RAPIDIRON-KIDS Study

RESEARCH PROTOCOL

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Table of Contents

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Version Tracking

Version	Date	Author(s)	Notes
1.0	April 7, 2022	RAPIDIRON Working Group	Original Version
2.0	April 7, 2022	RAPIDIRON Working Group	Minor amendments made to content and format
2.1	May 12, 2022	RAPIDIRON Working Group	Adjusted exclusion criteria; removed some assessments due to implementation feasibility issues; updated statistical plan
2.2	May 30, 2022	RAPIDIRON Working Group	Addition of Pediatrics site Investigators of Bagalkot and Raichur and Investigators of MRHRU, Sirwar, Raichur
2.3	September 20, 2022	RAPIDIRON Working Group	Minor amendments made to content, removed offspring blood draws at 24 and 36M to reduce participant burden

Acronyms

AE	Adverse event
ASHA	Accredited Social Health Activist
BSID	Bayley Scales of Infant Development
CBCL/1.5-5	Child Behavior Checklist for Ages 1.5-5
DQ	Developmental quotient
DSMB	Data and Safety Monitoring Board
Hb	Hemoglobin
HIF-1α	Hypoxia induced growth factor
ICFI	Infant Child Feeding Index
ID	Iron deficiency
IDA	Iron deficiency anemia
IGF-1	Insulin-like growth factor-1
ISAA	Indian Scale for Assessment of Autism
IV	Intravenous
JNMC	Jawaharlal Nehru Medical College
LBW	Low birth weight
MRHRU	Model Rural Health Research Unit
MRI	Magnetic resonance imaging
NICHD	National Institute of Child Health and Human Development
NFHS	National Family Health Survey
PI	Principal Investigator
PPH	Postpartum hemorrhage
RCT	Randomized controlled trial
RIMS	Raichur Institute of Medical Sciences
SAE	Serious adverse event
SNMC	S. Nijalingappa Medical College
TIBS	Total iron binding saturations
TSAT	Serum transferrin saturation
WHO	World Health Organization
WHODAS	World Health Organization Disability Assessment Schedule

Abstract

Background: Anemia is a worldwide problem with iron deficiency being the most common cause. When anemia occurs in pregnancy, it increases the risk of adverse maternal, fetal and postnatal outcomes. It induces preterm births and low birth weight (LBW) deliveries, long-term developmental sequelae and an increased risk of earlier onset of postnatal iron deficiency. Anemia rates are among the highest in South Asia, and India's National Family Health Survey (NFHS-5) for 2019-21 indicated that over half of pregnant women, and more than 65% of children, in the country are classified as anemic. [1] In 2021, the *RAPIDIRON Trial* [2] was initiated in two states in India, with the goal of assessing if a dose of intravenous (IV) iron given to anemic women during pregnancy will result in a greater proportion of participants with normal hemoglobin concentrations in the third trimester and a lower proportion of participants with low birth weight deliveries compared to oral iron. As a follow-up to the *RAPIDIRON Trial,* the *RAPIDIRON-KIDS* study will follow the previously randomized mothers as well as their offspring after birth to assess neurodevelopmental, hematological, and health outcomes. This study's overarching goal is to determine if the offspring born to *RAPIDIRON Trial* mothers in the IV iron groups, compared to the oral iron group, will achieve superior neurodevelopment, iron stores, and growth at specific time points during the first three years of life.

A. Specific Aims

Hypotheses and Specific Aims

This study has two primary hypotheses as follows:

(1) Infants born to *RAPIDIRON Trial* maternal participants from the intravenous (IV) iron arms will have higher hemoglobin and ferritin levels at birth (determined by cord blood) and at 4 months of age compared to infants born to mothers in the oral arm; and
(2) Offspring born to *RAPIDIRON Trial* participants who received IV iron treatment will have higher developmental quotients (DQs) on the cognitive domain of the Bayley Scales of Infant Development (BSID) at 2 years of age compared to offspring born to *RAPIDIRON Trial* participants given oral iron treatment.

The *RAPIDIRON-KIDS Study* is designed to determine if offspring born to *RAPIDIRON Trial* maternal participants in the IV iron groups, compared to the oral iron group, will achieve superior neurodevelopment, iron stores, and growth at specific postnatal timepoints during the first 3 years of life. Differences will be assessed between offspring based on the iron deficiency anemia (IDA) treatment of the mother.

The specific aims of this study are:

- To provide evidence that a single-dose of IV iron (either ferric carboxymaltose or iron isomaltoside, also known as ferric derisomaltose) given to pregnant women in the early second trimester of pregnancy during the *RAPIDIRON Trial* will prove more effective for prevention of neonatal and postnatal iron deficiency in the offspring than the oral iron given to pregnant women per the parent trial protocol;
- To assess if the offspring of women in the parent trial IV iron arms have better neurodevelopmental outcomes compared to the offspring of women treated with oral iron; and
- To determine longer-term hematologic effects in previously randomized mothers by obtaining ongoing hematologic indices, documented history of transfusion and hospitalization, and quality of life based on the use of a validated instrument.

Long-Term Objective

The long-term objectives of the *RAPIDIRON-KIDS Study* are to improve the iron status and health outcomes in both mothers and their offspring as well as to improve growth and neurodevelopment in children. Assuming that the primary hypotheses of both this follow-up study and the parent *RAPIDIRON Trial* prove true without raising safety concerns and the parent trial presents evidence of economic feasibility of single-dose IV iron for treatment of IDA in pregnancy, the researchers involved in these studies intend to aggressively disseminate the research results to increase the probability that the Government of India's Ministry of Health and Family Welfare will endorse broader and appropriate scale-up of a single-dose IV iron infusion as frontline treatment for IDA in pregnancy for women with moderate IDA. Further, dissemination of positive findings for these related studies can be globally promoted as supportive of goals to improve care and outcomes in women during pregnancy and the postpartum period and reduce the rates of anemia, iron deficiency, poor growth, and the occurrence of developmental disabilities in children.

B. Background and Significance

Based upon the National Family Health Survey (NFHS)-5 for 2019-21, over half of the pregnant women in India are anemic. [1] The major cause of anemia is iron deficiency, which is considered one of the most common micronutrient deficiencies in the world according to the World Health Organization (WHO). [3] Additionally, iron deficiency anemia (IDA) is a major contributing factor to the high rate, >65%, of children (6 – 59 months of age) who are classified as anemic in India (with hemoglobin level of <11g/dL). [1]Optimal fetal, neonatal, and childhood brain growth and development require adequate iron. However,

women with moderate to severe anemia during the late second and early third trimesters of pregnancy are often unable to make up their iron deficit. Thus, despite active transport via the placenta, insufficient iron may be transmitted to the developing fetus with consequent negative sequelae, including long-term neurodevelopmental impairment of the offspring.

