TITLE: A single-center, double-blinded, randomized, 12 week, superiority study in infants and young children to compare the efficacy of NovaFerrum® versus Ferrous Sulfate in the treatment of nutritional iron deficiency anemia (BESTIRON).

TRIAL REGISTRATION: Clinicaltrials.gov (NCT01904864)

PROTOCOL VERSION
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Gensavis Pharmaceutical, LLC is providing funding for both trial drugs as well as central organizational costs. The design, management, analysis and reporting of the study are entirely independent of the manufacturers of NovaFerrum®.

ROLES AND RESPONSIBILITIES:
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INTRODUCTION

BACKGROUND AND RATIONALE
Iron deficiency and iron deficiency anemia (IDA) affect millions of young children world wide.[1] In the United States, IDA affects approximately 3% of young children while iron deficiency without anemia is estimated to have a prevalence of 8-10% of infants and children.[2] Established strategies to prevent iron deficiency are often unsuccessful as evidenced by only modest decreases in the prevalence of iron deficiency during the past several decades.[2] Risk factors for IDA in infancy and early childhood include prematurity, exclusive breast feeding for greater than six months without supplemental iron, prolonged bottle feeding, obesity, low socio-economic status (SES), and early, excessive, and/or prolonged intake of cow's milk.[3-5] Peak incidence of IDA occurs from 12 to 48 months. The long-term consequences of iron deficiency and IDA include neuro-developmental impairment that appears to persist, at least, into the teenage years.[6-8]

Because IDA is usually preventable, the focus of the pediatric research community has been on early diagnosis, risk factors, and prevention. Recent recommendations regarding iron deficiency from the American Academy of Pediatrics emphasize the diagnosis and prevention of IDA with no attention given to treatment.[9] A 1958 review by Nathan Smith indicated that among 170 iron preparations useful in the prevention and treatment of IDA, none was more effective than ferrous sulfate, most were more costly, and many are more dangerous.[10] To date, his observations remain germane -- i.e. iron salts (especially ferrous sulfate drops or elixir) are the most frequently employed iron preparation for treatment of IDA during infancy and early childhood. However, incorrect dosing, inadequate instructions for caregivers, bad taste, and adverse effects (constipation, stained teeth, etc.) often result in suboptimal adherence and poor response to therapy.

Many iron preparations other than ferrous sulfate, including carbonyl iron and iron polysaccharide agents, are available over the counter as “supplements” or by prescription. However, to our knowledge no controlled studies have been performed to show that such alternatives are superior to ferrous sulfate. A Brazilian study comparing carbonyl iron to ferrous sulfate demonstrated significantly improved ferritin level in patients receiving carbonyl iron, but no significant difference in change in hemoglobin value between the two groups.[11] Iron polysaccharides differ from iron salts in that polar oxygen groups in the sugars form coordination complexes
with iron atoms that result in well hydrated microspheres which remain in solution over a wide range of pH
values, theoretically allowing for improved absorption and tolerability.[12] Accordingly there is a rationale for
polysaccharide iron complex preparations to be as effective as ferrous sulfate preparations and to result in fewer
adverse GI effects as well.

The recommended dosing schedule of elemental iron required for the treatment of IDA has been inconsistent in
the literature, ranging widely from 2 to 6 mg/kg/day and administered from one to three times daily. The
absorptive capacity of normal duodenum for iron, however, is approximately 25 mg of elemental iron, so higher
doses of iron may not lead to improved absorption or faster resolution of anemia.[13] Furthermore, ferrous
sulfate may cause fewer adverse gastrointestinal effects when given in low dosages on an empty stomach at
night although this has not been validated by formal studies. Even weekly dosing of oral iron has been utilized in
developing countries as a means of treating IDA and has been efficacious in improving hematologic parameters
in children with IDA.[13]

