Trial title: Optimizing Early Child Development in the Primary Care Practice Setting: Pragmatic Randomized Trial of Iron Treatment for Young Children with Non-anemic Iron Deficiency (OptEC)

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BACKGROUND:

PREVENTIVE CARE AND SCREENING DURING WELL-CHILD VISITS IN PEDIATRIC PRIMARY CARE PRACTICE

Providing preventive services during well-child visits is a defining component of pediatric primary care practice.[1] Physicians are amongst the only professionals who see nearly all preschool children, and thus they are in a unique position to provide this care; however, a recent US study evaluating the quality of ambulatory care delivered to children showed that children received only 40.7% of indicated preventive care, according to 57 quality indicators.[2] It has been proposed that failure to deliver preventive services could be more detrimental to children than any other age group given the potential for early investments to establish optimal trajectories for health and well-being.[3] There have been recent calls to 'rethink' pediatric primary care with an emphasis on the development of scientific evidence.[4,5] Given the limited time allowed for each physician-patient encounter, priority should be placed on those topics both clinically important and uniquely responsive to the personal intervention of the physician.[5] A recent survey asked US pediatric primary care providers to identify important yet inadequately addressed research questions; these included understanding the effectiveness of services provided as part of well-child care.[6]

There are critical gaps in scientific evidence regarding the effectiveness of screening in pediatric practice. A recent study of 42 well-child care preventive interventions recommended by major organizations identified 21 screening recommendations for which there are significant gaps in evidence, leading the authors to consider this a "challenge to our profession".[7] High quality evidence is often based on the results of randomized controlled trials, and our research group has identified a paucity of randomized controlled trials (RCTs) in the field of pediatric medicine. Over the 20 year period 1985-2005 in leading general medical journals, pediatric RCTs increased by only 0.4 RCTs/year, in contrast to adult RCTs which increased by almost 5 RCTs/year; [8] in the same time period in leading specialty journals, pediatric RCTs increased by only 17 RCTs/year, in contrast to adult RCTs which increased by 90 RCTs/year.[9] These findings have lead the editor of a leading pediatric journal to conclude that "We believe that the data presented by Cohen et al should be a wake-up call to pediatric investigators, department chairs, and research center directors. Advancement of the health of children and adolescents requires sophisticated, high-quality research in which RCTs are a keystone.[10]

High quality child health research, including high quality randomized controlled trials, is urgently needed. However, there are methodological challenges. Moyer and Butler have identified that "The time scale is long for many important outcomes; many conditions of interest are adult diseases with behavioral precursors in childhood".[7] Given this challenge, there are meaningful child health outcomes that can be measured in shorter time periods.

We believe that interventions which optimize early child development should be given high priority in child health research. Further, we believe that there is an urgent need to examine the role of laboratory screening of young children for iron deficiency, followed by evaluating the effectiveness of treatment for those identified with iron deficiency, as three decades of research

suggests that this may be an unrealized and unique opportunity to prevent poor developmental outcomes in children.

The over-arching aim of this new research collaboration is to optimize early childhood development in the primary care physician practice setting. The focus will be on advancing the evidence base for a screening intervention aimed at improving early childhood development. Specifically, we will determine the effectiveness of laboratory screening for iron deficiency followed by oral iron treatment to improve developmental outcomes. This aim has been selected because the question is highly relevant to child health, is pragmatic and responsive to physician care in primary care practice settings, and is feasible and efficient to study within our newly developed practice based research platform called TARGet Kids!.

EARLY CHILD DEVELOPMENT AND IRON DEFICIENCY

Prevalence of Iron Deficiency and Iron Deficiency Anemia – Nationally representative data from the US indicates a peak prevalence in children 1-3 years of age 9% and 3%, for iron deficiency and iron deficiency anemia respectively.[11,12] Although there are no similar nationally representative data for Canadian children, regional studies suggest similar rates,[13-22] leading experts to conclude that "iron deficiency is an inadequately addressed and significant public health problem among Canadian infants and children".[23]

Opportunities for Early Detection – The state of non-anemic iron deficiency (NAID) is the latent period which, if left untreated, may lead to symptomatic iron deficiency anemia (IDA). This natural history provides an opportunity for early detection.

Current Recommendations regarding Screening for IDA or NAID in Primary Care Practice – The American Academy of Pediatrics (1998) recommends screening with a hemoglobin or hematrocrit at 9-12 months of age and then 6 months later, and, for patients at high risk, once a year from age 2-5 years.[24] However, both the Canadian Task Force on the Periodic Health Examination (1994) and the US Preventive Task Force (2006) have concluded that the evidence is insufficient to recommend for or against routine screening for IDA in asymptomatic children.[25,26] These guidelines have focused on IDA, but have not addressed screening for NAID.

Developmental Outcomes of Young Children with Iron Deficiency Anemia – Studies of outcomes of IDA have been largely conducted in developing countries due to the high prevalence. Poor developmental outcomes of IDA, which persist long term despite iron therapy, have been summarized in two recent reviews of longitudinal observational studies.[27,28] In addition, case-series and case-control studies suggest an association between IDA and childhood stroke (including a publication from our research group).[29-34] A Cochrane systematic review examined the effectiveness of iron treatment to improve developmental outcomes.[35] Short term treatment (5-11 days) examined in five trials (published between 1978 and 1993), including 180 children with IDA, showed no differences in developmental outcomes.[36-40] Two studies (published in 1986 and 1993), including 160 children examined longer term treatment (more than 30 days) and demonstrated improvements in developmental outcomes for the groups receiving oral iron treatment versus placebo.[41,42] Although the authors of the Cochrane review concluded

that there is urgent need for further randomized trials in children with IDA with long term follow up, there have been no recent trials; most investigators currently consider the question of the association between IDA and development to lack equipoise.