A study of Chinese pregnant women found that daily oral iron, initiated at or prior to 20 weeks gestation and continued until delivery, improved oral iron parameters; however, 45% of the babies born to these women were nevertheless iron deficient, suggesting that oral iron supplementation may not optimally reach the developing fetus. [4] Published evidence confirms that iron deficiency in infancy is associated with a statistically significant increase in cognitive and behavioral abnormalities which may persist for decades despite iron repletion. [5] While one of the primary outcomes for the parent *RAPIDIRON Trial* is the rate of low birth weight (LBW) delivery (a leading cause of under-5 mortality and an independent risk for poorer neurodevelopment), iron deficiency anemia in pregnancy is associated with other adverse pregnancy outcomes including increased preterm birth and perinatal and neonatal mortality as well as iron deficiency leading to poor neurodevelopment of the offspring. [6] The risks of fetal underloading include: (1) abnormal fetal brain development and function; (2) a higher incidence of significant psychopathologies later in childhood or adulthood including autism, schizophrenia, and neurocognitive disorders; and (3) an earlier onset of postnatal iron deficiency, which carries its own attendant neurodevelopmental consequences including anxiety, depression, and hyperactivity/attention disorders. [6] The long-term effects occur in spite of a diagnosis of iron deficiency in infancy and prompt treatment, implying that prevention of neonatal and postnatal iron deficiency through adequate fetal iron loading is the preferred course of action. Apart from the costs associated with treatment of acute illness, it is the long-term effects that are the true cost to society in terms of lost educational potential, remediation costs during childhood, and ultimately lost job potential. [6]

We are unable to find published data on the duration of effect of IV iron administered to pregnant women. Further, additional research is required to determine if an increase in hemoglobin level over time translates into health-related consequences and overall life quality. Following women who were previously randomized for treatment of iron deficiency anemia with a single dose IV iron infusion or oral iron will allow us to detect differences in outcomes based on the treatment approach and determine if there is a time when differences among the arms no longer exist. This information is especially important if differences are found at the time of the next pregnancy.

The Cochrane Collaboration reported a paucity of quality trials that assessed clinical maternal and neonatal effects of iron administration to anemic pregnant women. The Cochrane reviewers indicated the need for large, high-quality trials that assess clinical outcomes and treatment effects. [7] This need has not been adequately met, especially in terms of assessing the efficacy of newer treatment approaches, specifically the use of single-dose IV iron during pregnancy to reduce LBW rates and to adequately iron load the fetus to preserve hematologic and brain development. The ongoing *RAPIDIRON Trial* addresses the identified need from the maternal and newborn perspective through six weeks postpartum, but hematologic, iron status, and neurodevelopmental follow-up of the offspring is urgently needed to assess the intergenerational impact of maternal iron and anemia management and to ensure that the offspring achieve full developmental potential. Together, the parent *RAPIDIRON Trial* and the *RAPIDIRON-KIDS Study* will increase the likelihood that India's anemia guidelines and recommendations of respected health organizations will continue to undergo refinement. We anticipate that new and improved anemia and iron deficiency treatment approaches that are convenient, cost-effective, and feasible can be more rapidly accepted, implemented, and scaled-up adequately to meet a great need.

Description of Parent Trial

The RAPIDIRON Trial

Reducing Anemia in Pregnancy in India: the RAPIDIRON Trial [2] is a 3-arm, randomized-controlled trial designed to assess if a single dose of an intravenous (IV) iron formulation (ferric carboxymaltose in intervention arm 1 or iron isomaltoside, also known as ferric derisomaltose, in intervention arm 2),

administered early in the second trimester of pregnancy for treatment of moderate iron deficiency anemia (IDA), will result in a greater percentage of pregnant participants in the IV iron arms achieving a normal for pregnancy hemoglobin (Hb) concentration of ≥11 g/dL in the third trimester (at either a 30-34 week antenatal visit or based on blood collected prior to delivery) when compared to the percentage of participants randomized to an active, comparator arm (arm 3) provided oral arm.

The second primary outcome for the parent trial is low birth weight (LBW) under 2500 grams, which is one of several adverse pregnancy outcomes associated with IDA. It is hypothesized that LBW delivery rates for participants randomized to the IV iron arms will be significantly lower when compared to the LBW delivery rate of participants randomly assigned to the oral iron arm. Additionally, numerous secondary outcomes will be explored.

Although a much larger number of pregnant women will be screened for eligibility at two timepoints for *RAPIDIRON*, it is expected that 4,320 pregnant participants will be randomized and treated for IDA consistent with the plan for the study arm to which they are assigned. This sample size should be sufficient to assure availability of approximately 1,332 complete data sets per arm even if up to 7.5% of study participants fail to complete study milestones.

An initial eligibility requirement for consent to participate in the *RAPIDIRON Trial* is hemoglobin concentration of 7 – 10.4 g/dL when measured in the first trimester or early in the second trimester of pregnancy. However, for randomization and treatment, requirements include:

- Iron deficiency anemia, defined for this study as moderate anemia with hemoglobin concentration level between 7 – 9.9 g/dL as well as test results indicating that serum transferrin saturation (TSAT) is <20% and/or ferritin is <30 ng/mL; and
- A live singleton, intrauterine fetus and dating ultrasound that indicates a pregnancy that would allow IDA treatment initiation between the beginning of week 14 and prior to 17 weeks 0 days gestation.

Exclusion criteria for the parent trial include:

- Fetal anomaly, if detectable when an initial ultrasound is done to date the pregnancy (subsequent discovery of a fetal anomaly is not viewed as an exclusion criterion);
- Maternal history of cardiovascular disease, hemoglobinopathy, or other disease or condition considered a contraindication for treatment, including conditions recommended for exclusion by the manufacturers of oral or IV iron to be used in this study; and
- Any condition that, in the opinion of the consenting physician, warrants study exclusion.

Pregnant participants randomly assigned to an IV iron arm receive a single-dose IV iron formulation having 1000mg of iron. However, participants weighing under 50kg receive a lower dose of iron as determined by a formula used by both manufacturers of the IV iron formulations employed in the trial. As indicated in the *RAPIDIRON Trial* protocol, the IV iron infusion should be given between 14 and 17 weeks fetal gestational age.

C. Research Team and Preliminary Studies

Since 2001, Dr. Richard Derman has been a Principal Investigator (PI) for the Global Network for Women's and Children's Health Research, Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), in partnership with Jawaharlal Nehru Medical College (JNMC), Belagavi, Karnataka, India. The first collaborative study, implemented in the Belagavi District of Karnataka, was a single-site, randomized, placebo controlled, community-based trial of oral misoprostol for prevention of postpartum hemorrhage (PPH). This study's findings, published in the *Lancet* in 2006, indicated a reduction in PPH of nearly 50%; severe PPH showed an 80% reduction. [8] Importantly, the study resulted in worldwide scale-up of misoprostol use for PPH prevention. Since then, eleven additional Global Network studies have been completed. Additionally, Belagavi Women's and Children's Health

Research Unit (or Global Network Site 8) has also completed, or is participating in, more than 20 other studies funded by various private and public sources – e.g., the Bill & Melinda Gates Foundation, the Thrasher Research Fund, the Children's Investment Fund Foundation, and the World Health Organization (WHO) – which recently named JNMC a WHO Collaborating Centre for Research in Maternal and Perinatal Health.

