NYU Langone Health

Official Title:	Ferric Carboxymaltose to Improve Skeletal Muscle Metabolism in Heart Failure Patients With Functional Iron Deficiency
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Study Title: Ferric Carboxymaltose To Improve Skeletal Muscle Metabolism in Heart Failure Patients with Functional Iron Deficiency

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Regulatory Sponsor: NYULMC

Source of Funding: American Regent, Inc.
One Luitpold Drive, P.O. Box 9001,Shirley, NY 11967
631-924-4000

Study Product: Ferric Carboxymaltose (Injectafer) marketed by American Regent Inc. (Shirley NY)

Protocol Number: s17-00444

IND Number: IND Exemption Letter Requested

Study Summary

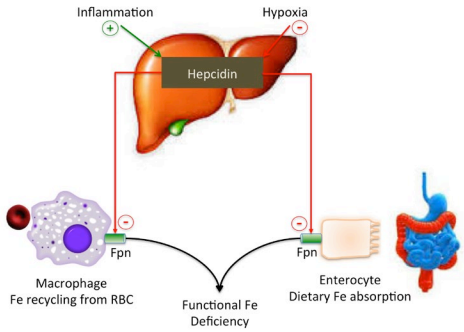
Title	Ferric Carboxymaltose To Improve Skeletal Muscle Metabolism in Heart Failure Patients with Functional Iron Deficiency
Short Title	FCM To Improve Sk. Muscle Metabolism in HF Patients with Fe Def.
Protocol Number	NYULMC IRB number: s17-00444
Phase	Phase 2 study of FDA-approved drug
Methodology	Double-blind, placebo-controlled single center randomized clinical trial
Study Duration	2 years
Study Center(s)	Single Center (NYULMC)
Objectives	To compare the effects of ferric carboxymaltose vs. placebo on skeletal muscle mitochondrial oxidative capacity, submaximal exercise tolerance, and health-related quality of life in HF patients with functional iron deficiency.
Number of Subjects	50 subjects will enrolled to identify 32 subjects eligible for randomization
Diagnosis and Main Inclusion Criteria	Clinically stable ambulatory chronic heart failure patients with functional iron deficiency without contraindication to study procedures (MRI and intravenous infusion of ferric carboxymaltose)
Study Product, Dose, Route, Regimen	Ferric carboxymaltose (Injectafer®) 750 mg intravenously, or normal saline (placebo)
Duration of administration	A single dose of ferric carboxymaltose or placebo will be administered.
Reference therapy	Placebo
Statistical Methodology	The change in the primary and secondary endpoints from baseline to week 4 will be compared between the two treatment arms by two-sided two sample t-test or Wilcoxon rank sum test. Significance will be decided at alpha=0.05.

INTRODUCTION

Statement of Compliance: This trial will be conducted in accordance with the ICH E6, the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46), and the NHLBI Terms of Award. The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without documented approval from the Institutional Review Board, except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study will have completed Human Subjects Protection Training and provided all required Conflict of Interests documentation before participation in research.

Background and Significance: Heart failure affects an estimated 6 million persons in the US, and is associated with high rates of hospitalization (1.1 million per year), high health care costs (>$30 billion Medicare costs on an annual basis), and high mortality risk (30-50% over 5 years).[1] The pathophysiology of heart failure is fundamentally determined by the failure of the circulatory system to deliver oxygen/nutrients sufficient for metabolic needs, and is best explained by a complex interplay between intrinsic abnormalities of myocardial contractile function and extra-cardiac factors that regulate oxygen utilization in metabolically active tissues. In patients with heart failure, reduced cardiac output reserve, impaired regulation of skeletal muscle blood flow, and abnormalities in skeletal muscle mass and mitochondrial function (e.g. reduced oxidative phosphorylation) all appear to be important determinants of exercise intolerance and may offer novel targets for therapy.[2]

