to subjects.

N/A- Participants will receive compensation following completion of the in-person study visit (see section 6.1 for details).

14.0 Withdrawal of Subjects

14.1 Procedures

As this is a prospective cohort design with no new investigational drugs or products, we do not anticipate many circumstances under which subjects will be withdrawn from the study without their consent. If the PI feels it is in the interest of the child or parent/guardian to not enroll them or to discontinue data collection with them prematurely, the PI will make that decision. We also do not see the need for any procedures regarding orderly termination from the study.

However, we recognize that research participation is completely voluntary; subjects may choose to withdraw consent/assent at any time. Should a participant indicate they would like to formally withdraw from the study, they will be asked to connect with the Principal Investigator (PI), Study Coordinator, or trained research staff via phone (or if currently at a study visit, in person).

If the research participant wishes to withdraw or refuses participation in the study, the staff will attempt to complete a "refusal questionnaire" with the family, to document their main reason in writing, and to

collect a few additional details about families that withdraw or refuse withdraw (e.g. any participation barriers, etc.). Some families may refuse this questionnaire; if so, staff will refrain from completing it.

If the participant wishes to withdraw all previously submitted data/PHI from the study, in addition to discontinuing future participation, the participant will be asked to submit this request in writing via mailed letter to the Principal Investigator (Dr. Sarah Keim). Dr. Keim's updated address will be included in the informed consent/assent documents, but will also be offered again at the time of withdraw procedures if needed by the participant. If a subject also wishes to have their DNA withdrawn, then Dr. Bartlett's Lab will destroy the DNA sample using appropriate molecular methods.

15.0 Risks to Subjects*

15.1 List the reasonably foreseeable risks, discomforts, hazards, or inconveniences to the subjects related the subjects' participation in the research. Include as may be useful for the IRB's consideration, a description of the probability, magnitude, duration, and reversibility of the risks. Consider physical, psychological, social, legal, and economic risks.

The intervention that was tested in the original Omega Tots trial may have lasting effects on child development and behavior. It is currently not foreseeable if that prior intervention may have effects at ages 8½ - 10 ½ years that are negative, that is a question to be answered by the research.

All of the research related procedures, surveys, and questionnaires, are designed to meet the definition of "minimal risk" in the federal regulations [§45 CFR 46.102(i)] and to be reviewed by IRBs under §45 CFR 46.404 "Research not involving greater than minimal risk." Minimal risk as defined in the federal regulations means "that the probability and magnitude of harm or discomfort anticipated in the research are not greater, in and of themselves, than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests." In addition, the Study Staff is committed to minimizing risks even when the risks are minimal.

The informed consent process takes place in private. It is possible some participants may feel uncomfortable or upset when answering survey questions. However, questionnaires are structured to avoid creating discomfort for the research participants; and participants are reminded at each data collection encounter that participation is voluntary, they have the right to withdraw from the research project at any time, and they may refuse to answer or may skip any question.

The risks of collecting and storing linked clinical data and biospecimens are primarily psycho-social. Developing coding strategies to mitigate these risks is a fundamental ethical requirement of the study. Potential harm could result from a breach of confidentiality. One of the primary concerns is that employment and insurance discrimination might result from exposure of information about health history, genetic makeup, or familial predisposition to disease. The risk is minor, especially since unique participant identifiers will not be stored with biospecimens. Moreover, the study will maintain confidentiality of the data in its databases. Finally, the genetic data to be studied in this phase of research have not been shown to be highly predictive of risk for any particular disease. Data submitted to the study does include participant identifiers, as defined by the U.S. Health Insurance Portability and Accountability Act of 1996 (HIPAA). All participant data will be maintained in secure databases and files by number code. The study will follow all necessary measures to assure the data are protected and secure from unauthorized access. Only IRB-approved study personnel will have access to the file that links names to identification numbers. Data will be summarized in aggregate for reporting purposes. All electronic information is stored on the

secure network and available only to study personnel. All paper files will secured in a locked room in the Center for Biobehavioral Health. Based on the research team's experience with previous studies, these methods of data confidentiality and protection have been effective.

Experienced research staff will perform biospecimen (i.e. saliva) collections with participants, via saliva tube. This method was selected in lieu of phlebotomy, in order to decrease pain and discomfort of research participants; phlebotomy is a participation barrier and we are keen to maximize enrollment, minimize discomfort and reduce study risks. Saliva collection procedures involve minimal discomfort. Saliva collection will be described using colorful drawings, and collectors will pantomime the procedure to ensure complete comprehension prior to beginning administration, to reduce as much mental or physical discomfort as possible. An alternative, sponge collection method will be offered if the child has difficulties with the saliva collection. The parent may also be present to support the child during saliva collection, if the child finds it unpleasant. All staff involved in collection of biospecimens will be credentialed accordingly by trained investigator staff.

The genetic tests are highly unlikely to identify new information on disease mutations. This research will not include mapping participant's DNA (whole genome sequencing). Families will be encourage to ask the study team if they have questions.

Although we do not conduct medical tests (the kind that can be ordered by a doctor), and we do not believe any of our tests have medical value, it is possible that, in the future, other researchers may decide that our tests may be medically important.

Child abuse protections will be in effect as study staff are mandated reporters trained to identify signs of abuse or neglect. Standard operating procedures have been developed and implemented under the Principal Investigator's leadership of past studies to protect the well-being of children. The same protocol will be implemented for this study. Study staff will be trained in mandated reporting procedures based on curriculum developed especially for studies in the Center for Biobehavioral Health by the NCH Department of Social Work and the PI in conjunction with guidelines and procedures of the Franklin County Department of Children's Services. Safety oversight will be under the direction of the PI. If medical intervention is required in the case of an adverse event, children may seek care at Nationwide Children's Hospital or their personal physician.