RAPIDIRON-KIDS Study Co-Investigators at Thomas Jefferson University include Dr. Rupsa Boelig, Dr. Benjamin Leiby, and Dr. Vanessa Short. Dr. Rupsa Boelig is an Assistant Professor of Obstetrics and Gynecology with a dual appointment in the Division of Maternal Fetal Medicine, where she serves as the Director of Research, and the Department of Clinical Pharmacology and Experimental Therapeutics. Her clinical background includes four years of residency training in obstetrics and gynecology followed by an additional four years of subspecialty training in maternal fetal medicine, with a two year concurrent assignment facilitated by a US National Institutes of Health Clinical Pharmacology fellowship. Dr. Boelig has been involved in the design and implementation of the *RAPIDIRON Trial* and will serve in the same capacityfor the *RAPIDIRON-KIDS Study*. Dr. Benjamin Leiby is a Professor and head of the Biostatistics division, skilled in the analysis and interpretation of biomedical and epidemiological data. He has collaborated widely with basic scientists, clinicians, and epidemiologists in many specialties. Dr. Leiby helped develop the parent *RAPIDIRON Trial* and works closely with the JNMC research team on the Data Management System as well as the implementation of the statistical analysis plans. Dr. Vanessa Short is a perinatal epidemiologist and an Assistant Professor in the Department of Obstetrics and Gynecology at Sidney Kimmel Medical College/TJU. Dr. Short currently serves as Director of Research at TJU's Maternal Addiction Treatment, Education and Research Program, where she leads her own federally and intramurally-funded research projects within the realm of maternal and child health, including the conduct of multi-year cluster randomized clinical trials. Dr. Short frequently collaborates with Dr. Derman and other faculty on maternal and child health-related trials in low-income and middle-income countries. She is currently providing services as a member of the *RAPIDIRON* research team, and she will work on the *RAPIDIRON-KIDS Study* providing input into the design and implementation of the trial and assisting with interpretation of study data and dissemination of findings.

The *RAPIDIRON-KIDS Study* will involve oversight from a research support team at Thomas Jefferson University and collaboration with researchers from JNMC Women and Children's Health Research Unit. Key personnel from JNMC include: Dr. Shivaprasad S. Goudar, a Professor of Physiology and JNMC's Senior Foreign Investigator; Dr. Mrutyunjaya Bellad, a Professor of Obstetrics and Gynecology, and the Site Principal Investigator of the parent *RAPIDIRON Trial*, who has participated in numerous studies being implemented by the Women's and Children's Health Research Unit based on the JNMC campus since it was established twenty years ago; Dr. Roopa Bellad, a Professor of Pediatrics who will serve as Co-Site Principal Investigator; and Dr. Yogesh Kumar, Associate Professor of Community Medicine at JNMC, who is the current Project Coordinator of the *RAPIDIRON Trial.*

Critical input into the design of the *RAPIDIRON-KIDS Study* has been provided by co-investigators who are recognized experts in neonatology, hematology, and pediatric neurodevelopment. Dr. Michael Georgieff, Executive Vice Chair and Professor, Department of Pediatrics at the University of Minnesota School of Medicine, is an internationally known researcher whose laboratory studies focus on the biological basis for the negative neurodevelopmental effects of fetal/neonatal iron deficiency and anemia. His work has contributed to an understanding of the neurological effects of iron deficiency prior to birth and the mechanisms by which long-term effects can be carried into adulthood. [9, 10] Dr. Michael Auerbach, Clinical Professor of Medicine, Georgetown University School of Medicine, is involved in an academic medical practice in hematology and oncology. He is recognized as a global leader with vast experience in the use of IV iron. He conducted the first US prospective study of IV iron use in pregnancy, the results of which indicated that IV iron is safe, less toxic, and more effective than oral iron. [11] Dr. Zubair H. Aghai, MD, FAAP is a Neonatologist in the Division of External Specialty Pediatrics, Department of Pediatrics at Alfred I. duPont Hospital for Children of the Nemours Foundation. He is also a Professor of Pediatrics and the Director of the Neonatology Fellowship Program at the Sidney Kimmel Medical

College of Thomas Jefferson University Hospital. Dr. Aghai has expertise in clinical and translational research in neonatal infection, inflammation, and nutrition, with a special interest in neurodevelopmental follow-up. He is active in several research projects including those funded by the National Institutes of Health. He is also collaborating with sites in India on an NIH R01 global health research project to improve maternal and child health in low- and lower-middle-income countries.

D. Research Design and Methods

Design of the RAPIDIRON-KIDS Study

Table 1 below indicates the plan for assessing participating infants and post-delivered women for this study. The parent *RAPIDIRON Trial* will be the source of much of the information for the first two visits listed. The visits at 4 months, 12 months, 24 months, and 36 months of age will involve assessments done specifically for the *RAPIDIRON-KIDS Study*. The study outcomes are as follows:

Offspring Primary Outcomes:

(1) Iron status – indicated by hemoglobin and ferritin at birth and at 4 months
(2) Developmental quotient (DQ) for cognitive domain of the Bayley Scales of Infant Development (BSID) at 24 months

Secondary Outcomes (Offspring):

- Iron status – indicated by hemoglobin, and reticulocyte hemoglobin content at 12 months and other indices at birth and four months
- Performance on Preferential Looking assessment at 4 and 12 months
- Scoring of performance on motor and language domains of BSID IV at 24 months and scoring of performance on all domains and 36 months
- Performance on Behavioral Rating Scale at 24 months
- Performance on Ages & Stages Questionnaire -3 at 12 months
- Internalizing and Externalizing behaviors on the Child Behavior Checklist for Ages 1.5-5 (CBCL/1.5-5) at 24 and 36 months
- Risk of autism diagnosis on Indian Scale for Assessment of Autism (ISAA) at 36 months
- Growth parameters (weight, length/height, and head circumference) at 4, 12, 24, and 36 months
- Hospitalizations and etiologies during the first three years of the child's life
- Infant feeding practices measured via Infant and Child Feeding Index (ICFI)

Secondary Outcomes (Mothers):

- Iron status (Hb, ferritin and TSAT) at 4, 12, and 24[1] months
- Quality of life indices at 4 and 12 months measured via the World Health Organization Disability Assessment Schedule 2.0 (WHODAS 2.0)
- Hospitalizations through three years post-delivery
- Documented blood transfusions
- Infant feeding practices measured via Infant and Child Feeding Index (ICFI)

[1] If no difference in iron status between *RAPIDIRON* treatment arms is detected at 12 months (once 12 month sample analysis is complete), we will no longer collect 24 month maternal blood samples.