Developmental Outcomes of Young Children with Non-Anemic Iron Deficiency – A graduate student and the principal investigator have recently undertaken a systematic review of the literature regarding the effectiveness of oral iron treatment to improve the developmental and hematologic outcomes of young children with NAID. From the titles of 743 articles, full text review was completed on 46, and two randomized controlled trials were found for preschool aged children with NAID treated with oral iron 3-6 mg/elemental iron/kg daily versus no treatment.[42,43] For both studies, the primary objective was to study children with IDA; however, both included children with NAID randomized to oral iron or no treatment, and this data was available for the review. The first study (n=29, conducted in Indonesia, published in 1993) showed no statistically significant difference between groups in the post-treatment developmental score.[42] The second study (n=40, conducted in Turkey, published in 2004) showed a statistically significant difference between groups in the post-treatment mental developmental score, but not in the psychomotor developmental score.[43] Meta-analysis was not possible due to significant heterogeneity. Both studies showed moderate risk of bias due to insufficient information regarding allocation concealment and inadequate reporting of adjustment for co-variates (notably, socio-demographic variables); furthermore, mothers were not blinded in the second trial as placebo was not used. We have concluded that the effectiveness of oral iron treatment in children with NAID to improve developmental outcomes remains in question, and with this equipoise it is ethical and urgent to conduct such a trial. The results of a high quality, adequately powered trial, conducted in a developed country will begin to establish an evidence base for screening for iron deficiency with an aim to improve developmental outcomes.

MEASUREMENT ISSUES

This section will provide a rationale for our selection of measures for this study. Our objective was to select scientifically sound, parsimonious and pragmatic measures of infant cognition, social and emotional behavior, and iron deficiency which may be obtained in the primary care practice setting.

Assessment of Infant Cognition – There are several tests of infant cognition from which to choose.[44] The Mullen Scale of Early Learning (MSEL) [45] has been used extensively in several pediatric conditions including autism spectrum disorders, [46] profound hearing loss,[47] genetic conditions,[48-51] biliary atresia,[52] language delay,[53] and congenital hypothyroidism.[54] Although the second edition of the Bayley Scales of Infant Development (BSID-II) have been used extensively in research and clinical practice for developmental follow up of preterm infants,[44] and in several studies of IDA,[36-40,42,43] there have been concerns regarding limitations of the scale, including lack of separate standardized scores for non-verbal performance (for example, low Mental Developmental Index scores may reflect a specific delay in communication skills, cognitive abilities, or both) and the unconventional structure of the test.[44] The third edition of the Bayley Scales (BSID-III) attempts to address this limitation; however a recent study demonstrates that the BSID-III scale underestimates developmental delay in 2-year-old premature children.[55]

Furthermore, the MSEL may be used to test children from birth to 5 years 8 months, whereas the BSID may be used up to 42 months only. For all of these reasons, the MSEL has been selected as the test of infant cognition to be used in this study.

Assessment of Social and Emotional Behaviour – Although measures of infant cognition have been the primary outcomes for studies of IDA and NAID, there is a body of literature supporting an association between IDA/NAID and altered infant social-emotional behavior, including shyness and frustration, and poor engagement, soothability, and affect.[56-58] Several measures of social-emotional behavior have been used in these studies; however, there is no clearly established measure. Our research team has recently studied 3-5 year old children using a questionnaire called the Child Behaviour Questionnaire (CBQ) and found that children who scored highly on the 'negative affect' scale of the CBQ were also at high nutrition risk, according to a validated questionnaire of nutrition. The 'negative affect' scale includes constructs identified as important in studies of children with IDA, including soothability, fearfulness, and sadness. The constructs of regulation captured in the CBQ questionnaires appear closely related to social-emotional development in young children. Therefore, we have selected the version developed for children aged 18-36 months called the Early Childhood Behavior Questionnaire (ECBQ) for inclusion in this proposal.[59]

Laboratory Assessment of Iron Deficiency Anemia (IDA) and Non-anemic Iron Deficiency (NAID) – There appears to be agreement regarding the hemoglobin cut off of $\geq$110 g/L to distinguish anemia from non-anemia in children 1-3 years of age. The most significant controversy is focused on measures of iron stores. In a systematic review of 55 studies of adults regarding the diagnostic test properties of various measures of iron compared with the gold standard of bone marrow aspirate, serum ferritin was identified as the single most powerful test, with a likelihood ratio of 51, for a serum ferritin of < 15 μg/L.[60] No similar research has been done in children. Population based research from the National Health and Nutrition Examination Survey requires 2 of 3 abnormal tests of iron to meet the definition of iron deficiency, which avoids the sole use of serum ferritin, as it may be falsely elevated during acute illness. [11,12] In our office based research, we exclude children with acute illness, and use serum ferritin as our primary measure of iron (mean corpuscular volume is used as a secondary measure).[61,62] The most commonly used cut-off for serum ferritin is >12 μg/L for distinguishing iron sufficiency and iron deficiency in children 1-3 years. For the purposes of this proposal, we will use the terms iron sufficiency (IS), iron deficiency anemia (IDA) and non-anemic iron deficiency (NAID), with laboratory definitions described in methods.

ESTABLISHMENT OF THE TARGet Kids! RESEARCH NETWORK

The investigators have established a program of research aimed at advancing the scientific basis for preventative primary healthcare for young children. The following progress has been made:

Establishment of a community based primary care research network called TARGet Kids! – Since September 2008, a conceptual framework of *TARGet Kids!* has been established (See Appendix 1: *TARGet Kids!* overview), a steering committee formed, practice sites selected, equipment purchased, procedures standardized, research staff trained, partnerships established with the Applied Health Research Centre (AHRC) at the St. Michael's Hospital for data management and Mount Sinai Services (MSS) for laboratory measurement. Electronic linkages

between physician practices, AHRC and MSS have been established to allow secure real time transfer of data to our central database. *TARGet Kids!* is the only primary care child health research network in Canada.

***Subject recruitment and data collection through TARGet Kids!* –** More than 3000 children, 1-5 years of age have been enrolled in *TARGet Kids!*, with collection of non-invasive measures including questionnaires and physical measures, and more recently laboratory testing has been completed in almost 1000 children. This work provides evidence of feasibility of *TARGet Kids!* as a platform for the current study. (See Appendix 2: Subject recruitment and data collection)

***Developing a focus in child development* –** Since September 2008, *TARGet Kids!* data collection includes the measurement of child temperament through a parent-completed questionnaire (Child Behaviour Questionnaire). We have found that children, 3-5 years, with high 'negative affect' are at high nutrition risk. In the current application, we seek to expand our focus in child development. To reach this goal we have established collaborations with researchers in child development. Including a focus in child development in the overall *TARGet Kids!* initiative is a strategic opportunity, given the importance of healthy developmental trajectories as a critical outcome measure for children.