The knowledge to be gained from this investigation outweighs these minimal risks. We do not anticipate any risks to others who are not study participants, nor any additional or unforeseeable risks. This study has the potential to inform future dietary recommendations for children, especially those who were born preterm.

16.0 Potential Benefits to Subjects

The intervention that was tested in the original Omega Tots trial may have lasting effects on child development and behavior. It is currently not foreseeable if that prior intervention may have effects at ages 8 ½ - 10 ½ years that are positive, that is a question to be answered by the research.

There are no known potential benefits from follow-up participation. Participation in this study will aid in acquiring new knowledge that might help other children who are born prematurely and their families in the future. There are no other known benefits expected from participation in this study. However, the child

may enjoy the small book or gift to thank them for their time, or find some of the data collection procedures entertaining (e.g. iPad assessments).

17.0 Data Management and Confidentiality

17.1 Data analysis plan

Statistical power and sample size for Aim 1:

All 377 children randomized in Omega Tots will be eligible for follow-up. We estimate power based on a conservative estimate of 300 children (80%) attending the age 8 ½ - 10 ½ visit. To evaluate differences between the treatment arms, 300 children divided equally to each arm provides >80% power to detect a 0.32-SD mean difference or larger (Cohen's d≥0.32) in each of our primary outcome measures: DAS-II GCA (general cognitive ability), CELF-Core Language (language), or Toolbox Executive Function factor (EF)(α=0.05) at age 8 ½ - 10 ½ (Hypothesis 1). This effect size is the same or smaller than what the DINO trial observed on the BRIEF at age 7, and the short (ECBQ) and follow-up (DP-3 at age 2) results from Omega Tots. These effect sizes are also similar in magnitude to the (beneficial) effect observed in the Infant Health and Development Program early intervention trial at age 8 on IQ and reading and math achievement among infants born at 2000-2500g. Sub-group analyses will necessarily be exploratory because only a much larger sample would be fully powered to test the interactions of interest. But, these are worth exploring because in the original Omega Tots trial we observed 2 statistically significant sub-group effects. Although it would be ideal to employ a repeated measures analysis using the 3 available time points (trial, age 2, age 8 ½ - 10 ½), robust developmental measures that are comparable at all time points are not available. We will pursue longitudinal analyses for the secondary outcomes where repeated measures exist, however.

Statistical analysis for Aim 1:

Analyses for Aim 1 will follow intent to treat principles, the most rigorous approach to RCT data. By using intent to treat we are able to detect lasting effects of DHA+AA supplementation if they are present at the magnitude we anticipate. Children who were randomized but discontinued taking treatment or were lost to follow-up will be included in analyses. Our main goal will be to estimate the effect of treatment assignment on our 3 primary outcomes at age 8½ - 10 ½ (DAS-II GCA, CELF-5 language, Toolbox EF) using the following mixed model, consistent with the analysis of our Omega Tots trial: $y_{it} = \beta_0 + \beta_1 Time_t + \beta_2 Time_t x Trt_i + \varepsilon_{it}$,, where implicitly it is assumed that there is no main effect of Trt_i, y_{it} is the outcome of interest at time t, $Time_t$ is coded as 0 for the outcome at baseline (i.e., the covariate for our purposes here) and 1 for the post-randomization measure of the outcome obtained at age 8 ½ - 10 ½, and the primary effect of interest is β_2, which corresponds to the difference between groups on the outcome at age 8 ½ - 10 ½. In this model, the baseline measure of the outcome is constrained to have equal means across groups by assuming there is no main effect of the intervention, Trt_i. This constraint yields a statistical test for β_2 analogous to the test of the treatment group main effect via ANCOVA controlling for the baseline measure without requiring list-wise deletion due to missing data. Specifically, our model is estimated using maximum likelihood estimation, and therefore allows for outcome data at either time point to be missing at random. We will account for clustering due to twins/triplets in our sample by adding a random effect at the family level. Exploratory pre-specified sub-group analyses will proceed by testing interaction terms added to the models for sex, birthweight (<1500g vs ≥1500g), socioeconomics (household income dichotomized at the median), and daily dietary intake of DHA plus EPA (below vs above the median).

Interactions with p<.20 will be considered statistically significant, and stratified results will be reported consequently. Analyses will use the MIXED procedure, SAS 9.4.

Statistical power, sample size, and analysis for Aim 2 (i.e. genetic biospecimens):