In efforts to improve adherence to treatment for IDA, the CDC published ‘Recommendations to Prevent and
Control Iron Deficiency in the United States’ in 1998, which suggested ‘treatment dosing of 3 mg/kg/day.[14]
This recommended dose was based on a panel of physicians’ expert opinion. A randomized controlled trial in
rural Ghana compared ferrous sulfate administered as a single daily dose versus three times daily dosing, and
showed similar success rates in correction of anemia.[15] Thus supporting a single daily dose as an effective
regimen in the treatment of IDA. Another study in India, comparing ferrous ascorbate to colloidal iron using a
dose of 3 mg/kg/day elemental iron demonstrated correction of anemia over a 12 week study period, further
supporting the efficacy of a once daily dose of iron.[16] Finally, few studies have reported the specific
hematologic responses to iron therapies employed in children with IDA. A 1985 study compared the side effects
of ferrous sulfate to placebo in a double-blind, randomized clinical trial and found no increase in gastrointestinal
or other complications.[17] However, no description of the hematologic response to therapy was provided. More
recently, a 2009 study described a randomized clinical trial evaluating adherence to ferrous sulfate drops vs.
ferrous fumarate sprinkles in low-income families of 6 month old infants without anemia.[18] Although adherence
to both regimens was similarly poor, no details regarding hematologic response to iron were described.

Approximately two-thirds of the 50 to 60 new cases of iron deficiency seen in the hematology clinic at Children’s
Medical Center each year are such young children with nutritional deficits. A recent study of our institutional
experience described suboptimal treatment results and assessed the diverse reasons that such children present
with this condition and why they often exhibit inadequate response to current treatments.[19] Iron deficiency
anemia is frequently taken “for granted” by primary care physicians and hematology-oncology specialists as
well. Unfortunately, few published studies characterize optimal means of delivering effective treatment.

Of the two iron preparations being compared in this clinical trial, one of them (ferrous sulfate) is considered by
most clinicians, textbooks and review articles as the “gold standard” agent for treatment of iron deficiency. Given
that iron polysaccharides are theoretically better absorbed than iron salts and have fewer adverse effects, we
will compare this type of iron preparation to ferrous sulfate. Thus, the other agent being tested is NovaFerrum®,
a new oral iron polysaccharide complex manufactured by Gensavis Pharmaceuticals, LLC in Corpus Christi,
Texas, which is specifically formulated to improve palatability and absorption in young children.

To begin to address this problem of the sparse scientific literature supporting current therapies for IDA in infants
and children, we will conduct a superiority study of a once daily dose of 3 mg/kg elemental iron given as either
ferrous sulfate drops or NovaFerrum® in infants and young children with nutritional iron deficiency. In an effort to
improve compliance, a simple yet potentially effective oral iron therapy has been adopted. The chosen dose is
within the current range of recommended treatment doses by the CDC, pediatric textbooks and guidelines, and
has demonstrated efficacy for treatment of IDA in at least one other well designed study.[14, 16] To further
optimize this study, it will be double-blinded to eliminate physician bias regarding one iron preparation over the
other. It is expected that the successful conduct of this research will better define how oral iron therapy is
optimally delivered – regarding preparation, dosing, and duration – to young children with this common disorder.
OBJECTIVES

Primary Aim:
To compare the efficacy of NovaFerrum® to ferrous sulfate for the treatment of nutritional IDA in infants and young children as determined by increase in hemoglobin concentration

Hypothesis: NovaFerrum® has greater efficacy than ferrous sulfate in increasing hemoglobin concentration during a twelve week course of treatment to subjects with iron deficiency anemia.

Secondary Aims:
(1) To compare the adverse effects of treatment for IDA between ferrous sulfate and NovaFerrum®
(2) To compare normalization of iron stores as demonstrated by laboratory measures of IDA (ferritin, TIBC, reticulocyte hemoglobin content) between subjects treated with ferrous sulfate and NovaFerrum®
(3) To compare the adherence to study medication between subjects on ferrous sulfate and NovaFerrum®
(4) To demonstrate efficacy of a once daily dosing regimen lasting 3 months in the treatment of nutritional IDA

TRIAL DESIGN
This study is a randomized, controlled, double-blinded single center superiority trial to compare the efficacy of NovaFerrum® to ferrous sulfate for the treatment of nutritional IDA in infants and young children.

METHODS

I. STUDY SETTING
This study will be conducted at a single academic hospital. Subjects will be initially evaluated in either the Hematology clinic or inpatient setting at Children’s Medical Center.