RATIONALE FOR A FOCUS OF RESEARCH TO OPTIMIZE EARLY CHILD DEVELOPMENT THROUGH SCREENING IN THE PRIMARY CARE PHYSICIAN PRACTICE SETTING

Early detection of non-anemic iron deficiency presents an important opportunity to provide effective interventions and improve child developmental outcomes. Physicians in primary care settings are ideally suited to provide this screening. There are critical gaps in knowledge regarding the effectiveness of screening for NAID, which this proposal can address. This proposal brings together a unique collaboration between primary care practitioners and research teams with established expertise in preventive child health research, child development, iron deficiency, clinical epidemiology and clinical trials methodology, analysis and data management. *TARGet Kids!* is now an established research platform which provides a unique opportunity to evaluate screening in primary care physician practice settings. Integrating the current studies into the *TARGet Kids!* platform is timely and highly efficient.

STUDY OBJECTIVES

Over-arching objectives –The over-arching aims of this new research collaboration are to improve the early childhood developmental outcomes and advance the evidence base for screening of children in primary care practice settings.

Specific objectives – In young children 18 to 36 months of age, to assess *laboratory screening for iron deficiency* followed by a comparison of the *effectiveness* of four months of oral iron treatment plus dietary counseling with placebo plus dietary counseling, to *improve developmental and laboratory outcomes* in children with non-anemic iron deficiency (NAID).

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Research Question (PICOT Format) – In young children 18 to 36 months of age, identified with non-anemic iron deficiency (NAID) based on screening at the time of their well-child visit, is four months of oral iron treatment plus dietary counseling better than placebo plus dietary counseling, to improve developmental and laboratory outcomes?

Hypothesis – We hypothesize that for young children 18 to 36 months of age with non-anemic iron deficiency (NAID) four months of oral iron treatment plus dietary counseling is better than placebo plus dietary counseling to improve developmental and laboratory outcomes.

TRIAL DESIGN

Design – To assess laboratory screening and the effectiveness of treatment for NAID, a cohort of children will be screened and those identified with NAID will be invited to participate in a pragmatic, placebo controlled, blind, parallel group, randomized trial.

Pragmatic-Explanatory Continuum – The primary purpose of this trial is to inform clinical decision making, and if possible, secondarily to contribute to an explanation of the causal association between iron deficiency and child development. Therefore, according to the framework described by Thorpe (co-investigator) and other leading trial methodologists, the trial has been designed along the pragmatic end of the pragmatic-explanatory continuum.[63] Specifically, eligibility criteria, participant compliance, follow-up intensity, and primary analysis will follow pragmatic approaches ("Does this intervention work under usual conditions?"); practitioner expertise and adherence, intervention and outcomes will follow approaches mid-way along the pragmatic-explanatory continuum ("Can this intervention work under ideal conditions?") with research assistants undertaking these aspects of the trial. The investigators will report the trial results according to the 2008 CONSORT guidelines for pragmatic trials.[64]

TRIAL DURATION

The trial will run for approximately 4 years.

METHODS

Study Setting – The sampling frame will be parents/caregivers of all young children attending their well-child visit with their primary care physician. The study will be set in the offices of practices participating in the *TARGet Kids!* research network (See Appendix 1: *TARGet Kids!* overview)

Eligibility for Participation in the Screening Cohort –

Inclusion criteria:

1) Age between 18 and 36 months attending any well child visit.
2) Informed parental consent

Exclusion criteria:

1) Previously diagnosed: developmental disorder, genetic, chromosomal or syndromic condition, chronic medical condition, (with the exception of asthma and allergies), including chronic anemia, iron deficiency or recent oral iron supplementation or treatment
2) Prematurity with a gestational age less than 35 weeks; low birth weight less than 2500 grams
3) Attending the office for an acute illness, such as a viral illness, or other health concern other than well-child assessment
4) Any contraindications to receiving elemental iron (i.e. NHP, comparator or placebo)
5) The use of any NPH containing the same medicinal ingredient(s) as the investigational product
6) English not spoken to the child in the home or in a child care setting

Eligibility for Randomization – All participants in the screening cohort will undergo laboratory testing and children will be assigned to one of three categories based upon the results of their hemoglobin and serum ferritin:

1) NAID (non-anemic iron deficiency) - hemoglobin $\geq$110 g/L and serum ferritin $\leq$12 µg/L
2) IDA (Iron deficiency anemia) - hemoglobin <110 g/L and serum ferritin $\leq$12 µg/L
3) IS (Iron sufficiency) - hemoglobin $\geq$110 g/L and serum ferritin >12 µg/L

Interventions – Children with NAID will be randomized to receive either oral iron treatment 6 mg elemental iron/kg/day or placebo (equivalent volume) once daily or bid or tid, at the discretion of the prescribing study doctor based on the patient's tolerance and preference, for four months. Dietary advice will be provided to both the groups. Dietary advice will include recommendations regarding maximum daily cow's milk intake, varied solid food intake including high iron containing foods, and avoidance of foods which reduce iron absorption. As this is a pragmatic trial, no specific strategies will be introduced to improve adherence. To monitor adherence at the end of the trial, parents will be asked to return bottles and the amount of iron administered will be calculated based on the volume of solution remaining. No specific criteria will be developed for discontinuation or modification of the interventions, as the dose of iron is within the safe and recommended dosages for children.

Investigational Agent

Active Arm: Ferrous Sulfate Oral Solution Drops USP (brand Fer-In=Sol by Mead Johnson); Strength: 75mg Ferrous Sulfate/mL = 15mg Fe++/mL; NPN # 00762954; Commercial supply donated in kind by Mead Johnson. Blinded over-labeling will be done by the SickKids Research Support Pharmacy.

Placebo Arm: The placebo is developed by the Hospital for Sick Children Compounding Pharmacy. Contains preserved water (preserved with sodium benzoate), simple syrup NF, denatonium benzoate (for bitter/medicinal taste) and food colouring (see detailed worksheet); similar in colour and taste to active; packaged in 50mL bottles identical to active Fer-in-Sol (empty bottles donated in kind by Mead Johnson).