To address the first part of Aim 2, we apply the standard method for estimating SNP-based heritability, which assumes the common SNP effect is a random effect in a mixed linear model and with variance decomposed into variance components for genetic and non-genetic effects. The generic model is therefore specified as: $\boldsymbol{y = \beta_0 + X\beta + g + \varepsilon}$, where g is the random genetic effect and further the model is constrained by $\mathbf{V = A\sigma^2_g + I\sigma^2_e}$ where V is the overall variance decomposed into genetic and non-genetic (i.e. environmental) components; the matrix **A** is the genetic relationship matrix, which is similar to the concept of a distance matrix in geospatial modeling, though in this case the distance is genetic similarity calculated from the genome-wide SNP data. Therefore, genetic effects are associated with the covariance matrix for common SNPs (called **A**) and non-genetic effects with the identity matrix (called **I**). Using the identity matrix for this purpose assumes no shared environment effects, which is valid since only unrelated persons are included in the calculation. We routinely assess age, sex, and socioeconomic status as covariates, and always include ancestry principal components. SNP-based heritability can be performed on case-control datasets, population cohorts or other collections of unrelated individuals. Thus, while traditional heritability assumes zero genetic covariance between "unrelated" persons, observed SNP-based genetic covariance will rarely if ever be zero. The cohort includes mostly unrelated individuals (55 sets of twins/triplets). Rather than model the relationships among related individuals and lose power from increased degrees of freedom, we will apply a resampling procedure. Sampled datasets with 1 twin chosen at random ensures the datasets in each replicate contain only unrelated persons but the average over the sampled datasets converges on estimates that use information from all children. In our cohort we will have >80% power to detect SNP-based heritabilities as low as 21%, after accounting for the resampling procedure. While fatty acid markers have not explicitly been tested this way before, the relationship between genetics and many metabolism biomarkers in blood is much higher than a heritability of 21%, giving further reassurance that the study is adequately powered. The test of *en masse* SNP effects in the context of this clinical trial takes the form similar to Aim 1, $\boldsymbol{y_{it} = \beta_0 + \beta_1 Time_t + \beta_2 Trt_i + \beta_3 g + \beta_4 Trt_i x g + \beta_5 Time_t x g + \beta_6 Time_t Trt_i + \beta_7 Time_t x Trt_i x g_i + \varepsilon_{it}}$, while the constraints on **V** are still imposed. The matrix **A** will be estimated using the software GCTA and the model will be tested using R or Python depending on whether numerical issues arise in the numerical matrix calculations. We can perform this model using structural equation models implemented in the R library genomicSEM. This modeling platform will allow us to use *en masse* SNPs in the models described above.

To address the second part of Aim 2, our primary tests will be conducted using 2 linear mixed models similar to that described above in Aim 1. We will estimate the effect of treatment assignment on our 3 primary outcomes at age 8 ½ - 10 ½ (DAS-II GCA, CELF-5 language, Toolbox EF) except that the interaction of treatment by time will include one SNP at a time. These two genetic variants come from the two previously identified SNPs in the literature that we selected as the primary SNPs of interest for this study (SNPS rs1535, rs174575 in the *FADS2* gene). We will be conducting a test of null hypothesis that each SNP has no interaction affecting the outcome. Using the model terminology from Aim 1, $\boldsymbol{y_{it} = \beta_0 + \beta_1 Time_t + \beta_2 Trt_i + \beta_3 SNP_i + \beta_4 Trt_i x SNP_i + \beta_5 Time_t x SNP_i + \beta_6 Time_t Trt_i + \beta_7 Time_t x Trt_i x SNP_i + \varepsilon_{it}}$, where SNP_i is defined as two groups based on the presence/absence of the rare allele at that locus. Last, although we will make efforts to have DNA from every child, some parents may refuse or sample quality could

occasionally be compromised. We will treat missing genetic data just as for other missing data (see Aim 1 – Statistical Analysis).

Analysis plan for secondary outcomes:
Secondary outcomes will be analyzed as for the plan listed above for Aim 1, with the exception that binary outcomes will use logistic or log-binomial models. Secondary outcomes have been specified under section 4.0 Study Endpoints. Results will not be adjusted for multiple comparisons because of the pitfalls of that approach (see Rothman, Epidemiology 1990).

Exploratory analysis of genetic data:
The genetic data in this study, when combined with publicly available SNP by gene transcription reference datasets allows investigators to impute gene transcription values using bioinformatics software such as PrediXcan, FUSION, or TIGAR. All methods use the estimated change in gene transcript per SNP allele to provide an overall estimate of the relative gene expression of the given gene when provided genomewide SNP data. The main advantage of analyzing surrogate gene expression values in this context is that we may interrogate specific genes of physiological or metabolic interest one by one with the clinical outcomes. This procedure generates mechanistic hypotheses for testing in future studies on both humans and model organism. The analysis is necessarily exploratory due to sample size limitations. To prevent excessive loss of power due to transcriptome-wide testing (i.e., testing all genes) we will limit out analysis to genes related to cellular fatty acid processing. The same model form from Aim 1 would be ideal, though in this case some variable selection is likely to be needed. We prefer LASSO regression to select genes with main effects, and test only those genes in the mixed model from Aim 1.

Repeated measures:
When the same or similar outcome measure has been made repeatedly during the original trial plus follow-up study (e.g., weight, global cognitive composite), the analyses described above under Aim 1 will be augmented to accommodate additional timepoints.

Additional analyses:
Fatty acid levels at trial baseline, the end of the trial, the change in values between those timepoints, and calculated dietary intakes of long-chain fatty acids at ages 8 ½ - 10 ½ will be correlated with the outcome measures. This analysis will not be per intent to treat.

17.2 Data quality, storage, and security

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party. The IRB or other authorized representatives may inspect all documents and records required to be maintained, including but not limited to, data collection records for the subjects in this study. Records will be retained indefinitely, and safeguarded using password protected and encrypted devices (computers, cell phones) and through de-identification processes during storage, use, and transmission.

Questionnaire data and scores from some neuropsychological tests will be entered directly into a secure iPad using REDCap, a customizable data collection and study management tool suited to computer-

assisted in-person collections which we have used in many studies. The Toolbox and other computerized assessments have their own secure interfaces and back-end databases for data storage. The Clinical Research Coordinator and PI will assure quality by observing a sample of data collections, restricting entry of and checking for out-of-range values, and staff refresher training. The NCH Research Data and Computing Core will provide REDCap support to design and manage the databases and will maintain secure server storage backed up nightly.