Table 1. Schedule of RAPIDIRON-KIDS Study

Assessment Visit #	1	2	3	4	5	6
Age at Assessment	Birth	6 weeks	4 months	12 months	24 months	36 months
Iron Status						
Hemoglobin	X		XX	XX	XXX**	
Reticulocyte Hgb content	X		XX	XX	XXX	
Ferritin	X		XX	XXX	XXX	
%TSAT	X		XX	XXX	XXX	
Neurobehavioral						
Preferential Looking			X	X		
Ages & Stages Questionnaire 3				X		
Bayley Scales of Infant Development IV					X	X
Behavioral Rating Scale					X	
India Scale for Assessment of Autism						X
Child Behavior Checklist for Ages 1.5-5 (CBCL/1.5-5)					X	X
General Growth & Breastfeeding Practices						
Weight	X	X	X	X	X	X
Length	X	X	X	X	X	X
Head circumference	X	X	X	X	X	X
Breastfeeding	XXX	XXX	XXX	XXX	XXX	XXX
Infant Child Feeding Index (ICFI)	X	X	X	X	X	
Quality of life indices (WHODAS 2.0)		XXX	XXX	XXX		
Rates of hospitalization (post-delivery)	XX	XX	XX	XX	XX	XX
Rates of transfusions (post-delivery)	XXX	XXX	XXX	XXX	XXX	XXX

	RAPIDIRON
	RAPIDIRON-KIDS

X = offspring

XX = offspring and mother

XXX = mother only

*Note: The RAPIDIRON study will provide much of the information for the first two visits (as made clear by the different color boxes and key). Cord blood is the source of iron status for Visit 1.

**See footnote on page 14.

As previously noted, if a participating mother experiences an intercurrent pregnancy within 36 months post-delivery, a blood sample will be collected and analyzed to obtain a complete set of iron indices.

Domains and Timing of Assessments

Primary Outcomes, Related Hypotheses, and Rationale:

(1) Iron status as indicated by hemoglobin and ferritin at birth and at 4 months of age
Hypothesis: Infants born to RAPIDIRON mothers from the IV iron arms will have higher hemoglobin and ferritin levels at birth and at 4 months of age compared to infants born to mothers in the oral iron arm.

- *Rationale:* Hemoglobin concentration is used to assess the presence of anemia and to calculate iron in the red cell mass. Serum ferritin concentration primarily assesses iron stores and is important for calculating total body iron along with other parameters. The sample obtained at birth – using cord blood – directly assesses fetal loading, which is the direct goal of maternal iron therapy. Iron status at 4 months allows assessment of whether fetal iron loading results in longer lasting improvements in hematologic and iron status during a period of rapid neurodevelopment. The timing of assessments is also based on prior studies that show improved hemoglobin 2-4 months after birth. However, assessing iron status at 4 months of age has been recommended by many experts, including a noted neonatologist-researcher who has expertise related to the impact of iron deficiency on young children and serves as a Co-Investigator for both the parent *RAPIDIRON Trial* and this follow-up study. He noted that the World Health Organization recommends exclusive breastfeeding with no supplemental foods until an infant reaches six months of age. However, infants born at term who have delayed cord clamping, are breastfed and not supplemented with iron will start to "run out" of fetal iron stores beginning at four months of age, which puts their brain at risk for the effects of iron deficiency. But, if oral supplementation of an infant begins at about four months to address the deficiency, it can result in a shift toward a more pathogenic microbiome. Babies are relatively immunocompromised and such a shift can place them at greater risk for diarrheal illness earlier in life when they are less able to tolerate infection. If the above *RAPIDIRON* hypothesis is true, babies born to mothers in the IV iron arms will not have a need for postnatal oral iron therapy as early in life as the infants whose mothers received prenatal oral iron. The infants with mothers who received IV iron are expected to be loaded with prenatal IV iron, to be born with greater iron stores, and to have greater residual total body iron, as indexed by hemoglobin and ferritin concentrations, as well as greater storage iron, as indexed by ferritin concentration at birth and 4 months of age.

(2) Developmental quotient (DQ) on the cognitive domain of the Bayley Scales of Infant Development (BSID) at 2 years of age (24 months)
Hypothesis: Offspring born to RAPIDIRON participants who received IV iron treatment will have higher developmental quotients (DQs) on the cognitive domain of the Bayley Scales of Infant Development (BSID) at 2 years of age compared to offspring born to RAPIDIRON mothers given oral iron treatment.

- *Rationale:* Bayley Scales of Infant Development (BSID) is a validated tool for assessment of general developmental abilities routinely used in large-scale randomized controlled trials (RCTs) to detect deficits in the motor, language, and cognitive domains. The BSID will be applied at 24 months as this is considered the optimal time for testing. The test scores are age normed to a median of 100 (similar to an IQ test) and components are referred to as developmental quotients (DQs). Iron deficiency anemia has been shown to reduce DQ at one year and beyond.

Secondary Outcomes for the Offspring:

Content experts that suggested the timing of assessment of iron status in infants also provided information utilized for determining the schedule associated with assessing secondary outcomes. From a neurodevelopmental perspective, four months is the first age at which reliable assessments of the integrity of the cortex are possible. Cortical maturation is critical for motor, cognitive, and social well-being.

Prior to four months, infants' behaviors are primarily subcortical and reflexic (as characterized by the presence of primitive reflexes). At four months, cortical control is evidenced by controlled motor movements, social smiling, and novel visual object discrimination memory. Based on the *RAPIDIRON* hypotheses, infants loaded with prenatal IV iron will be neurologically more mature with faster speed of processing and better discrimination memory performance at four months than infants whose mothers received prenatal oral iron.

Neurobehavioral Development
A secondary hypothesis of this study is that infants born to women randomized to the IV iron arms of the parent *RAPIDIRON Trial* will have superior neurobehavioral development compared to infants born to women randomized to the oral iron arm. To test this secondary hypothesis, standard generalized tests of development and brain region domain-specific tests that are expected to be influenced by iron status will be administered at 4, 12, 24, and 36 months of age.

The tests and ages applied include:

- *Preferential Looking Time* – a behavioral test that assesses recognition learning and memory. This is a test of the integrity of the hippocampus brain region and will be applied when the child is 4 and 12 months of age. The test leverages the brain development principle of novelty preference. The hippocampus is rapidly developing in the last trimester through the first 18 postnatal months. Iron deficiency reduces hippocampal capacity. Infants are presented with an object (or picture of an object) and are expected to familiarize themselves with it. After a time delay, the familiar object is presented along with a novel object. The infant is expected to spend more time (>60% of total looking time) observing the novel object.
    - *Hypothesis: Infants born to mothers randomized to IV iron arms will spend more time looking at the novel object compared to those in the oral iron arm.*
- *Ages & Stages Questionnaire®, 3rd Edition* (ASQ-3) – a questionnaire designed to obtain parental input regarding a child's general developmental milestones which can be used for children from one month to five and a half years of age. Mothers will be expected to provide answers for the age-appropriate questionnaire that will be used when the child is 12 months of age.
    - *Hypothesis: Infants born to mothers randomized to IV iron arms will have more advanced developmental milestones compared to those in the oral iron arm. The age-equivalent stage will be positively correlated with iron status at birth.*
- *Bayley Scales of Infant Development Version 4 (BSID)*– this is a validated assessment of general developmental abilities which is routinely used in large-scale randomized controlled trials to detect deficits in the motor, language, and cognitive domains. The BSID will be administered at 24 and 36 months. The test scores are age normed to a median of 100 (like an IQ test), and components are referred to as developmental quotients (DQs). IDA has been shown to reduce DQs at one year of age.
    - *Hypothesis*: *Infants born to mothers randomized to IV iron arms will have higher DQs on the subscales of these tests at 24 and 36 months compared to those in the oral iron arm.*
- *Behavioral Rating Scale* – this was devised by the pioneer iron researcher Betsy Lozoff. [12] It is coded from a video recording of a child undergoing the BSID and it assesses engagement, tractability, exploration, hesitancy, and activity level. All domains that are dopamine-dependent and reported by Lozoff and others as perturbed by iron deficiency effects on dopamine metabolism will be administered during visits to children at 24 months of age.
    - *Hypothesis*: *Infants born to mothers randomized to IV iron arms will have higher scores for exploration, tractability, and engagement, and less evidence of hesitancy and hyperactivity at 24 months of age compared to children in the oral iron arm when assessed at the same age.*
- *Child Behavior Checklist for Ages 1.5 to 5 years (CBCL/1.5-5)* – this test will be administered to the parent/caretaker who spends the most time with the child and requires 20-30 minutes for completion. Parents rate items on a three-point Likert scale assessing internalizing and

