Protocol for the Heart Failure Clinical Research Network

Oral Iron Repletion effects ON Oxygen UpTake in Heart Failure: **IRONOUT-HF**

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1.1 List of Abbreviations

Abbreviation	Definition
6MWD	6-minute walk distance
6MWT	6-minute walk test
ACC	American College of Cardiology
AE	Adverse event
AHA	American Heart Association
ARB	Angiotensin receptor blocker
BB	Beta blocker
CC	Coordinating Center
CI	Cardiac index
CO	Cardiac output
COPD	Chronic obstructive pulmonary disease
DCRI	Duke Clinical Research Institute
ECG	Electrocardiogram
eCRF	Electronic case report form
EDC	Electronic data capture
EF	Ejection fraction
Fe	Iron
HF	Heart failure
HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
HFN	Heart Failure Clinical Research Network
HHS	Department of Health and Human Services
HR	Heart rate
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ITT	Intention to treat
KCCQ	Kansas City Cardiomyopathy Questionnaire
LV	Left ventricular
NT-proBNP	N-terminal pro-B-type natriuretic peptide
NHLBI	National Heart, Lung, and Blood Institute
NYHA	New York Heart Association
PE	Physical examination
QOL	Quality of life
RAAS	Renin-angiotensin-aldosterone system
RCT	Randomized clinical trial
ROS	Reactive oxygen species
SAE	Serious adverse event
SAR	Suspected adverse reaction
SBP	Systolic blood pressure
SV	Stroke volume
VO_2	Volume of oxygen

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2 EXECUTIVE SUMMARY

Title	**IRONOUT-HF**: Oral **I**ron **R**epletion effects **ON O**xygen **U**p**T**ake in **H**eart **F**ailure
Indication	Chronic NYHA class II-IV heart failure with reduced left ventricular ejection fraction (LVEF ≤0.40) and iron deficiency (with or without anemia).
Location	Approximately 20 clinical centers in the United States and Canada
Brief Rationale	Therapeutic options to further improve functional capacity and symptoms in HF beyond neurohormonal antagonism are limited. Studies have demonstrated impaired oxidative capacity of skeletal muscle among HF patients, which may contribute to symptoms of breathlessness and persistent fatigue. In addition to its role in erythropoiesis, iron (Fe) plays a critical role in skeletal muscle's oxygen (O_2)-storage capacity (myoglobin) and systemic aerobic energy production. As Fe deficiency is common in patients with symptomatic HF, repletion of iron stores may improve submaximal exercise capacity among these patients beyond the effects on erythropoiesis. While intravenous Fe repletion in HF patients with mild Fe-deficiency (i.e. Ferritin <100 or Ferritin 100-299 with transferrin saturation <20%) with or without anemia global well-being and functional status, *oral* Fe repletion has not been studied. Furthermore, the efficacy of *oral* Fe to replete iron stores in a similar population and its impact on functional capacity, measured objectively by peak VO_2, remains unknown.
Primary Objective	Multi-center, randomized, double-blind, placebo-controlled study for 16 weeks duration. To determine if oral iron polysaccharide is superior to oral placebo in improving the peak VO_2as assessed by cardiopulmonary exercise testing (CPET) of a broad population patients with HFrEF and Fe deficiency at 16 weeks. Approximately 20 clinical centers in the United States and Canada.
Secondary Objectives	To determine the impact of oral Fe repletion on: a. Submaximal exercise capacity: O_2 uptake kinetics and ventilatory efficiency as measured by CPET and 6 minute walk distance b. Plasma NT-proBNP c. Health status: KCC Questionnaire (KCCQ)
Study Design	
Treatment	Twice-daily oral polysaccharide iron complex 150 mg versus matching oral placebo for 16 weeks.
Primary Endpoint	Peak VO_2 (ml/min)

Secondary Endpoints	1. O_2 uptake kinetics and ventilatory efficiency as assessed by CPET 2. 6 Minute walk distance 3. Plasma NT-proBNP level 4. KCCQ quality of life score

3 INTRODUCTION

3.1 Background and Significance

Current Paradigm for HF Treatment.
Understanding the critical role of neurohormonal activation in heart failure (HF) has led to the development of life-saving treatments including ACE inhibitors, angiotensin receptor blockers, aldosterone antagonists, and β-blockers.[2, 3] However, efforts to further improve functional capacity and symptoms in HF by intensifying neurohormonal antagonism have not been successful.[4,5] Novel approaches beyond neurohormonal antagonism must be pursued.

The Influence of the Periphery on Exercise Capacity in HF
Limitation in exercise capacity is a cardinal manifestation of HF that is closely related to poor quality of life and mortality.[6, 7] Neither abnormal central hemodynamics nor the degree of LVSD[8] adequately explain the early onset of anaerobic metabolism and impaired peak VO_2 in HF.[8-10] HF patients are also limited by abnormal skeletal muscle oxygenation and impaired oxidative capacity. Morphologic and histochemical changes in skeletal muscle include a shift to type II fibers[11] and a reduction in oxidative enzymes,[11] which contribute to an early transition from oxidative to glycolytic metabolism. Impaired skeletal muscle oxidative metabolism[12] and glycolytic end products mediate exaggerated stimuli to ventilation through ergoreflex signaling.[13] These findings have led to the "muscle hypothesis" as a dominant mechanism to explain breathlessness and persistent fatigue in HF.[9, 14]

The Central Role of Fe in O_2 Delivery and Utilization.
Fe plays a critical role in systemic O_2 delivery and utilization (**Table 2.1**).[15-18] Fe also contributes to erythropoiesis and is an obligate component of other enzymes critical in vascular homeostasis and cardiac function. Skeletal muscle dysfunction in iron deficiency mirrors skeletal muscle abnormalities in HF.[18, 19] Iron deficiency decreases O_2-carrying capacity of both blood (reduced hemoglobin) and skeletal muscle (reduced myoglobin) and attenuates mitochondrial O_2 utilization (reduced aerobic enzyme activity)(**Fig 2.1**).[17-19]

The observed functional benefits in studies of iron repletion summarized below are likely multifactorial. Elegant animal studies have suggested that during Fe repletion, improvements in hemoglobin levels and peak VO_2 evolve in parallel, whereas enhancements in endurance capacity track with the increase in aerobic enzyme activity.[17, 18, 20] If iron repletion is shown to improve exercise capacity in HF patients *beyond its effects on erythropoiesis,* it will profoundly impact the treatment of HF.

Table 2.1. Fe Containing Proteins that are Altered in HF

Name of Protein	Functional Site	Status in HF	Biological Functions
Hemoglobin	Red blood cell	↓	O_2 transport
Myoglobin	Cytoplasm of muscle cells	↓	Facilitation of O_2 transport
Oxidative enzymes	Mitochondrial inner membrane	↓	Substrate oxidation→NADH, $FADH_2$
Respiratory chain proteins	Mitochondrial inner membrane	↓	Electron transfer of O_2→NADH, $FADH_2$
Soluble Guanylate Cyclase	Vascular smooth muscle cells, cardiomyocytes	↓	Nitric oxidestimulation of cGMP synthesis

NADH indicates the reduced form of nicotinamide adenine dinucleotide; FADH2 indicates the reduced form of flavin adenine dinucleotide; cGMP indicates cyclic guanosine monophosphate.

