ASPIRIN: NEURODEVELOPMENTAL FOLLOW-UP TRIAL

NCT04888377

Aspirin Supplementation for Pregnancy Indicated Risk Reduction in Nulliparas (ASPIRIN): Neurodevelopmental Follow-up Trial

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ACRONYMS

ACOG	American Congress of Obstetricians and Gynecologists
ASA	Acetylsalicylic acid
ASPIRIN	Aspirin Supplementation for Prevention of Inherent Risk in Nulliparas Trial
ASQ	Ages & Stages Questionnaire® Third Edition
BSID-III	Bayley Scales of Infant Development-III
BSID-IV	Bayley Scales of Infant Development-IV
DCC	Data Coordinating Center
DMC	Data monitoring committee
DMS	Data Management System
DRC	Democratic Republic of Congo
ERC	Ethical review Committee
GA	Gestational age
GN	Global Network for Women's and Children's Health Research
HDP	Hypertensive disorders of pregnancy
JNMC	Jawaharlal Nehru Medical College
IRB	Institutional Review Board
IUGR	Intrauterine growth restriction
LBW	Low birth weight
LDA	Low dose aspirin
LMIC	Low- and middle-income country
LMP	Last menstrual period
MDI	Mental Development Index
MNH	Maternal and Newborn Health
NICHD	*Eunice Kennedy Shriver* National Institute of Child Health and Human Development
NIH	National Institutes of Health
NSAID	Non-steroidal anti-inflammatory drug
PIH	Pregnancy induced hypertension
PMNCH	The Partnership for Maternal, Newborn, & Child Health
PROM	Premature rupture of membranes
PTB	Preterm birth
RCT	Randomized controlled trial
RTI	Research Triangle Institute International
SAE	Serious adverse event
SFI	Senior Foreign Investigator
SGA	Small for gestational age
SPTB	Spontaneous preterm birth
VLBW	Very low birth weight
WHO	World Health Organization

ABSTRACT

Background: Over 52.9 million children under five years of age in low and middle-income countries (LMIC) are estimated to have neurodevelopmental delays[1]. Though neurodevelopmental delay is complex in its origins, preterm birth[2], hypertensive disorders of pregnancy (HDP)[3] and chronic inflammation[4] are known primary drivers. Recently the ASPIRIN[5] trial demonstrated that low dose aspirin (LDA) administered antenatally decreases the rate of preterm birth, preterm (HDP) and perinatal mortality. LDA has also long been known to decrease chronic inflammation. Therefore, antenatal LDA may prevent neurodevelopmental delay through its effect on preventing preterm birth & HDP and inhibiting chronic inflammation. Currently data of neurodevelopmental outcomes following antenatal LDA exposure can be summarized as: 1. multiple (37+) observation trials that have linked maternal inflammation with developmental delays/behavioral issues[4], 2. Numerous studies showing that offspring of HDP pregnancies are at significant risk of cognitive delay[3], 3. follow-up trials of children whose mothers were part of an antenatal LDA trial that the assessment occurred too early (e.g. 12 or 18 months) or used outdated assessments (e.g. parental perception) but show either no difference or improvement[6–8] and 4. registry studies that have associated maternal LDA use with slight increases in cerebral palsy[9] and hearing loss[10]. Despite the recent positive findings of the ASPIRIN trial, broad acceptance of low dose aspirin (LDA) may be tempered by concerns about effects on longitudinal neurodevelopment. In contrast, if LDA is found to mitigate these risks, women would be further motivated to take LDA and professional societies to recommend LDA as a universal part of prenatal care.

Hypothesis(es) and Aims:

Primary Hypothesis: Children exposed antenatally to low dose aspirin will have scores on the Bayley Scales of Infant Development-III (BSID-III) examination at 36 months of life(+/-3months) that are not inferior to their peers who were not exposed (i.e., by no more than a margin of 4 points).

Sub-Hypothesis: Conditional on showing non-inferiority is that children exposed antenatally to low dose aspirin will have higher scores on the BSID-III examination compared to their peers who were not exposed.

Secondary Hypothesis: The secondary hypothesis will be that children exposed antenatally to low dose aspirin will have similar scores on the Ages & Stage's Questionnaire® Third Edition (ASQ-3).

Sub-Hypothesis: Conditional on showing non-inferiority is that children exposed antenatally to low dose aspirin will have higher scores on the ASQ-3 examination compared to their peers who were not exposed.

Design: This will be a prospective masked matched cohort study of children between 33 and 39 months (mean 36 months) of age whose mothers were randomized in the ASPIRIN trial (1:1 Aspirin-Placebo), who will be evaluated using the BSID-III. Additionally, the Family Resources and Context questionnaire will be performed to adjust for the local context and the ASQ-3 will be administered as a secondary screen. Recognizing the significant role that preterm birth plays in neurodevelopment we will include 100 (50 in each group) children who were delivered before 37 weeks.

Inclusion Criteria: The following are the inclusion criteria: 1. Children whose mothers participated in the ASPIRIN trial whose parents/guardians are able to consent, 2. No evidence of congenital anomalies that would affect development (e.g., blindness) and 3. Other conditions that would preclude them from completing a BSID-III.

Exclusion Criteria: Children not meeting the inclusion criteria will be excluded. Likewise, children may be excluded at the discretion of the site investigator out of concern for the child's wellbeing (e.g. distress with testing).

Power & Sample Size: Assuming the BSID-III score has a mean scaled score of 100 and standard deviation of 15, a non-inferiority margin of 4 points is clinically significant, and the true effect of LDA is not more than a 1-point decrease, a total sample size of 620 children with a 1:1 allocation (310 in each group) will have 80% power for a one-sided test for non-inferiority with α=0.05.

Primary Outcome: The primary outcome will be the results of the BSID-III- specifically the motor, cognitive and language components.

Potential Impact: The ASPIRIN trial results prove that LDA can improve maternal-child outcomes; nonetheless, women often underestimate their risk of preterm birth and are reticent to take medication during pregnancy. By demonstrating the safety and potential superiority of antenatal LDA on neurodevelopment, women will be more willing to accept LDA as part of routine care and obstetrical providers and countries to recommend its routine use.

1 STATEMENT OF PROBLEM

1.1 PRIMARY HYPOTHESIS OR QUESTION:

Children exposed antenatally to low dose aspirin will have scores on the Bayley Scales of Infant Development-III (BSID-III) examination at 36 months of life (+/-3months) that are not inferior to their peers who were not exposed (i.e., by no more than a margin of 4 points).

1.2 EPIDEMIOLOGY:

An estimated 250 million children under the age of 5[11] worldwide are at risk for not achieving their developmental potential[11]; 52.9 million children under five years of age in low- and middle-income country (LMIC) settings have neurodevelopmental delays[1]. Compounding the issue is preterm birth (more common in LMICs [12]) which has consistently been identified as a cause of neurodevelopmental delay [2,13]. A recent review reported that out of the estimated 13 million preterm infants who survive beyond the first month, 0.9 million will suffer long term neurodevelopmental impairment, with 345,000 moderately or severely affected [14]. This burden places a significant strain on the families, healthcare systems and societies that provide care for these children.

1.3 ANTECEDENTS OF NEURODEVELOPMENT:

Neurodevelopment of children is the aggregate outcome of complex exposures, both antenatally and post-delivery. Antenatal factors such as maternal nutrition[15], inflammation[16], and stress may all play important roles in both cognitive development of children and attainment of optimal growth[17,18]. As a single risk factor, preterm birth is strongly linked to abnormal growth, cognition and motor development [14,19 20]. Inflammation, which is a common pathway of preterm birth[21], has been further implicated in neurodevelopment. In a review of 37 papers, Nist and Pickler[16] identified a series of inflammatory mediators (interleukin (IL)-6, IL-1β, IL-8, and tumor necrosis factor (TNF)-α) that were all associated with neurodevelopmental delay in children.

Specific to LMIC settings, Jiang and colleagues examined known markers of inflammation (C-Reactive Protein, Soluble CD-14, IL-6 & Others) and found a negative correlation between elevated levels of inflammatory markers and outcomes of the Bayley exam[22,23]. Additionally, they noted that elevated IL-4 levels (which help differentiate T Helper cells) were positively correlated with improved neurological outcomes.

Poor nutrition and growth limitations in the fetus/infant have also been associated with impairments in neurodevelopment [15]. In Guatemala, a cohort study found stunting, which is reflective of perinatal nutrition, to be prevalent in 47% of newborns; 53% at 3 months of age and 56% at 6 months of age [24]. This study concluded that growth failure in infants was predicted by growth failure in fetal life, thus indicating the importance of maternal interventions. Data from other Global Network participating sites (Guatemala, Democratic Republic of Congo, Zambia and Pakistan) also found strikingly high rates of stunting ranging from 44% to 66%, among infants and toddlers [25]. Malnutrition, which also includes being overweight and obese, are known inflammatory processes that can potentially impact neurodevelopment. These conditions are increasingly more

common to low and middle income countries [26]. Other newborn exposures including exclusive breastfeeding, stunting, as well as caregiver factors such as maternal/parental education, mental health, and home environment. Poverty additionally contributes to the attainment of optimal neurodevelopment. As such, any study of neurodevelopment should at least document these potential confounders.

1.4 IMPLICATIONS OF ASPIRIN ON NEURODEVELOPMENT:

1.4.1 Biologic Rationale:

In a review of 37 papers, Nist and Pickler[16] identified a series of inflammatory mediators (interleukin (IL)-6, IL-1β, IL-8, and tumor necrosis factor (TNF)-α & others) that were all associated with neurodevelopmental delay in children. Specifically both inflammatory mediators and hypertensive disorders have been shown to negatively affect neutrophins and other neuroproteins that are critical in the neuronal programming and development[27]. Thus, there is direct biological plausibility of maternal inflammation hindering normal child neurodevelopment. Of particular interest is that many of these same inflammatory mediators have been implicated as playing crucial roles in spontaneous preterm birth and maternal hypertensive disorders of pregnancy (HDP).