Both active and placebo arms will be packaged in identical bottles with blinded labels. A participant information sheet that contains information regarding the study drug will be provided to parents who agree to participate in the trial.

Outcomes – A complete course of iron therapy is 4 months. Changes in developmental and hematologic status may be expected within this time frame. Outcomes will be measured at two time periods: baseline and 4 months after enrollment. The primary outcome will be the developmental score using the Mullen Scales of Early Learning (MSEL). The secondary outcomes will include the behavior measure (Early Childhood Behavior Questionnaire) and two laboratory measures (hemoglobin and serum ferritin levels). The analysis metric will be the mean difference in outcome between the treatment and control groups at 4 month follow up, adjusting for the baseline measures.

Outcome Measures –

The following measures will be used in **all children enrolled in the screening cohort**:

1) *TARGet Kids! child and family characteristics questionnaire* – Data on child characteristics (including socioeconomic status and ethnicity) as well as family factors (family structure, child care and familial illnesses), diet and physical activity, and health will be obtained from a detailed 60 item questionnaire (adapted from the Canadian Community Health Survey[65]) which is completed by a parent at the time of enrolment. Questionnaires have been extensively pilot tested since September 2008, and numerous modifications have been made to ensure understandability and reduce incomplete responses.
2) *The Early Childhood Behavior Questionnaire (ECBQ)* – The ECBQ assesses the following dimensions in children 18-36 months: Activity level/Energy, Attentional Focusing, Attentional Shifting, Cuddliness, Discomfort, Fear, Frustration, High-intensity Pleasure, Impulsivity, Inhibitory Control, Low-intensity Pleasure, Motor Activation, Perceptual Sensitivity, Positive Anticipation, Sadness, Shyness, Sociability, Soothability. Parents are asked how often they have observed each behavior during the last two weeks, and respond on a 7-point scale (never, very rarely, less than half the time, about half the time, more than half the time, almost always, always). The Very Short Form consists of 36 items in 3 broad scales: Negative Affect (12 items), Surgency (12 items), Effortful Control (12 items). For each scale, an average is calculated, so that the child has a score for each of the 3 scales[59].
3) *Laboratory measures* – 3 mL of blood is obtained to complete all of the *TARGet Kids!* laboratory measures. This is well within the acceptable amount for this age group. The current study focuses on laboratory measures of iron status, including hemoglobin, mean corpuscular volume, and serum ferritin. (Separately funded *TARGet Kids!* studies focus on laboratory measures of vitamin D and cardiometabolic status). Standardized reference ranges will be used to determine if the result is normal or abnormal.
4) *Physical measurements* – Measurement of height, weight and waist circumference of subjects and their accompanying parent, along with child head circumference, is performed using standardized anthropometric protocols.[66] Weight is measured using a precision digital scale (±0.025% SECA, Hamburg Germany) and standing height is measured using a stadiometer (SECA, Hamburg Germany). Body Mass Index (BMI) is calculated as weight in kilograms divided by height in meters squared and categorized as percentile categories using WHO Child Growth curves (which, unlike CDC growth curves, include percentiles for children under 2 years

of age and are able to monitor the rapid rate of growth in early infancy).[67,68] Blood pressure is assessed according to the guidelines of the National High Blood Pressure Education Program.[69]

Additional measures for children enrolled into the OptEC trial

Mullen Scales of Early Learning (MSEL) – The MSEL assess the cognitive functioning of young children from birth to 68 months. The assessment is based on the child's responses to activities prepared by the trained examiner. Five skill areas are measured: Gross Motor and four cognitive skills (summarized into an Early Learning Composite score) - Fine Motor, Visual Reception, Receptive Language, and Expressive Language. The raw scores for each scale can be converted into age-adjusted normalized scores. The four 'cognitive' skills score can be converted into a normalized ELC score, with a mean of 100 and a standard deviation of 15.[45] A participant information sheet that contains information regarding developmental testing will be provided to parents who agree to participate in the trial.

Pre-study screening and Baseline Measurements

The baseline data for this trial will be the same data collected during the TARGet Kids! visit of a child. In the TARGet Kids! Program parents/caregivers of eligible children are invited to participate by letter two weeks before their scheduled well-child visit. At the time of the physician office visit, parents will complete the written consent form, questionnaires (*TARGet Kids!* child and family characteristics questionnaire, ECBQ) and physical and laboratory measurements as described above. The overall research plan is shown in a schematic (See Appendix 3: Schematic of study plan). A Parent Letter containing a short description of the OptEC trial will be given to parents of children enrolled in the TARGet Kids! Initiative and are potential candidate for the OPtEC trial. This letter will state that incase found eligible the parents will be contacted by phone to invite them to participate in the OptEC trial.

Recruitment Strategy

TARGet Kids! Network has 6 collaborating primary pediatrician and family medicine practices. This trial will be integrated into the current TARGet Kids data collection system and the baseline data for this trial will be the same data collected during the TARGet Kids! visit of a child. After the physicians receive the lab test results research assistants (RA) will be instructed to contact potential participants over the phone. That the RA can contact TARGet Kids participants over the phone for participation into future studies have been incorporated into the TARGet Kids consent form. When the RA approach the parents over the phone to consider participating in this trial the RA will at the same time introduce the OptEC Trial to the parents and request them to return to the physician's office if they agree to participate. The RAs will be provided with a scripted telephone dialogue in order to standardize the patient recruitment process done over telephone. A Screening Form will be used to screen and enroll participants in the trial. At the physician's office the RA will review the consent form with the parent. The clinic nurse will review the iron status of the child with the parent and obtain informed written consent. The family will have the opportunity to ask questions to the RA, the pediatrician and/or office nurse/staff at any time.

Participant Timeline

Participants will be assessed at two time periods: baseline and four months after the baseline visit. The baseline assessment will be carried out in two steps. Step 1: The baseline TARGet Kids! Data collection; Step 2: According to the lab test results those who agree to participate in the OptEC trial will be asked to come back to the physicians office where the developmental testing will be performed. Data (questionnaires, physical measurements, developmental testing and laboratory testing) will be collected at both the baseline and follow-up periods. There is no requirement for a run-in period or washout period.