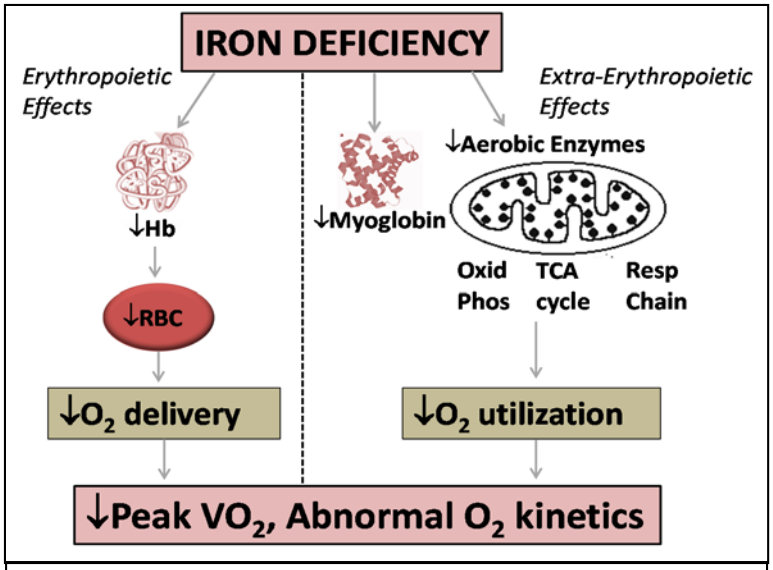


Fig 2.1 FeDef has erythropoietic and extra-erythropoietic effects on O_2 delivery and utilization.

3.2 Preliminary Studies

Epidemiology and Pathophysiology

The prevalence of Fe deficiency in HF is estimated to be 38-50%.[21] Anemia is also present in more than one third of HF patients and is associated with increased LV mass, more re-hospitalization, and higher mortality.[22-24] Among HF patients with anemia, the majority (71%) have depleted Fe stores as measured by bone marrow aspiration.[25]

Studies in animals and humans without HF have demonstrated that Fe deficiency anemia reduces indices of work capacity (i.e. peak VO_2) by 10-50%.[18, 26, 27] The correction of Fe deficiency in both anemic and non-anemic patients without HF improves symptoms, quality of life, and exercise performance.[18] Improvements in PGA, quality of life and 6MWD were apparent within 4 weeks of initiation of Fe repletion and were sustained for at least 24 weeks. Beyond the recognized influence of Fe deficiency on erythropoiesis and O_2 utilization in the periphery, in animal models, Fe deficiency has also been associated with direct myocardial effects as described below.[28]

Direct myocardial effects of iron repletion

Beyond the recognized influence of iron deficiency on erythropoiesis and O_2 storage and utilization in skeletal muscle, iron deficiency has also been associated with ultrastructural changes in cardiomyocytes such as mitochondrial swelling and abnormal sarcomere structure.[28] Heart failure patients have lower myocardial iron content and transferrin receptor concentrations than controls,[32] and severe iron deficiency leads to LV systolic dysfunction.[33, 34]

The influence of iron repletion (IV only) on echocardiography-derived LVEF in patients with chronic HFrEF has been reported in 3 small studies and two meta-analyses. Two of the studies[35, 36]and both meta-analyses,[37, 38] reported significant improvement in LVEF with iron supplementation (mean improvement +5%). Separation of ΔLVEF in the treatment vs. placebo groups in Toblli et al was evident at 3 months into the trial. A third study by Okonko et al.[31] demonstrated no significant difference between IV iron repletion and placebo on change in LVEF. In another recent single-center study,[39] a large, detectable improvement in echo-derived

LV strain rate was reported after 3 months of IV iron supplementation in 40 patients. LV strain has a proven association with HF outcomes.[40, 41] These echocardiographic findings reinforce the hypothesis that iron repletion may directly improve cardiac function in addition to promoting improved oxygen delivery and utilization.

Trials of Intravenous Fe repletion: Studies of iron repletion in humans with HF are summarized in **Table 2.2.** Two small single-center studies of intravenous Fe repletion demonstrated improvement in Fe stores and functional capacity (**Table 2.2**). The one multicenter trial of intravenous Fe repletion (FAIR-HF), conducted in HF patients with mild Fe-deficiency (i.e. Ferritin <100 or Ferritin 100-299 with transferrin saturation <20%) with or without anemia, demonstrated improvement in co-primary endpoints of *subjective* patient global assessment and NYHA class.[1] However, 8-12 weekly injections of intravenous iron (200mg) were required to achieve Fe repletion, followed by monthly injections during the maintenance phase of this trial. Intravenous iron infusions are expensive (~$4,000/injection for the least expensive preparation, iron sucrose 200mg, based on cost estimates from one HFN CC) and pose logistical challenges for outpatients based on the frequency of required visits and lack of infrastructure within HF clinics to give IV iron infusions. While a higher-dose IV iron preparation is now available (Ferumoxytol [Feraheme], 510 mg elemental iron), this preparation has been associated with hypersensitivity reactions in 3.7% of patients, with anaphylaxis, cardiac arrest, and hypotension being among the observed side-effects.

Rationale for Oral Fe Repletion: Oral Fe is safe, inexpensive and readily available in over-the-counter preparations. However, to date there have been no studies of oral Fe repletion to treat Fe deficiency anemia in patients with HF. A single study suggested that oral Fe repletion improved Fe stores and exercise capacityin patients with congenital heart disease.[29] In non-HF populations, IV and oral Fe repletion have resulted in similar sustained increases in circulating ferritin levels, transferrin saturations, and hemoglobin concentrations.[30] Of note both oral and IV Fe repletion studies demonstrating benefits from Fe repletion have been ≤ 16 weeks in duration, with the exception of FAIR-HF, in which the trial duration was 24 weeks but clinical benefits were apparent by 4 weeks. However, the efficacy of *oral* Fe to replete Fe stores in a similar population to FAIR-HF and its impact on functional capacity, measured objectively by peak VO_2, remains unknown. Furthermore, the effects of oral Fe repletion on LV function and prognostic biomarkers, as well as the role of regulatory molecules, such as hepcidin, in modulating response to oral Fe repletion warrant further investigation.

Table 2.2 Previous studies of Fe repletion in heart failure

Drug	Authors/ Journal	Subjects Studied	Fe-Def Definition	N	Time	Primary Endpoint	Findings
IV Fe Sucrose	Tobilli *JACC 2007*	NYHA 3-4 LVEF<0.35	Ferritin <100 ng/ml and/or Tsat<20%	40	5 wks	Δ Hb, Tsat, Cr Clearance	↑ PGAS, ↑6MWD ↓ NT-BNP, ↑LVEF
IV Fe Sucrose	Okonko *JACC 2008*[31]	NYHA 2-3 LVEF<0.35	Ferritin <100 ng/ml or 100-300 with Tsat<20%	35	16wks	Δ peak VO_2	↑ PGAS, ↓ NYHA ↑ Peak VO_2αΔ Tsat
IV Fe Carboxym	Anker *NEJM 2009*	NYHA 2-3 LVEF<0.4 Hb 9.5-13.5	Ferritin <100 ng/ml or 100-300 with Tsat<20%	459	24wks	Δ Global Assessment Score	↑ PGAS, ↓NYHA class ↑6MWD Similar benefit in Hb<12 vs. Hb>12

Oral Fe Fumarate	Taylor Int J Card 2010	Congenital Heart Disease	Ferritin <30 ng/ml or Ferritin<50 w/Tsat<15%	25	12wks	∆6MWT, VO2, QOL	↑ 6MWT, ↑ QOL, ↑ VO_2 (trend)

IV indicates intravenous, PGAS patient global assessment scale; 6MWD six minute walk distance; Hb hemoglobin, Tsat transferrin saturation; QOL quality of life

4 OBJECTIVES AND HYPOTHESIS

4.1 Primary Objectives

To determine if oral Fe polysaccharide is superior to oral placebo in improving functional capacity as measured by change in peak VO_2 by CPET, of a broad population of patients with HFrEF and Fe deficiency at 16 weeks.