Aspirin has been shown to predominantly affect both the COX-1 pathway which is involved in thrombosis and the COX-2 pathway, which affects inflammation through the production of Aspirin Triggered Lipoxins [28,29] Various Lipoxins have been shown to affect placental development and play roles in the development of preeclampsia and preterm birth[30]. Lipoxin A4 has been particularly singled out in playing a crucial role in placental inflammation and acts not only to inhibit new inflammation but to aid in the resolution of existing inflammation. More specifically, aspirin has been shown to inhibit the production of IL-6, IL-1B, CRP and TNF-α all of which have been shown to negatively affect child neurodevelopment and be involved in preeclampsia and preterm birth. Of equal interest, Aspirin inhibition of COX-2 has been shown to increase the production of neuroprotection DI and Resolvin D1 which have been suggested to lower the risk of Alzheimer's disease and ischemic brain injury[31].

1.4.2 Hypertensive Disorders of Pregnancy and Neurodevelopmental Delay:

The origins of prescribing LDA in pregnancy began with an attempt to decrease hypertensive disorders of pregnancy. Through studies of over 50,000 pregnant women[5,32], it has now become well established that LDA decreases the risk of both term and preterm HDP. The offspring of women whose pregnancies are complicated by preeclampsia/HDP have consistently shown both short and long term neurodevelopmental delays and behavioral issues regardless of the gestational age at birth[3,33]. Nonetheless, studies showing that the prescription of LDA during pregnancy improves poor neurodevelopmental outcomes due to HDP are absent.

1.4.3 Clinical Data of Neurodevelopment and Fetal Exposure to LDA:

1.4.3.1 Systematic Follow-up of Prospective RCT's of LDA:

Data regarding fetal exposure to LDA is noted to consist of the following:

- The largest study to examine longitudinal neurological follow up of children born to women randomized to prenatal aspirin was a follow up trial of children whose mothers participated in the United Kingdom's Collaborative Low-dose Aspirin Study in Pregnancy (CLASP) trial that included 9,364 women. Amongst this cohort, the child's medical practitioner provided information on development for 4,168 children at 12 months of life and 4,365 parents answered a standardized questionnaire at 18 months of age. No differences in development, motor function or anthropometric measurements were identified [8].
- Marret and colleagues examined 656 preterm children (125 LDA exposed) born to 584 women before 33 weeks who were enrolled in a French RCT of prophylactic LDA [6]. They examined children using the Kaufman Assessment Battery for Children at 5 years of age. The percent of low simultaneous processing scores (<70) was lower in the group with LDA (7% vs 19% without LDA; P = .04). They also found that LDA exposure trended towards improvement in total behavior difficulties (aOR: 0.44 [95% CI: 0.19-1.02]) and hyperactivity (aOR: 0.43 [95% CI: 0.17-1.05]).
- In a letter to the Lancet, Parazzini et. al. provided information regarding neurodevelopment of 788 children (427 aspirin exposed) whose mothers had participated in the Italian Aspirin in Pregnancy Study [34]. They provided a questionnaire to parents of children at 18 months that addressed both neurodevelopment (Sonnander's questionnaire) and fetal growth. Though statistical analysis was not provided, the presented tables provide adequate information to perform simple univariate analysis. Children exposed to aspirin had lower rates for less than the 10th percentile for height (8.4% vs. 12.2%-P = 0.047) and they had significantly improved gross and fine motor function (3.5% vs. 7.2%-P=0.02). Weight at less than the 10th percentile and language attainment were similar. These results must be viewed cautiously as the trial was not blinded, relied on maternal report and 27% of the children were lost to follow up.

1.4.3.2 Population-based Cohorts:

- The Extremely Low Gestational Age Newborns (ELGAN) study examined 877 extremely premature (<33 weeks) and surveyed mothers about antenatal exposures at the time of birth. Both neonatal ultrasound of the neonatal brain and neurological follow up at 24 months of corrected age was performed. The neurological follow up was standardized and blinded but did not use a widely recognized methodology.[35] An association with maternal aspirin use was associated with a slightly increased risk of quadriparetic cerebral palsy following adjustment for multiple confounders (OR 3.0, 95% CI 1.3 to 6.9)[36]. Data regarding the duration of exposure, dosage taken and indication for use was not presented. The total number of women who took LDA was noted to be 49. There was no association with LDA and ultrasound findings that have been linked to cerebral palsy nor any association with diparetic cerebral palsy.
- Petersen and colleagues examined both prenatal exposures to analgesics (acetaminophen, ibuprofen, and aspirin) and the risk of cerebral palsy by examining two large linked Dutch cohort studies (Norwegian Mother and Child Cohort Study and Danish National Birth Cohort)[9]. They examined the risk of cerebral palsy by exposure amongst 185,617 children. They found that aspirin exposure was associated with an elevated risk of bilateral spastic cerebral palsy (aOR 2.4, 95% CI: 1.1–5.3). Nonetheless the data were limited by very low rates of antenatal

LDA exposure (3.1%). In contrast, acetaminophen use occurred in 49% of women and was associated with a OR of 1.3 (95% CI:1.0–1.7) for unilateral cerebral palsy. A significant limitation of this study is that these associations may be confounded by indication. Both acetaminophen and aspirin are commonly utilized to treat fever. Infections in pregnancy have been consistently associated with cerebral palsy[36].

- Foch et al recently published a case control study examining maternal drug exposures during pregnancy comparing children who either had a normal hearing evaluation (N=28,046) or an abnormal hearing evaluation (N=1,245). They found that children who had an abnormal hearing loss were more likely to have had an in utero exposure to LDA (OR 1.61, 95%CI 1.09-2.37)[10].

1.4.3.3 Summary of These Trials and the Potential to Improve Neurological Outcomes:

As detailed above, there is biologic plausibility and limited clinical evidence that by mediating inflammation and preterm birth during pregnancy, we may be able to improve neurodevelopmental outcomes. However, there is conflicting information on the potential of harm versus benefit regarding motor function and hearing. Though several studies have suggested harm, they are confounded by indication and report small increases of risk of rare conditions. Nonetheless the aggregate data are sparse, utilize short duration of follow-up, are not based on well accepted instruments of development, do not account for potential confounding factors and rely predominantly on indirect observation. None of these studies have utilized the BSID-III, the currently best validated and accepted method for evaluating neurodevelopment. It is equally important to address that perinatal inflammation may be unique in LMIC communities, wherein the incidence and causes of inflammation may be different. Similarly, cultural variation may also have unique implications for differences in longitudinal development.

The Global Network's ASPIRIN study provides a unique opportunity to study the impact of LDA with earlier and validated exposure and infant outcomes at 33 to 39 months of age. This follow up study will likewise provide the infrastructure and a stable cohort to potentially examine children's outcomes at 5 years of age, when issues like behavior and IQ can be more accurately assessed. In summary, the follow-up of infants born to participants in the ASPRIN trial will allow us to: a) analyze the safety of low dose aspirin during pregnancy relative to neonatal health, b) study the impact of LDA on neurodevelopment, growth and morbidity, and c) potentially establish a longitudinal cohort to allow meaningful exploration of issues related to child behavior in future investigations.

1.4.3.4 General Safety of Aspirin During Pregnancy:

To date, in excess of over 50,000 women have participated in research trials of aspirin[37, 5]. Based on the available data, aspirin appears to have a favorable safety profile for both mothers [38] and their newborns [39]. Specifically in terms of the neonate, differences in anomalies [40], post-natal intracranial hemorrhage or complications in circumcision have not been detected despite robust numbers being reported [39]. More recently, follow-up safety data from the ASPIRIN trial demonstrated comparable adverse events and usage of medical services among women who took aspirin compared to those who took placebo. Finally, an analysis of the impact of aspirin randomization on hemoglobin revealed no impact amongst women enrolled in the ASPIRIN study[41].

1.5 POPULATION IMPLICATIONS FOR THE CHANGING PATTERNS OF PRENATAL ASPIRIN USE:

The necessity to examine the impact of LDA on longitudinal development is fueled by the fact that the utilization of LDA during pregnancy is poised to dramatically change. Low-dose aspirin has increasingly been recommended by professional societies (ACOG[42] and Royal College of Obstetricians and Gynaecologists) and the World Health Organization (WHO) for women at risk for preeclampsia during pregnancy [43]. Following the recent publication of the ASPRE trial (Combined Multimarker Screening and Randomized Patient Treatment with Aspirin for Evidence-Based Preeclampsia Prevention)[44] and the current Global Network's ASPIRIN trial[5], the number of potentially exposed newborns is anticipated to grow.

1.6 PUBLIC HEALTH IMPLICATIONS:

Although the benefits of LDA in preventing preeclampsia and now preterm birth have been proven, the ultimate public health impact will be decided by the willingness of pregnant women to choose and comply with this therapy. Though complex and influenced by familial and cultural inputs, these decisions are largely based upon the individual patient's perception of their risk/benefit ratio. Prior research has demonstrated that mothers (particularly first-time mothers) underestimate or minimize their risks and have a strong reluctance to take ongoing medications during pregnancy[45]. While both current recommendations and outcomes from the ASPRE and the ASPIRIN trial suggest significant fetal benefit, to fully inform individual patient decision making, systematic longitudinal follow-up studies are warranted. If LDA is found to lack detrimental effects for offspring or to have potential cognitive benefits, women will be much more likely to view low dose aspirin as an acceptable intervention.

1.7 SUMMARY:

Though the recent ASPRE and ASPIRIN trials have established low dose aspirin as a strong preventative measure in the prevention of the major causes of maternal and perinatal morbidity (preterm birth and preterm preeclampsia), the implications on longitudinal newborn outcome remain less clear.

2 METHODS

2.1 STUDY DESIGN:

We propose a matched (1:1) cohort study of children whose mothers were randomized to either low dose aspirin or placebo. As preterm infants are at higher risk they will be oversampled (N=100) and matched for gestational age within one +week and equally distributed between mothers who received LDA and those who received placebo.

2.2 STUDY POPULATION:

The study population will consist of approximately 620 children, between 33 and 39 months of age, whose mothers were included in the ASPIRIN trial. Among those, one hundred children who were born preterm (<37 weeks) will be selected as they have a higher risk of developmental delays. They will be equally distributed between the LDA and placebo arms. They will be matched by site and within 1 week of delivery.