II. ELIGIBILITY CRITERIA
Parents must provide written, informed consent before any study procedures occur.

<table>
<thead>
<tr>
<th>INCLUSION CRITERIA</th>
<th>RATIONALE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Age ≥ 9 to &lt; 48 months</td>
<td>Nutritional iron deficiency is rarely seen at other ages</td>
</tr>
<tr>
<td>2. IDA documented by hematologic indices (hemoglobin, MCV, reticulocyte hemoglobin content), serum ferritin, and total iron binding capacity (See Appendix for definition of IDA).</td>
<td>Iron deficiency without anemia but depleted iron stores is extremely common, but only children with frank anemia will be enrolled in order to conclusively assess efficacy of treatment</td>
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</table>

<table>
<thead>
<tr>
<th>EXCLUSION CRITERIA</th>
<th>RATIONALE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Iron deficiency likely or definitely due to blood loss from the intestine or other sites.</td>
<td>Ongoing gastrointestinal or other blood loss would confound the effects of oral iron treatment.</td>
</tr>
<tr>
<td>2. Evidence of response to recent/current oral iron therapy (30 days or less), as determined by increase in hemoglobin by ≥ 1.0 gm/dL and MCV by 5 fL above measurements prior to iron therapy</td>
<td>Enrolling subjects already responding to iron therapy confounds the outcome assessment</td>
</tr>
<tr>
<td>3. History or evidence of intestinal malabsorption</td>
<td>Oral iron cannot be expected to be effective in malabsorption states</td>
</tr>
<tr>
<td>4. History of prior intravenous iron therapy</td>
<td>Effects of intravenous iron would confound treatment responses</td>
</tr>
<tr>
<td>5. Major co-morbidity such as a serious chronic medical condition unrelated to iron deficiency apparent on history, physical examination, or laboratory tests</td>
<td>Other medical conditions may result in malabsorption, bleeding, renal disease, inflammation or other confounders that would affect observed changes in the primary outcome independent of the intervention</td>
</tr>
<tr>
<td>6. Other causes of anemia (sickle cell disease, thalassemia, other hemolytic anemia, bone marrow failure, etc.) apparent by history, physical examination, and/or laboratory tests.</td>
<td>Children with anemia due to other causes would not be expected to respond to iron</td>
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<tr>
<td>7. High likelihood of suboptimal adherence by parents with study requirements (previous missed clinic visits)</td>
<td>Poor adherence will undermine the ability to determine the relative efficacy of the two study medications</td>
</tr>
<tr>
<td>8. Inability to tolerate oral medications</td>
<td>Some children with feeding or swallowing disorders, severe neurological problems and/or inability to easily take liquids are unsuitable candidates</td>
</tr>
<tr>
<td>9. History of birth at &lt; 30 weeks gestation</td>
<td>Prematurity independently increases the risk of iron deficiency and increases the likelihood for medical comorbidities that may influence the response to iron therapy</td>
</tr>
<tr>
<td>10. Other medical or social factors at discretion of treating physician</td>
<td>Unforeseen circumstances that will undermine the ability to determine the relative efficacy of the two study medications</td>
</tr>
</tbody>
</table>

### III. STUDY PROTOCOL

**a. Laboratory definition of iron deficiency anemia:** Please see Appendix I.

**b. Baseline Assessment:**

**i. History and Physical Examination:**

1. Detailed history regarding medical disorders predisposing to iron deficiency and diet (breast feeding, iron fortified formula, whole cow’s milk), including timing and total daily amount as well as additional food items.
2. Review of existing medical records (including blood counts and iron studies) submitted by primary care provider or recorded on Children’s electronic medical record.
3. History of pica, failure to thrive and/or other sequelae from iron deficiency.
4. Details regarding prior or current oral iron therapy (dose, preparation, timing, response) or recent blood transfusions.
5. Comprehensive physical exam (PE) (baseline visit) consisting of vital signs, general appearance, head, eyes, ears, nose throat (HEENT), cardio-respiratory, abdominal, extremities and skin; focused PE (Weeks 4 and 12) consisting of vital signs, general, cardio-respiratory, abdominal and skin.

**ii. Laboratory:**

1. Complete blood count (CBC), reticulocyte count and reticulocyte hemoglobin content.
2. Serum ferritin, iron and total body iron capacity (TIBC).