Hypothesis: In a broad population of HFrEF patients with Fe deficiency, compared to oral placebo, therapy with oral Fe polysaccharide will be associated with improvement in functional capacity at 16 weeks as assessed by CPET.

4.2 Secondary Objectives

1. To determine the impact of oral Fe repletion on:
   a. Submaximal exercise capacity: O_2 uptake kinetics and ventilatory efficiency as measured by CPET and 6MWD
   b. Plasma NT-pro BNP
   c. Health Status: KCC Questionnaire (KCCQ)

4.3 Exploratory Objectives

1. To determine if the following subgroups of patients may derive differential benefit from oral Fe polysaccharide:
   1) Patients with or without anemia. Anemia is defined as hemoglobin <12 g/dl.
   2) Patients with or without venous congestion, based on the following criteria:
      a) JVP>10cm
      b) Lower extremity edema
   3) Patients with and without an RER>1.1 during maximum incremental exercise
2. To determine change in renal function (creatinine, cystatin C) with iron polysaccharide vs. placebo
3. To determine if Fe repletion impacts markers of the RV-PV unit reserve capacity (i.e. VE/VCO2 slope) as assessed on CPET.
4. To determine if oral iron repletion influences clinical outcomes: time to death and HF hospitalization

5 BASIC STUDY DESIGN

IRONOUT-HF is a multi-center, randomized, double-blinded, placebo-controlled superiority trial of oral Fe polysaccharide compared to matching placebo with the primary endpoint of change in

peak VO_2 measured by CPET at 16 weeks.

5.1 Screening Phase

Screening will be conducted in outpatients with chronic symptomatic HFrEF. Pre-screening informed consent may be obtained from prospective participants in order to draw blood and measure Fe levels. Willing participants who are found to have Fe deficiency and meet other entry criteria will be consented for the trial.

5.2 Baseline Evaluation Phase and Randomization

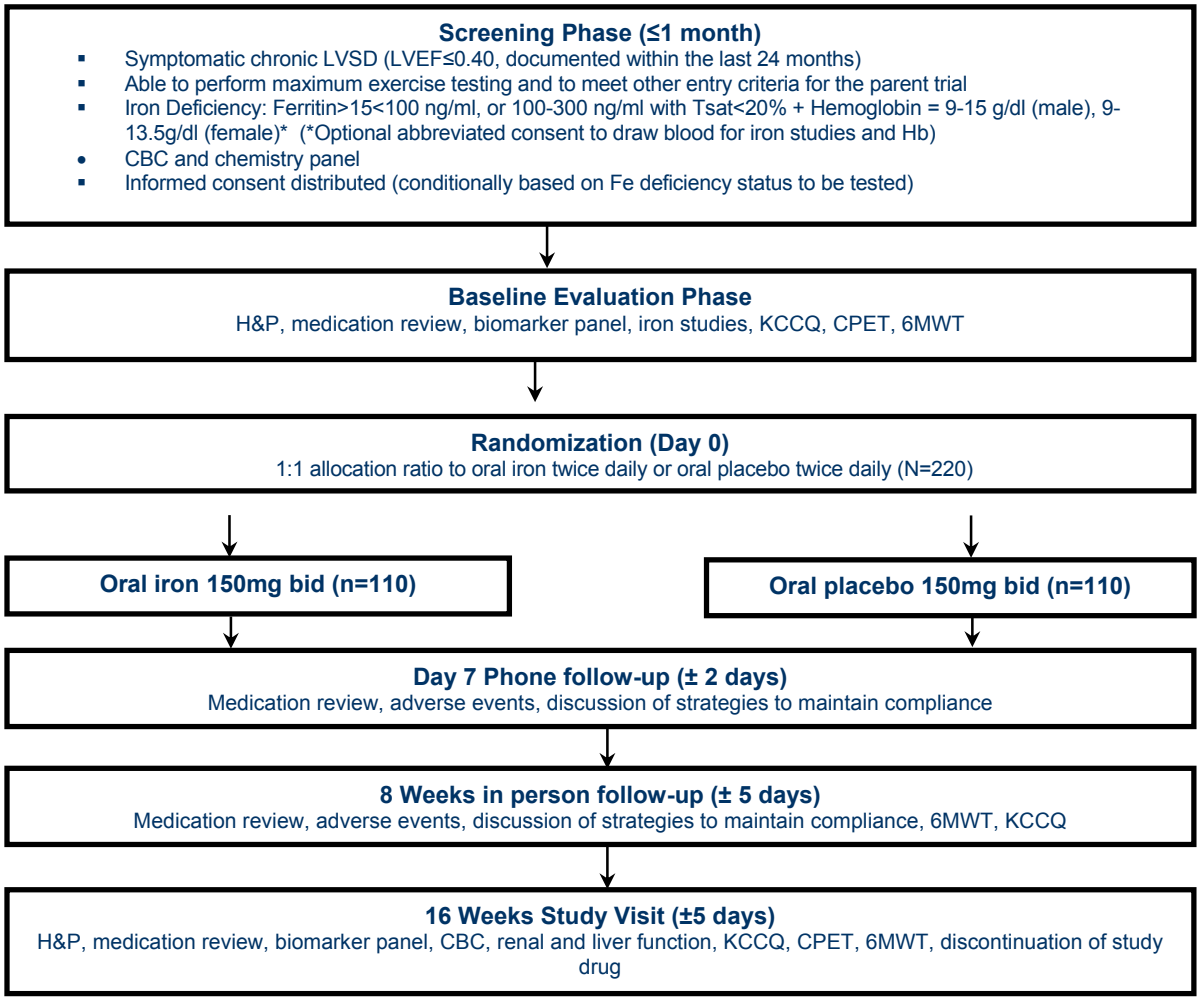
After providing written informed consent, research participants will complete all baseline procedures, including: clinical evaluation, blood samples, and CPET. Participants who fulfill all the inclusion criteria and none of the exclusion criteria will be randomized. Eligible participants will be randomized with a 1:1 allocation ratio to either oral Fe polysaccharide 150mg twice daily or matching placebo.

During randomization visit, participants will be counseled on optimal study drug administration (after 2 hours of fasting when possible while avoiding concomitant ingestion of proton pump inhibitors, H_2 blockers, dairy products or calcium supplements). Arrangements will be made for an initial phone call follow-up 7 days after randomization. Participants will receive a written description of the study with contact numbers of study staff to present to their usual care provider(s).

5.3 Follow-up Phase

Randomized participants are followed carefully by study staff during the 16 week study with visits performed as outlined in Appendix A. Phone follow-up will occur at day 7. The 8 week and 16-week follow-up visits will be conducted in person.

6 STUDY FLOW DIAGRAM



7 STUDY POPULATION AND ELIGIBILITY CRITERIA

7.1 Study Population

Patients with chronic NYHA class II-IV heart failure who have reduced left ventricular ejection fraction (LVEF ≤0.40) and Fe deficiency (with or without anemia) will be suitable for this study.