Iron is an essential trace element for oxidative metabolism. Many of the proteins responsible for oxygen transport to skeletal muscle (hemoglobin, guanylyl cyclase), oxygen storage (myoglobin) and oxygen utilization in skeletal muscle (cytochromes and iron-sulfur enzymes involved in electron transport in mitochondria) require iron as an essential co-factor for normal enzyme activity.[2] Iron homeostasis is regulated by hepcidin, a liver-derived peptide hormone that modulates macrophage transfer of iron from enterocyte to the reticuloendothelial system by interaction with the iron channel protein ferroportin.[3] Hepcidin secretion is modulated by metabolic sensors of tissue oxygen tension, and by inflammatory mediators such as interleukin-6 (Figure 1, adapted from ref 3). In patients with heart failure, chronic inflammation and hepatic congestion are associated with abnormal iron homeostasis characterized by increased hepcidin secretion and consequent functional iron deficiency with or without anemia.[2] Functional iron deficiency is present in 35-50% of heart failure subjects and is associated with exercise intolerance and greater mortality risk in patients with concomitant anemia (HR 1.71, 95% CI 1.24-2.36, p=0.001) or without concomitant anemia (HR 1.44, 95% CI 1.11-1.87, p=0.006).[4-6]



Two randomized clinical trials of the effects of intravenous iron on measures of functional capacity have been published. The Ferinject Assessment in Patients with Iron Deficiency and Chronic Heart Failure (FAIR-HF) study was a randomized, double-blind placebo-controlled trial that compared the effects of treatment with intravenous ferric carboxymaltose vs. placebo for 6 months on measures of functional capacity in 459 patients with symptomatic chronic heart failure, hemoglobin level 9.5-13.5 g/dl, and functional iron deficiency.[7] Ferric carboxymaltose was administered weekly to achieve the replacement dose based on a clinically used formula, and then given every 4 weeks based on serial measurements of serum ferritin and transferrin saturation. When compared with placebo, subjects who received ferric carboxymaltose improve symptoms, improved 6-minute walk distance and improved quality of life after 6 months (all p<0.001). These benefits were consistent across pre-specified subgroups, including subjects without anemia, and subjects with higher than median serum ferritin at study entry. Intravenous iron therapy was well-tolerated, with no difference in study discontinuation or adverse events when compared with placebo. The CONFIRM-HF (FerricCarboxymaltOse evaluatioN on perFormance in patients with IRon deficiency in coMbination with chronic Heart Failure) study was another randomized, double-blind, placebo controlled trial that compared the effects of treatment with intravenous ferric carboxymaltose vs. placebo for 12 months on 6-minute walking distance in 304 patients with symptomatic chronic heart failure.[8] The entry criteria and treatment regimens were similar to the FAIR-HF study, except the intravenous iron was given in doses ranging 500-750 mg via intravenous bolus. When compared with placebo, subjects who received ferric carboxymaltose demonstrated improved 6-minute walk distance (p<0.001), improved symptoms (p<0.001),

improved quality of life (all p=0.01) and reduced hospitalization risk (p=0.009) at 52 weeks of follow-up. These benefits were consistent across subgroups including subjects without anemia, and subjects with higher than median serum ferritin at study entry. Intravenous iron therapy was well-tolerated, with no difference in study discontinuation or adverse events when compared with placebo. Taken together, these two studies provide concordant evidence of improved exercise capacity in response to intravenous iron therapy even in patients without evidence of anemia.

PRELIMINARY DATA

We measured post-exercise phosphocreatine recovery time as a validated index of skeletal muscle mitochondrial oxidative capacity with a novel spectrally selective three-dimensional turbo spin echo (3D-TSE) 31P MRS/MRI protocol developed at NYU School of Medicine in eight non-anemic HF patients and five non-heart failure control subjects. In three of the HF patients with functional iron deficiency, the 31P MRS/MRI protocol was repeated one week after a single dose of ferric carboxymaltose 500 mg. In agreement with previous studies using surface coils, we showed that the post-exercise phosphocreatine recovery time was prolonged in HF subjects when compared with control subjects (Figure 1), both for the whole muscle of the lower extremity (MRS Global and MRI Global) and also for individual muscle groups. In three HF subjects with functional iron deficiency, intravenous administration of ferric carboxymaltose 500 mg increased serum ferritin (mean 67 to 783 ng/mL), increased 6-minute walk distance (mean 342 to 369 m), decreased post-exercise phosphocreatine recovery time (mean 51 to 39 seconds, Figure 3), but did not change hemoglobin level (mean 14.6 to 14.0 g/dL). Intravenous iron was well-tolerated. There were no adverse events related to study drug or study procedures. These data were presented in abstract form at the 2015 International Society for Magnetic Resonance in Medicine meeting.