**c. Treatment Interventions:**

i. Patients who meet eligibility criteria and whose parents provide written informed consent will be randomized in a 1:1 ratio to receive either ferrous sulfate drops (15 mg/ml) or NovaFerrum® drops (15 mg/ml).

ii. Subjects will be assigned to receive a single daily dose of 3 mg/kg elemental iron (Please see Appendix II for study drug dosing.)

iii. Timing of study drug will be at bedtime.

iv. Families will be instructed not to re-dose the medication. (If the child spits up medication after ingesting, families are not to give the medication again. If the medication is spilled on child’s shirt in process of giving, it is acceptable to give a new dose.)

v. As a part of standard care, subjects will be advised to reduce cow milk intake to a maximum 20 oz. daily and not to give any milk after medication administration. Subjects will be made aware of iron rich foods. There will be no other dietary modification prescribed by the study.

vi. Other iron containing medications (including vitamins) will be discontinued.

vii. Families will be asked to complete a daily diary entry documenting administration and adverse effects and return the diary at follow-up clinic visits. The diary will include the subject’s specific dosing, timing of medication and appointment schedule.

**d. Assessment via phone contact at Weeks 2, 6, and 10 after initiation of therapy:**

i. Phone contact with parents will be made biweekly between scheduled visits
ii. Review adverse effects, promote strict adherence, and reminder of next scheduled visit.

e. Assessment During Follow-up Visits at Weeks 4, 8, and 12 after initiation of therapy:

i. Review of interval history and diet by direct questioning of the parents and review of
   diary regarding adherence, adverse effects of iron therapy (e.g., refusal to take,
   spitting/vomiting, abdominal pain, constipation, black stools, stained teeth). The diary
   will also contain distractor items to assess background “noise”.

ii. Recording of other medications or intercurrent illnesses.

iii. Laboratory studies at each follow-up visit: CBC, reticulocyte count, reticulocyte

    hemoglobin content, serum ferritin, serum iron and total iron binding capacity.

iv. Lead level will be checked at week 4.

v. Focused physical exam at 4 and 12 week visits

vi. At 12 week visit, family will be asked if they have been mixing the medication with food

    and/or drink.

IV. MODIFICATIONS

a. RISKS

All study subjects will have documented iron deficiency anemia and will be treated with an oral
iron preparation in the standard dose. Both study medicines, labelled as “supplements’ by the
FDA, are approved for this purpose, and when used appropriately, are without significant risks.
See table below for expected effects.

i. Adverse effects:

<table>
<thead>
<tr>
<th>Severity</th>
<th>Description</th>
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<tbody>
<tr>
<td>Minor</td>
<td>Unpleasant taste (Common)</td>
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<td></td>
<td>Temporary staining of teeth (Common)</td>
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<tr>
<td>Constipation (Common)</td>
<td></td>
</tr>
<tr>
<td>Dark stools (Common)</td>
<td></td>
</tr>
<tr>
<td>Nausea (Common)</td>
<td></td>
</tr>
<tr>
<td>Vomiting (Less common)</td>
<td></td>
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<tr>
<td>Abdominal pain (Less common)</td>
<td></td>
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<tr>
<td>Diarrhea (Less common)</td>
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<tr>
<td>Serious</td>
<td>None</td>
</tr>
</tbody>
</table>

ii. Serious complication: Accidental ingestion of a large amount of an oral iron preparation
by the patient may result in acute iron poisoning which can manifest as severe diarrhea,
adocis, shock, and death. Iron ingestion is one of the most common causes of
accidental drug related death in children. Therefore, study medications will be
administered in a child-proof container, and parents will be given strict instructions to
keep the study medicine in a safe location out of the child’s reach.

iii. Other expected childhood ailments, unrelated to the study treatment, include upper
respiratory infections and other non-specific viral infections, asthma, ear infections and
environmental allergies.

b. BENEFITS

Study subjects may discontinue their participation at any time. However, information regarding
the potential benefits to continued iron treatment will be made explicitly clear prior to their
discontinuation.

i. Whichever iron preparation the child receives, he or she will be taking an approved iron
medication, according to the contemporary care standards, with regard to daily dose
and monitoring.

ii. Subjects will also derive benefit from the regular structured follow-up and careful
monitoring of hematologic parameters and adverse effects as well as interval telephone
calls to promote compliance and answer questions.

iii. Successful treatment of the subjects’ iron deficiency is expected to improve their overall
well being, energy level and color, and possibly correct or prevent any cognitive and
behavioral abnormalities resulting from iron deficiency.
c. Alternative care will be offered to the subjects, which might include less or longer follow-up and fewer laboratory studies.
d. Every effort will be made to retain study subjects in the trial for the entire 12 weeks to enable complete follow-up data collection.