7.2 Inclusion Criteria

1. Age ≥18 years
2. Previous clinical diagnosis of heart failure with current NYHA Class II-IV symptoms LVEF≤0.40 within 2 years prior to consent, and ≥3 months after a major change in cardiac status (i.e. CABG or CRT).
3. Serum ferritin between 15-100 ng/ml or serum ferritin between 100-299 ng/ml with transferrin saturation <20%
4. Hemoglobin 9.0-15.0 g/dL (males), 9.0-13.5 (females) at time of enrollment
5. Evidence-based medical therapy for HF (including beta-blocker and ACE-inhibitor/ARB unless previously deemed intolerant and diuretics as necessary) with ≤100% change in dose for 30 days prior to randomization
    a. Changes in diuretic dose guided by a patient-directed flexible dosing program are considered stable medical therapy
6. Willingness to provide informed consent

7.3 Exclusion Criteria

1. Presence of a neuromuscular, orthopedic or other non-cardiac condition that prevents the patient from exercise testing on a cycle/treadmill ergometer and/or inability to achieve an RER ≥ 1.0 on screening/baseline CPET
2. Severe renal dysfunction (eGFR< 20 ml/min/1.73m^2)
3. Severe liver disease (ALT or AST > 3x normal, alkaline phosphatase or bilirubin >2x normal)
4. Gastrointestinal conditions known to impair Fe absorption (i.e. inflammatory bowel disease)
5. Known active infection as defined by current use of oral or intravenous antimicrobial agents
6. Documented active gastrointestinal bleeding
7. Active malignancy other than non-melanoma skin cancers
8. Anemia with known cause other than Fe deficiency or chronic disease
9. Fe overload disorders (i.e. hemochromatosis or hemosiderosis)
10. History of erythropoietin, IV or oral Fe therapy, or blood transfusion in previous 3 months.
11. Current ventricular assist device
12. Anticipated cardiac transplantation within the next 4 months
13. Primary hypertrophic cardiomyopathy, infiltrative cardiomyopathy, acute myocarditis, constrictive pericarditis or tamponade
14. Previous adverse reaction to study drug or other oral Fe preparation
15. Known or anticipated pregnancy in the next 4 months

8 TREATMENT INTERVENTIONS

8.1 Intervention

Polysaccharide Iron Complex 150 mg or placebo will be administered twice daily. Pills should be taken separately from meals (1 hour prior to or 2 or more hours after meals). Subjects should avoid taking antacids, dairy products, tea, or coffee within 2 hours before or after this medication because they will decrease its effectiveness. Drug administration with orange juice or other products rich in Vitamin C may enhance absorption and therefore will be encouraged.

Polysaccharide Iron Complex 150mg capsules are a highly water soluble complex of Fe and a low molecular weight polysaccharide. Fe polysaccharide is considered to be a dietary supplement, and it has a human over-the-counter drug label, it is not an FDA approved drug. Polysaccharide Iron Complex is relatively nontoxic, thus permitting a higher therapeutic dosage (150 - 300 mg elemental Fe daily) than other Fe preparations. There is no staining of teeth and no metallic aftertaste.

Permitted dose adjustment: For all participants, Fe polysaccharide should be initiated with a dose of 150mg twice daily. At each of the follow-up visits, the dose can be reduced to once daily if required for participant tolerability. If a dose is missed, the twice daily regimen should be resumed with the next scheduled dose. An extra dose or increase in dose should not be taken to make up for the missed dose.

8.2 Potential Side Effects and Risk Reduction Plan

Polysaccharide Fe preparations are well tolerated. However, side effects may include constipation, diarrhea, nausea, vomiting, dark stools, and abdominal pain. These effects are usually transient.

8.3 Drug Dispensing

Drug dispensing will be managed by the CC in collaboration with the contracted drug supply vendor. At the baseline visit, participants will receive a sufficient supply of study drug to permit twice daily dosing throughout the study.

Patients will be instructed to take the medication as required by the protocol.

8.4 Drug storage

The study drug should be stored at controlled room temperature 15°-30°C (59°-86°F). Temperature excursions are to be reported immediately to the CC. Study drug will be dispensed in a light-resistant container. Excessive moisture should be avoided. Study drug must be kept out of reach of children.

8.5 Drug accountability

Participants are instructed to return all used, partly used and unused trial product (study drug and packaging material) at each study visit. Returned trial product(s) must be stored separately from the non-allocated trial product(s) until drug accountability has been reconciled. The Investigators will be responsible for monitoring all received, used, partly used and unused trial product(s).

8.6 Drug Destruction

Used and unused study drug can be destroyed at the site according to accepted pharmacy practice and both local and national guidelines, using the site's destruction procedure. A copy of the drug destruction procedure should be maintained in the pharmacy section of the Regulatory Binder.

Study drug destruction should be documented in the comments section of the Subject Specific Drug Accountability Log.

8.7 Randomization, Stratification and Blinding

Randomization will be stratified by anemia status (anemia is defined as hemoglobin <12g/dL). A permuted block randomization method stratified by site will be used to ensure relatively equal distribution of subjects to each arm within each clinical site. Subjects will be randomized using procedures determined by the Coordinating Center (CC).

Blinding of the study, with respect to treatment groups will be preserved by the use of over-encapsulation of Fe polysaccharide capsules. The Investigator may be asked at the end of the trial if he/she had obtained any information that may have led to the potential unblinding of treatment

8.8 Unblinding

Given the safety profile of Fe polysaccharide, it is anticipated that there should be no need to un-blind the study drug for any reason. Any suspected study drug-related events should be treated as though the patient received active therapy.

Unblinding should be a very rare occurrence. The potential physiologic actions of the therapy are well characterized. The investigative sites will be given access to the treatment code for their participants for emergency unblinding only by calling the CC. In the rare event of necessary unblinding, the CC Medical Monitor must be contacted to discuss the case.

Randomization data are kept strictly confidential, accessible only to authorized persons, until the time of un-blinding.

8.9 Concomitant Medication

Study participants should be treated with standard HF therapies as per recommended guidelines. Medications should be adjusted during and after a hospitalization as dictated by the guidelines including attempted up-titration of neurohormonal antagonists if not at goal or maximally tolerated doses. Adjustment of diuretics should be performed as appropriate for volume status. All research participants are not allowed to consume open-label prescription or over the counter (OTC) Fe supplementation (oral or IV) during the course of the study.

9 RECRUITMENT AND SCREENING PROCEDURES

9.1 Common Recruitment Procedures

All individuals followed at participating Heart Failure Network (HFN) centers with chronic, symptomatic HFrEF who are thought to be able to perform maximum incremental exercise testing may be considered for recruitment.

Potentially eligible research participants will be identified by investigators reviewing the patients' medical records. The primary physician will be contacted with full explanation of the protocol and consultation regarding the suitability of the patient. If the primary physician agrees, patients will be approached for participation of the study and to undergo further screening evaluations.

9.2 Pre-screening

The recruitment strategy to ensure effective screening of potential research participants is to identify patients with Fe deficiency among the HFrEF patients population. This can be accomplished by reviewing clinically available Fe studies performed during routine clinical practice. In addition, a pre-screening informed consent may be obtained from prospective participants in order to draw blood and measure Fe levels, and hematology labs. Individuals who agree to undergo screening evaluations who are found to meet inclusion criteria for Fe deficiency will be approached to consider participation in the IRON-OUT study.

9.3 Screening Phase (≤ 1 month)

Suitable candidates will be approached by investigators regarding the study and be provided with detailed study information and a copy of the informed consent form. Informed consent will be obtained from eligible candidate to undergo further evaluations and potential enrollment as required by the local institutional review boards. There can be no changes in the protocol without the prior agreement of the Heart Failure Network (HFN) Steering Committee.

Screening procedures include the following:

- Written informed consent for the study protocol. (Section 17.3)
- Complete medical history:
    - HF etiology, duration, and severity

    - NYHA class assessment
    - History of bleeding, Fe exposure or erythropoietin stimulating agents
    - Medication review
    - ~~Other exposures within the time-frames specified in Figure 10.1~~
- Physical examination
- Documentation of LVEF ≤0.40:
    - Clinically available echocardiography within 24 months of informed consent may be used to qualify the patient for study inclusion.
    - However, if the patient had an intercurrent cardiac procedure that may alter the LVEF significantly (e.g. CRT) since the last echocardiography, the LVEF assessment used for inclusion must be obtained at least 3 months since the procedure.
- Phlebotomy for: (processed at local laboratory)
    - Fe studies* (Fe, ferritin, transferrin/TIBC, transferrin saturation)
    - Complete blood count*
    - Complete chemistry panel* (sodium, potassium, chloride, carbon dioxide, BUN, creatinine, glucose, ALT, AST, alkaline phosphatase and total bilirubin)

**Clinically available or pre-screening laboratory studies*

9.4 Estimated Enrollment Period

This study will enroll approximately 220 participants at approximately 20 clinical centers in the U.S. and Canada. It is projected that 16 patients per month will be enrolled, for a total anticipated enrollment period of approximately 14 months.