The study staff is also responsible for data management, quality review, analysis, and reporting of the study data under the direction of the investigator team. Paper study records will be kept in secure, locked locations in the NCH Center for Biobehavioral Health (CBH). Electronic files will be maintained at NCH. Electronic REDCap databases will be housed on the secure networked server at NCH and only accessible to study staff from NCH. Participant names and contact information will be stored separately from participant data (identified by code number only) and only be accessible to key personnel. Original source documents will be maintained indefinitely.

17.3 Describe how Biospecimens

Biospecimen handling procedures will be conducted according to proper NCH guidance, as follows:

a) All staff will be trained by Dr. Chris Bartlett's team regarding proper safety equipment, collection techniques, and disposal techniques. A Lab Safety manual will be kept within the collection area at all times, to document the aforementioned procedures if a reference is needed.

b) The Lab Safety manual will also include general lab spill procedures, and emphasize to contact the Environmental Services staff main line (daily hours 6:00 a.m. -3:00 p.m, ext. 51801) or the after-hours 24/7 pager as needed (614-690-1165).

c) Following collection procedures outlined above (see section 6.2, i and 6.3, l), saliva samples will be stored in a dry, dark secure drawer per Dr. Bartlett's instruction, until they are transferred to his team for analysis purposes. Blood was collected and stored during the original trial from about half of the sample, and have been stored in the Keim Lab -80°C freezer.

d) Saliva samples will be transferred to Dr. Bartlett's team at the time of analysis. Dr. Bartlett's team will be responsible for the transfer and receipt of all bio specimens, as they have expertise in this field. Dr. Bartlett's molecular genetics laboratory is experienced in DNA extraction, using protocols his lab developed expressly to maximize the yield of high-quality DNA suitable for genetics assays. Stored blood from the original trial will be used in lieu of saliva samples for those children that have samples, per the consent form signed by the family at the trial enrollment visit.

e) DNA will be retained after the study is complete and will be stored indefinitely for future analysis and use, per the participant consent document.

f) Specimens will be maintained in a secure location in the NCH Center for Biobehavioral Health or Battelle Center for Mathematical Medicine and will be labeled with a numeric code and no identifying information. The numeric code will be linked to the other study data. Only the study investigators and their staff IRB-approved to work on this research and internal and external human subjects' protection officials and auditors (IRB, FDA,

NIH) will have access to the specimens. De-identified study data (but not biospecimens) will be available to scientists external to the study team upon securing appropriate Data Use Agreements and per the informed consent form.

18.0 Provisions to Monitor the Data to Ensure the Safety of Subjects

This study is designed to be Minimal Risk to subjects. Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party. The IRB, FDA, or other authorized representatives may inspect all documents and records required to be maintained, including but not limited to, data collection records and pharmacy records for the subjects in this study.

19.0 Provisions to Protect the Privacy Interests of Subjects

Participants' privacy interests are of the utmost importance, and will be emphasized across all stages of the recruitment, informed consent and enrollment, and study visit phases (see sections 13, 14, and 15 for details).

As stated previously, subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party. The IRB, FDA, or other authorized representatives may inspect all documents and records required to be maintained, including but not limited to, data collection records and pharmacy records for the subjects in this study.

Questions and concerns regarding privacy interests, or any other study related matters, will always be encouraged and answered thoughtfully by trained research staff. Moreover, a section on our informed consent will be included entitled, "WHOM SHOULD I CALL IF I HAVE QUESTIONS OR PROBLEMS?" Participants will be directed to the Principal Investigator's and Study Coordinator's direct contact information, as well as information for the Nationwide Children's Hospital Institutional Review Board, (IRB, the committee that reviews all research involving human subjects at Nationwide Children's Hospital).

Additional details about how the team is permitted to access information regarding subjects is outlined in sections 15 & 17.

20.0 Compensation for Research-Related Injury

N/A – This study is designed to be Minimal Risk to subjects.

21.0 Economic Burden to Subjects

N/A – participants will not be responsible for any costs.

22.0 Consent Process

For "in-person" visits (as described in section 6.0), informed, written consent will be acquired from one parent/legal guardian of the child; informed, written assent will be acquired from the participating child if the child is enrolling at an age ≥9. A copy of these documents may be found in the supplemental materials. Procedures will take place at the caregiver-child dyad's baseline visit at NCH, in a quiet, private space. Trained research staff will follow the *"SOP: Informed Consent Process for Research (HRP-090),* and procedures detailed previously (see also section 11.0 for vulnerable populations, 13.0 for recruitment procedures and 14.0 for withdrawal procedures). The child and parent will have the opportunity to discuss the study with his/her family or think about it prior to agreeing to participate. The child or parent may withdraw consent/assent at any time.

For "surveys only" visits via online, postal mail, or phone (as described in section 6.0), we are requesting a waiver of written documentation of consent. A *HRP-509 NCH Information Sheet* will be included with the survey if it is completed online or through mail. If the survey is conducted over the phone, staff will read through the informed consent form and obtain verbal authorization from the family to use PHI. A written version of the consent form will be mailed or emailed to parent, depending on their preference. We will obtain written documentation for authorization to store PHI and samples for future research.