V. ASSESSMENTS OF MEDICATION ADHERENCE
a. Study visit attendance
b. Volume of unused medication returned at each visit
c. Medication administration and documentation in patient diary
d. Estimation by questioning of parent and review of treatment diary regarding number of doses missed or deviations from the protocol-directed dosing.
e. Phone contact with parents will be made biweekly between scheduled visits (e.g. Weeks 2, 6, and 10) to assess adverse effects, promote strict adherence and remind them of the next scheduled visit.

VI. TREATMENT FAILURE
a. Changing the time of iron dosing (i.e. from evening to late afternoon), will be considered protocol deviation and not treatment failure.
b. At the 4 week visit, a lack of response in hemoglobin concentration (hemoglobin rise <0.5 gm/dL) will be addressed with increased emphasis on adherence.
c. At the 8 week visit if the hemoglobin is <0.5 gm/dL above baseline assessment, the subject will be considered a treatment failure and removed from the study. Further treatment will be at discretion of attending hematologist.

VII. WITHDRAWAL/TERMINATION
a. Subjects that 'No Show' for 4, 8, and 12-week visits and are unable to be contacted/rescheduled. Data already collected will be used in the analysis.
b. Stated non-compliance with study drug by the parent
c. Alternative diagnosis discovered
d. In the investigator’s medical opinion, it is best to withdraw the subject from the protocol

VIII. STOPPING RULES
a. If more than one subject has the serious complication of iron poisoning, the study will be discontinued for assessment of child-proof containers and safety.
b. The study will be discontinued if one of the products is recalled by the manufacturer or Food and Drug Administration (FDA).

IX. CONCOMITANT CARE
a. Patients who initially receive RBC transfusion for their anemia prior to enrollment may participate in the trial if all eligibility criteria are met.
b. Subjects who continue to have iron deficiency or IDA upon study completion will receive treatment at discretion of their attending hematologist.

X. OUTCOMES
a. Primary Outcome: The primary outcome will be the change in the peripheral blood hemoglobin concentration in grams/deciliter upon serial measurements at 0, 4, 8, and 12 weeks post-initiation of treatment.
b. Secondary Outcomes:
   i. Proportion of subjects with complete response to treatment
   ii. Change in iron measurements between the study groups
   iii. Side effects of ferrous sulfate vs. NovaFerrum®
   iv. Drop out/lost to follow-up rates.
   v. Resolution of signs and symptoms of iron deficiency (pica, irritability, pallor, lethargy).
   vi. Adherence as measured by attendance to study visits, patient diary and returned medication measurement.
XI. PARTICIPANT TIMELINE

<table>
<thead>
<tr>
<th>Study Procedures</th>
<th>Baseline screening / Consent</th>
<th>2 wks</th>
<th>4 wks*</th>
<th>6 wks</th>
<th>8 wks*</th>
<th>10 wks</th>
<th>12 wks*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screening history for eligibility</td>
<td>x</td>
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<tr>
<td>*Laboratory Studies</td>
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<tr>
<td>**Comprehensive Medical History</td>
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<tr>
<td>*Comprehensive Physical Exam</td>
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<tr>
<td>*Focused Physical Exam</td>
<td>x</td>
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<tr>
<td>Dispense Study Medication</td>
<td>x</td>
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<tr>
<td>Telephone call to assess adherence</td>
<td>x</td>
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<tr>
<td>Adverse Event Review</td>
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<tr>
<td>Collect diaries, review responses, etc</td>
<td>x</td>
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</table>

*Laboratory studies include: CBC, Retic, Retic-He, Ferritin, Iron, TIBC; Lead (at week 4 visit – repeat only if abnormal)
**Medical history includes: prior hospitalizations, laboratory results, medications, dietary and review of systems; social and family history.
*Physical exam: Comprehensive PE consists of vital signs, general, HEENT, cardio-respiratory, abdominal, extremities and skin; Focused PE consists of vital signs, general, cardio-respiratory, abdominal and skin.