10 BASELINE EVALUATION AND RANDOMIZATION VISIT

All participants who completed all screening assessments and have not met any exclusion criteria will undergo baseline evaluation and subsequent randomization.

A complete schedule of assessments throughout the study is provided in Appendix A.

10.1 Baseline Evaluation Phase (≤ 1 month)

Baseline assessments include:

- Clinical evaluation:
    - Interim history
    - NYHA class assessment
    - Medication review
    - Physical examination
- Phlebotomy for core laboratory blood samples:
    - Fe studies
    - Fe bioavailability markers
    - Biomarkers
    - HFN Bio-repository ± HFN Genetic samples (if consented)

- Cardiopulmonary Exercise Test (CPET)
- 6MWT
- Kansas City Cardiomyopathy Questionnaire (KCCQ)

10.2 Randomization (Day 0)

All eligible study participants who successfully completed all screening and baseline evaluations will be randomized within 30 days of informed consent using procedures determined by the CC. Allocated study medication kits will be dispensed to the patient as detailed in Section 8.3.

All randomized participants will receive a written description of the study with contact numbers of study staff to present to their provider(s). Arrangements will be made for an initial phone call within 7 days of starting study medication.

11 FOLLOW-UP EVALUATIONS

11.1 Day 7 Phone Follow-up (± 2 days)

Participants will receive a phone call from study staff at Day 7 to monitor compliance and tolerance to study medication. Specific queries regarding gastrointestinal symptoms will be included. Study staff will be discouraged from specifically asking questions regarding stool discoloration. Phone scripts will be provided by the Coordinating Center.

11.2 Week 8 Clinic Visit (± 5 days)

Participants will be seen in clinic and will undergo the following study procedures:

- Clinical evaluation:
    - Interim history
    - NYHA class assessment
    - Medication review and study medication adherence assessment
    - Physical examination
    - 6MWT
- Kansas City Cardiomyopathy Questionnaire (KCCQ)
- Dispense study medication kits

11.3 Week 16 Clinic Visit (± 5 days)

Participants will be seen in clinic and will undergo the following study procedures:

- Clinical evaluation:
    - Interim history
    - NYHA class assessment
    - Medication review and study medication adherence assessment
    - Physical examination
- Phlebotomy for core laboratory blood samples:

    - Fe studies and hemoglobin
    - Fe bioavailability markers
    - Biomarkers
    - HFN Bio-repository ± HFN Genetic samples (if consented)
- Phlebotomy for local laboratory blood samples:
    - Complete blood count
    - Renal function and liver function (BUN, creatinine, ALT, AST, alkaline phosphatase and total bilirubin)
- Cardiopulmonary Exercise Test (CPET)
- 6 minute walk test
- Kansas City Cardiomyopathy Questionnaire (KCCQ)
- Discontinue study medication

12 OUTCOME DETERMINATIONS

12.1 Primary Endpoint

1. Peak VO_2 (ml/min) as measured by CPET.

12.2 Secondary Endpoints

1. O_2 uptake kinetics and ventilatory efficiency as assessed by CPET
2. 6 Minute walk distance
3. Plasma NT-proBNP
4. KCCQ quality of life

12.3 Pre-specified Subgroups

Pre-defined subgroup analyses of the primary endpoint (change in peak VO_2 at 16 Weeks) for the following sub-population of patients:

1. Patients with or without anemia. Anemia is defined as hemoglobin <12 g/dl.
2. Patients with or without venous congestion, based on the following criteria:
    a. JVP>10cm
    b. Lower extremity edema

13 METHODS TO PROMOTE ADHERENCE

13.1 Adherence to Study Procedures

Protocol training and adherence will be a major focus of the Investigator training. Based on our experience in prior studies, identifying and correcting non-adherence is best accomplished in a stepped approach. The CC will contact each site to offer per-participant feedback on adherence; will review episodes of non-adherence and reemphasize the importance of adherence; and will provide adherence reports to the Executive Committee.

14 PARTICIPANT SAFETY AND ADVERSE EVENTS

14.1 Institutional Review Boards

All HFN sites will submit the study protocol, informed consent form, and other study documents to their IRB for approval—the approval letter for each clinical center will be stored at the CC. Any amendments to the protocol, other than minor administrative changes, must be approved by each IRB before they are implemented.

14.2 Definitions

14.2.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence associated with the use of a drug in a subject, whether or not considered drug or biologic related. An AE can therefore be any undesirable sign, symptom or medical condition occurring after starting study drug, even if the event is not considered to be related to the pharmaceutical product. Study drug includes the drug under evaluation, and any reference or placebo drug given during any phase of the trial.

Pre-existing medical conditions/diseases that were present prior to starting study treatment are only considered adverse events if they worsen after initiating study therapy(any procedures specified in the protocol).

14.2.2 Suspected Adverse Reaction

A suspected adverse reaction (SAR) is any adverse event for which there is a reasonable possibility that the drug caused the event. “Reasonable possibility” suggests there is a causal relationship between the drug and the adverse event. “Suspected adverse reaction” implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

14.2.3 Serious Adverse Events

An AE or SAR is considered serious if the Investigator or sponsor believes any of the following outcomes may occur:

- Death
- Life-threatening AE: Places the subject at immediate risk of death at the time of the event as it occurred. It does not include an AE that, had it occurred in a more severe form, might have caused death.
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- Inpatient hospitalization or prolongation of hospitalization.
- Congenital anomaly or birth defect.
- Important medical events that may not result in death, be life threatening, or require hospitalization may be considered a serious adverse event when, based upon

appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition above.

This determination is based on the opinion of either the investigator or sponsor (e.g., if either believes it is serious, it must be considered serious).

14.2.4 Laboratory Test Abnormalities

For laboratory test abnormalities that meet the definition of a serious adverse event (SAE), which required the subject to have the investigational product discontinued or interrupted, or required the subject to received specific corrective therapy, the clinical diagnosis rather than the laboratory term will be used by the reporting investigator (e.g., anemia versus low hemoglobin value).

14.2.5 Assessment of Adverse Event Severity

The determination of adverse event severity rests on medical judgment of a medically-qualified investigator. The severity of AEs will be graded using the following definitions:
Mild: awareness of sign, symptom, or event, but easily tolerated.

Moderate: discomfort enough to cause interference with usual activity and may warrant intervention.

Severe: incapacitating with inability to do usual activities or significantly affects clinical status, and warrants intervention.

14.2.6 Assessment of Causal Relationship

A medically-qualified investigator must assess the relationship of any AE to the use of study drug, based on available information, using the following guidelines:

- **Not related:** There is not a reasonable causal relationship to the investigational product and the adverse event.
- **Unlikely related:** No temporal association or the cause of the event has been identified, or the drug or biologic cannot be implicated.
- **Possibly related:** There is reasonable evidence to suggest a causal relationship between the drug and adverse event.
- **Related:** There is evidence to suggest a causal relationship, and the influence of other factors is unlikely.

The Investigator reports causality, but the sponsor retains the final decision on causality when filing to the FDA.