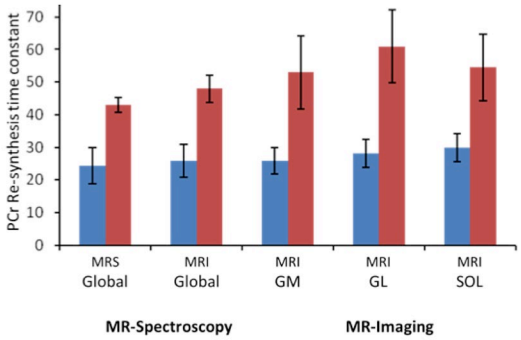


Figure 1. Mean±SEM data for comparison of global (31P-MRS) and regional (31P-MRI, gastrocnemius medial (GM), gastrocnemius lateral (GL), and soleus muscle (SOL)) post-exercise PCr recovery time constant between 5 healthy control subjects (blue bars) and 8 HF subjects (red bars).

Significance: Abnormal iron homeostasis due to chronic inflammation and/or nutritional deficiencies affects over 1 billion persons worldwide. Functional iron deficiency is present in 50% of heart failure patients and is associated with more severe symptoms and greater risk for adverse outcomes with or without anemia.[2] Although the effects of iron deficiency on erythropoiesis are well characterized, there are no existing human data on the impact of iron deficiency on skeletal muscle metabolism, and only limited data on the extra-medullary biological effects of intravenous iron therapy.

STUDY OBJECTIVES

A single-center, prospective double-blind parallel group randomized study is proposed to compare the effects of ferric carboxymaltose vs. placebo on skeletal muscle mitochondrial oxidative capacity, submaximal exercise tolerance, and health-related quality of life in HF patients with functional iron deficiency. The goal of this study is to provide novel “proof-of-concept” data to demonstrate biological plausibility to support the use of ferric carboxymaltose in HF patients and other disease populations with functional iron deficiency.

32 eligible subjects will be randomly assigned (1:1) to ferric carboxymaltose 750 mg or placebo (normal saline) and will undergo serial assessment before and 4 weeks after study drug administration to address the following study endpoints:

Primary Endpoint: To compare the effects of ferric carboxymaltose 750 mg vs. placebo on change in skeletal muscle mitochondrial oxidative capacity from baseline to 4 weeks (post-exercise phosphocreatine recovery time measured non-invasively with 31P-magnetic resonance spectroscopy).

Secondary Endpoints. To compare the effects of ferric carboxymaltose 750 mg vs. placebo on:

- Change in 6-minute walk test distance from baseline to 4 weeks
- Change in Kansas City Cardiomyopathy Questionnaire score from baseline to 4 weeks
- Change in laboratory markers of iron stores and complete blood count from baseline to 4 weeks

STUDY DESIGN

The proposed study design is a prospective, single-center, randomized, double-blind, parallel group placebo-controlled study (Figure 2). Primary and secondary endpoints will be assessed before and 4 weeks after administration of ferric carboxymaltose or placebo. It is anticipated that the study will require 2 years to complete enrollment.

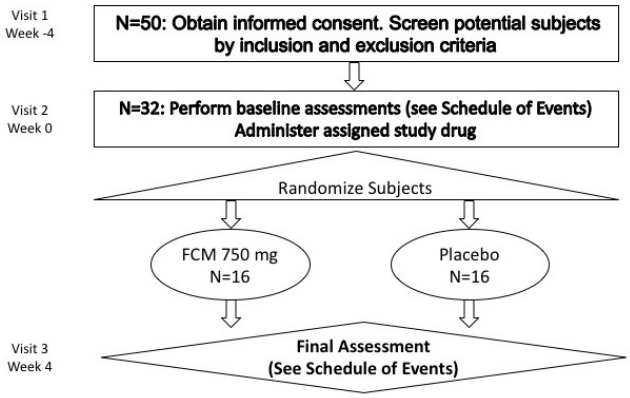


Figure 2. Study design overview. FCM=ferric carboxymaltose

Study population: Up to 50 subjects will be recruited from the clinical practice of the NYU Advanced Cardiac Therapeutics group at NYU Langone Medical Center. 36 eligible subjects will be randomized. Study entry criteria are listed in the Human Subjects section below.