Children of a parent who is cognitively impaired or mentally ill are not eligible if the parent is not able to understand fully the research project and to grant informed consent. While English-language ability was a criterion for parents in the original trial, it is possible a child will not be under the custody of an adult who does not speak English. In these cases, we will follow prescribed procedures to administer an abbreviated consent form with the aide of a qualified medical interpreter to enable participation in the study. However, measures for which validated versions are unavailable in the primary language of the adult will not be administered and a protocol deviation will be filed.

A copy of the informed consent & assent documents will be given to the family for their records. The rights and welfare of the participants is protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

23.0 Process to Document Consent in Writing

We have requested a waiver of documentation of consent for the individuals completing the survey via mail, online, or phone.

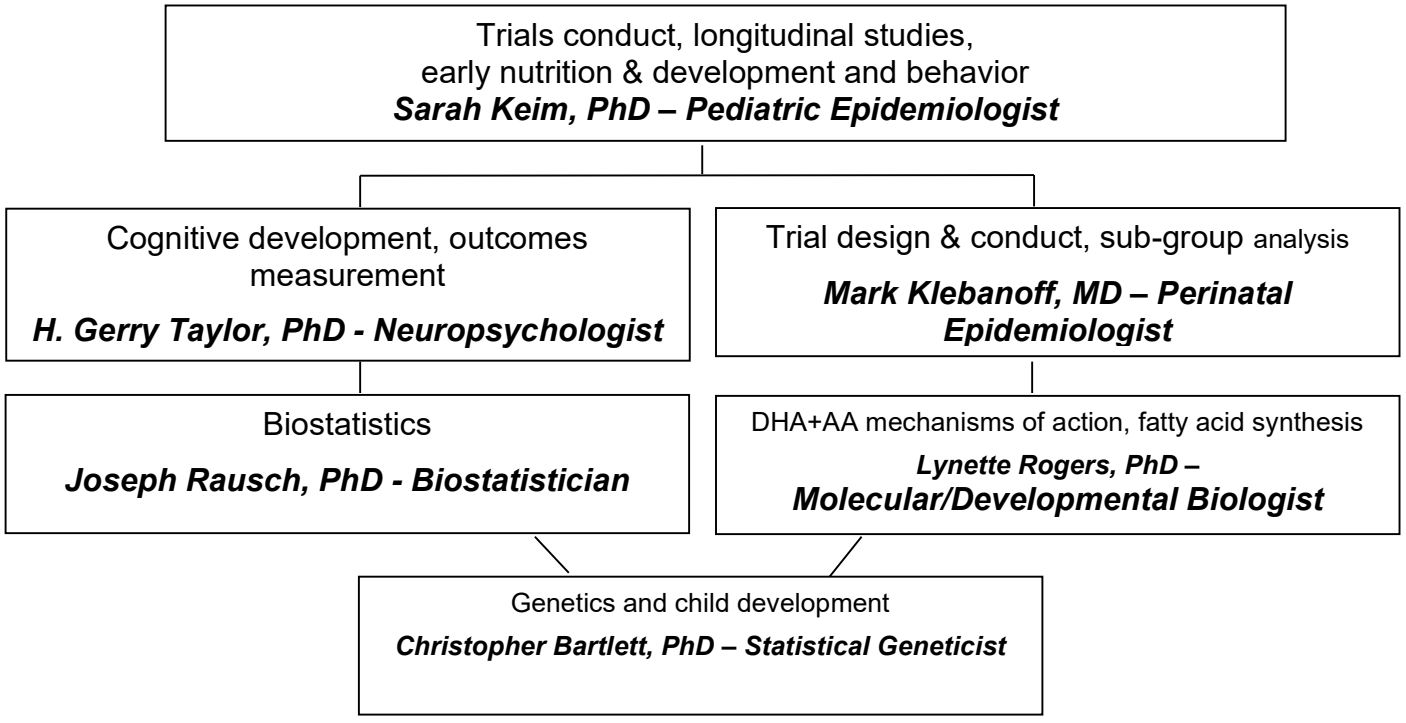
24.0 Setting

Our one, comprehensive study visit with parents and children will be held at Nationwide Children's Hospital, in the Center for Biobehavioral Health, child-friendly observation rooms (x9) with video recording equipment built for assessment, observation, and interviews. No additional institution sites will be used. Alternative locations for study visit data collection may be required due to original participants relocating out of the area or being unwilling to come to NCH for the visit. In these instances, modified visits may be conducted in participant homes, satellite NCH facilities, or nearby community centers, or caregivers will be asked to complete associated surveys to offer a degree of involvement in the study.

25.0 Resources Available

Facilities and resources described below are readily available to the study team, and will help ensure the success of the proposed study.

Investigator collaborations:
The proposed study team, organizational structure, and staffing approach have been constructed based on our proven approach with the original Omega Tots trial but modified to meet the needs of the proposed follow-up study. Part of that experience has been to further enhance our existing collaborations and to attain high operational efficiency and cost effectiveness. Clinical studies such as this require partnerships between clinicians and researchers, and the research team must include diverse expertise related to outcomes, measurement and analysis. Each proposed member contributes unique complementary expertise such that all of these aspects are reflected in an integrated whole that meets the needs of this multi-disciplinary research effort, as follows:



The investigators form the leadership committee for the study, chaired by Dr. Keim. She will convene the committee on a regular basis to review enrollment and data collection progress, data quality parameters,

timeline progress, and milestones for disseminating results. All of the investigators are located in either the Near East Office Building or the Research Building III which are part of the Main Campus of Nationwide Children's Hospital, within 10 minutes' walk of each other. In the occasional event that a collaborator is traveling, all conference rooms and offices are equipped with Skype for Business video conferencing capabilities. All of the involved investigators are devoted 100% time to research, no formal teaching responsibilities, so availability for meetings is flexible. To assist the investigators, the Study Coordinator will record minutes and action items and track progress across investigator meetings. The multi-disciplinary investigator team has a strong track record of collaboration to successfully carry out the proposed study.