14.2.7 Expectedness

The expectedness of an AE or SAR shall be determined according to the specified reference document containing safety information (e.g., most current investigator's brochure or product

label). Any AE that is not identified in nature, severity, or specificity in the current study drug reference document(s) (e.g., investigator's brochure) is considered unexpected. Events that are mentioned in the product label as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but not specifically mentioned as occurring with the particular drug under investigation are considered unexpected.

14.3 Anticipated Adverse Events and Procedure Effects

The following AEs are anticipated, disease-related events in patients with HF with reduced EF (HFrEF):

- Arrhythmias
- Sudden cardiac death
- Acute coronary syndrome
- Unplanned hospitalization, ER visit or clinic visit for worsening HF
- Cerebrovascular event
- Venous thromboembolism
- Lightheadedness, presyncope or syncope
- Worsening renal function

All anticipated disease related events, will not be captured as AEs/SAEs during the study, but will be entered on the appropriate eCRF module.

14.3.1 Recording and Reporting of AEs/SAEs

Information about all adverse events, whether volunteered by the subject, discovered by investigator questioning, or detected through physical examination, laboratory test or other means, will be collected and recorded on the Adverse Event eCRF

For this study, all AES/SAEs occurring from signed informed consent to week-16 study visit will be captured on the AE/SAE eCRF. Unless exempted as described above, all AEs/SAEs, whether or not deemed drug-related or expected, must be reported by the investigator or qualified designee within 1 business day of first becoming aware of the event. The investigator/qualified designee will enter the required information regarding the AE/SAE into the appropriate module of the InForm eCRF, which will automatically result in distribution of the information to Duke Clinical Research Institute Safety Surveillance (DCRI SS). If InForm is temporarily unavailable, the event, including the investigator-determined causality to study drug, should be reported via a paper back-up SAE form to DCRI SS. Upon return of the availability of EDC system, the AE/SAE information must be entered into the eCRF.

Follow-up

When additional relevant information becomes available, the Investigator will record follow-up information according to the same process used for reporting the initial event as described above. The Investigator will follow all reportable events until resolution, stabilization or the event is otherwise explained.

Investigators are also responsible for promptly reporting AE/SAEs to their reviewing IRB/EC in accordance with local requirements.

DCRI Safety Surveillance will follow all SAEs until resolution, stabilization, until otherwise explained or until the last subject completes the final follow-up, whichever occurs first. DCRI Safety Surveillance will report all SAEs to the Heart Failure Network trial team within 1-2 business day(s) of receipt and notify the Data Safety Monitoring Board (DSMB) chair monthly.

The DSMB will review detailed safety data approximately every 6 months throughout the study.

Suspected Unexpected Serious Adverse Reaction

AEs that meet the criteria of serious, related to study drug, and unexpected per investigator brochure or product labeling, qualify for expedited reporting to the regulatory authorities. DCRI will notify the FDA and all participating investigators in a written IND safety report of an SAR that is serious and is unexpected, based on the opinion of the investigator and DCRI safety medical monitor, as soon as possible, but not later than 15 calendar days after the event is confirmed to be a serious, unexpected SAR and qualifies for expedited reporting. DCRI will identify all safety reports previously filed with the IND concerning a similar SAR, and will analyze the significance of the SAR in light of the previous, similar reports. Follow-up reports will be sent to investigators to inform and update them about an important suspected adverse reaction if it significantly affects the care of the subjects or conduct of the study.

The site investigator will be responsible for reporting adverse events and unanticipated problems involving risks to subjects to their local IRBs/IECs in accordance with local regulations.

Day Zero
Day zero (0) is the calendar day that DCRI is first notified of an event. Day zero can also be the date the event qualified for expedited reporting as determined by the DCRI Safety Medical Monitor.

Pregnancy
Pregnancy occurring during a clinical investigation, although not considered a serious adverse event, must be reported to DCRI within the same timelines as a serious adverse event. The pregnancy will be recorded on the appropriate paper pregnancy tracking form. The pregnancy will be followed until final outcome. Any associated AEs or SAEs that occur to the mother or fetus/child will be recorded in the AE/SAE eCRF, within InForm.

15 STATISTICAL CONSIDERATIONS

15.1 Overview

A statistical analysis plan will be completed before the data are analyzed in a blinded fashion. All test statistics will be two-sided. Statistical tests with p-value <0.05 will be considered statistically significant. The trial results will be reported according to guidelines specified in the CONSORT statement. A flow diagram describing screening, recruitment, randomization, dropout, and vital status will be included in the primary manuscript. Adverse events and efficacy data will be presented by the two treatment groups. Adherence, dropout, and lost-to-follow-up will be carefully examined across the two treatment groups. All primary analyses will be based on intent-to-treat (ITT) principles using all randomized participants. Analyses will be performed using SAS software (SAS Institute, Inc, Cary, NC).

15.2 Analysis of the Primary Endpoint

A general linear model with the change in peak VO_2 measured at 16 weeks as the response variable and predictor variables including a treatment indicator and the baseline measure of peak VO_2 will be used in the primary analysis. The treatment effect will be determined by the point estimate and 95% confidence interval for the treatment indicator variable.

A secondary analysis of the peak VO_2 outcome will be conducted using a repeated measures analysis. For this analysis all measurement of peak VO_2(including baseline) will be treated as response variables. The following covariates will be included in the regression model treatment group, time period, treatment group * time period interaction, baseline VO_2 and baseline Fe level. An unstructured correlation matrix will be assumed for the repeated measures within subjects. The mixed model repeated measures analysis will be estimated using SAS PROC MIXED.

15.3 Analysis of Secondary and Exploratory Endpoints

Summaries of continuous variables will be displayed using the mean, standard deviation, median, and 25^{th}-75^{th} percentiles. For nominal variables, the number and percentages in each category will be presented. General linear models and nonparametric approaches will be used to analyze the continuous outcomes. For binary outcomes, Chi-square tests and Fisher's exact test will be used for unadjusted comparisons. For adjusted comparisons, logistic regression analysis will be used to compare oral Fe vs. placebo with the estimated odds ratio and associated 95% confidence interval.

For the composite exploratory endpoint of time to death and heart failure hospitalization, unadjusted time-to-event comparisons will be conducted using Kaplan-Meier survival estimates and log-rank tests. For adjusted analyses, Cox proportional hazards regression models will be used to estimate hazard ratios. Heart failure hospitalization is defined as an event that occurred primarily because of the documented presence of at least:

- Clinical manifestations of worsening heart failure
- Use of additional or increased pharmacologic or mechanical interventions directed at the treatment of heart failure

15.4 Analysis of Safety Data and Statistical Monitoring Plan

Interim data analysis for efficacy and futility will not be conducted due to relatively small size and short duration of this clinical trial. Safety data, summarized at the treatment level, will be assessed approximately every 6 months by the NHLBI-appointed DSMB. The safety analyses will be based on the entire ITT population. Safety will be evaluated by comparing the occurrence of AEs and changes in laboratory values of the active arm compared to placebo. The number and percentage of participants experiencing AEs will be tabulated by treatment group, body system, and preferred term. The percentages between treatment groups will be compared using Fisher's exact test.

15.5 Sample Size Justification

The proposed sample size of 220 subjects will be adequate to address the primary objectives of the study. As in the HFN's PhosphodiesteRasE-5 Inhibition to Improve CLinical Status And

EXercise Capacity in Diastolic Heart Failure (RELAX) clinical trial, the assumed minimally important difference for peak VO_2 is 1.0 ml/kg/min. Based on prior studies within the HFN, a conservative estimate of 2.0 ml/kg/min for the standard deviation for peak VO_2 (ml/kg/min) will be assumed. Using a two-sample t-test a sample size of 172 subjects (86 per group) will provide 90% power to detect the minimally important difference. These calculations assume a common standard deviation of 2.0 ml/kg/min and a two-sided type I error of 0.05. Allowing for 20% missing data would result in a sample size of roughly 108 per group or a total sample size of approximately 220 subjects.