2.2.1 Inclusion Criteria:

The following are the inclusion criteria:

a. For a prior study sponsored by the Global Network for Women's and Children's Health Research, the child's parents gave permission to recontact.
b. The child's parent/guardian is willing and able to provide consent for the child and willing also to participate in the child's assessment (e.g., by providing answers to ASQ-3 and the Family Resources and Context questionnaire).
c. The child will be between 33 and 39 months of age at time of assessment.
d. The child does not have a significant congenital anomaly (e.g., blindness or deafness) that would affect development or preclude them from completing the BSID-III. This will be left at the discretion of the Senior Foreign Investigator (SFI) with the ability to consult with the Working Committee as needed.
e. Other significant medical conditions that would preclude the child from completing a BSID III are absent. This will be left to the discretion of the Senior Foreign Investigator who will have the ability to consult with the Working Committee as needed.

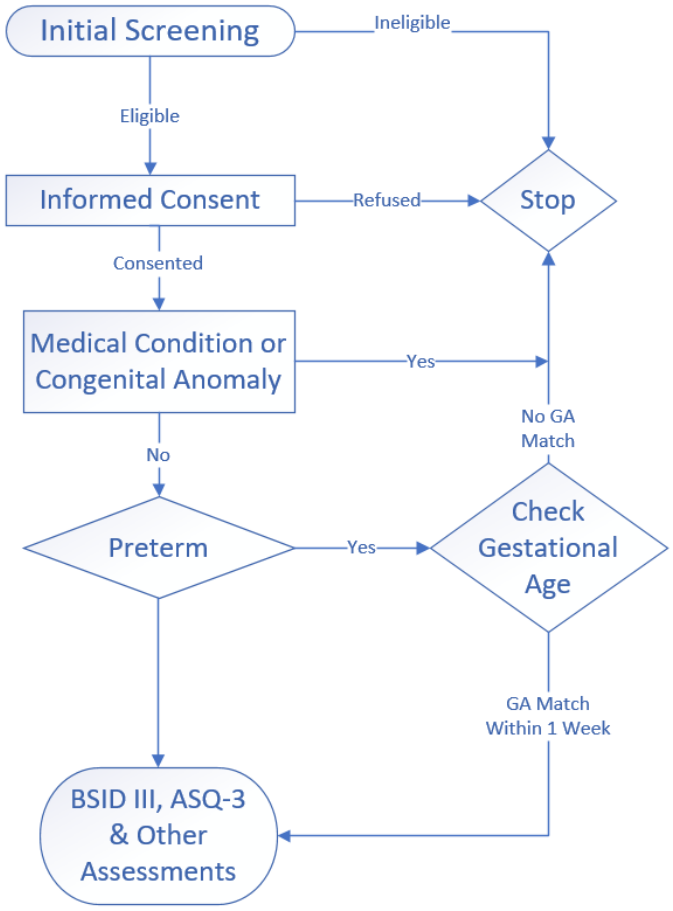
2.2.2 Exclusion Criteria:

Children not meeting the inclusion criteria will be excluded. Likewise, children may be excluded at the discretion of the site investigator out of concern for the child's wellbeing (e.g., the child has a current respiratory infection or emotional distress during testing).

2.3 SEQUENCE OF STUDY ACTIVITIES:

The sequence of study activities is shown in **Figure 1**.

Figure 1. Flowchart of Study Activities



2.4 Detailed Study Procedures:

2.4.1 Initial Screening:

Initial screening will be done by the Data Coordinating Center (DCC). ASPIRIN trial participants who agreed in their initial consent to be contacted for a future study will be identified by the DCC and this information will be supplied to the local sites. Secondly, the DCC will review the delivery dates to identify children who are near 36-months of age. Assuming that both criteria are met, the DCC will examine whether the child's mother was in the aspirin group or the placebo arm. Finally, the gestational ages will be reviewed. The DCC will issue pairs of eligible children (1 Aspirin: 1 Placebo) which are matched within the site and are matched in gestational age within one week. The DCC will track successful recruitment of pairs. When successful recruitment of a pair cannot be obtained, a child meeting the gestational age, allocation and site requirements will be substituted to ensure that balance occurs across these groups. As children will be older than 2 years of age, we will not correct for preterm birth.

2.4.2 Blinding/Masking:

Throughout the study, research staff and local health providers will be blinded to treatment status of the mother. Data regarding the subject's allocation will be held and protected centrally and will not be made available to participants or their families.

2.4.3 Location of Participants:

As contact information was obtained during the initial study, this will be utilized to reach out to participants. Local centers will determine the optimal method of contact. This can include in person visits or phone calls. If the parents/guardians decline participation, no further efforts will be made to encourage study participation. To ensure that research is not coercive, no more than three attempts at contacting potential participants will be made.

2.4.4 Consenting:

Before a child participates in any research activity, the research staff must obtain the informed consent to voluntarily take part in the study from the child's mother, father or guardian. Consent will be obtained from parents or guardians ≥ 18 years of age or minors 14-17 years of age in countries where married or pregnant minors (or their authorized representatives) are legally permitted to give consent. When approaching guardians who are minors, we will follow the in-country policies for human research protection and the guidelines approved by the local ethical review committees (ERCs). In the case of minors, this may require that written consent is obtained from a parent/guardian or husband, with written assent from the minor guardian.

The research staff will give adequate opportunity to the individual providing consent for a child's participation and/or immediate family members to read the consent form and ask questions. Recognizing that literacy levels will vary and may be a challenge, the consent process will include a verbal review of the consent form. If the individual requested to provide consent cannot read, the form will be read aloud to the individual by a person unaffiliated with the study. Alternatively, the Program Coordinator or a designate may read the consent, but in the presence of a witness who is unaffiliated with the research study. Potential consenters will be given an opportunity to discuss the study procedures and ask questions. Fair balance will be maintained while describing the risks and benefits of participation in the study. No undue pressure will be placed on the potential consenter to enroll the child in the trial. It will further be explained that lack of participation will not affect the usual and anticipated standard of care.

If the individual providing consent (a parent/guardian) is unable to sign their name, he/she will be asked to use her/his thumbprint to indicate written approval. In both cases, the unaffiliated person will also sign the consent form. Both the research staff and the study participant retain signed copies of the form. The potential participant may also take the form home to discuss with the family before signing.

The parent/guardian may refuse to agree to the child's participation in the trial at the time of recruitment. This will be recorded in the Screening and Recruitment Form. He/she may also choose withdrawal from the study at any time after enrollment. This will be recorded on study forms. Similarly, if an enrolled child becomes ineligible prior to assessment, the child will be withdrawn from the study and this will be recorded.

All research staff responsible for obtaining consent will be trained and certified in the protection of human subjects and the study-specific consent procedures. A model written informed consent form, developed according to the requirements of the U.S. Office of Research Protections (OHRP), is found in Appendix 1. Each site may modify the model consent to conform to local standards, but the OHRP required elements must be maintained. The research sites will also be responsible for translating the consent form into the appropriate language(s) for their local context.

Global Network countries with legislation regarding the need to videotape consents will comply with the country regulations; however, this is not part of the consent form requirements. This will not be required by protocol but rather decided by each site so as to comply with country rules and regulations.

2.4.5 Screening for Medical Eligibility & Anthropometric Measurements:

Upon completion of the consenting process, an assessment of medical eligibility will be undertaken using the Medical History form. This form contains a basic medical history and immediate assessment to ensure that the child has no significant medical problems that would preclude or interfere with the performance of the evaluation. Similarly, if the site coordinator is concerned about the child's wellbeing on the date of assessment, she/he can either be rescheduled for a time that is deemed more appropriate or removed from the study. Examples would include an acute rhinitis, asthma exacerbation or emotionally distressing event. If the child is scheduled more than two times but not assessed, the child will be excluded from the study. Decisions about medical eligibility will be made by the SFI and, if requested, in collaboration with the working committee.

In addition, anthropometric measurements will be recorded on this form. Measurements will be done by qualified staff who will be trained in accordance with prior protocols using standardized equipment. Those who were train in the Women First: Preconception Nutrition protocol will be considered as certified.

2.4.6 Performance of the BSID-III Assessment:

The BSID-III assessment will be performed by only trained and qualified examiners. To be considered trained, an examiner will have been qualified in a prior study that utilized the BSID-III assessment forms or a health professional experienced in use of the Bayley assessment tools. If not previously trained, examiners must be willing to be trained and undergo a period of observation. Specifically, we will be performing the motor, cognitive and language components of the BSID-III. We will not include the social and emotional domains intentionally for the following reasons: 1. The social and emotional domains are lengthy and therefore result in significant participant burden and may negatively impact ascertainment of the other three domains, 2. The available qualified and trained staff have historically not performed these components and to introduce them now would be a significant challenge, and 3. The internal reliability of these sections are subject to greater social/cultural influences, particularly in a study involving different countries/culture. Results of the BSID-III will be recorded on the BSID-III assessment form.

2.4.7 Ages & Stages Questionnaire-3:

The Ages & Stages Questionnaire 3 (ASQ-3) is a broadly accepted and validated screening tool for neurodevelopment. Like the BSID-III, ASQ-3 has been utilized and validated in multiple languages

and cultural contexts. The ASQ-3 will be administered by research staff to parents. Where literacy issues may be present, staff will be trained on how to help administer the questionnaire to obtain accurate information without introducing bias. Following completion of the questionnaire by the child's parents, staff, will score the questionnaire and those results will be recorded on the ASQ form.

2.4.8 Family Care Indicators Questionnaire:

This questionnaire is based upon family care indicators developed in 2002 with UNICEF involvement for the purpose of assessing fundamental practices or qualities that support and encourage learning and development. The Family Care Indicators questionnaire was developed to be implemented across diverse countries and different cultural backgrounds. Moreover, it has been explicitly field-tested and validated for this purpose[46]. The questionnaire also has questions to judge psycho-social wellbeing developed by UNICEF working groups for a psychosocial evaluation project[47.] The results of this will be recorded on the Family Care Indicators form.

2.4.9 Infant andYoung Child Feeding & Breastfeeding History:

The Infant Feeding Index form will be administered by the assessment staff. This form will collect information on breastfeeding history, feeding practices, and food frequency, and recent dietary history.