Study visits: The study will require 3 visits over approximately 8-10 weeks for each subject: Visit 1: Screening; Visit 2: Baseline primary and secondary endpoint measurements, randomization, and study drug administration; Visit 3: final assessment of primary and secondary endpoint measurements. Additional details of the procedures at each study visit are described below.

Study agent and control description: Ferric Carboxymaltose (Injectafer®, 750 mg iron/15 ml sterile single use vial) will be provided by the American Regent pharmaceuticals (Shirley, NY) to the NYU Investigational Pharmacy and stored in the NYU Investigational pharmacy at 20°C to 25°C according to manufacturer instructions. An IND exemption will be requested from the FDA. Ferric carboxymaltose is nonionic polynuclear iron (III) hydroxide carbohydrate complex that binds iron tightly and requires macrophage processing for release of iron into body stores.[9, 10] Ferric carboxymaltose has been demonstrated to be safe and effective in the treatment of iron deficiency anemia and is FDA-approved for treatment of iron deficiency in adult patients. The most common adverse reactions recorded in clinical trials (>2%) are nausea, hypertension, flushing, hypophosphatemia, and dizziness. The dosing and route of administration used in this study protocol are consistent with the FDA-approved prescribing information for this drug (see appendix for FDA-approved package insert) and prior studies of this agent in heart failure populations.

Study drug administration: Intravenous ferric carboxymaltose 750 mg will be mixed under sterile conditions in 100 ml of normal saline for final concentration of 7.5 mg/ml and administered via volumetric infusion pump over 15 minutes. Placebo will consist of 100 ml of normal saline solution, and administered via volumetric infusion pump over 15 minutes. The research pharmacy will dispense study agent based on the randomized treatment allocation sequence.

Study Agent blinding procedure. Since ferric carboxymaltose is a dark brown solution, the study agent will be administered by un-blinded research nursing personnel of the NYU Clinical Translational Science Institute who are not otherwise involved in the research study procedures. A licensed physician safety monitor not otherwise involved in study procedures will be available to monitor the infusion procedure. Blinded research personnel will not be present during the study agent infusion. Opaque covers over the study agent infusion equipment and curtains will be used to maintain blinding for the study participants.

Study Drug Randomization Procedure: A variable size blocked randomization allocation scheme will be generated by an independent biostatistician and held by the NYU research pharmacy. When an eligible subject arrives at the research site for participation in study visit 2, a study drug order will be placed in the electronic medical record, and a randomization form will be hand-delivered to the NYU Investigational Pharmacy. The Investigational Pharmacist will confirm subject eligibility and assign the treatment allocation based on the sequence of the randomization code. The Investigational Pharmacist will process the study agent order in the electronic medical record, dispense assigned study drug to the un-blinded CTSI research nurse. Case report forms to track the treatment allocation for each participant (ID number and randomization code number) will be

completed by the NYU investigational pharmacist, and verified by the unblinded CTSI research nurse assigned to administer study drug. Blinded research personnel will not have access to the randomization case report form. Except in case of emergency, treatment allocation will not be revealed to the investigation team until the last enrolled subject has completed all of the study procedures and the database is locked.

Study Assessments

Schedule of Assessments. The Schedule of Assessments (Table 1) provides a list of the study procedures for each study visit. Each study visit will be conducted within a time window of ±10 days. Details of the study assessments are as follows:

Table 1. Schedule of assessments

	Screening Visit 1	Baseline Assessment, Randomization & Study Drug Administration Visit 2	Final Assessment Visit 3
Study Week (±10 days)	-4	0	4
Informed consent	X		
Screening procedures	X		
Randomization		X	
6-minute walk test	X	X	X
Kansas City Cardiomyopathy Questionnaire		X	X
^{31}P MRS/MRI Study		X	X
CBC and iron biomarkers		X	X
Vital Signs		X	X
Heart Failure Symptoms		X	X
Cardiovascular examination		X	X
Ferric Carboxymaltose/Placebo Study Drug Administration		X	
Adverse event monitoring		X	X
Concomitant Medications		X	X
Safety blood monitoring			X

Screening procedures. Screening procedures include obtaining of written informed consent, vital signs, medical history, heart failure symptoms, medications history, cardiovascular examination, ankle brachial index, 6-minute walk test, and screening blood collection (comprehensive metabolic panel, serum phosphate, complete blood count, serum iron, total iron binding capacity, transferrin saturation, serum ferritin, and serum beta-HCG for women of childbearing potential)

Vital Signs: Height (cm) will be measured at the screening visit. Weight (kg), seated systolic and diastolic blood pressure in the right arm (mmHg), seated heart rate (beats per minute), and resting oxygen saturation on room air (%) will be measured at each study visit.