Randomization, Stratification, Blocking and Sequence Generation
Randomization will be stratified by clinic site. Block randomization will be generated with blocks of variable sizes. An advantage of block randomization is that group sizes are similar at the end of each block enrolled. The biostatistician will generate the randomization sequence for each clinic site using computer-generated random numbers.

Allocation Concealment
Allocation concealment is the process that prevents any trial participant or investigator from knowing in advance the treatment to which subjects will be assigned, and seeks to prevent selection bias. In this study, allocation concealment will be achieved by having the Pharmacy Department at the Hospital for Sick Children prepare the treatment and placebo in sealed, serially numbered bottles of similar appearance and weight according to the allocation sequence. A disadvantage of block randomization is that it may lead to study personnel guessing the allocation sequence; to reduce this possibility and to enhance allocation concealment the block sizes will be varied.

Blinding
Parents, attending physicians, laboratory personnel, and study personnel conducting the outcome assessments, data analysts and investigators will be blind to the group allocation. Study medication and placebo will be supplied in bottles that look identical, and the appearance, consistency and taste of the liquid will be similar. Group allocation will be concealed until the final data analysis is performed.

Non-randomized Children
Since all children will undergo laboratory screening at baseline, two additional groups of children (IDA, IS) will be identified and followed without randomization. Children with IDA will all receive oral iron treatment 6 mg elemental iron/kg/day in 2 divided doses for four months plus dietary counseling, which would be considered standard of care. Children with iron sufficiency (IS) will not receive any intervention. Children with IDA and IS will be included in the analysis plan for comparison with the NAID groups.

Data Collection Methods
Questionnaire data and physical measurements will be collected by the Research Assistants who are embedded in the practices. The Research Assistants have been trained to ensure accuracy of data collection and the questionnaires have been extensively pilot tested. The TARGet Kids! Health and Nutrition Questionnaire will be used to collect data only at baseline, a follow-up questionnaire will be used at the follow-up visit and the ECBQ will be used both at baseline and

follow-up visit. Blood work will be obtained by trained personnel according to the arrangements established at each of the practice sites; for example at some sites the Research Assistant is also a trained Phlebotomist and at some sites the clinic Nurse performs phlebotomy. Blood testing will be performed once during the TARGet Kids! visit and then after four months during the follow-up visit. The Research Assistant is responsible for ensuring the blood is delivered to Mount Sinai Services (MSS) for laboratory testing. MSS provides customized laboratory and research services to pharmaceutical and biotech companies, and researchers (http://www.mountsinaiservices.com/pages/Home). Laboratory results are sent electronically to the data management centre (see next section) as well as faxed to the practicing physician who is responsible for communicating the results to the participants. Children eligible for the randomized trial will return to the physician's office where the Mullen Scales of Early Learning will be administered by a trained psychometrist under the supervision of one of the co-investigators who is a developmental psychologist (Dr. Eva Mamak). This will take approximately 30-40 minutes. At the 4 month follow up visit, the Research Assistant is responsible for ensuring that the questionnaires, physical and laboratory measures are completed. The psychometrist will complete the MSEL.

Concomitant Medication

Concomitant interventions permitted include over the counter multivitamins which do not contain iron; those prohibited include additional over the counter iron.

Premature Withdrawal Criteria

1) Participant developing hypersensitivity to the provided syrup
2) Participant becoming extremely sick
3) Participant hospitalized for more than a week without signs of improvement

Probable lost to follow-up patients

Children who fail to show-up for their 4 month follow-up visit will be contacted initially on alternate days up to a period of one month and then on a monthly basis until the end of the trial period (4 years) by the RAs over telephone.

Unblinding Procedures

If a subject in the randomized study deteriorates or has persistent, severe bothersome side effects then unblinding may be necessary. Emergency unblinding should only be done when the clinical treatment of the patient will be different by knowing which arm of the study the patient was on. The physician caring for the subject should contact the principal investigator or co-investigator first to discuss the unblinding procedure. The study investigators should remain blinded if possible.

Adverse event reporting

All adverse events will be reported to the Hospital for Sick Children Research Ethics Board according to the Hospital for Sick Children's adverse event reporting requirements. All adverse drug reactions to the study medication will be reported to Health Canada within 15 calendar days or for death or life-threatening events, within 7 calendar days. In the latter case, a follow-up

report must be filed within 8 calendar days. Adverse reactions will be managed according to the Hospital for Sick Children's standard clinical management practices.

Sample Size

From previous research, it is anticipated that the mean MSEL developmental score for children with NAID will be 90, and the standard deviation is $\pm$ 15.[41-43] To detect a 6 point difference in post-treatment MSEL developmental score between treatment and placebo group, with a power of 80% and a significance level of 5% will require a total sample size of 150 (75 per group). With an estimated prevalence of NAID of 10%, it is anticipated that screening approximately 1500 children will identify 150 children with NAID to be randomized over a 4 year period. Expecting potential drop-outs and withdrawals to be 0-20% a total of 180 NAID children will be randomized. For the non-randomized children (IDA and IS), it is anticipated that the mean MSEL developmental score for children with IDA will be 85 and the mean developmental score for IS children will be 100 with a standard deviation of $\pm$ 15.[41-43] From the screening cohort of 1500 it is anticipated that 1-2% will have IDA (n=25) and an equal number of randomly selected children with IS (n=25) will be sampled for comparison. To randomly select children with IS, once a child with IDA is identified the immediate next child identified with IS and agrees to participate will be enrolled in the trial.

Statistical Analysis

From the screening cohort, the baseline characteristics of the three groups (NAID, IDA, IS) will be compared with descriptive statistics and significance testing. Categorical variables will be compared with a chi-square test and that continuous variables will be compared with an ANOVA (or non-parametric equivalent). For the participants with NAID randomized to treatment or placebo groups, no significance testing will be performed on the baseline characteristics); however, we will note any imbalances that have arisen by chance which may be clinically meaningful. All children with NAID randomized to treatment or placebo will be analyzed in the group to which they were randomized following the intention to treat principle. In the primary analysis, the difference in developmental and hematologic measures in children with NAID randomized to treatment versus placebo will be assessed using linear regression (ANCOVA method) with the initial baseline measures included as the adjusting variable[70]. In a secondary analysis, additional co-variates of clinical or statistical significance (including, parent education, family income) will be included in the model. The primary analysis will be a fixed effects model, ignoring stratification by clinic site. A secondary analysis will include a confirmatory analysis using a mixed effects model with interventions and MSEL scores as the fixed effect and clinic site as the random effects. Although efforts to ensure complete data collection and participant follow up will be maximized, analytic strategies to handle missing data will include imputation techniques, if appropriate. If >20% of participants are lost to follow-up a per protocol analysis will be carried out in addition to the intention to treat analysis.