2.4.10 Referral Process for Children Found to be at Risk:

Children who have a BSID-III score below <85% or an ASQ-3 below 2 standard deviations will be noted, and their parent(s) provided with this information. Because complex factors can affect individual scores, parents will be encouraged to consider further evaluation of their child.

2.4.11 Study Governance and Logistics:

Oversight of the trial will be handled by two principal groups with different focuses:

a. **Composition and Role of the Steering Committee:** The steering committee (SC) is comprised of the DCC, the BSID-III quality oversight individual, and Global Network Senior Foreign Investigators and US investigators associated with each of the participating sites (see pages 5-7). The steering committee will have primary responsibility for overall study design, approval of study materials and procedures, and oversight of study implementation. The SC will convene via conference call at least once per quarter and in person (pandemic permitting) in conjunction with a Global Network Steering Committee meeting to discuss the study and implementation issues. During the early phases of the study more frequent meetings may be needed. If the budget allows, members of the group may conduct site visits to bolster enthusiasm, provide hands-on training and education to the participating staff, and address site-specific issues, if any.
b. **Composition of the Working Group:** The working group will consist of the lead investigators (Drs. Goudar, Hoffman, and Derman), the BSID-quality consultant (Dr. Michele Lobo), and representatives of RTI International, including the Data Coordinating Center. They will meet via conference call at least monthly to monitor study progress and ensure proper implementation of the trial. They will have primary responsibility of the following tasks:

i. Protocol development and revision as needed
ii. Manual of operations development and revision as needed
iii. Oversight of data forms and database development
iv. Tracking ethics approvals and renewals
v. Monitoring recruitment and individual site progress
vi. Monitoring of BSID-III examiners
vii. Ongoing review of data quality
viii. Review of serious adverse events and protocol violations
ix. Budgetary oversight
x. Review of findings and analysis
xi. Manuscript drafting and submission
xii. Coordination with the steering committee
xiii. Handling of any and all operational concerns

Members of the group may also conduct site visits to research sites, as the budget allows, to bolster enthusiasm, provide hands-on training and education to the participating staff, and address site-specific issues, if any.

2.4.12 Role of Senior Foreign Investigators:

The Senior Foreign Investigators (SFIs) will be responsible for developing site-specific implementation plans, ensuring study staff are properly trained, and providing oversight of the study at the site level. As members of the study SC, convene via conference call at least once per quarter and meet in person with other members of the study's Steering Committee (if it is feasible to take advantage of an in-person Global Network Steering Committee meeting) to discuss study implementation issues.

2.4.13 Institutional Review Boards and Trial Registration:

Human subjects review will be undertaken by the IRB of Thomas Jefferson University and the ethical review committees of the foreign sites. This effort will be coordinated across the varying groups by the Data Coordinating Center. To ensure transparency, the study will be registered at Clinicaltrials.gov and at other required sites as stipulated by review committees of the foreign sites.

2.4.14 Data Monitoring Plan:

All participating foreign sites will transmit data to the Data Coordinating Center, located at RTI International. The data will be used to evaluate protocol adherence and site performance (e.g., recruitment, loss to follow-up, data quality). The DCC will provide standardized progress reports to Steering committee and the site investigators monthly to monitor outcome variables and data audits/edits.

2.4.15 Training and Implementation of Neurodevelopmental Testing:

Significant experience and trained staff already exist at a number of the sites due to participation in the BRAIN HIT trial[48] and the Women First: Preconception Maternal Nutrition trial[49]. Both trials had qualified individuals and the infrastructure to ensure training and ongoing validity of testing. Additionally, it is recognized that individuals may have been trained in the performance of these types of tests as part of clinical practice.

BSID-III Training: To be qualified as a BSID-III examiner for the purpose of this study, the examiner must meet the following criteria:

a. Demonstrate credentialling or consistent experience in administering Bayley Scales of Infant Development:
   - Prior successful administration of BSID-II or BSID-III in a Global Network study (BRAIN-HIT or Women First) or in another research trial using the Bayley Scales
   - Certification of training in administration of Bayley Scales by other approved means
   - Use of the BSID-III in association with clinical care
b. Attend either a virtual webinar on performance or watch a recording of a webinar on correct performance of the BSID-III featuring key metrics

BSID-III Monitoring: Key to ensuring that high quality BSID-III testing is being done, the following provisions have been developed to ensure longitudinal follow up occurs. This is noted to include the following:

a. BSID-III examiners are asked to film one of their first 5 BSID-III examinations. This is to be labeled with the participant number, examiner number and no other identifiers. Feedback will be provided by the central BSID-III study credentialler. The credentialler will provide feedback to the examiner in each of the domains used for this study that are designed to serve as positive coaching.
b. Following each review, the credentialler will then determine the window to the next reevaluation. The choices for duration can be either one within the next 5 exams or the next 10. She/he may also choose to have the next cycle of reevaluations performed by either the site lead for BSID-III training or by the central credentialler.
c. If after 2 cycles significant concerns are raised by the central credentialler, the examiner may be asked not to continue in the study.

Key to the success of this approach is to take a positive coaching approach as has been utilized in the past. This process will use an IT infrastructure and central review process that has been utilized in a number of prior studies of both neurodevelopment[48] and ultrasound[50] in the recent past.

2.4.16 Data Management Procedures:

Data will be collected prospectively using hard copy forms or Android tablets. Regardless of data capture methodology, all data will be kept confidential. Each participant will be assigned a unique study ID which will be used to identify the participant. Only the screening log will contain the name (which is not transmitted). If hard copy forms are used, they will be retained in a secure location for possible editing or queries at the central data entry site. Data will be entered into computers using the Data Management System (DMS) developed by RTI and the assigned study number. The DMS will also allow site staff to produce project reports and backup the study

database. Electronic data will be transferred from each data management computer to a single Research Unit Data Center (RUDC) in each country, creating a complete data repository. At least once a week, data will be transmitted from the RUDC to the DCC at RTI. The data center will conduct training on the DMS system, as needed, and will maintain the central database for the study. Precision and accuracy of actual data collected will be checked by chart review (random 5%) and internal procedures using the computer program. Monthly audits and incomplete data reports will be performed by a review team consisting of at least of the SFI and the country coordinator. Data editing and error resolution will be performed monthly. In addition, a sample of participants will be visited to confirm their participation, with procedures determined per site. These activities will be shared between the site and the data center.

2.5 PRIMARY OUTCOME:

The BSID-III provides systematic assessment of development in the three primary domains of cognition, language (receptive and expressive) and motor skills (fine & gross). The BSID-III forms for these domains are validated tools which can be used for assessment of children at about 36 months of age and are well accepted by the research community. Equally important, this tool has been validated in a diverse group of languages and cultures including low/middle income countries [51]. Specifically, Bayley forms have been validated in the following countries of relevance: Democratic Republic of Congo, Pakistan, Zambia, India and Guatemala.

Foundational to utilizing the Bayley forms will be a requirement for both demonstration of competence in performing the examination and an ongoing assessment of correct implementation through periodic reassessment. For this proposed trial we will utilize the existing staff, credentialing process and oversight mechanisms of the Women First: Preconception Maternal Nutrition trial.

2.6 SAFETY MONITORING:

The risk implicit in the BSID-III is noted to be minimal. Little risk is anticipated. Nonetheless, we will monitor non-completed studies, and case report forms will include any adverse events that have occurred during the visit. This will include an open-ended question about issues that occurred during the visit. Review will occur on an ongoing basis. Adverse events will be submitted on Serious Adverse Event forms.

2.7 SITE PREPARATION:

In preparation for study implementation, the sites will meet with health authorities and conduct community sensitization activities to ensure that study procedures are appropriate for the local context and to encourage commitment and engagement at the facility and community level. Site

preparation activities will focus on:

- Identifying and hiring study staff;
- Identifying potential implementation challenges and developing culturally-appropriate solutions;.
- Educating health workers and community members on the purpose of this investigation.

2.8 POTENTIAL RISKS AND BENEFITS TO PARTICIPANTS:

No direct benefits to the current participants are noted beyond possible consideration for time and effort as allowed by local IRB standards. The risks are noted to be nominal as no invasive procedures are planned.

3 ANALYTICAL PLAN

3.1 STATISTICAL ANALYSIS PLAN:

Prior to conducting formal analyses baseline demographic characteristics and key clinical measures will be compared between the mothers who participated in the two treatment arms using contingency table approaches for categorical variables and analysis of variance models and t-tests for continuous variables. For all of these analyses, comparisons will be made within Global Network sites and overall across the sites controlling for site in the models.

3.1.1 Primary Analysis:

The primary outcome of BSID-III score is assumed to have a mean scaled score of 100 and standard deviation of 15[47]. Assuming a non-inferiority margin of 4 points is clinically significant, and the true effect of LDA is not more than a 1 point decrease, a total sample size of 620 children with a 1:1 allocation (310 in each group will have 80% power for a one-sided test for non-inferiority with α=0.05. Conditional on non-inferiority being observed, this sample size will also provide over 90% power at α=0.05 to detect a different of 4 points between LDA exposed and non-exposed children based on a two-sided test for the secondary hypothesis.

3.1.2 Secondary and Exploratory Analysis:

In addition to the planned formal primary analysis, we also anticipate conducting exploratory outcomes for a number of other binary outcomes. The analytic approach for these exploratory analyses, will be comparable to that planned for the primary and formal secondary analyses but will focus on estimation of effect sizes and generation of hypotheses. Initially, contingency tables will be used to generate estimates of the risk of each outcome for the two treatment arms, both overall and separately by site and across all sites, and the relative risk associated with treatment will be estimated by site and across sites controlling for site. For each outcome a series of generalized linear models analogous to those described for the primary outcome to generate unadjusted estimates of risk, estimates of risk adjusted for potential confounders, and estimates of risk adjusted to account for any possible differences in risk between the arms.