Medical History: The following information will be obtained from review of the medical record and/or patient interview at Visit 1: 1) Left ventricular ejection fraction (date and imaging mode); 2) Etiology of heart failure (ischemic or non-ischemic); 3) Duration of heart failure (years); 4) NYHA Class (I-IV); 5) Co-morbid diseases (hypertension, hyperlipidemia, coronary artery disease, chronic kidney disease, chronic lung disease, atrial fibrillation); 6) Medications by pharmacological class: angiotensin converting enzyme inhibitors, angiotensin receptor blockers, beta-adrenergic receptor blockers, digitalis glycosides, loop diuretics, mineralocorticoid receptor antagonists, statins, aspirin, thienopyridines, warfarin, and novel oral anticoagulant agents; 7) Demographic information including age, sex, ethnicity; 8) Contact information including home, work and mobile phones, street address, email address (to be purged after completion of study visits).

Heart Failure Symptoms: The following information will be obtained from review of medical record and/or patient interview: 1) Functional capacity; 2) Orthopnea, paroxysmal nocturnal dyspnea, edema; 3) Medical resource use (doctor visit, emergency department visit, hospitalization); 4) Medication change; and 5) Use of appropriate measures to protect against pregnancy in women of childbearing potential.

Cardiovascular examination: Limited physical examination to assess evidence of congestion including assessment of jugular venous pressure, lung exam, cardiac exam, abdominal exam for hepatomegaly, and extremity exam for edema and perfusion.

Safety Blood Monitoring: Venous blood will be collected from a forearm vein with standard sterile venipuncture technique for analysis of biomarkers of iron stores (serum iron, total iron binding capacity, serum ferritin), complete blood count, comprehensive metabolic panel, serum phosphate, and beta-HCG (for women of childbearing potential) according to the schedule of assessments (Table 1). All laboratory analyses will be performed by the New York State licensed CLIA-compliant NYU Langone Medical Center Tisch Hospital Clinical Laboratory.

Kansas City Cardiomyopathy Questionnaire. The KCCQ is a self-administered disease-specific health status instrument composed of 23 items that quantify the domains of physical limitation, symptoms, self-efficacy, social limitation, and quality of life limitation from heart failure.[11] Participants will complete the questionnaire form under the supervision of the research staff. The questionnaires will be scored by an automated system provided by the distributor of the questionnaire.

Six-minute walk test: The 6-minute walk test is a validated quantitative measure of functional capacity in patients with heart and lung diseases. This submaximal self-paced test will be conducted according to

American Thoracic Society guidelines at Visits 1 and 2. Subjects will be asked to walk along a 30 meter marked path in a corridor at a self-selected pace. Subjects will receive standardized instruction before and during the walking test. The distance walked after 6 minutes (meters) will be recorded.

31P-MRS and MRI protocol: We will use a spectrally selective three-dimensional turbo spin echo (3D-TSE) protocol for measurement of phosphocreatine recovery kinetics on a 3T Siemens MRI scanner as previously described (Siemens Medical Solutions, Erlangen, Germany).[6] Each subject will perform the same rhythmic plantar extension exercise protocol twice, for sequential acquisition of MRS and MRI measurements respectively. For the exercise protocol, the subject will lay supine on the scanning table with the right leg inside the volume coil. The exercise consists of repeated plantar flexion movements using resistance bands at a frequency of one repetition per second for 1-2 minutes. Data are collected continuously for 2 minutes before the exercise (baseline), during exercise, and 5 minutes after the exercise for the MRS and MRI experiments. The phosphocreatine recovery kinetics will be calculated as previously described.[12]

Ankle brachial index. Systolic blood pressure in the brachial artery and in the posterior tibialis artery or dorsalis pedis artery will be determined with a Doppler ultrasound flow probe. The ankle brachial index is calculated as the ratio of the ankle:arm pressures.