XII. SAMPLE SIZE

Power Calculation: Using the trend in hemoglobin concentration over time as the primary outcome, we estimate the trend for subjects on NovaFerrum® and ferrous sulfate. The estimates for baseline mean hemoglobin concentration and standard deviation are derived from unpublished data from Catherine Daniel and George Buchanan, M.D. who analyzed the variability in response to oral iron and presented the data at the 2011 Annual Meeting of the American Society of Hematology (manuscript in preparation). We assume that the ferrous sulfate group will have a mean baseline hemoglobin value of 7.8, and a mean change in hemoglobin of 0.27 gm/dL per week while receiving ferrous sulfate. The standard deviation of hemoglobin measurements is estimated to be 1.5. We assume the NovaFerrum group to have a similar baseline value and standard deviation, but the change in hemoglobin is 0.35 gm/dL per week. The above assumptions imply that at the 12th week the mean hemoglobin values are 11 gm/dL and 12 gm/dL in the ferrous sulfate and NovaFerrum group, respectively. We further assume a within-subject correlation of 0.25. The patient population (predominantly low socioeconomic status, Spanish-speaking) may be prone to drop out, so therefore we will estimate a 25% dropout rate. Using a linear mixed model, by enrolling 40 subjects in each group (80 in total), we can detect a difference of 0.083 in the slope (change in hemoglobin per week) between the two groups with a two-sided type I error of 5% and a power of 80%.

<table>
<thead>
<tr>
<th>ESTIMATES FOR POWER CALCULATION</th>
<th>Baseline</th>
<th>4 weeks</th>
<th>8 weeks</th>
<th>12 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>NovaFerrum®</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Hgb</td>
<td>7.8</td>
<td>9.2</td>
<td>10.6</td>
<td>12</td>
</tr>
<tr>
<td>Standard Dev</td>
<td>1.5</td>
<td>1.5</td>
<td>1.2</td>
<td>1.0</td>
</tr>
<tr>
<td>Ferrous Sulfate</td>
<td></td>
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</tr>
<tr>
<td>Mean Hgb</td>
<td>7.8</td>
<td>8.9</td>
<td>9.9</td>
<td>11</td>
</tr>
<tr>
<td>Standard Dev</td>
<td>1.5</td>
<td>1.5</td>
<td>1.2</td>
<td>1.0</td>
</tr>
</tbody>
</table>

XIII. RECRUITMENT

a. Information about the clinical trial will be provided via direct communication by study investigators with physicians at Children’s Medical Center including ED physicians, hospitalists, and general pediatrics attending on the inpatient medicine services and outpatient continuity clinic as well as house officers. Furthermore, physicians at Parkland COPC clinics, Los Barrios Unidos, MyChildren’s clinics and various private practices will be invited to refer patients for participation in the trial. The Dallas Area Pediatric Society will also be provided information about the clinical trial.
b. Information regarding the trial will be available on the University of Texas Southwestern clinical trials website and at clinicaltrials.gov.

c. Potential subjects will be identified through review of new patient referrals to Children’s Medical Center Dallas, inpatient admissions and/or consultations for iron deficiency anemia.

d. Each subject will receive financial compensation for participation. Payment will be made at completion of the study visits at week 4 ($25), 8 ($25), and 12 ($50) based on attendance and return of study medication at each visit.

e. Expected recruitment rate will be 1-2 participants per week.

f. Duration of recruitment period is expected to be approximately 18 months.

g. Assessment of study recruitment and retention will be performed when 40 participants have been enrolled on the study, or at approximately 9 -12 months.

XIV. ASSIGNMENT OF INTERVENTIONS

a. ALLOCATION

i. Sequence generation: Participants will be stratified by degree of anemia between those with baseline hemoglobin \( \geq 8 \) gm/dL and those with baseline hemoglobin \(< 8 \) gm/dL. Within each stratum, subjects will then be randomly assigned to either experimental group with a 1:1 allocation by a computer generated randomization schedule using permuted blocks of 4.

ii. Concealment mechanism: Allocation concealment will be ensured by an investigational pharmacist and will not take place until the patient has been recruited into the trial, after all baseline measurements have been completed.

iii. Implementation: An investigational pharmacist will perform the sequence generation and allocation concealment mechanism. Trial investigators will perform enrollment and implementation of study group assignments and will not be involved in the allocation process.

b. MASKING

i. Masking: All members of the study team (trial participants, care providers, outcome assessors, data analysts), with exception to the investigational pharmacist, will be masked to treatment allocation.

ii. Emergency unblinding: Given that both study drugs contain the same amount of elemental iron, accidental overdose will be treated the same way regardless of which preparation the subject is taking, and there will be no need to unmask the assigned intervention.