Facilities overview:
NCH research laboratories and other facilities are located in the Wexner Institute for Pediatric Research, Research Building II, Research Building III, Near East Office Building, and the JWest building. These structures are contiguous with clinical and educational facilities. Together, these buildings have over 675,000 square feet of lab and office space dedicated to research. The lower levels are occupied by the animal facilities. The first floors house general administrative offices, including grants administration, accounting, and operations; Research Information Services; purchasing; glass washing facilities; and the Biopathology Center. The remaining floors consist of research laboratories, investigator offices, and ample space for support personnel and functions. Researchers are also supported by a collective of shared resources (i.e. scientific core facilities) that offer leading-edge analyses, processes, and systems that offer an array of options for basic, clinical, and translational investigation. The primary cores relevant to this proposal are described below.

Laboratory:

Battelle Center for Mathematical Medicine (Dr. Bartlett's lab) – Dr. Bartlett has an appointment in The Battelle Center for Mathematical Medicine (BCMM), located in Research Building III of the main campus at Nationwide Children's Hospital. Established in 2006, the mission of the BCMM is to bring together a range of mathematical, statistical, and computational specialties and extensive computer hardware and software to bear on basic and clinical biomedical research to improve clinical care and foster research in pediatrics. Building upon existing expertise in statistical modeling in genetics, bioinformatics, biophysics, and data visualization, the BCMM continues to recruit new faculty and is currently comprised of eight members. In addition to faculty, the BCMM houses a team of specialized computational staff, with expertise in database design, software engineering, and parallel computation.

Dr. Bartlett's laboratory occupies 1000 sq. ft. of exclusive laboratory space on the 4th floor. This includes bench space and desks to accommodate at least 6 people, and direct access to a tissue culture room with 2 BL-2 tissue culture hoods (shared with one other PI). The Bartlett lab is equipped with a refrigerator, a -20°C freezer, fume hood, spectrophotometer, gel boxes and power supplies, the MADGE gel system, a refrigerated microcentrifuge, Bunsen burners, water baths and other standard small pieces of equipment. Dr. Bartlett also has several instruments for high through-put genotyping equipment in his lab including 2 Eppendorf sliver gradient thermocycler blocks and a BioRad Tetrad 2 (4 blocks); both are suitable for long PCR programs required in highly multiplexed reactions. The lab also has a LuminexTM 200 flow cytometry platform that has been optimized for SNP genotyping and qPCR by MiraiBio who designed the software for these applications. Additionally, the lab has a forced air incubator with internal shaker on the bottom shelf for bacterial library work. A Millipore MilliQ Plus water purification system as well as autoclaves and dishwashing facilities are also located nearby. In addition to the general NCH computer

services (see Computer section below), each BCMM staff member is equipped with a Mac desktop that delivers advanced performance with two 3.4 GHz 64-bit Quad-Core Intel i7 CPUs, and each is equipped with a 30" HD Display. IT support is provided by Research Information Services (RIS) at The Research Institute, including networked printers and desktop systems administration support, HIPPA compliant data security protocols, and fully automated backup of all systems with archival storage of electronic information.

Dr. Bartlett's lab specializes in analyzing DNA samples for array genotyping and whole genome sequencing using Illumina platforms. His lab was among the first to publish protocols for extracting DNA from saliva for high throughput sequencing at a much reduced cost relative to commercial kits. And his group was among the first to establish a panel of barcode SNPs chosen for the triple purposes of unique sample identification, close relative determination and continental ancestry.

To complete the proposed specimen analyses, Dr. Bartlett's team will utilize both personal and allied laboratory facilities. Dr. Bartlett's team will use personal lab facilities at the BCMM to extract DNA from saliva samples for the proposed study. Dr. Bartlett's team will coordinate with allied lab facilities at the Institute for Genomic Medicine Clinical Laboratory Core to extract DNA from blood samples. Dr. Bartlett's team is prepared to coordinate with this core, as they have used their services for genetic analyses in previous studies.

All genotyping (i.e. blood & saliva) will be directed by the Bartlett lab.

Institute for Genomic Medicine Clinical Laboratory Core – The Institute for Genomic Medicine Clinical Laboratory Core supports NCH Principal Investigators through a variety of services, including specimen processing, banking, and distribution. Genomics services include whole genome sequencing for interrogating single-nucleotide variants (SNVs), insertions and deletions (indels), structural variants (SVs), and copy number variants (CNVs) in coding and non-coding regions of the genome. Other genomics services include whole exome sequencing (i.e. targeting only the coding regions of the genome), RNA sequencing coverage, and bioinformatics analysis. All analyses are performed using the latest genomics software (i.e. 10X Genomics, Oxford Nanopore, Pacific Biosciences). Researchers are encouraged to include the Genomic Medicine Clinical Laboratory Core in the planning stages of their project, as staff can assist with creating a budget, reviewing feasibility, and lending their biobanking proficiency to ensure quality specimen outcomes. This core is prepared to accommodate the unique needs of each project. Thus, the Genomic Medicine Clinical Laboratory Core is expertly equipped to meet the needs of the proposed study, and the needs of Dr. Bartlett's team as they arise.