Adverse Event Monitoring: The Principal Investigator is responsible for monitoring and reporting adverse events to the ethical review board. The definitions and procedures for classification and reporting of adverse events (AEs) are described in the Human Subjects section below. Subjects will be prompted to report any new problems or events since the start of the study at each visit.

Early Withdrawal Criteria: Subjects may be removed from participation based on the following criteria: subject request, intolerance of study drug or study procedures, intercurrent illness that alters risk of participation or interferes with study procedures, acquisition of new information that affects subject eligibility (e.g. pregnancy or prohibited concomitant medication), or other situations that would put the subject at increased risk, compromise adherence to study procedures, or confound interpretation of the study findings. Women of childbearing potential will be instructed to use accepted methods of contraception to avoid pregnancy during the study, and will have a blood pregnancy test at screening, and before all study visits with efficacy endpoint assessments.

Early study termination criteria: The study may be prematurely terminated or suspended by the Principal Investigator due to issues related to participant safety, study futility, failure to meet accrual, site performance, or data quality and completeness milestones, failure to adhere to ethical standards in the conduct of human subjects research, or other study protocol deviations.

DATA ANALYSIS

Data management and analysis: All data will be recorded on paper case report forms as primary source documentation and will be transferred to a secure REDCap electronic database protected by the NYU firewall. Case report forms and electronic data will use a randomly assigned Study ID number and will not include any identifiers. A separate study folder with study subject contact information will be kept in a locked cabinet in a locked room. PHI for each subject will be securely destroyed after completion of the study visits.

Hypothesis to be tested: The null hypothesis to be tested is that there is no difference between ferric carboxymaltose and placebo for the change in the primary and secondary study endpoints over 4 weeks.

Description of analysis sets:

Efficacy analysis dataset. Intention to treat (ITT) will be defined as all patients who have been randomized to study treatment and completed at least one follow-up visit with primary endpoint data collection. The ITT data set will be used for the primary and secondary efficacy endpoints and ancillary study endpoints.

Safety analysis dataset. All patients who received study drug, and for whom post-dose data are available, will be included in the safety analysis set.

Analysis plan for primary and secondary endpoints:
The objective is to compare the change in the primary and secondary endpoints over time between the two treatment groups.

Descriptive analysis techniques will be used to characterize study participants. Center and variability of continuous variables will be presented as means (SD) when they follow a normal distribution and as medians and interquartile range otherwise. Log transformation will be applied on variables with skewed distribution.

The primary endpoint is change in the phosphocreatine recovery time (s). Secondary endpoints are 1) change in six-minute walk distance (m), change in quality of life score (points), and change in hemoglobin (g/dL), serum ferritin (ng/ml), and transferrin saturation (%). The change in the primary and secondary endpoints from baseline to week 4 will be compared between the two treatment arms by two-sided two sample t-test or Wilcoxon rank sum test. Significance will be decided at alpha=0.05.

We will also construct multivariable regression models with treatment assignment as the main predictor and the change in the primary or secondary endpoint as the outcome variable while adjusting for patients' characteristics such as baseline value of the endpoint, age, sex, ethnicity, hemoglobin level, and measures of disease severity and their interactions with the treatment assignment if necessary. Pre-specified subgroup analyses are based on median age, sex, heart failure etiology (ischemic vs. non-ischemic), NYHA functional Class, median serum ferritin level, and sex-stratified median hemoglobin level.

Missing Data Consideration: We anticipate a low percentage of missing data due to the short duration of the study and relatively small data set. The missing data will be handled by intent-to-treat analysis in principle, with last value carried forward. We will compare baseline data in subjects with and without missing data to identify factors contributing to missingness. As sensitivity analyses, a "per protocol" analysis, and analysis with multiple imputation will be undertaken with five imputed datasets using the NORM software. Parameter estimates and their standard errors will be derived by averaging across the five imputations and by adjusting for their variance.