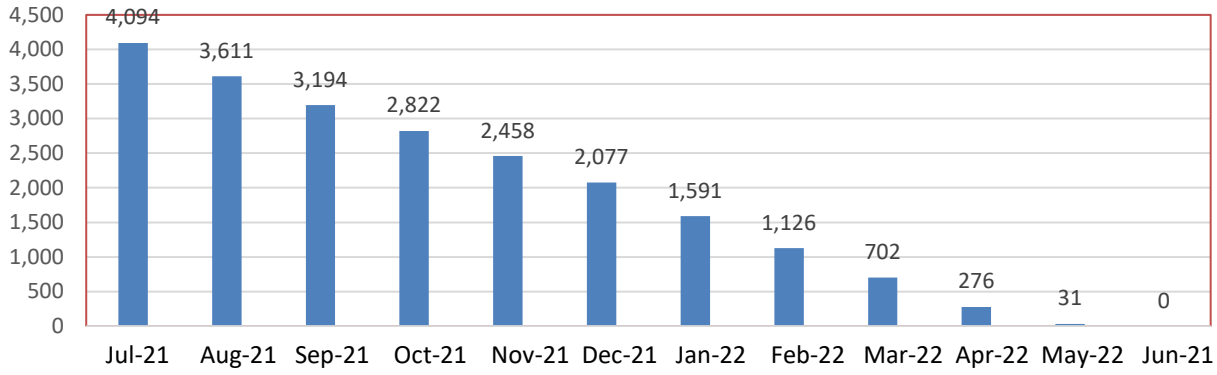
3.2 SAMPLE SIZE AND POWER ESTIMATES

The primary outcome of BSID-III score is assumed to have a mean scaled score of 100 and standard deviation of 15[47]. The second hypothesis of neurocognitive benefit of LDA will only be assessed conditional on a finding of non-inferiority for the primary hypothesis; thus, no further adjustment for multiplicity is required. Assuming a non-inferiority margin of 4 points is clinically significant, and the true effect of LDA is not more than a 1 point decrease, a total sample size of 620 children with a 1:1 allocation (310 in each group will have 80% power for a one-sided test for non-inferiority with α=0.05. Conditional on non-inferiority being observed, this sample size will also provide over 90% power at α=0.05 to detect a different of 4 points between LDA exposed and non-exposed children based on a two-sided test for the secondary hypothesis.

3.3 AVAILABLE POPULATION:

Children available across the sites will be identified as noted above. The number of available children at different time periods is graphically displayed below (Figure 2). As the work will occur over time, the pool of children who will potentially age out will grow over the duration of the study. Thus, the intent is to prioritize those children who are closer to aging out of the study parameters and those who were born prematurely. This will ensure the greatest chance of successful recruitment. Similarly, as a guiding principle, the attempt will be to try and identify children who are 36 months or greater to minimize variation in child age that may impact testing.

Figure 2: 39 Month Children Available



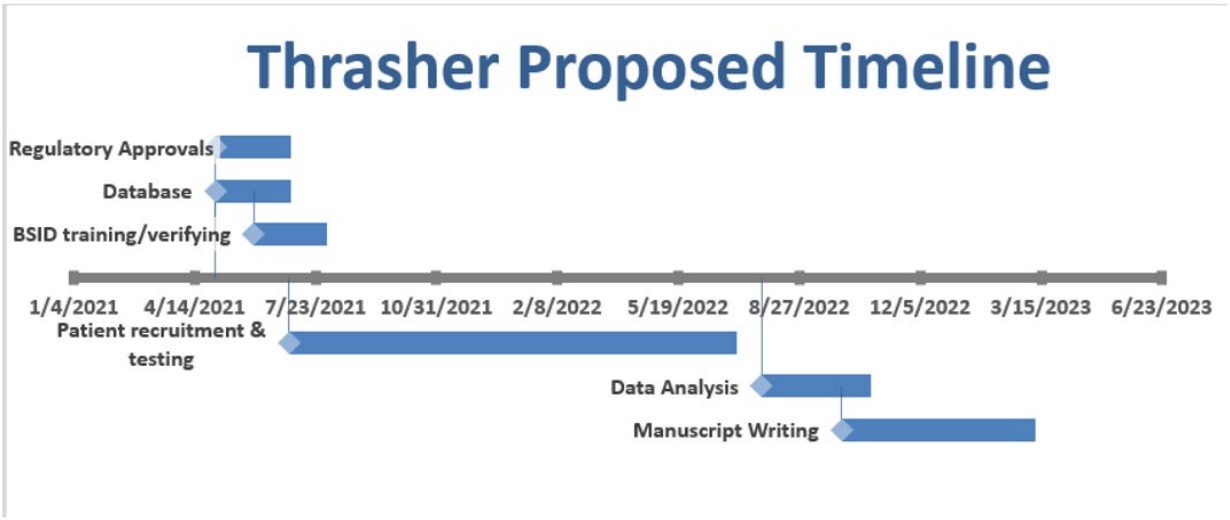
Numbers of available children by site are displayed in Table 1 which follows.

Table 1
Available Children N=4,094 as of July 1 2021

	Overall	DRC	Zambia	Guatemala	Belagavi, India	Pakistan	Nagpur, India
Infants < 40 months on July1, 2021, n (%)	4,571	450	382	792	940	716	814
39 months	531 (11.6)	43 (9.6)	52 (13.6)	98 (12.4)	113 (12.0)	49 (6.8)	128 (15.7)
38 months	444 (9.7)	40 (8.9)	51 (13.4)	65 (8.2)	92 (9.8)	68 (9.5)	101 (12.4)
37 months	419 (9.2)	42 (9.3)	39 (10.2)	74 (9.3)	83 (8.8)	67 (9.4)	67 (8.2)
36 months	399 (8.7)	46 (10.2)	38 (9.9)	72 (9.1)	73 (7.8)	98 (13.7)	37 (4.5)
35 months	434 (9.5)	50 (11.1)	37 (9.7)	78 (9.8)	83 (8.8)	67 (9.4)	66 (8.1)
34 months	544 (11.9)	54 (12.0)	30 (7.9)	95 (12.0)	102 (10.9)	112 (15.6)	93 (11.4)
33 months	531 (11.6)	50 (11.1)	39 (10.2)	90 (11.4)	110 (11.7)	84 (11.7)	92 (11.3)
32 months	468 (10.2)	57 (12.7)	35 (9.2)	80 (10.1)	103 (11.0)	66 (9.2)	83 (10.2)
31 months	480 (10.5)	45 (10.0)	41 (10.7)	79 (10.0)	106 (11.3)	65 (9.1)	90 (11.1)
30 months	284 (6.2)	21 (4.7)	15 (3.9)	56 (7.1)	63 (6.7)	34 (4.7)	56 (6.9)
29 months	37 (0.8)	2 (0.4)	5 (1.3)	5 (0.6)	12 (1.3)	6 (0.8)	1 (0.1)

3.4 PROJECTED RECRUITMENT TIME:

Each site will begin recruitment after their site-specific ethical and regulatory approvals are in place, the site has been adequately prepared, and training is completed. This will require a staggered start, as the timeline for these activities will vary per site. The projected timeline for this study is two years with over 1 year dedicated for recruitment. This includes a 2-month preparatory phase for regulatory approvals, 2 months for BSID training, a 12-month recruitment period, time for data cleaning and analysis. A graphical representation of the timeline is found below:



3.5 STUDY MONITORING PLAN:

3.5.1 Reporting Serious Adverse Events:

Serious Adverse events (SAEs) will be monitored continuously using a special form that will be required for any event that meets one or more of the following criteria: results in death, is life-threatening, requires hospitalization or prolongs existing hospitalization, results in persistent or significant disability or incapacity, any other serious or unexpected adverse event that the study investigator(s) feels should be reported.

3.5.2 Method and Timing for Reporting Serious Adverse Events

The Senior Foreign Investigator (SFI) must report the following SAEs by transmitting the form to RTI as follows:

<u>Within 48 hours of SFI's notification of the event:</u>

- All child deaths
- All SAEs with a definite or suspected/probable relationship to the intervention

<u>Within 7 days of SFI's notification of the event:</u>

- All life-threatening events
- All SAEs considered to have a probable or possible relationship to the intervention.

All completed SAE forms should also be entered into the DMS and transmitted within 7 days as a back-up to ensure no SAE is missed. Additional reporting procedures include:

- RTI will forward all SAEs to the US-based Principal Investigator (PI) for further assessment of relationship to study intervention.
- The PI and SFI will be responsible for reporting to their respective IRB and other regulatory authorities per their institutional policy.
- RTI will be responsible for reporting SAEs to the DMC bi-annually at a minimum. The frequency of reporting to the DMC may be increased if the reported events or interim data reviews by the DMC indicate that more frequent safety monitoring is needed.

Any SAE considered unrelated to the intervention is not required to be reported in an expedited manner. These events should be entered into the data management system and transmitted per routine procedures.

3.5.3 Data Monitoring Plan:

All participating sites will report data to the Data Coordinating Center, located at RTI International. The data will be used to evaluate protocol adherence and site performance (e.g., recruitment, and data quality). The DCC will provide standardized progress reports to the steering committee and the site investigators on a monthly basis to monitor outcome variables and adverse events.

3.5.4 Data Management Procedures:

Data will be collected prospectively using hard copy forms or Android tablets. Regardless of data capture methodology, all data will be kept confidential. Each participant will be assigned a unique study ID which will be used to identify the participant. Only the screening log will contain the name (which is not transmitted). If hard copy forms are used, they will be retained in a secure location for possible editing or queries at the central data entry site. Data will be entered into computers using the Data Management System (DMS) developed by RTI and the assigned study number. The DMS will also allow site staff to produce project reports and backup the study database. Electronic data will be transferred from each data management computer to a single Research Unit Data Center (RUDC) in each country, creating a complete data repository. At least once a week, data will be transmitted from the RUDC to the DCC at RTI.

The data center will conduct training on the DMS system, as needed, and will maintain the central database for the study. Precision and accuracy of actual data collected will be checked by chart review (random 5%) and internal procedures using the computer program. Monthly audits and incomplete data reports will be performed by a review team consisting of at least the SFI and the country coordinator. Data editing and error resolution will be performed monthly. In addition, a sample of participants will be visited to confirm their participation, with procedures determined per site. These activities will be shared between the site and the data center.

3.6 QUALITY CONTROL:

3.6.1 Training:

All study personnel must participate in training on the proper implementation of study procedures and the ethics of conducting research with human subjects before beginning any research activity. The SFI and project coordinator will ensure that all study personnel receive the appropriate training,

and obtain the required certification ensuring that they have met the training objectives. RTI will be responsible for developing a certification test and BSID-III proficiency. The SFI and project manager will be responsible for overseeing the certification process.