externalizing behaviors. Total scores, t-scores, and percentiles are available for each scale. A recent study reported that children with iron deficiency at birth had higher internalizing behavior problem scores at 5 years compared to those born iron sufficient {median (IQR) 9.0 (5.3, 12.0) compared with 5.0 (3.0, 10.0), p = 0.006; [adjusted estimate (95% CI): 2.8 (0.5, 5.1), p = 0.015] and total [24.5 (15.3, 40.8) compared with 1.0 (10.0, 30.0), p = 0.009; adjusted estimate (95% CI): 6.6 (0.1, 13.1), p = 0.047]}. [13] This will be administered at 24 and 36 months of age.

- *India Scale of Assessment for Autism* – this test is similar to the standard screening tool for autism (the Modified Checklist for Autism in Toddlers) and has been validated for the Indian population. Iron deficiency during early pregnancy is associated with a higher risk of autism in the offspring. This test will be applied at the 36 month visit.
    - *Hypothesis*: *Infants born to mothers randomized to IV iron arms will have a lower risk of autism diagnosis compared to those in the oral iron arm. The risk will be related to lower initial maternal iron status at entry into the study and randomization to the oral iron arm.*

Iron Status at 12 months

A secondary hypothesis of this study is that children born to women randomized to the two IV iron arms of the parent *RAPIDIRON Trial* will have better iron status at 12 months compared to children born to women randomized to the oral iron arm. To evaluate this hypothesis, a number of standard hematologic and iron indices will be assessed in all subjects. The trajectory of differences in iron status in children will be graphically presented from birth through 12 months.

The biomarkers include:

- Hemoglobin concentration to assess presence of anemia and to calculate iron in the red cell mass; and
- Reticulocyte hemoglobin content to assess iron dependent erythropoiesis.

The timing of the visits for iron assessments are:

- *Birth* – the sample to determine % TIBS will be obtained from cord blood (which will be available from the parent *RAPIDIRON Trial).* Assessment of indices related to fetal iron loading will also occur at birth since 88% of total body iron can be calculated from the cord blood hemoglobin and cord serum ferritin
    - *Hypothesis: Infants born to mothers randomized to IV iron arms will have higher total body iron than infants born to mothers randomized to the oral iron arm.*
- *4 months* – this is a critical time for an initial blood draw of infants to determine iron status
- *12 months* – this sample time is consistent with standard of care recommendations for infants from the World Health Organization and the American Academy of Pediatrics and coincides with the first global neurodevelopmental testing scheduled to occur during the study.
    - *Hypothesis: The incidence of iron deficiency (ID) or iron deficiency anemia (IDA) will be lower in the infants born to mothers randomized to receive an IV iron infusion compared to mothers that receive oral iron.*

Following infant development through 12 months of age will provide critical data regarding associations of early iron deficiency with autism and schizophrenia. It will also enable a better understanding of growth and hemoglobin trajectories related to overall neurocognitive development.

While there is a paucity of data on the longer-term impact of iron supplementation (especially the use of IV iron) given to women in pregnancy, this study will enable us to describe correlations between women recruited in the 3 arms of the *RAPIDIRON Trial* as well as their hematologic, clinical, and overall health impact. These results will be additive to the critical primary outcome data generated on the offspring of these women.

General Growth and Nutrition

A secondary hypothesis of the *RAPIDIRON Trial* is that IV iron treatment early in pregnancy will reduce iron-deficiency-associated low birth weight (LBW) primarily due to premature delivery or intrauterine growth restriction. Both are risks for low iron stores at birth and poor postnatal growth in the offspring. Offspring born to mothers in the oral iron group, compared to the IV iron group, are more likely to be iron deficient during their fetal life, at birth, and during infancy. Prolonged iron deficiency can negatively impact linear growth. Chronic iron deficiency during the rapid phase of growth in infancy and early childhood results in vulnerability for growth impairment. Iron plays an important role in tissue oxygen delivery and transport of electrons within the cells, and iron acts as a co-factor for essential enzymatic reactions. Iron deficiency anemia likely delays growth by creating a hypoxemic condition and inhibiting insulin-like growth factor-I (IGF-1). [14, 15] Hypoxemia due to iron deficiency anemia can also lead to an increase in hypoxia-induced growth factor (HIF-1α), which in utero has been shown to decrease human growth hormone mRNA levels. [16] In infants and young children with iron deficieny anemia, iron therapy increased serum IGF-1 levels and growth velocity. [17] Several other studies have reported lower body weight, height, head circumference, and growth velocity in anemic children and improvement in growth parameters with iron therapy. [18, 19, 20]

The following general growth and nutrition assessments will be performed:

- *Standard anthropometrics* – weight, length (height), and head circumference will be measured at each of the six timepoints and plotted on the WHO standard pediatric growth curves. From these data, weight for age, length for age, and head circumference for age Z-scores will be generated and growth velocities will be calculated.
- *Nutritional survey* – the Infant and Child Feeding Index (ICFI) is a composite index used to measure feeding practices, which has been previously used in the Indian context and allocates scores based upon breastfeeding, dietary diversity, meal frequency, etc. [21] Data regarding iron-supplementation and iron-fortified foods will also be collected. This survey will be conducted at the birth, 6 week, 4 month, 12 month, and 24 month visits.

Maternal Secondary Outcomes

While the *RAPIDIRON-KIDS Study* represents an opportunity to understand the impact of two different approaches for the treatment of IDA during pregnancy on offspring, it also enables continued contact with a sample of *RAPIDIRON* participants. The interaction with these mothers during this follow-up study is an important means of appreciating the longer-term effects of the treatments used during the parent trial. As indicated in Table 1, the maternal secondary outcomes to be explored include the following: iron status when the offspring is 4, 12, and 24 months old; breastfeeding practices; quality of life, as measured by the World Health Organization Disability Assessment Schedule 2.0 (WHODAS 2.0); and rates of hospitalization and transfusion subsequent to the conclusion of the *RAPIDIRON Trial*.