Data Management

The Applied Health Research Centre (AHRC) of the Keenan Research Centre, Li Ka Shing Knowledge Institute of St. Michael's Hospital, University of Toronto, will serve as the data management centre under the direction of Dr. Mamdani, Director of the AHRC (http://www.stmichaelshospital.com/research). AHRC employs state-of-the art web-based data

management software: Medidata RAVE™ (5.6.3). RAVE™ is a secure encrypted web-based clinical trial data management system which is fully configurable and incorporates sophisticated data validation rules to ensure high quality data is captured. RAVE™ is a web-based application allowing for remote web-based data entry directly from the participating practice sites, and facilitates real-time data access. Data from the *TARGet Kids!* initiative, launched in September 2008, has been entered into a RAVE™ database and will be leveraged for this study. The licensing fee has been paid through previous *TARGet Kids!* related grants.

Trial Monitoring

A Data Monitoring Committee is not deemed necessary, as the experimental intervention (oral iron treatment 6 mg elemental iron/kg/day given once daily or in 2 or 3 divided doses daily for four months plus dietary counseling) is standard of care for children in the same age group with IDA and the side effect profile is well known. In the current study of children with NAID, similar side effects are expected and will be collected. There will be no criteria or plans for early trial termination.

SUB-STUDIES OF THE OptEC TRIAL:

Kawsari Abdullah, MBBS is a Pediatrician trained in Bangladesh and currently a PhD student in the Clinical Epidemiology and Health Services Research program in the University of Toronto, Department of Health Policy, Management and Evaluation. Dr. Patricia Parkin is her supervisor. In accordance with the Department of Health Policy, Management and Evaluation rules for the PhD program she has submitted a proposal for her PhD thesis and the department has accepted her proposal. Among the three projects she will carry out for her PhD thesis two of them will be undertaken as sub-studies of the OptEC trial. Members of the thesis committee and co-investigators of the OptEC trial have approved the sub-studies for the PhD student.

Sub-study #1: Internal pilot study to estimate the sample size for the OptEC trial.

The estimates we have used for the calculation of sample size for the multi-centre OptEC trial are based on literature review It has been shown that sample size estimates based on literature review are likely to be considerably less than the true values because the literature tends to report more homogenous cases series than will be entered in a large clinical trial, especially an efficient one with inclusive entry criteria [71]. Furthermore prior estimates are often obtained from trials that may have different study conditions to the trial, for example different patient types, small number of centres, different treatment duration [72]. Thus the prior estimates obtained from these studies may not be representative of the current randomized trial. These potential problems can be overcome by the use of an internal pilot study which forms an integral part of the trial itself and not a separate study. The methodologic issues associated with internal pilot studies are described in the literature of the references.

Objectives of the internal pilot study:

1. To obtain a reliable estimate of the variance ($\sigma 2$) of the child development scores
2. To obtain the baseline to 4 month correlation coefficient of the developmental scores based on actual data from patients entered in the trial
3. To recalculate the sample size of the trial using the estimates of the internal pilot
4. To determine pre-randomization feasibility issues

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Sample size:
The minimum size for an internal pilot study should be 10 or more patients per intervention group. It has been shown that the larger the size of the internal pilot the more the accurate will be the estimates and so more appropriate will be the recalculated sample size [71-72]. Thus based on these facts the sample size may be recalculated after 30 participants have been enrolled in the randomized trial.

In order to recalculate the sample size for the OptEC trial using the estimates of the internal pilot study, we will not need to break the randomization code. As discussed with the study statistician this methodology is in alignment with proper conduct of clinical trials. None of the investigators will be unblinded to subjects' group allocation.

Sub-study #2: The effectiveness of oral iron therapy in improving the growth and haematological outcome of pre-school children with non-anemic iron deficiency (NAID): A randomized trial.

Research Question in PICOT format:
P = Pre-school children aged 18-36 months identified as having NAID (defined as serum ferritin <12 µg/L and hemoglobin >110 g/L) enrolled in the TARGet Kids! Research Network program
I = Oral iron (6mg elemental iron/kg/day in two divided doses) plus parental dietary counselling
C = Placebo plus parental dietary counseling
O = Hematological indices (serum ferritin and hemoglobin level), Growth parameters (weight and height)
T = Four (4) months

Objective of the study:
The objective is to evaluate whether oral iron therapy given to pre-school children (18-36 months) with NAID affects their hematological and growth outcome by comparing those receiving either oral iron therapy or placebo for four months (with both groups receiving parental dietary counseling).

Sample size:
The sample for this sub-study will be the first 40 NAID children enrolled in the randomized trial. Based on previous literature it has been shown that 1.2 cm improvement in children's height is a clinically significant improvement [73]. Thus it has been found that a sample of 40 will have 90% power to detect a difference of 1.2 cm improvement in height (with a SD=1), at 0.05% significance level.

Analysis:
An analysis of variance using multiple linear regression procedure to examine the association between the post-intervention differences in hematological and growth parameters of the two intervention groups will be carried out after adjusting for the baseline growth and serum ferritin measurements.

Group allocation for the first 40 subjects of the OptEC trial will be identified only for the hematological and growth parameters (secondary outcomes) for the analysis of the sub-study. This un-blinding will only be made known to co-investigator (KA) who will no longer be involved with the ongoing conduct of the trial. Group allocation for the primary outcome of the OptEC trial (developmental scores) will remain concealed and will not be analyzed for this sub-study. This methodology has been verified and accepted by the study statistician and other members of the OptEC trial team members.

ETHICAL CONSIDERATIONS

The *TARGet Kids!* research platform has been approved by the research ethics board at SickKids, as well as the other affiliated sites. Blood results are provided to the child's physician within 24 hours; developmental screening tests will be available immediately and detailed developmental assessment reports will be available in 4 weeks. The study will be approved by the Research Ethics Board prior to enrolling patients. Many parents and physicians may perceive the opportunity for a detailed developmental assessment and detailed laboratory testing to be a direct benefit of participation in this study.