3.6.2 Study Monitoring:

Major monitoring responsibilities of the PI/SFI, assisted by the country coordinator, are (1) confirming IRB approval; (2) monitoring the recruitment of subjects; (3) assessing and evaluating the quality of study implementation; (5) evaluating accuracy, precision, and completeness of data collected, entered, and transmitted (along with the DCC); (6) ensuring that all personnel are fulfilling their obligations; (7) maintaining morale and enthusiasm of the staff; (8) handling ad hoc problems and maintaining communication; (9) ensuring inter-site consistency; and (10) proposing improvements to the monitoring activities.

4 DATA FORMS

The following forms will be used for this study:

Form Description	Purpose	Key Data Elements	Data Source
Screening Log	To track screening and enrollment at facility or community level	Contact information, screening and enrollment date and status	Mother Report
Screening	To determine eligibility and record consent status	Consent and inclusion/exclusion criterion	Clinician Report
Medical History	To determine medical history and record anthropometric measures	Limited medical history by parent report, height and weight measurement of child	Parent or Guardian Report
Infant and Young Child Feeding Index	To determine the maternal breastfeeding experience and types of food the child receives	Feeding practices, frequency of feedings, breastfeeding information	Parent or Guardian Report
BSID-III	To record outcomes of BSID-III	Scoring and elements of the BSID-III	BSID-III
ASQ-3	To record outcomes of Ages and Stages Questionaire-3.	Scoring and elements of the ASQ-3	Parent or Guardian Report
Family Care Indicators	To record family care indicators and resources to understand the family context	Parent report of family resources and context	Parent or Guardian Report
Serious Adverse Events	To record and report serious adverse events	Description of Serious Adverse Events	Parent or Guardian Report
Closeout Form	To record participants completion status of study	Final form that records if participant completed or withdrew from the study	Study Staff

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6 APPENDIX 1. SAMPLE INFORMED CONSENT

Aspirin Supplementation for Pregnancy Indicated Risk Reduction in Nulliparas (ASPIRIN): Neurodevelopmental Follow-up Trial

INVESTIGATORS:
[LIST SITE INVESTIGATORS]

SPONSOR:
Thrasher Research Fund

You are being asked to have your child participate in a research study. This project is funded by the Thrasher Research Fund. This form provides you with information about the study. A member of the research team will describe the study to you and answer all of your questions. Please read the information below and ask questions about anything you don't understand before deciding whether or not to have your child take part. You may also request that the research staff read the form to you.

What is the purpose of the study?
The purpose of this study is to learn about your child's development (motor skills, language skills, etc.) following the child's mother having participated in a prior research trial of low dose aspirin during pregnancy. Prior trials of taking aspirin during pregnancy have been reassuring on development of children; however, the purpose of this study is to obtain information using different tests.

Who will be in the study?
A total of 620 children will be enrolled in this study from six sites in sub-Saharan Africa, South Asia, and Latin America. In [insert site name], no more than 248 children will be enrolled. Half of the children's mothers will have taken aspirin and half will have taken placebo, a pill that looks identical to aspirin but did not contain any medicine. This will allow the researchers to compare results of the two groups of children and determine if aspirin benefitted or had no effect on children and their development.

Your child qualifies for this study if he or she is a healthy child between 33 months of age of age to 39 months of age and whose mother participated in the ASPIRIN trial.

What will happen if you join this study?
Before participating, you will be provided with information about the study procedures and given an opportunity to ask questions. If you agree to have your child participate, you will be asked to sign this form to indicate your consent. The research staff will ask you some questions about your child's health, and your child's ability to talk, play and follow commands. They will also ask your child to do simple tasks and evaluate his or her ability to do them. Once these tests are done, this will complete your involvement in the study.

What are the risks and discomforts by being in this study?

No risks or discomforts are anticipated from this study. Your child will be asked to perform simple

activities. If your child is uncomfortable doing these activities or tires of them, they will not be required to continue.

What are the benefits of participating?

You will not receive any money from participating in this study, but your participation may provide important information that can be used in the future to prevent babies from being born too soon. Also, there is existing evidence that the use of aspirin in pregnancy can improve the infant's neurological outcomes.

If new information about the benefits or risks of aspirin use in pregnancy becomes available during this study, this information will be given to you by [Insert name of Senior Investigator] or his/her staff.

Will I have to pay for anything?

It will not cost you anything to be in the study.

Is my participation voluntary?

Taking part in this study is voluntary. You have the right to refuse to let your child participate or to withdraw approval for participation at any time. If you refuse to allow participation or decide to withdraw, you will not lose any benefits or rights to which you are entitled. These actions will not have any negative effect on the health care you or the child receive from your local health providers. You and the child will have the right to receive normal medical care.

Can I or my child be removed from this study?

Your child will be withdrawn from the study if the research staff thinks that his or her participation may cause him or her harm. The research staff may also remove him or her from the study for other reasons at their discretion. Also, the sponsor may stop the study at any time if there is good reason to do so.

What will happen if you or the participating child are injured by this research?

Although the risk of injury is expected to be very low, no research can provide an absolute guarantee that nothing bad will occur. Despite safety measures, your child's participation could result in a reaction or injury. If you or your child is injured as a result of study participation, emergency care will be provided and a referral made to a doctor for ongoing care, if needed. Ongoing care will not be paid for by the study. [Insert name of Research Institution] and the Thrasher Research Fund have not set aside funds to pay you for any such reactions, injuries or related medical care. However, by signing this form, you do not give up any of your legal rights.

What should you do if you have additional questions?

If you have questions about this study or a project-related injury, you should contact [investigator contact]. If you have questions about your or your child's rights as a project participant, please contact [insert ethics committee contact].

If you have any questions about the study, please call [insert senior investigator name/contact information].

Agreement to be in this study

I have read this paper about the study or it was read to me. I understand the possible risks and benefits of this study. I know that being in this study is voluntary and I choose to be in this study. I understand I will get a copy of this consent form.

Signature (or thumbprint):_____ Date:_____

(Mother)

Print Name:_____
(Mother)

Signature (or thumbprint):_____ Date:_____
(Parent/Guardian/Husband)

Print Name:_____
(Parent/Guardian/Husband)

STATISTICAL ANALYSIS PLAN

Aspirin Supplementation for Pregnancy Indicated Risk Reduction in Nulliparas (ASPIRIN): Neurodevelopmental Follow-up Trial

SAP VERSION: Version 1

SAP DATE: June 17, 2022

SPONSOR: Thrasher Research Fund

PREPARED BY: RTI International

3040 Cornwallis Rd

Research Triangle Park, NC 27709-2104

AUTHOR (S): Tracy Nolen, Marissa Trotta, Janet Moore

Contents

LIST OF ABBREVIATIONS

Acronym	Definition
DCC	Data Coordinating Center
DMC	Data Monitoring Committee
DRC	Democratic Republic of the Congo
EmONC	Emergency Obstetric and Neonatal Care
GA	Gestational Age
GEE	Generalized Estimating Equation
GN	Global Network
ITT	Intention to Treat
LMP	Last menstrual period
MDG	Millennium Development Goals
MNH	Maternal and Neonatal Health
PP	Per protocol
RCT	Randomized Clinical Trial
SAP	Statistical Analysis Plan
UN	United Nations

1. BACKGROUND AND PROTOCOL HISTORY

Background and Rationale

Over 52.9 million children under five years of age in low and middle-income countries (LMIC) are estimated to have neurodevelopmental delays[1]. Though neurodevelopmental delay is complex in its origins, preterm birth[2], hypertensive disorders of pregnancy (HDP)[3] and chronic inflammation[4] are known primary drivers. Recently the ASPIRIN[5] trial demonstrated that low dose aspirin (LDA) administered antenatally decreases the rate of preterm birth, preterm (HDP) and perinatal mortality. LDA has also long been known to decrease chronic inflammation. Therefore, antenatal LDA may prevent neurodevelopmental delay through its effect on preventing preterm birth & HDP and inhibiting chronic inflammation. Currently data of neurodevelopmental outcomes following antenatal LDA exposure can be summarized as: 1. multiple (37+) observation trials that have linked maternal inflammation with developmental delays/behavioral issues[4], 2. Numerous studies showing that offspring of HDP pregnancies are at significant risk of cognitive delay[3], 3. follow-up trials of children whose mothers were part of an antenatal LDA trial that the assessment occurred too early (e.g. 12 or 18 months) or used outdated assessments (e.g. parental perception) but show either no difference or improvement[6–8] and 4. registry studies that have associated maternal LDA use with slight increases in cerebral palsy[9] and hearing loss[10]. Despite the recent positive findings of the ASPIRIN trial, broad acceptance of low dose aspirin (LDA) may be tempered by concerns about effects on longitudinal neurodevelopment. In contrast, if LDA is found to mitigate these risks, women would be further motivated to take LDA and professional societies to recommend LDA as a universal part of prenatal care.

Protocol History

The protocol was initiated in April 2021.

No formal interim analyses were planned nor completed for this study and as such, the first formal assessment for efficacy is planned for after completion of primary follow-up.

2. PURPOSE OF THE ANALYSES

This statistical analysis plan (SAP) contains detailed information about statistical analyses to be performed to assess the primary and secondary hypotheses and associated sub-hypotheses outlined in the protocol (i.e. children exposed antenatally to low dose aspirin will have a) scores on the Bayley Scales of Infant Development-III (BSID-III) examination at 36 months of life (+/-3months) that are not inferior to their peers who were not exposed (i.e., by no more than a margin of 4 points) and b) similar scores on the Ages & Stage's Questionnaire® Third Edition (ASQ-3) with sub-hypotheses looking for superiority conditional on showing non-inferiority). The results of these analyses will be included in the study manuscript(s). Additional exploratory analyses may be performed to support further manuscript development. These analyses will not require an update to the SAP, but abbreviated analysis plans will be prepared prior to conducting those analyses.