E. Statistical Methods

Sample Size

We anticipate that at least 2,000 pregnant women will be recruited for the primary *RAPIDIRON Trial* across the three sites in Karnataka state and project that at least 600 complete data sets will be available for each of the three arms for analysis. Thus, an adequate number of infants per arm should be eligible for enrollment in the *RAPIDIRON-KIDS Study*. However, as demonstrated in the summary of the preliminary power analyses below, the aims of the *RAPIDIRON-KIDS Study* can be achieved by obtaining complete data sets for approximately 147 mother-offspring dyads for each of the three study arms (or a total of 441 dyads). Since *RAPIDIRON Trial* participants will be asked to consent to this follow-up study prior to delivery, we expect that some of them will experience a stillbirth or neonatal death and, thus, not

provide evaluable information on the infants. In addition, we expect that cord blood data will not be obtainable or analyzable for a certain percentage of live births resulting in missing data on two of the primary outcomes. To account for this, we plan on initially consenting approximately 525 *RAPIDIRON Trial* participants (~175 per arm). Because Aims 1 and 2 answer different essential research questions, the study is powered with a type I error rate of 0.05 for each of those aims.

We propose to conduct a total of four primary hypothesis tests for Aim 1. Two of those tests will be conducted on data collected at birth with separate tests comparing offspring born to mothers across both IV iron arms to those born to mothers in the oral arm with respect to hemoglobin level and log-transformed ferritin levels at birth. If either of those tests at birth show differences between the two arms, the comparable tests will be conducted at 4 months post-delivery. In all cases, the null hypothesis is that the mean across the IV arms is the same as that in the oral iron arm. To control the type I error rate across these two tests, the alpha level for each individual comparison will be 0.025. Although information on the likely effect sizes for the proposed intervention are insufficient to provide explicit estimates of power for this study, limited comparisons of infant hemoglobin levels in south Asian and Africa suggest that the differences in both hemoglobin levels and log ferritin levels in infants born to mothers with and without IDA differ by at least 0.8 standard deviations. Because we want to assure that this intervention is powered to detect effects somewhat smaller than that observed in mothers with and without IDA, we want to assure that this study has sufficient power to detect an effect that is in the range of 0.3 to 0.4 standard deviations. We anticipate that approximately 85% of the enrolled sample will provide data for our primary iron outcomes at birth. Thus, we estimate a sample size of at least 441 infants (147 per arm) will provide information at birth with a slightly higher number at 4 months as this time is not subject to difficulties with obtaining data from the cord blood at delivery. When evaluated in the context of the proposed mixed effects model where we assume an intraclass correlation coefficient of 0.5, a sample size of 147 per arm (294 IV iron vs. 147 oral) gives more than 80% power to detect a difference in means at birth of 0.31 standard deviations at the alpha=0.025 level. Since demonstrating the effects at 4 months requires that the hypothesis tests at birth reject the null hypothesis, the overall study power to detect a difference of 0.3 standard deviations or greater at 4 months is greater than 80%.

The primary outcome for Aim 2 is the Bayley Developmental Quotient (DQ) measured at 2 years of age. The average score across the two IV iron arms will be compared to the oral arm at the alpha=0.05 level. We expect to obtain data for approximately 85% of consented participants at this time point for a sample of at least 147 per arm. This sample size gives more than 90% power to detect a difference in means of 5 units on the BSID mean score assuming a standard deviation of 15 at the alpha=0.05 level. Because effect sizes in the range of 5 to 7.5 are generally clinically meaningful for the BSID, the study is well powered to detect clinically meaningful differences.

Primary Data Analysis

Statistical analyses involve both formal hypotheses testing for specified primary outcome measures and supportive descriptive analyses of secondary outcome measures.

Hemoglobin and log transformed ferritin will be separately modeled using mixed effects linear regression. Both outcomes will be measured at birth and 4 months, and hemoglobin will also be measured at 12 months. The mixed effects models will treat time as a categorical value and include fixed effects for time, treatment, and time by treatment interaction. Correlation among repeated measurements for each subject will be modeled through structuring the variance-covariance matrix of the residuals. Inclusion of the time by treatment interaction will allow for estimation of mean iron indices at each measurement time for each group. Within these models, the primary hypothesis tests for aim 1 will be performed by comparing the average across the two IV iron groups to the oral iron group with respect to the mean values at birth and at 4 months using a significance level of 0.025.

A similar mixed-effects model will be used for modeling Bayley DQ cognitive domain in participating children at 2 and 3 years of age. The primary test for aim 2 will be performed by comparing the average across the two IV arms to the oral iron arm at 2 years using a significance level of 0.05.

Secondary Analyses

Secondary analyses will examine the effect of the three treatment regimens on each of the secondary outcome measures including iron measures (hemoglobin, ferritin, reticulocyte hemoglobin content and % total iron binding saturation), neurodevelopmental measures (total score and scores for individual domains such as communication, fine motor, gross motor, problem solving ability, and personal-social functioning), preferential looking time (log transformed), scores for the four factors associated with the Behavioral Rating Scale (object orientation, motor quality, negative affect, and positive social responsiveness), T-scores for the CBCL/1.5-5 syndrome scales (Emotionally Reactive, Anxious/Depressed, Somatic Complaints, Withdrawn, Attention Problems, Aggressive Behavior, and Sleep Problems), the India Scale of Assessment for Autism, and the standard growth metrics (weight for age, length for age, and head circumference for age Z-scores). As noted previously, data from a nutritional survey will be collected; these data will only be included in analyses as potential effect modifiers or moderators, not as outcome measures. Maternal outcomes that include hematologic indices, a history of transfusion and/or hospitalization, and assessment of life quality are incorporated into this study as secondary analyses.

Each of the outcome measures outlined above are continuous, and most are collected longitudinally on each of the infants enrolled in this follow-up study. Outcomes measured at only one time will be analyzed using two-sample t-tests. The secondary analyses based on these outcome measures will generally examine the trajectories over time on each of these measures using linear mixed models. A first set of models will be designed to describe how the trajectory for each outcome differs as a function of treatment arm and will include the outcome measure (or an appropriate log transformation) as the independent variable, fixed effects of treatment, time, and the treatment by time interaction, with infant as a random effect. Analogous models will be generated for the secondary analysis of maternal iron levels with the inclusion of the woman as a random effect. Model results will be used to generate point and interval estimates of treatment-specific mean levels of the outcome measures at each developmental time. For each of these outcome measures, there will be an omnibus hypothesis test that assesses evidence for a difference in outcome by treatment at any time point with step-down tests of time-specific differences in treatment. All hypothesis tests are considered to be descriptive with p-values used as indicators of strength of evidence of treatment differences with no plans for formal statistical inference for the secondary analyses. As such, no control for multiple comparisons is planned.

A second set of descriptive secondary analyses will examine the relationship between each of the neurodevelopmental outcomes and growth parameters and infant iron status at delivery measured as delivery total body iron. These analyses will utilize linear mixed models that evaluate the regression relationship between baseline iron levels and outcome measures over time, controlling for treatment arm. These models will also evaluate whether the relationship between baseline iron and neurodevelopmental outcomes are mediated by specific post-delivery nutritional measures.