Quality Assurance -

This project will be monitored by the Hospital for Sick Children Clinical Research Continuing Review program during the data collection phase of the project. The aim of Continuing Review is to ensure that all SickKids researchers are maintaining the highest ethical, scientific and safety standards for all study participants, and are in compliance with all relevant SickKids policies, provincial and federal legislation, and international guidelines such as ICH- Good Clinical Practice. All studies are categorized according to the Continuing Review Matrix based on the type of study and the level of risk (I to IV) to research subjects and the Clinical Research Monitor will review at minimum 10% of the research subjects' records for study eligibility, informed consent, adherence to study protocol, reporting of adverse drug reactions and adverse events, and data quality including computer database security and storage of records. Findings of the Continuing Review will be presented to the Research Ethics Board and lead PI in a written report, and specific recommendations arising from the report will be implemented in a timely manner.

RESEARCH TEAM

Patricia Parkin, MD is a specialist in Pediatric Medicine at SickKids and Senior Associate Scientist in the SickKids Research Institute. She leads the Pediatric Outcomes Research Team and has experience in observational and controlled trials in common problems of childhood in diverse clinical settings. She established the *TARGet Kids!* initiative along with **Catherine Birken**, MD, MSc and **Jonathon Maguire**, MD, MSc, who are also specialists in Pediatric Medicine, applied health researchers, PhD, C Psych, is a Psychologist at The Hospital for Sick Children and Investigator at Sickkids Research Institute with expertise in early child development and will supervise all cognitive testing. **Darcy Fehlings**, MD, MSc is a Developmental Pediatrician and Scientist at the Holland Bloorview Research Institute. **Colin Macarthur**, MBChB, PhD, is Vice President Research at Holland Bloorview Kids Rehabilitation Hospital with expertise in child health epidemiology. **Stanley Zlotkin**, MD, PhD is a Scientist in the Sickkids Research Institute with expertise in nutrition research, especially

iron deficiency anemia. **Muhammad Mamdani**, PharmD, MPH, is a pharmacist and clinical epidemiologist, and the Director of the Applied Health Research Centre and will provide oversight of the data management aspects of this study. **Kevin Thorpe**, MMath, is a Biostatistician at the Applied Health Research Centre, has expertise in clinical trials design, has developed the biostatistical plan for this study, and will perform the final analysis for this study. **Azar Azad**, PhD, is the Director of Mount Sinai Services, and will provide oversight of all aspects of the laboratory testing and reporting. **Parkin, Birken, Maguire, Fehlings, Macarthur** and **Thorpe** are all faculty teaching in the Controlled Clinical Trials course at the University of Toronto Department of Health Policy, Management and Evaluation. **Collaborators** include: *TARGet Kids!* steering committee members: Mark Feldman, MD, Head of Community Pediatrics, U of Toronto, and Head of the Section on Community Pediatrics of the Canadian Paediatric Society; Moshe Ipp, MD and Brian Chisamore, MD MHSc. *TARGet Kids!* collaborating practicing physicians: Dr. Sheila Jacobson, Dr. Michael Peer, Dr. Carolyn Taylor; Dr. Eddy Lau, Dr. Brian Chisamore, Dr. Sharon Naymark; Dr. Marty Perlmutar, Dr. Joanne Vaughn, Dr. Janet Saunderson, Dr. Patricia Neelands, Dr. Anh Do, Dr. Alannah Rosenthal.

TRIAL REGISTRATION

The trial will be registered with U.S. National Institutes of Health ClinicalTrials.gov, which is a registry of federally and privately supported clinical trials conducted in the United States and around the world.

KNOWLEDGE TRANSLATION

This proposal brings together a unique collaboration between primary care practitioners and research teams with established expertise in preventive child health research, autism and developmental research, child health epidemiology, and clinical trials methodology, analysis and data management. The primary care physician members of *TARGet Kids!* have participated in the early stages of shaping the research process to reach the overarching *TARGet Kids!* goals. Members of the *TARGet Kids!* network include decision makers and policy makers in primary care practice. There is no other current network of primary care practitioners and researchers focused on child health in Canada. Findings from this research will be disseminated directly to the physician participants and to their patients. An annual meeting of all the *TARGet Kids!* practices (physicians, nurses, office staff), research team (investigators, research assistants, students), and policy leaders (representatives from Section of Community Paediatrics, Family and Community Practice, and parent representatives) will occur. Parents of participants will receive the summary of their child's developmental assessment, anthropometric measures, and laboratory measures, leading to a direct benefit for individual participants. Further downstream dissemination to primary care physicians will occur through formal and informal venues at local levels, such as educational rounds (for example City Wide Paediatric Rounds, SickKids Annual Paediatric Update) and held by local physician groups. End of grant knowledge will be shared with the academic community through publication in relevant journals and presentations at national and international conferences Annual Meetings of the Pediatric Academic Societies, and the Canadian Paediatric Society), and locally through hospital rounds and presentations. Messages will be relevant to professionals working in the fields of pediatrics, family medicine, developmental pediatrics, nutrition, nursing, dietetics, and public health. Information will be disseminated to professionals by the research team with colleagues at the Canadian Paediatric

Society. The principal applicant is a member of the Canadian Task Force for Preventive Health Care and will participate in the upcoming guideline development for developmental screening and screening for iron deficiency anemia, which are on the priority list of topics for 2011-2012. Opportunities for coverage in lay publications and media will be sought using experienced knowledge broker at SickKids Department of Public Relations.

SUMMARY AND FUTURE DIRECTIONS

Early childhood is a critical time of development. There are large gaps in knowledge regarding the effectiveness of strategies for promoting healthy child development in the primary care physician practice setting. This study will focus on evaluating the effectiveness of screening and treating children with iron deficiency anemia and non-anemic iron deficiency on child developmental outcomes. Future research includes examining the longer term outcomes of this screening strategy, to more fully understand the implications for practice and policy. For example, the *TARGet Kids!* team has initiated communication with researchers at the Toronto District School Board, to explore opportunities to link (via unique identifiers, name and date of birth) data from preschool years (from *TARGet Kids!* data collection) to early school readiness (measured by the school board using the Early Development Instrument) and school performance (measured by the school board using standardized testing from the Education Quality and Accountability Office). The key strengths of this proposal include an important research question, engagement with the primary care health care system, a novel research platform- *TARGet Kids!,* and a highly qualified team of experienced and emerging investigators, with embedded knowledge translation between practicing physicians, investigators, parents and children.