For secondary endpoints, a sample size of greater than 86 subjects per group will provide greater than 90% power to detect differences greater than 0.5 standard deviations for continuous endpoints. These calculations are based on the two-side t-test and assume that the data are normally distributed with a common standard deviation. Similar sample sizes will provide greater than 80% power to detect differences larger than 0.43 standard deviations.

16 DATA MANAGEMENT PROCEDURES

16.1 Overview of Data Management

The CC will have primary responsibility for data management, including the development of data collection systems, data monitoring processes, and data storage and back-up. State-of-the-art technology will be used for the management of the network's data.

eCRF: The CC management team will develop eCRF modules necessary for IRONOUT-HFpEF. Common fields and data elements will be used across the HFN trials to promote data standardization and facilitate cross-network analyses. Study eCRF components will include an enrollment and demographics form; forms for recording relevant history, HF symptoms, physical exam results, laboratory results, baseline biomarker levels, and other baseline presenting characteristics; follow-up forms for use during regular follow-up visits; forms to track the participant's clinical course over time; and event forms for recording the circumstances and details surrounding the occurrence of a death or hospitalization.

EDC System: The data will be collected in a validated, 21 CFR Part 11-compliant EDC system. The DCRI has an internal team of skilled data managers and programmers that will design and produce a tailored network system that provides operational efficiency and meaningful reporting of metrics.

Data Management Process: The EDC system will be used for data entry and simple reports. All data will be entered into the eCRF by personnel at the clinical sites. Any out-of-range values and missing key variables will be flagged and addressed in real-time at the site during data entry. When a query is generated on a particular variable, a flag is raised in a database field; the system tracks the queries and produces reports of outstanding queries. Queries can also be generated from manual or statistical review of the data forms.

The CC will create reports to identify trends in the data that may require additional clarification

and training. These reports will be available to the sites and to the study leadership as we work with the sites to correct negative trends and eliminate future data errors. The CC will perform internal database quality-control checks during the study to identify systematic deviations requiring corrections.

Data Quality Control: A three-step approach to data quality control will be implemented.

1. Training: Prior to the start of enrollment, the Investigators and Study Coordinators will be trained on the clinical protocol and data collection procedures. Recent site surveys indicate that most Coordinators are very familiar with the EDC system, so training is typically targeted to a specific protocol. For Coordinators new to the InForm database, the CC will provide training with hands-on database interaction, demonstration of key EDC system functionality. Personnel at the clinical sites will enter the data mandated by the protocol into the eCRFs. The data will be extracted from the participant's medical charts and other source documents. All CRFs will be completed according to the current Good Clinical Practice (GCP) guidelines. The CC will conduct follow-up training and training for new study personnel as needed.

2. Monitoring: A CC monitor will visit sites during the enrollment period to ensure that data collection is being handled properly, to provide in-service training, and to address questions from site investigators and coordinators. Additional details will be outlined in the Clinical Monitoring Plan.

3. Managing data: A series of computerized data validation checks will be programmed by the CC to check for missing data, inconsistencies in the data or data that is out of range. After the data have been exported from the EDC system to SAS for statistical summarization and data analysis, further cross-checking of the data will be performed by the CC and queries issued through the EDC system for any discrepancies.

16.2 Data Security

Access to databases will be controlled centrally by the CC through user passwords linked to appropriate privileges. This protects the data from unauthorized changes and inadvertent loss or damage. Database and web servers will be secured by a firewall and through controlled physical access. Database back-up will be performed daily using standard procedures in place at the CC. All disk drives that provide network services, and all user computers, will be protected using virus-scanning software.

16.3 Publication Policy

Dissemination of preliminary information can adversely affect the objectivity of study data. For this reason, Investigators will be prohibited from performing subset analyses at any point prior to the conclusion of the study, and any data, other than safety data, cannot be used for publication or reporting outside of this study until the study is completed or discontinued by the DSMB or

HFN Steering Committee.

17 STUDY ADMINISTRATION

17.1 Data and Safety Monitoring Board (DSMB)

A DSMB has been appointed by the NHLBI for the HFN, and will function as the DSMB for this trial. This committee consists of a group of highly experienced individuals with extensive pertinent expertise in HF and clinical trials. The DSMB will advise the HFN Steering Committee regarding the continuing safety of current participants and those yet to be recruited, as well as the continuing validity and scientific merit of the trial. Safety data, summarized at the treatment level, will be assessed approximately every 6 months by the DSMB. The safety analyses will be based on the entire ITT population. Safety will be evaluated by comparing the occurrence of AEs and changes in laboratory values of the active arm compared to placebo.

17.2 Coordinating Center

The DCRI will function as the CC for this trial as specified by the National Institute of Health and NHLBI HFN grant.

17.3 Core Laboratories

17.3.1 Biomarker Core Laboratory

The University of Vermont will serve as the core laboratory for measurement of HFN biomarkers. Plasma specimens will be collected at the following study visits: Baseline and 16 Weeks. Samples will be processed at the clinical centers according to the procedures provided by the core laboratory. Samples will be shipped to the core laboratory on dry ice (Refer to Biomarker Core Laboratory Manual of Procedures).

17.3.2 Cardiopulmonary Exercise Testing Core Laboratory

Massachusetts General Hospital will serve as the core laboratory for cardiopulmonary exercise testing. Comprehensive site certifications will be performed for participating centers and CPETs will be electronically transferred to the MGH Core Laboratory. Detailed CPET procedures are outlined in the manual of operations.

Measurement Methods: The following procedures or measurements will be performed at various time points throughout the study (see Human Subjects for details). CPETs will be performed on each subject at baseline and 16 weeks. The lag in VO_2 increment following initiation of constant workload exercise, before steady state is reached, is the oxygen deficit (Fig 16.3.3).[45] Phase I of this period is a brief cardiodynamic phase that reflects increase in cardiopulmonary blood flow. The shallower phase II reflects O_2 extraction that we hypothesize is strongly influenced by Fe. The O_2 deficit will be measured as the time constant from the monoexponential equation: $VO_2(t) = VO_2(\text{baseline}) + A(1-e^{-t/\tau})$, where t is the time after the start of exercise, A is the amplitude of VO_2 response, and τ is the time constant.[46] Recovery O_2 kinetics (O_2 debt, denoted in **Fig 16.3.3**) correlate with recovery of energy stores as well as

blood and tissue O_2 stores after exercise.[47] Both O_2 deficit and debt are increased in HF and are related to symptoms during sub-maximum exercise.

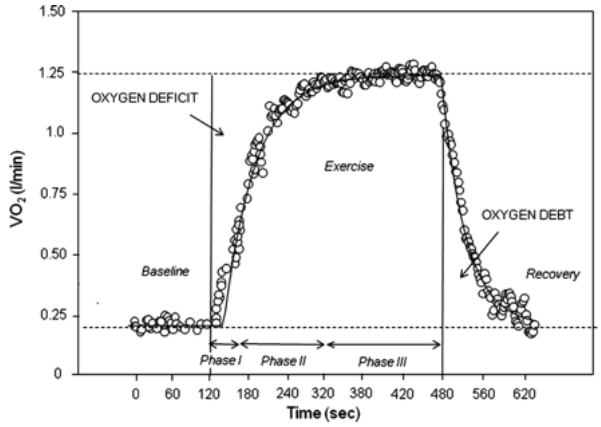


Fig 16.3.3 O_2 kinetics during low level CPET

The CPET will also measure the primary endpoint, peak VO_2 and will consist of a 10 watt/min incremental load performed according to the HF Network CPET Core Lab Manual of Operations to derive the primary endpoint ΔpVO_2, as well as VO_2 at AT and OUES.