Interim Analysis

An analysis of maternal hematologic indices will be completed when all women have passed the 12 month collection period. This analysis will include all available maternal blood data through 12 months. Based on the results of this analysis, the research team may decide to suspend collection of maternal blood at 24 months. Other extensive interim analysis is not planned. However, typical study monitoring activities could trigger such an analysis for one or more of the following reasons: recruitment proves problematic, making it difficult to perform an adequate number of tests and obtain measurements at desired time points; study withdrawals, investigator-recommended discontinuations, or ‘loss-to-follow-up’ among children enrolled in the study are significant; and/or there is a poor ratio for data obtained vs. data expected for important study variables. If undertaken, the interim analysis would consider if continuation

of the study, despite existence of challenges such as those mentioned, could nevertheless provide enough valid evidence to judge whether the primary study hypotheses remain valid. If the answer is no, the study could be prematurely stopped.

Safety Analyses

All safety analyses will be descriptive based on the safety cohort with no planned formal inference for safety. All adverse events will be summarized by system organ class by arm with point and interval estimates of the risk of each of these events estimated by either exact binomial methods or normal approximations to the binomial as appropriate for the incidence of the event.

Descriptive statistics will also be generated for maternal and infant mortality and for incidence of serious adverse events (SAEs).

Analysis Cohorts

Intent-to-treat (ITT) cohort: study participants (mothers or infants, depending on the particular analysis) will be included using group assignments for the mother as randomized.

Delivery iron status cohort: Analyses that examine outcomes as a function of delivery iron status will include enrolled maternal participants with sufficient delivery data to determine iron status of the infant at delivery.

Assignment of participants into the various cohorts will be determined based on a masked data review prior to initiation of analysis.

F. Gender/Minority/Pediatric Inclusion for Research

The parent study, the *RAPIDIRON Trial*, was initiated in mid-March 2021 in two states of India; thus, its participants are classified as adult, female Asians and their offspring will likewise be considered Asian. Pregnant women utilizing facilities classified in the Indian public health system as primary or community health centers will have the opportunity to participate if they meet specified eligibility criteria. No pregnant woman will be excluded because of socioeconomic status or other personal characteristic that could be considered to qualify such women as minorities. Similarly, the offspring for whom consent will be sought – from the *RAPIDIRON Trial* participant – will not be excluded due to minority or family economic status. Additionally, there will be no gender bias in the inclusion of the participants for this follow-up study. By definition, newborn infant participants are children (thus, there is pediatric inclusion).

G. Human Subjects

1. Study Population

Participants of this study are mother-infant dyads consisting of women, residing in Karnataka state, who were randomized and treated for IDA during the *RAPIDIRON Trial* and their newborns.

Inclusion Criteria:

- Informed consent of the *RAPIDIRON Trial* participant for their study inclusion and that of their offspring for the *RAPIDIRON-KIDS Study*
- Expressed intent of the *RAPIDIRON Trial* participant to remain in the designated Karnataka research area for delivery and during the follow-up period for the *RAPIDIRON-KIDS Study* to enable participation in study visits
- For the offspring – live-born singleton infants of Karnataka maternal participants randomized and treated in the *RAPIDIRON Trial*, if consent is provided by the mother

Exclusion Criteria:

- Unwillingness of maternal participant to provide *RAPIDIRON-KIDS Study* consent for herself and her offspring

2. Sources of Research Material

Results of blood tests will be a source of data for this research. The *RAPIDIRON Trial* will provide the hemoglobin (Hb) for the last blood sample taken for a participating woman prior to delivery, as well as iron indices at 6 weeks postpartum. The *RAPIDIRON Trial* protocol also calls for the collection of cord blood subsequent to delivery to assess Hb, transferrin saturation, ferritin, and reticulocyte indices of the newborns. For the purposes of the *RAPIDIRON-KIDS Study*, blood samples of approximately half a teaspoon of blood (2-3 mL) will be collected from the mother at the 4, 12, and 24 month study visits, and 0.5 – 0.8 mL collected via heel stick from the offspring at the 4 and 12 month study visits to determine hemoglobin and iron indices.

The visits for the *RAPIDIRON-KIDS Study* also include performing assessments as listed in Table 1 (some of which overlap with the parent study). As part of the *RAPIDIRON Trial*, infant weight and length will be collected at birth, and maternal reports of labor and delivery practices and complications will be recorded. For the *RAPIDIRON-KIDS Study,* we will collect child head circumference at birth and administer the ICFI to the mother/parent to collect information regarding breastfeeding practices, general nutritional intake, and iron supplementation. Additionally, we will collect child height and head circumference at 6 weeks, 4, 12, 24, and 36 months of age. We will administer the ICFI at 6 weeks, 4, 12, and 24 months of age. We will also collect child weight at 4, 12, 24, and 36 months of age (as it will be collected via the *RAPIDIRON Trial* at the 6-week visit). The World Health Organization Disability Assessment Schedule 2.0 (WHODAS 2.0) will be utilized to assess the mother's quality of life when the offspring has reached 6 weeks (as part of the *RAPIDIRON Trial)*, 4 months, and 12 months of age. Data will also be collected to monitor rates of transfusion and hospitalization among the maternal participants through the end of the study.

As part of the *RAPIDIRON-KIDS Study*, we will conduct a variety of neurobehavioral assessments with the offspring using validated tools, as follows:

- The Bayley Scales of Infant Development (BSID) is a validated tool for assessment of general developmental abilities which is routinely used in large-scale randomized controlled trials (RCTs) to detect deficits in the motor, language, and cognitive domains. This will be conducted at the 24 and 36 month visit.
- The Ages & Stages Questionnaire, 3rd Edition (ASQ-3) is designed to obtain parental input regarding a child's general developmental milestones. For the ASQ-3, mothers provide answers for the questionnaires, and this will be conducted at the 12 month visit.
- The Preferential Looking Time test assess recognition learning and memory by presenting an infant with an object (or a picture of an object) which they are expected to familiarize themselves with. After a time delay, the familiar object is presented along with a novel object, and the time spent observing each object will be measured (with the expectation that they will spend more time observing the novel object to demonstrate recognition memory). This test will be conducted at the 4 and 12 month visits.
- The Behavioral Rating Scale is coded from a video recording of the offspring undergoing the BSID and assesses engagement, tractability, exploration, hesitancy, and activity level. At the 24-month visit, the child will be recorded undergoing the BSID. Trained staff will code the recordings, with a random sub-sample sent to the Masonic Institute of the Developing Brain to be coded again for quality assurance.
- The Child Behavior Checklist for Ages 1.5 to 5 years (CBCL/1.5-5) is completed by the parent/caretaker who spends the most time with the child, and involves rating items on a three-

point Likert scale assessing internalizing and externalizing behaviors. This requires 10-20 minutes for completion and will be conducted at the 24 and 36 month visits.
- The India Scale of Assessment for Autism is similar to the standard screening tool for autism (Modified Checklist for Autism in Toddlers) and has been validated for the Indian population. This will be conducted at the 36 month visit.

Personnel will be trained prior to initiating assessments and will be required to achieve competence in scoring performance, recording results, and completing and submitting data forms for entry into the data management system. Arrangements will be made for the use of suitable space for assessments and quality monitoring will ensure appropriate conduct of the research. Specific data forms will be used for recording data for participating mothers and offspring, and these forms will be available at locations where needed.