Trial title: Optimizing Early Child Development in the Primary Care Practice Setting: Pragmatic Randomized Trial of Iron Treatment for Young Children with Non-anemic Iron Deficiency (OptEC)

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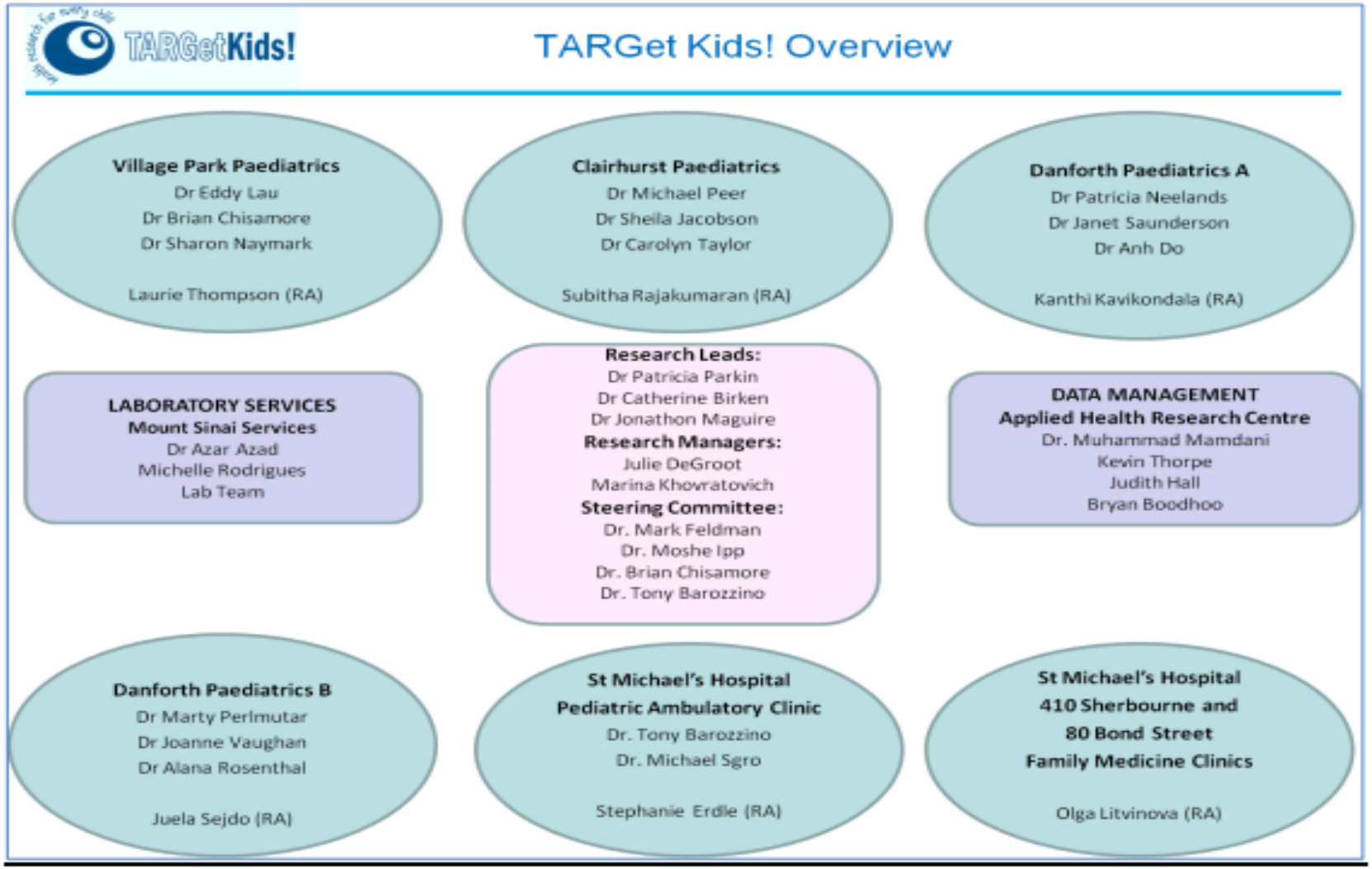
Appendix 1: TARGet Kids Overview



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Appendix 2: Subject Recruitment and Data Collection

Subject recruitment and data collection for children 12-36 months, September 2008 to January 2011:

Site	Date Launched	Number
Village Park Pediatrics	September 2008	626
Clairhurst Pediatrics	August 2008	754
St. Joseph's Family Medicine*	February 2009	2
Mount Sinai Family Medicine*	October 2009	2
Danforth Pediatrics (1st floor)	May 2009	168
Danforth Pediatrics (4th floor)	June 2009	306
St. Michael's Family Medicine	August 2010	7
St. Michael's Ambulatory Pediatric Clinic	To be announced	
Total		1865

*Discontinued due to poor recruitment rates

Laboratory tests completed for children aged 12-36 months, September 2008 to January 2011:

Laboratory Test	Number	mean	SD	min	max	1st quantile	median	3rd quantile
Hemoglobin g/L	608	122.1	8.84	75	151	117	122	128
Serum Ferritin µg/L	579	28.47	19.13	1.82	138	15.55	23.5	36.7
MCV fL	610	77.91	4.65	55.5	89	75.9	78.4	80.97
C Reactive Protein mg/L	578	1.662	6.70	0.09	122.1	0.2	0.3	0.7

Trial title: Optimizing Early Child Development in the Primary Care Practice Setting: Pragmatic Randomized Trial of Iron Treatment for Young Children with Non-anemic Iron Deficiency (OptEC)

Appendix 3: Schematic of study plan

Study Schematic of Optimizing Early Child Development in the Primary Care Physician Practice Setting: Pragmatic Randomized Trial of Iron Treatment for Young Children with Non-anemic Iron Deficiency