Analytical Chemistry and Small Molecule Analytic Facilities (Dr. Rogers' lab) – Dr. Rogers' lab provides analytical chemistry services in support of clinical and basic research. Liquid and gas chromatography with mass spectroscopy capabilities are available for monitoring and quantifying compounds of interest, with a particular focus on small molecules. Services are available for method development and validation of new assays. Dr. Rogers' team conducted previous analyses on fatty acid concentrations in red blood cell samples from the children in the original trial.

Dr. Rogers' lab is located in the Research Building III, and includes the following available instrumentation:

- Liquid Chromatography – Mass Spec/Mass Spec (LC-MS/MS) currently equipped for small molecule analyses such as drugs, drug metabolites, and lipids.
- Gas Chromatography – Mass Spec (GC-MS) equipped to analyze small molecules not amenable to liquid chromatography separation.
- Gas Chromatography (GC) equipped with a flame ionization detector, currently used for fatty acid analysis or abundant sterols.
- High Pressure Liquid Chromatography two independent units available with UV/VIS, diode array, and fluorescence detection.
- Multiplex analysis for protein molecules (ELISA based format) using an MSD platform, samples can be analyzed for chemokines, cytokines, hormones, and other signaling molecules.

Nationwide Children's Hospital patient care facilities - Nationwide Children's Hospital is one of the largest children's hospitals in the United States, with 427 licensed beds and 91 leased beds. Nationwide Children's Hospital is the only tertiary care facility with pediatric subspecialty services in the Columbus area, and it serves a population of over 5 million. With over 1 million patient visits per year, it captures more than 80% of the market share. A new 12 story main hospital opened in 2012, adding 750,000 square feet of clinical space and increasing the total to nearly 2 million square feet of state of the art inpatient and outpatient facilities on campus.

The source population in Columbus for the proposed study includes patients of the Nationwide Children's Hospital Division of Neonatology. The Division is highly supportive in working to follow up with former patients. Neonatology manages 255 Neonatal Intensive Care Unit beds and cares for preterm children to age 2, with >3,000 follow-up visits/year, including ongoing developmental assessment for clinical purposes. Team staff will continue to work with Neonatology to maintain a patient database, including contact information and medical record data. Partnerships with the Division of Neonatology make the environment particularly well-suited to the proposed research, and permit incorporation of these clinical data with the study-collected data for richer analyses.

Center for Biobehavioral Health clinical research facilities - The Center for Biobehavioral Health facilities for family study visits are located in the JWest Building. Participant study visits take place in child-friendly, private observation rooms, built for behavioral research with integrated digital video and sound recording. Visit-specific, clinical research facilities to be used for the proposed study at JWest include:

- 8 child-friendly, private observation rooms built for behavioral research with integrated digital video and sound recording and one-way mirrors. Observation rooms are easily scheduled daily. These rooms greatly facilitate assessment and result in higher quality data than less conducive spaces like clinical exam rooms.
- Biospecimen processing facility, including centrifuge, two -20°C and three -80°C freezers, and biohazard controls.
- Secure storage for assessment technology and supplies.

NCH staff are supported by the following clinical research cores:

Clinical Research Services (CRS) – Clinical Research Services supports the initiation of all clinical research studies, providing staff and/or services to manage a study from beginning to end according to Good Clinical Practice and federal, state, and institutional regulations and guidelines. Services include

clinical research project consultation; study design assistance; protocol development and feasibility; regulatory/IRB document preparation services; clinical research budget development, including research pricing; data collection for subject tracking to ensure proper invoicing/revenue capture; clinical research training; and overall study management provided by certified and extensively trained clinical research coordinators. Clinical Research Services can also facilitate survey research, large data set analyses, investigational drug/device (IND) submissions to FDA and psychometrics. Clinical Research Services is located in the main hospital facility, and is funded by the CTSA grant. Clinical Research Services will provide robust, intellectual and collaborative support for the initiation and management of the proposed study.

Computer:

All Nationwide Children's employees receive 24/7 access to assigned desktop computers at their respective office buildings, including 24/7 wired internet access. NCH staff also have access to secure, wireless laptops during business hours. All wired and wireless computers at NCH include the following:

- Data analysis software (i.e. SAS, STATA, SPSS)
- VPN external connectivity
- Microsoft Office packages (i.e. Excel, Powerpoint, Word, Outlook, Access).
- Secure connection to Nationwide Children's Hospital EPIC electronic medical record system
- Microsoft Lync and Skype for Business, web-conferencing software and hardware
- Software for genetic analyses (i.e. GENETIX, KELVIN, KELVIZ, MoFlow, StickWRLD)

NCH staff are supported by the following technology service cores:

Research Data and Computing Core – The Research Data and Computing Core is composed of a multidisciplinary team dedicated to enhancing and expanding research through the scientific application of information management and informatics innovation. The Core possesses capabilities in custom research computer application development including: research study database design and development, image analysis, and data extraction; mining; curation; and integration. The Core also provides bioinformatics support, creates workflow pipelines, and provides IT infrastructure. The Research Data and Computing Core will develop and test the RedCap database and data entry interface for the proposed study, along with the NIH Toolbox measure used for parent and child assessments.

Research Information Services (RIS) – Research Information Services supports research projects by providing 24/7 hardware and software support, data security, and backup services. This team also ensures the use of encrypted emails via Microsoft Outlook. The Research Information Services team will safeguard data collected during the proposed study, along with digital communications amongst research team members.