3. Recruitment and Consent Procedures

Community sensitization occurred in communities served by the primary and community health centers participating in the *RAPIDIRON Trial* and additional sensitization will occur in the Karnataka research area to promote awareness of plans for implementation of the *RAPIDIRON-KIDS* study. Sensitization generally consists of research staff holding meetings with community leaders, health officials, and other relevant parties. Accredited Social Health Activities (ASHAs) who live within the communities that constitute the research are may be asked to participate in the study (with authorization of the supervising health official), for example, by accompanying a mother and child to a center of engagement in the screening and consent process, or by ensuring that a participating child and mother keep an appointment for a blood draw or for developmental testing. ASHAs receive incentive-based fees for their work associated with government-defined responsibilities which include transporting mothers and children to health facilities for needed care. In prior situations when approval has been granted for their involvement in research designed to improve women's and children's health, their assistance has been a key factor in the success of the research.

ASHAs who have received education relevant to anemia and iron deficiency can be an additional source of information about this study. However, consent will be obtained by a trained facility health care provider or research staff member who is a physician or other certified health care provider having adequate health-related training to provide information about the nature and purpose of the study and is able to answer questions during the consent process.

Current *RAPIDIRON* participants will be given information about the study and an opportunity to consent as early as the 6th *RAPIDIRON* visit (26-30 weeks of pregnancy) and will be able to provide consent up until delivery, as long as they are not yet in active labor.

4. Description of Risks, Assessment of Likelihood and Seriousness

This *RAPIDIRON-KIDS Study* involves no intervention, and thus the risks associated with study participation can be considered minimal but include the possibility that drawing blood for analysis could cause bruising and, rarely, infection. No serious risks are associated with the various assessments indicated in Table 1, although some discomfort or anxiety on the part of the child or a mother cannot be ruled out but can often be ameliorated by individuals appropriately trained to conduct assessments.

5. Procedures for Protecting Against or Minimizing Potential Risks

Care will be taken to reduce the risk of bruising. Sterile technique will be utilized during blood draw to reduce risk of infection. Personnel conducting sociodemographic, neurodevelopmental, and other assessments will have adequate background and relevant training to ensure competency.

6. Potential Benefits, Importance to Participants and Others

The possible benefits to participants may be earlier diagnosis of iron deficiency due to a screening schedule which encompasses more monitoring opportunities than is associated with usual practice. Both mothers and infants whose hemoglobin values fall below a pre-designated cutoff will be appropriately referred as per standard of care. Further, if neurodevelopmental testing indicate possible disorder, parents will be told about resources for confirming a diagnosis and for seeking supportive services (if they exist locally).

In combination with the parent study, the *RAPIDIRON Trial,* it is hoped that this follow-up study will result in the expanded use of a more effective approach for the treatment of IDA and decrease the risk that offspring will suffer the consequences of an ineffective IDA treatment approach. Further, if the IV iron treatment approach is determined feasible and adopted widely as a frontline treatment for IDA in pregnancy, the number of future beneficiaries of these studies can be multiplied.

7. Discussion of Why Risks are Reasonable in Relation to Benefits

The *RAPIDIRON-KIDS Study* can be considered an observational study with minimal risk. However, if the study results in earlier diagnosis and earlier treatment of iron deficiency, and delivery of services to address developmental disorders, then participating children and mothers may receive greater benefit than would be the case if such problems were recognized later in life.

H. Data and Safety Monitoring Plan

1. Data and Safety Monitoring Plan (DSMP)

For the *RAPIDIRON-KIDS Study*, a serious adverse event (SAE) involves mothers (and their infants) who were treated for iron deficiency anemia as participants in the *RAPIDIRON Trial* and who provide consent for themselves and their offspring to participate in the *RAPIDIRON-KIDS Study*. To qualify as an SAE, the event must fulfill at least one of the criteria below:

- Results in a maternal or neonatal death, or fetal death >20 weeks gestation;
- Is life-threatening;
- Requires an unanticipated hospitalization or prolongs an existing hospitalization
- Results in persistent or significant disability or incapacity; or
- Represents other serious or unexpected adverse events that a study investigator feels should be reported, including need for resuscitation.

Specific forms will be utilized to report an SAE. For an SAE, the reporting form should be sent to the person designated to receive it within 7 days of the notification of the event. Reportable adverse and serious adverse events will be shared with the DSMB. If there is an unanticipated problem recognized as posing a risk to participants or others and it is likely to reoccur, a designated researcher should obtain additional information on the unanticipated event and compile a report to forward to the study Principal Investigator, Chief Quality and Safety Officer, and the Chair of the DSMB. The Chair will review the report and determine significance of the event to the study and the need for action to avoid harm to participants. The Chair may put the report on the agenda of the next DSMB meeting or communicate with members to address the unanticipated problem sooner.

At each scheduled DSMB meeting after completion of the primary randomized trial, SAE data and a report summarizing retention and participant status will be prepared for review and discussion. Following its deliberations, the DSMB will issue any recommendations it may have and specify that the study should continue based upon absence of safety concerns or concerns about data quality or excessive loss-to-follow-up.

2. Data and Safety Monitoring Board (DSMB)

It is envisioned that the independent data and safety monitoring for this *RAPIDIRON-KIDS Study* will be provided by the same Data and Safety Monitoring Board (DSMB) that oversees the primary randomized controlled *RAPIDIRON Trial.* The Board Chair has expertise in high-risk obstetrics and global health research. The other members have expertise that spans obstetrics and gynecology, neonatology/pediatrics, biostatistics, clinical trials design and implementation, and clinical management of anemia. All members of the DSMB are free of significant conflicts of interest (i.e., financial, intellectual, professional, or regulatory) to enable performance of their duties in an unbiased manner.

The duties to be performed by the DSMB specific to this study include:

- Review all serious adverse events (SAEs) and unanticipated problems that infants and mothers incur to determine if they pose ongoing risk for continuation in the *RAPIDIRON-KIDS Study;*
- Assure that the *RAPIDIRON-KIDS Study* activities are conducted in a manner to minimize risk to infants and mothers and assess maternal and infant burden of participation to confirm that the study is conducted in a safe and ethical manner;
- Assess data quality and participant retention to assure that the benefit of study continuation is consistent with participant burden; and
- Prepare reports that outline DSMB recommendations and provide them to the study Principal Investigator, the Senior Foreign Investigator, and the Chief Quality and Safety Officer.

After data from the primary RCT have been collected for outcomes through 42 days post-delivery (which constitutes completion of the clinical component of the *RAPIDIRON Trial*), the DSMB will continue to meet approximately annually to review the data from this follow-up study. The DSMB may revise the annual meeting schedule if the workload justifies. If a scheduled meeting is considered too remote to review an SAE and determine relevant action, information may be distributed to DSMB members via email and input sent to the DSMB Chair who can communicate a recommendation representing consensus among DSMB members to senior key personnel of the study. The Chief Quality and Safety Officer will be the intermediary in supplying appropriate data to the DSMB for review.

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