3. STUDY OBJECTIVES AND OUTCOMES

3.1 Primary Hypothesis and Associated Outcomes

Our primary hypothesis is that children exposed antenatally to low dose aspirin will have scores on the Bayley Scales of Infant Development-III (BSID-III) examination at 36 months of life(+/-3months)

that are not inferior to their peers who were not exposed (i.e., by no more than a margin of 4 points). Conditional on showing non-inferiority is that children exposed antenatally to low dose aspirin will have higher scores on the BSID-III examination compared to their peers who were not exposed.

The primary outcome of this study is scores on the BSID-III examination at 36 months of life(+/-3months). The BSID-III provides systematic assessment of development in the three primary domains of cognition, language (receptive and expressive) and motor skills (fine & gross). The examination will be performed by only trained and qualified examiners. The cognitive score will be used as the primary endpoint with the other scores considered secondary.

3.2 Secondary Hypothesis and Associated Outcomes

Our secondary hypothesis is that children exposed antenatally to low dose aspirin will have similar scores on the Ages & Stage's Questionnaire® Third Edition (ASQ-3). Conditional on showing non-inferiority is that children exposed antenatally to low dose aspirin will have higher scores on the ASQ-3 examination compared to their peers who were not exposed.

The secondary outcome of this study is the scores on the ASQ-3, a broadly accepted and validated screening tool for neurodevelopment. The ASQ-3 will be administered by research staff to parents. Where literacy issues may be present, staff will be trained on how to help administer the questionnaire to obtain accurate information without introducing bias. Following completion of the questionnaire by the child's parents, staff, will score the questionnaire and those results will be recorded on the ASQ form. All ASQ subscales will be analyzed.

3.3 Other Outcomes of Interest in this Study

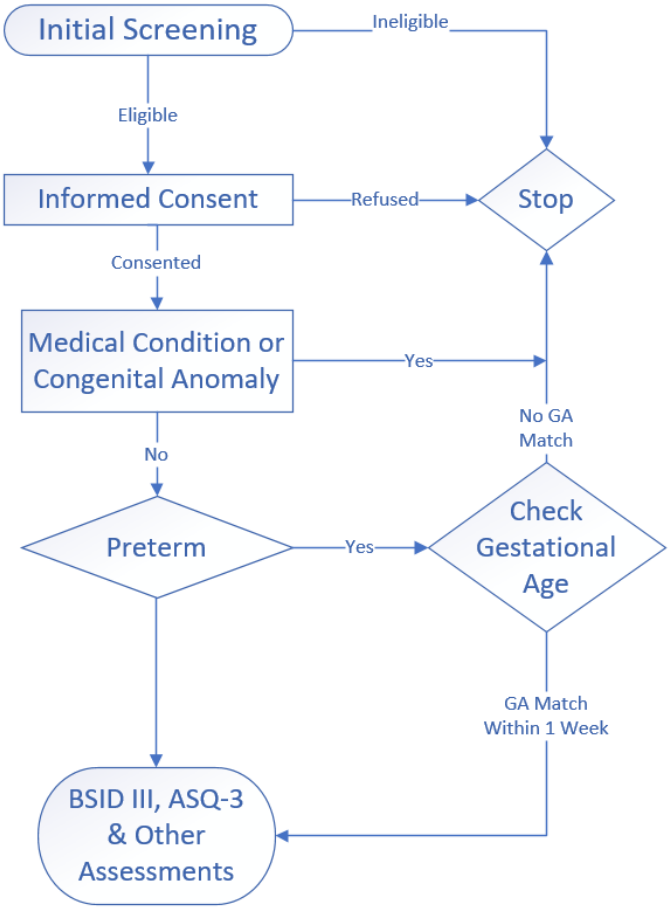
Study analyses will examine a number of additional outcomes to assess whether use of LDA daily beginning between 6 0/7 weeks and 13 6/7 weeks has an effect on those outcomes. Specific maternal and fetal/child outcomes may include: anthropomorphic measures, binary classifications for BSID and ASQ.

4. STUDY METHODS

4.1 Overall Study Design

For the ASPIRIN Follow-up Study, we designed a cohort study of children whose mothers were randomized to either low dose aspirin or placebo in the ASPIRIN trial. As preterm infants are at higher risk they will be over-sampled and the distribution of gestational age will be reviewed to ensure approximately equally distribution between mothers who received LDA and those who received placebo. Gestational age matching was originally proposed but ultimately determined to be operationally challenging given the limited population available for follow-up and short timeline until they aged out of ability to participate in this study.

Study Schematic



4.2 Study Population

The study population will consist of approximately 620 children, between 33 and 39 months of age, whose mothers were included in the ASPIRIN trial. Among those, children who were born preterm (<37 weeks) will be prioritized for inclusion as they have a higher risk of developmental delays. Enrollment will be monitored to ensure approximately equal distribution between the LDA and placebo arms.

Inclusion criteria

- For a prior study sponsored by the Global Network for Women's and Children's Health Research, the child's parents gave permission to recontact.
- The child's parent/guardian is willing and able to provide consent for the child and willing also to participate in the child's assessment (e.g., by providing answers to ASQ-3 and the Family Resources and Context questionnaire).
- The child will be between 33 and 39 months of age at time of assessment.
- The child does not have a significant congenital anomaly (e.g., blindness or deafness) that would affect development or preclude them from completing the BSID-III. This will be left at

the discretion of the Senior Foreign Investigator (SFI) with the ability to consult with the Working Committee as needed.

- Other significant medical conditions that would preclude the child from completing a BSID III are absent. This will be left to the discretion of the Senior Foreign Investigator who will have the ability to consult with the Working Committee as needed.

Exclusion criteria

Children not meeting the inclusion criteria will be excluded. Likewise, children may be excluded at the discretion of the site investigator out of concern for the child's wellbeing (e.g., the child has a current respiratory infection or emotional distress during testing).

4.3 Participant Selection

Participant selection was done by the study sites. However, the Data Coordinating Center provided lists of children to prioritize based on gestational age. Additionally, the Data Coordinating Center create and reviewed reports of enrollment by site, sex and gestational age to ensure balanced enrollment between treatment groups.

4.4 Masking and Data Lock

Masking was maintained from the larger ASPIRIN Trial through follow-up. As such, throughout the study, research staff and local health providers will be blinded to treatment status of the mother. Data regarding the subject's allocation will be held and protected centrally and will not be made available to participants or their families.

Data Lock: Data will be locked at the site level with final analysis data sets generated and locked once data from all sites have been received and all queries processed. For each site, data collection for the protocol will stop approximately 2 months after the last child was randomized into the study at that site. Site will transmit all data forms to the central site within one months of this date, and data will be locked two to three months post last follow-up visit. The study will not be unmasked until after data lock.

5. ANALYSIS POPULATIONS

Intention to Treat (ITT) Population

The primary analysis population will be the intention to treat population, which includes all eligible and enrolled follow-up participants. All participants will be assigned to the arm to which they were randomized irrespective of treatment received. Unless specified otherwise, all participants with data available will be included in analyses using this population. Missing data will be considered missing at random and not included. This population will also be used for many of the secondary analyses.

Per Protocol (PP) Population

This population is based on the main study PP population and will be used to conduct sensitivity analyses for the primary outcome. This population will exclude all or part of the data obtained from any eligible, randomized participants that did not receive at least 90% of the full amount of intended

randomized study therapy or are considered to have substantially deviated from the protocol in a manner that may impact study outcome or treatment receipt. The population will also exclude individuals who were randomized after 10 weeks, 6 days gestational age. Participants will be grouped by actual treatment received. Treatment receipt reasons and substantial deviations that will lead to exclusion include:

- Documented receipt of aspirin while on study outside of assigned study drug as identified by a reported protocol deviation
- Not receiving treatment after randomization: All data from these participants will be excluded.
- Receipt of less than 90% of planned total doses after randomization where the number of planned doses will be calculated as those expected between randomization and the earliest of end of pregnancy and 36 0/7 weeks GA irrespective of if study drug administration was prematurely discontinued for any reason prior to that point: All data from these participants will be excluded.

Receiving more than the planned total doses of study drug will generally NOT be an exclusion criterion for the PP population. Additional exclusion reasons may be identified after completion of participant enrollment. Any participant deviations that will exclude participants or their data from the PP analyses will be identified by the study team, prior to review of efficacy and safety data, as a part of a masked data review to reduce the opportunity for introducing of bias and will be documented in an addendum to this analysis plan.

6. SAMPLE SIZE DETERMINATION

The primary outcome of BSID-III score is assumed to have a mean scaled score of 100 and standard deviation of 15.47. Assuming a non-inferiority margin of 4 points is clinically significant, and the true effect of LDA is not more than a 1 point decrease, a total sample size of 620 children with a 1:1 allocation (310 in each group will have 80% power for a one-sided test for non-inferiority with α=0.05. Conditional on non-inferiority being observed, this sample size will also provide over 90% power at α=0.05 to detect a different of 4 points between LDA exposed and non-exposed children based on a two-sided test for the secondary hypothesis.

Analyses of secondary outcomes will be exploratory in nature and generally will focus on estimation of effect sizes and generation of hypotheses.

7. STATISTICAL / ANALYTICAL ISSUES

7.1 General Rules

All statistical computations will be performed and data summaries will be created using SAS 9.3 or higher. If additional statistical packages are required, these will be discussed in the study report. For summaries of study data, categorical measures will be summarized in tables listing the frequency and the percentage of participants in each study arm; continuous data will be summarized by presenting mean, standard deviation, median, minimum, and maximum; and ordinal data will be summarized by only presenting median and the limits of the interquartile range.

7.2 Adjustments for Covariates

While this is a cohort study conducted on a subset of participants from a randomized trial, covariates may be adjusted for in the analyses if treatment imbalance is present. Covariates for consideration include gender, breast feeding, stunting, weight, and family care indicators. These factors will also be explored in secondary analyses for modifying/mediation effects.

7.3 Missing Data Approaches:

The primary reason for missing data will be participant withdrew consent or the child aging out. All missing data will be excluded from primary analyses.

7.4 Multicenter Studies

For this multicenter study, randomization of study participants was stratified within site. Consequently, for all test-based analyses of treatment effect and model-based primary and secondary analyses, site will be included as a fixed effect in the models. As exploratory analyses, analyses, particularly those providing estimate of treatment effect, will be conducted by region: Africa, Asia, and Guatemala.