XV. DATA COLLECTION, MANAGEMENT AND ANALYSIS

a. DATA COLLECTION METHODS

Clinical information will be collected from each patient’s electronic medical record at all visits while on study. This information will include the following: name, gender, race, ethnicity, date of birth, current medications, social history, patient medical and hospitalization history; family history, physical exam and laboratory results. Additional information to be collected from self reported diaries include adherence data and adverse reaction data. Returned diaries will be maintained in a research binder, while medical history, lab results, and medications will be recorded in the electronic medical record and then on electronic Case Report Forms (eCRF). Contact phone numbers for parents and relatives will be collected for the interim visit phone calls.

b. DATA MANAGEMENT

The eCRF will be housed in UT Southwestern REDCap. REDCap is a self-managed, secure, web-based data support system. The data is backed up offsite nightly and hosted in a secure environment maintained by Information Resources. This password protected study database will include a subject’s personal identifiers and all Protected Health Information (PHI) such as medical history. All personnel who will be accessing the data will be trained in REDCap and have individual user ID and passwords. Subjects will have a study ID number that will be utilized in lieu of personally identifiable information for all research data provided to statisticians.
c. STATISTICAL METHODS

The primary hypothesis to be tested is that, compared to ferrous sulfate, NovaFerrum® produces a superior hematologic response in infants and young children with nutritional IDA. The primary outcome will be the serial change in hemoglobin compared to baseline. The anticipated magnitude and variability for the primary outcome is detailed in the power calculation (see section IX). The primary analysis will consist of a linear mixed regression model with the interaction term of treatment and time as the primary predictor variable in the model.

\[ y_{ij} = \beta_0 + \beta_1 x_i + \beta_2 t_j + \beta_3 x_i t_j + e_{ij} \]

In the above formula, we use \( y_{ij} \) to denote the measurement of outcome from patient \( i \) at time \( t_j \), \( x_i \) the indicator of treatment (0 for ferrous sulfate and 1 for NovaFerrum®) for each patient, and \( e_{ij} \) the residual effect which is assumed to be correlated within but independent between subjects. Our primary interest is to test the null hypothesis \( H_0: \beta_3 = 0 \), the rejection of which suggests that subjects receiving NovaFerrum® would have a different slope.

Of the secondary outcomes, the proportion of subjects with a complete response, the proportion of subjects with various side effects, and the proportion of dropouts will be compared by the \( \chi^2 \) test. The serial change in non-hemoglobin laboratory measures of response (e.g., ferritin, reticulocyte count) to iron deficiency will be modeled with the same linear mixed regression method as the primary outcome (change in hemoglobin). As secondary analyses, age, ethnicity, primary language, source of primary care, distance from Children’s Medical Center Dallas will be included in a multivariable mixed linear regression model of serial change in hemoglobin and other laboratory parameters of IDA. A time-to-event analysis will compare the resolution of all signs/symptoms of IDA between NovaFerrum® and ferrous sulfate groups. For adherence, the proportion of missed doses according to the patient’s medication diary and the proportion of missed study visits will be compared between groups with the \( \chi^2 \) test. The % volume of study medication returned at each visit will be compared using the Wilcoxon Rank Sum test.

As for dropouts: The generalized estimation equation approach (GEE) will be employed to estimate model parameters from the linear mixed model, which accommodate incomplete data and is robust against deviation from the normality assumption.

For protocol deviation: The efficacy analysis will be conducted on the intention-to-treat (ITT) population, which groups subjects based on their treatment assigned at randomization.

XVI. MONITORING

After 15 subjects are enrolled, local hospital compliance personnel will audit pharmacy and consenting procedures for proper conduct and documentation. Additional audits may occur without warning as warranted by the initial review or spontaneously.