26.0 Multi-Site Research

N/A – this is not a multicenter study.

27.0 Protected Health Information Recording

1.0 Indicate which subject identifiers will be recorded for this research.

- ☒ Name
- ☒ Complete Address
- ☒ Telephone or Fax Number
- ☐ Social Security Number (do not check if only used for ClinCard)
- ☒ Dates (treatment dates, birth date, date of death)
- ☒ Email address, IP address or url
- ☒ Medical Record Number or other account number
- ☐ Health Plan Beneficiary Identification Number
- ☐ Full face photographic images and/or any comparable images (x-rays)
- ☐ Account Numbers
- ☐ Certificate/License Numbers
- ☐ Vehicle Identifiers and Serial Numbers (e.g. VINs, License Plate Numbers)
- ☐ Device Identifiers and Serial Numbers
- ☐ Biometric identifiers, including finger and voice prints
- ☐ Other number, characteristic or code that could be used to identify an individual
- ☐ None (Complete De-identification Certification Form)

2.0 Check the appropriate category and attach the required form* on the Local Site Documents, #3. Other Documents, page of the application. (Choose one.)

- ☒ Patient Authorization will be obtained. (Include the appropriate HIPAA language (see Section 14 of consent template) in the consent form OR attach the HRP-900, HIPAA AUTHORIZATION form.)
- ☒ Protocol meets the criteria for waiver of authorization. (Attach the HRP-901, WAIVER OF HIPAA AUTHORIZATION REQUEST form.)
- ☐ Protocol is using de-identified information. (Attach the HRP-902, DE-IDENTIFICATION CERTIFICATION form.) (Checked "None" in 1.0 above)
- ☐ Protocol involves research on decedents. (Attach the HRP-903, RESEARCH ON DECEDENTS REQUEST form.)
- ☐ Protocol is using a limited data set and data use agreement. (Contact the Office of Technology Commercialization to initiate a Limited Data Use Agreement.

***Find the HIPAA forms in the IRB Website Library, Templates.**

Attach the appropriate HIPAA form on the "Local Site Documents, #3. Other Documents", page of the application.

3.0 How long will identifying information on each participant be maintained?

Identifying information will be maintained for 20 years following study closure. Ongoing follow-up of this cohort beyond mid childhood is a priority for this line of research.

4.0 Describe any plans to code identifiable information collected about each participant.

All participant data will be maintained in secure databases and files by number code. The study will follow all necessary measures to assure the data are protected and secure from unauthorized access. Only key personnel will have access to the file that links names to identification numbers. All electronic information is stored on the secure network and available only to study personnel. All paper files will secured in a locked room in the Center for Biobehavioral Health. Based on the research team's experience with previous studies, these methods of data confidentiality and protection have been effective. Data will be summarized in aggregate for reporting.

5.0 Check each box that describes steps that will be taken to safeguard the confidentiality of information collected for this research:

☒ Research records will be stored in a locked cabinet in a secure location

☒ Research records will be stored in a password-protected computer file

☒ The list linking the assigned code number to the individual subject will be maintained separately from the other research data

☒ Only certified research personnel will be given access to identifiable subject information

6.0 Describe the provisions included in the protocol to protect the privacy interests of subjects, where "privacy interests" refer to the interest of individuals in being left alone, limiting access to them, and limiting access to their information. (This is not the same provision to maintain the confidentiality of data.)

Participants' privacy interests are of the utmost importance, and will be emphasized across all stages of the recruitment, informed consent and enrollment, and study visit phases (see sections 13, 14, and 15 for details).

As stated previously, subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party. The IRB, FDA, or other authorized representatives may inspect all documents and records required to be maintained, including but not limited to, data collection records and pharmacy records for the subjects in this study.

Questions and concerns regarding privacy interests, or any other study related matters, will always be encouraged and answered thoughtfully by trained research staff. Moreover, a section on our informed consent will be included entitled, "WHOM SHOULD I CALL IF I HAVE QUESTIONS OR PROBLEMS?" Participants will be directed to the Principal Investigator's and Study Coordinator's direct contact information, as well as information for the Nationwide Children's Hospital Institutional Review Board, (IRB, the committee that reviews all research involving human subjects at Nationwide Children's Hospital).

28.0 Confidential Health Information

1.0 Please mark all categories that reflect the nature of health information to be accessed and used as part of this research.

- ☒ Demographics (age, gender, educational level)
- ☒ Diagnosis
- ☒ Laboratory reports
- ☐ Radiology reports
- ☒ Discharge summaries
- ☒ Procedures/Treatments received
- ☒ Dates related to course of treatment (admission, surgery, discharge)
- ☐ Billing information
- ☒ Names of drugs and/or devices used as part of treatment
- ☐ Location of treatment
- ☐ Name of treatment provider
- ☐ Surgical reports
- ☐ Other information related to course of treatment
- ☐ None

2.0 Please discuss why it is necessary to access and review the health information noted in your response above.

Whether the child has been diagnosed with particular health conditions/diseases that are a consequence of preterm birth and /or may be prevented or ameliorated due to the intervention will be collected to supplement and verify parent report of such diagnoses/outcomes.

3.0 Is the health information to be accessed and reviewed the minimal necessary to achieve the goals of this research? ☒ Yes ☐ No

4.0 Will it be necessary to record information of a sensitive nature? ☐ Yes ☒ No

5.0 Do you plan to obtain a federally-issued Certificate of Confidentiality as a means of protecting the confidentiality of the information collected? ☐ Yes ☒ No