7.5 Multiple Comparisons and Multiplicity

There is only one formal hypothesis test for this study. As such, a statistical test will be conducted at a 5% type I error rate (two-sided) for the primary efficacy measure, and no adjustments for multiplicity will be made. All analyses of secondary outcomes are exploratory in nature; therefore, p-values and confidence intervals are provided for descriptive purposes only. All p-values provided for any baseline and demographic characteristics and safety parameters will be for descriptive purposes only. As such, unless otherwise specified, p-values presented will be on a per analysis basis, with no further control for multiple tests.

7.6 Masked Data Review

A masked data review is unlikely to be needed for this study. Details of any masked data review conducted and resulting decisions will be described in a SAP addendum.

8. STUDY PARTICIPANTS, TREATMENT EXPOSURE AND COMPLIANCE

8.1 Participant Disposition

Participant eligibility status will be summarized by study arm and overall disposition of study participants will be described using a standard CONSORT diagram.

8.2 Protocol Deviations

Any protocol deviations reported will be reviewed to determine if any data need to be excluded from analysis.

8.3 Demographic and Baseline Characteristics

Demographic and baseline clinical characteristics for the study participants will be summarized by treatment group using the general analysis rules describe above for the ITT and PP populations.

Variables of interest include the same characteristics as from the main trial: maternal age, maternal education, gravidity, maternal weight and BMI, previous pregnancy loss, number of antenatal care visits, delivery attendant, delivery location, and type of delivery.

9. NON-INFERIORITY AND EFFICACY ANALYSES

9.1 Overview of Efficacy Analysis Methods

Efficacy analyses will be performed using the ITT population. Sensitivity analyses will be performed using the per-protocol population as detailed below. The data will be summarized by treatment arm, overall and by region. All model-based analyses and test-statistics examining the treatment effect will adjust for covariates as specified in sections 7.2 and 7.3. Additional details are presented in the sections below.

9.2 Definition of Analysis Variables

The following table defines each of the effectiveness and process analysis variables.

Variable	Type	Definition
Primary Outcome		
BSID-III scores	Continuous	The unit of analysis is the child enrolled in the study. The three associated outcomes will be the mean scaled composite cognitive, language and motor BSID-III scores assessed and reported at 36 months of life (+/- 3 months). The cognitive score will be considered primary. Raw scores will be summarized but not analyzed. Analysis Population: ITT
Low BSID-III scores	Binary	The unit of analysis is the child enrolled in the study. The three associate outcomes will be whether the child scored less than an 85 on each of the composite cognitive, language and motor BSID-III scaled composite scores assessed at 36 months of life (+/- 3 months). Cognitive scores < 54 and motor scores < 45 will have missing associated binary outcomes. Analysis Population: ITT
Secondary Outcome		
ASQ-3 scores	Continuous	The unit of analysis is the child enrolled in the study. The 5 associated outcomes will be the scores of the components of the ASQ-3 assessment, including communication, gross motor, fine motor, problem solving and personal-social. Analysis Population: ITT

Variable	Type	Definition
Low ASQ-3 scores	Binary	The unit of analysis is the child enrolled in the study. The outcome will be whether the child scored 2 standard deviations less than the mean on each of the communication, gross motor, fine motor, problem solving and personal-social ASQ-3 scores assessed at 36 months of life (+/- 3 months). Established, published cut-offs using US standards will be used as there are no region specific cut-offs established for the study sites. Analysis Population: ITT

9.4 Primary Analysis

The primary analysis will compare the BSID-III composite scores between the two treatment arms using a non-inferiority test. For each of the three BSID-III composite scores, a mean difference between treatment arms and its associated 90% confidence interval will be calculated. If the lower bound of the 90% confidence interval is greater than -4, then the treatment is assumed to be non-inferior to placebo. If non-inferiority is assumed, a one-sided t-test will be run to test the benefit of treatment compared to placebo. All analyses will be modeled based controlling for site as the original randomization stratification factor. For each outcome, the adjusted mean difference between treatment groups and the associated 90% confidence interval based on an ANCOVA model will be calculated and presented. Secondary models will include region and a region by treatment interaction in order to get region specific treatment effect estimates.

Prior to conducting formal analyses baseline demographic characteristics and key clinical measures will be compared between the mothers who participated in the two treatment arms using contingency table approaches for categorical variables and analysis of variance models and t-tests for continuous variables. For all of these analyses, comparisons will be made within Global Network sites and overall across the sites controlling for site in the models.

9.5 Secondary Outcome Analyses

For ASQ and any other continuous outcomes, modeled-based analyses similar to the primary analysis will be used to generate estimated treatment effects (i.e., ANCOVA models used to obtain adjusted mean differences controlling for site). For binary outcomes, robust Poisson regression models will be used controlling for site in order to obtain relative risk estimates and associated confidence intervals. All models will be repeated including terms for region and its interaction with treatment to get region specific estimates of treatment effect.

10. SAFETY ANALYSIS:

No formal safety analyses are planned as a part of this study, although SAEs will be summarized. No formal hypothesis tests will be conducted, but descriptive p-values generated using Cochran-Mantel-Haenszel statistics with stratification by GN site will be generated.

11. CHANGES TO THE ANALYSES PLANNED IN THE PROTOCOL

No changes.

12. REFERENCES

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14. ATTACHMENTS

Updates to these tables will not require an analysis plan amendment.

Table 1 Participant Distribution

Table 1. Participant Distribution

Variable	Overall		DRC		Zambia		Belagavi, India		Nagpur, India		Pakistan		Guatemala	
	Aspirin	**Placebo**	**Aspirin**	**Placebo**	**Aspirin**	**Placebo**	**Aspirin**	**Placebo**	**Aspirin**	**Placebo**	**Aspirin**	**Placebo**	**Aspirin**	**Placebo**
Screened														
Ineligible														
Eligible														
Enrolled														
BSIDS III completed														
Within age required														
ASQ completed														
Within age required														

Table 2: Covariate Summaries

Variable	Overall		DRC		Zambia		Belagavi, India		Nagpur, India		Pakistan		Guatemala	
	Aspirin	Placebo	Aspirin	Placebo	Aspirin	Placebo	Aspirin	Placebo	Aspirin	Placebo	Aspirin	Placebo	Aspirin	Placebo
Gender, n (%)														
Male														
Female														
Gestational age at delivery, n (%)														
< 37														
>= 37														
Breastfed after delivery														
Yes														
Months breastfed, mean (sd)														
No														
Stunting														
Yes														
No														
Family Care Indicators														
Books, mean (sd)														
Variety of play materials score (Q3-Q5), mean (sd)														
Sources of play materials score (Q7-Q13), mean (sd)														
Play activitites score (Q14-Q23), mean (sd)														

Table 3: BSID-III Outcome Descriptive Statistics

Variable	Overall		DRC		Zambia		Belagavi, India		Nagpur, India		Pakistan		
	Aspirin	Placebo	Aspirin	Placebo	Aspirin	Placebo	Aspirin	Placebo	Aspirin	Placebo	Aspirin	Placebo	A
Evaluated on BSID III													
Age at Examination, N													
Mean (SD)													
Median (P25, P75)													
Minimum - Maximum													
Cognitive Test Results													
Language Test Results													
Motor Test Results													
Scaled Scores													
Cognitive Scaled Score, N													
Mean (SD)													
Median (P25, P75)													
Minimum - Maximum													
Receptive Language Scaled Score, N													
Mean (SD)													
Median (P25, P75)													
Minimum - Maximum													
Expressive Language Scaled Score, N													
Mean (SD)													
Median (P25, P75)													
Minimum - Maximum													
Summed Language Scaled Score, N													
Mean (SD)													
Median (P25, P75)													
Minimum - Maximum													
Fine Motor Scaled Score, N													

Mean (SD)													
Median (P25, P75)													
Minimum - Maximum													
Gross Motor Scaled Score, N													
Mean (SD)													
Median (P25, P75)													
Minimum - Maximum													
Summed Motor Scaled Score, N													
Mean (SD)													
Median (P25, P75)													
Minimum - Maximum													
Composite Scores													
Cognitive Composite Score, N													
Mean (SD)													
Median (P25, P75)													
Minimum - Maximum													
Language Composite Score, N													
Mean (SD)													
Median (P25, P75)													
Minimum - Maximum													
Motor Composite Score, N													
Mean (SD)													
Median (P25, P75)													
Minimum - Maximum													
Low Composite Scores													
Cognitive Composite Score <85													
Language Composite Score <85													
Motor Composite Score <85													

Table 4: ASQ Outcome Descriptive Statistics

Table 5a: BSID-III Adjusted Mean Estimates

Variable	Adjusted[1] Mean (95% CI)		
	Aspirin	**Placebo**	**Difference**
BSID III Composite Scores			
Cognitive Composite Score			
Language Composite Score			
Motor Composite Score			
BSID III Scaled Scores			
Cognitive Scaled Score			
Receptive Language Scaled Score			
Expressive Language Scaled Score			
Summed Language Scaled Score			
Fine Motor Scaled Score			
Gross Motor Scaled Score			
Summed Motor Scaled Score			

Table 5b: BSID-III Adjusted Risk Ratio Estimates

	Aspirin n (%)	**Placebo n (%)**	**RR (95% CI)**
BSID III Composite Scores			
Cognitive Composite Score <85			
Language Composite Score <85			
Motor Composite Score <85			

1. Mean estimates and 95% confidence intervals from a regression model adjusting for site, gender, breast feeding, stunting and family care indicators.

Table 6a: ASQ Adjusted Mean Estimates

Variable	Adjusted[1] Mean (95% CI)		
	Aspirin	**Placebo**	**Difference**
ASQ Total Score			
Communication total score			
Gross motor total score			
Fine motor total score			
Problem solving total score			
Personal-social total score			

Table 6b: ASQ Adjusted Risk Ratio Estimates

	Aspirin n (%)	**Placebo n (%)**	**RR (95% CI)**
ASQ Scores Below Cut Off			
Communication total score < 27.93			
Gross motor total score < 30.68			
Fine motor total score < 35.39			
Problem solving total score < 16.71			
Personal-social total score < 32.53			

1. Mean estimates and 95% confidence intervals from a regression model adjusting for site, gender, breast feeding, stunting and family care indicators.

Figure 1: Consort Diagram