ETHICS AND DISSEMINATION

I. RESEARCH ETHICS APPROVAL

This protocol and template informed consent forms will be reviewed and approved by the sponsor and UT Southwestern IRB with respect to scientific content and compliance with applicable research and human subjects regulations. Subsequent to initial review and approval, the investigators will make safety and progress reports to the IRB at least annually and within three months of study completion.

II. PROTOCOL AMMENDMENTS

Any modifications to the protocol, which impact the conduct of the study, or potential risk/benefit of the participants, will require a formal amendment to the protocol with IRB approval.
III. CONSENT OR ASSENT
Informed consent will be obtained after information has been provided to patients’ families along with a discussion of the risks and benefits along with opportunity for questions. Consent forms will be provided for all parents involved in the trial.

IV. CONFIDENTIALITY
Records of each patient’s participation in this study, including the original informed consent document, will be kept in a locked file cabinet in the Center for Cancer and Blood Disorders at Children’s Medical Center Dallas. Access to local research files is limited to treating physicians and nurses, data management personnel and the Institutional Review Board. These entities may need to view data for quality assurance and data management purposes. Confidentiality of all medical records would be maintained by correct identification of the appropriate person before viewing the medical record and ascertaining the reason for the viewing of the medical records.

V. DECLARATION OF INTERESTS
Jacquelyn Powers, MD, No financial or other competing interests.
George Buchanan, MD, No financial or other competing interests.
Timothy McCavit, MD, Salary support from Gensavis Pharmaceuticals.
Leah Adix, CCRP, No financial or other competing interests.
Jennifer Evans, CPNP, No financial or other competing interests.

Gensavis Pharmaceuticals, LLC, manufacturers of NovaFerrum®, is funding the entire conduct of this study including partial salary support for Timothy McCavit, MD. The above authors have independently written this protocol without input from Gensavis Pharmaceuticals, LLC and will analyze all data without influence from this sponsor.

VI. ACCESS TO DATA
All trial investigators will be given direct access to the data sets.

VII. ANCILLARY AND POST TRIAL CARE
Participants who continue to have iron deficiency upon study completion will receive treatment at discretion of their provider.

VIII. DISSEMINATION POLICY
Every attempt will be made to reduce the interval between completion of data collection and the release of the study results. We expect to take about 3 to 4 months to compile the final results and submit them for presentation at a prominent society meeting and publication in a high profile peer reviewed journal. The study results will also be released to the participating physicians, referring physicians, subjects and the general medical community.
REFERENCES


APPENDICES

Appendix 1 - Laboratory Definitions of Iron Deficiency Anemia and Complete Response at Study Exit

<table>
<thead>
<tr>
<th>Laboratory Tests Performed at Each Study Visit</th>
<th>Definition of Iron Deficiency Anemia (week 0)</th>
<th>Definition of Complete Response at Study Exit (week 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Hemoglobin concentration</td>
<td>≤ 10 gm/dl</td>
<td>≥ 11.0 gm/dl</td>
</tr>
<tr>
<td>*Mean cell volume (MCV)</td>
<td>≤ 70 fl</td>
<td>&gt; 70 fl</td>
</tr>
<tr>
<td>*Reticulocyte hemoglobin content (cHR or Retic-He)</td>
<td>≤ 25 pg</td>
<td>&gt; 28 pg</td>
</tr>
<tr>
<td>+Serum ferritin</td>
<td>≤ 15 ng/ml</td>
<td>&gt; 15 ng/ml</td>
</tr>
<tr>
<td>+Total iron binding capacity (TIBC)</td>
<td>≥ 425 µg/dL</td>
<td>&lt; 425 µg/dL</td>
</tr>
</tbody>
</table>

*Required for study eligibility
+Low ferritin AND/OR elevated TIBC required for study eligibility

Appendix 2 - Study Drug Dosing

<table>
<thead>
<tr>
<th>Patient's Weight (kg)</th>
<th>Daily Iron Dose (mg)</th>
<th>Daily Volume of Medication (ml)</th>
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<tbody>
<tr>
<td>5</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>7.5</td>
<td>22.5</td>
<td>1.5</td>
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<tr>
<td>10</td>
<td>30</td>
<td>2</td>
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<td>12.5</td>
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<tr>
<td>15</td>
<td>45</td>
<td>3</td>
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<td>75</td>
<td>5</td>
</tr>
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<tr>
<td>30</td>
<td>90</td>
<td>6</td>
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