18 ETHICAL AND REGULATORY CONSIDERATIONS

18.1 Ethics and Good Clinical Practice

This study must be carried out in compliance with the protocol and in accordance with DCRI standard operating procedures. These procedures are designed to ensure adherence to Good Clinical Practice, as described in the following documents:

1. ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996.
2. US 21 Code of Federal Regulations dealing with clinical studies (including parts 50 and 56 concerning informed consent and IRB regulations).
3. Declaration of Helsinki, concerning medical research in humans (Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects, Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996).

By signing the protocol, the investigator agrees to adhere to the instructions and procedures described in it and thereby to adhere to the principles of Good Clinical Practice to which it conforms.

18.2 Institutional Review Board/Independent Ethics Committee

Before implementing this study, the protocol, the proposed informed consent form and other information to subjects, must be reviewed by a properly constituted Institutional Review Board/Independent Ethics Committee (IRB/IEC). A signed and dated statement that the protocol and informed consent have been approved by the IRB/IEC must be provided to the CC before study initiation. Any amendments to the protocol, other than administrative ones, must be approved by this committee.

18.3 Informed Consent Procedures

18.3.1 Informed Consent

The Investigator or designee must explain to each subject (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each subject must be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

This informed consent should be given by means of a standard written statement, written in non-technical language. If a patient agrees to participate in the IRON-OUT study, they will review and sign the site-specific Internal Review Board (IRB) approved informed consent form (ICF). The subject should read and consider the statement before signing and dating it, and should be given a copy of the signed document. If written consent is not possible, oral consent can be obtained if witnessed by a signed statement from one or more persons not involved in the study, mentioning why the patient was unable to sign the form. No patient can enter the study before his/her informed consent has been obtained.

The ICFs are part of the protocol, and must be submitted by the investigator with it for IRB/IEC approval. The CC will supply proposed informed consent forms, which comply with regulatory requirements, and are considered appropriate for the study. Any changes to the proposed consent form suggested by the Investigator must be agreed to by the CC before submission to the IRB/IEC, and a copy of the approved version must be provided to the CC after IRB/IEC approval.

18.3.2 Confidentiality and HIPAA Requirements

All information collected on study participants will be stored in a confidential manner using the procedures in place at each participating center. Only approved study personnel will have access to data collected as part of the study. Study participants will be identified by a participant ID number on all study documents. Data will be transmitted to the CC in a secure manner, and stored securely at the CC using standard Duke Clinical Research Institute (DCRI) operating procedures.

18.3.3 Protections of Human Subjects

Protections for human subjects of research are required under Department of Health and Human Services (HHS) regulations at 45 CFR 46. Subpart A of the HHS regulations constitutes the Federal Policy (Common Rule) for the Protection of Human Subjects, which has been adopted by an additional 16 Executive Branch Departments and Agencies.

Each institution engaged in (non-exempt) HHS-supported human subjects research must provide a written Assurance of Compliance, satisfactory to the Office for Protection from

Research Risks, that it will comply with the HHS human subjects regulations—45 CFR46.103(a).

18.3.4 Summary of the Risks and Benefits

Blood draws: The risks of drawing blood include bleeding at the puncture site, bruising and pain. These occur in a very small portion of the population.

Cardiopulmonary exercise testing: The risk of cardiovascular events (myocardial infarction, malignant arrhythmia) or death with CPET in heart failure patients is <5/10,000. CPET is routinely performed in patients with advanced heart failure awaiting heart transplant or mechanical circulatory support. Low-level cardiopulmonary exercise testing during which CPET parameters will be assessed will involve a similar degree of exertion to that of 6-minute walk tests. Sites will be obligated to adhere to current AHA/ACC guidelines for exercise stress testing with regard to indications for the test supervisor to stop the test (i.e. ventricular arrhythmia, >10mmHg fall in systolic blood pressure).

Gastrointestinal symptoms from Fe polysaccharide administration: The most common adverse effects of oral Fe are gastrointestinal related and include constipation, diarrhea, nausea, vomiting, dark stools, and abdominal pain. Gastrointestinal adverse reactions are dose-related and typically decrease over time.

19 REMOTE MONITORING

The study will be monitored remotely by representatives of the DCRI or its designee according to the prospective clinical monitoring plan for the following purposes:

- To enable real-time monitoring of compliance with study protocol inclusion and exclusion criteria is enabled via triggers and range checks programmed in the InForm database.
- To assist site personnel who will verify data identified within query reports against source documents through frequent telephone and email contact.
- To verify that written informed consent was obtained before initiation of any screening procedures that are performed solely for the purpose of determining eligibility for the clinical study and/or prior to the participant's randomization to a procedure.

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21 APPENDICES

21.1 Appendix A. Schedule of Assessments

Measurement	Screening*	Baseline*	1 Week**	8 Weeks	16 Weeks
Informed Consent	x				
History & Physical	x			x	x
Laboratory Evaluation[a]	x				x
Current Medications	x	x	x	x	x
Central Lab and Repository		x			x
CPET[b]		x			x
6MW Test		x		x	x
KCCQ[c]		x		x	x
Study drug dispensing		x		x	
Review adverse events			x	x	x
Study drug compliance			x	x	x

*Screening and Baseline assessments must occur within 30 days prior to randomization.

**This visit performed by telephone.

[a] Screening Labs: Fe Studies (FE, Ferritin, Tranferrin/TIBC, Transferrin Saturation), CBC, Complete Chemistry panel (Sodium, Potassium, Chloride, Carbon Dioxide, BUN, Creatinine, Glucose, AL, AST, Alkaline Phosphatase & Ttoal Bilirubin)
16-Week Labs: CBC; Renal and liver function (BUN, Creatinine, ALT, AST, Alkaline Phosphatase & Total Bilirubin

[b] CPET indicates cardiopulmonary exercise test, maximum incremental ramp protocol

[C] KCCQ indicates Kansas City Living with Cardiomyopathy

21.2 Appendix B. Kansas City Cardiomyopathy Questionnaire

The KCCQ is a self-administered, 23-item questionnaire developed to provide a better description of health-related QOL in patients with HF. It quantifies physical limitation, symptoms, QOL, social interference and self-efficacy. The survey requires 4-6 minutes to complete, and is scored by assigning each response an ordinal value, beginning with 1 for the response that implies the lowest level of functioning and summing items within each domain. Scale scores are transformed to a 0 to 100 range by subtracting the lowest possible scale score, dividing by the range of the scale and multiplying by 100. A clinical summary score will be calculated by combining the functional status with the QOL and social limitation domains.

21.3 Appendix C.6-Minute Walk Test

Because usual daily activities generally require much less than maximal exertion, the measurement of submaximal exercise capacity may provide information that is complementary to that provided by maximal exercise testing.[18] 6MWT is the most common of the fixed-time tests; it measures the distance walked on level ground in 6 minutes. In this test, the participant is asked to walk along a level corridor as far as he or she can in 6 minutes. The participant can slow down or even stop, may be given a carefully controlled level of encouragement, and is told when 3 and 5 minutes have elapsed. The 6MWT is moderately predictive of maximal oxygen consumption, and independently predicts morbidity and mortality in HF.[19,20] For a complete description of the indications, contraindications, technical aspects, safety issues, and interpretation of the 6MWT, the investigator is referred to the 2002 guidelines published by the American Thoracic Society.

21.4 Appendix D. New York Heart Association Functional Classification

Class	NYHA Classification
I	Patients with cardiac disease but without resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue, palpitations, dyspnea, or anginal pain.
II	Patients with cardiac disease resulting in slight limitations of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitations, dyspnea, or anginal pain.
III	Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary physical activity causes fatigue, palpitation, dyspnea, or anginal pain.
IV	Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of cardiac insufficiency or of the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.