4.14: Sample size calculation:
Our pilot data demonstrated that the post-exercise phosphocreatine recovery time constant decreased by >10 seconds in all 3 non-anemic HF subjects with functional iron deficiency who received ferric carboxymaltose with mean±SD decrease 11.4±3.4 seconds. Table 2 presents the minimal detectable difference between treatment groups over time based on estimates of a range of standard deviations of the between-group difference and assuming 0-20% dropout, power=0.90, two-tailed alpha=0.05. Table 2 indicates that the proposed sample size provides adequate power to detect moderate to large changes in the post-exercise phosphocreatinine recovery time, even when accounting for 20% drop-out. A 10-second change in phosphocreatine recovery time is likely meaningful, since our pilot data suggest that a 10-second decrease would place many HF subjects back to the normal or near normal range. The sample size of 16 subjects per treatment arm will provide >80% power to detect a 35 m difference in six minute walk distance (consistent with a moderate improvement in submaximal exercise capacity, based on data from FAIR-HF and other assumptions as above) and a 20-point difference in the KCCQ score (consistent with a large improvement in health status, based on previous data from FAIR-HF and other assumptions as above).[11]

Table 2. Estimates of minimal detectable between-group difference for post-exercise phosphocreatine recovery time based on power 0.9 with 2-tailed alpha=0.05.

Drop-out Rate	N/group at 4 weeks	Detectable Difference (proportion of SD)	Estimated SD of between group difference (seconds)	Minimal Detectable Difference (seconds)
0%	16	1.15	2	2.3
			4	4.6
			6	6.9
			8	9.2
20%	13	1.3	2	2.6
			4	5.2
			6	7.8
			8	10.4

HUMAN SUBJECTS

1. Risks to Human Subjects

a) Human Subjects Involvement, Characteristics, and Design

This is a prospective single center double-blind placebo-controlled randomized clinical trial in human subjects with chronic heart failure. Since iron metabolism varies greatly among different mammalian species, it is necessary to study human subjects in order to obtain relevant information that might eventually favorably impact human health.

The study entry criteria are as follows:

Inclusion Criteria:

- Age 21-75 years
- Symptomatic NYHA Class II-III heart failure >3 months
- Guideline-recommended heart failure treatment for > 3 months
- Hemoglobin >10 g/dl for men and >9 g/dl for women
- Functional iron deficiency (defined as serum ferritin level <100 ng/ml or between 100 and 299 ng/ml with transferrin saturation <20%)
- Left ventricular ejection fraction <40%, or left ventricular ejection fraction ≥40% with left atrial enlargement (left atrial volume index >28 ml/m^2) and/or left ventricular hypertrophy (left ventricular mass index >95 g/m^2 (women) or >115 g/m^2 (men) determined by echocardiogram within last 24 months.
- Able and willing to provide written informed consent

Exclusion Criteria:

- Presence of implantable defibrillator, permanent pacemaker, other metal implant not compatible with 3TMRS/MRI, or other contraindication to 3T MRS/MRI procedures
- Heart failure due to infiltrative cardiomyopathy, restrictive cardiomyopathy, or hypertrophic cardiomyopathy
- Weight <50 kg or >120 kg
- Height < 5 feet 4 inches (1.63 m)
- Coronary or cerebral atherothrombotic events in the past 6 months
- Hospitalization of emergency room visit for heart failure within past 3 months
- ICD shock in last 3 months
- Known peripheral artery disease or ankle-brachial index <0.9 at screening visit
- Exercise primarily limited by angina, lung disease or neuromuscular disease
- Systolic blood pressure <100 or >160 mmHg
- Heart rate <50 or >110 min^{-1}
- Estimated glomerular filtration rate <30 ml/min
- Liver function tests >3 times upper limit of normal
- Serum phosphate below normal limit
- Pregnant or breast-feeding women
- Women of child-bearing potential unwilling to use recommended contraception methods during the study
- Treatment with intravenous iron in past year
- Treatment with erythropoiesis stimulating agents in the past year
- Known intolerance of intravenous iron
- History of anaphylaxis
- Participation in another clinical trial within last 30 days.

Recruitment strategy. We will use admission and emergency department logs, clinic appointment logs, and other existing electronic hospital information systems to identify potentially eligible subjects from within our clinical practice. Clinical personnel in the NYU Heart Failure program meet on a weekly basis to identify potential study subjects. An experienced research coordinator will initiate contact with the attending physician

to obtain additional information on potential eligibility and permission to approach the subject. Based on historical data from the NYU Heart Failure Quality Improvement Program case identifications over the past year, there are approximately 1200 annual discharges with primary HF diagnosis from NYU Langone Medical Center (Tisch, Bellevue, and Manhattan VA hospitals). If we conservatively estimate that 50% of those admissions are re-admissions, then approximately 1800 unique heart failure cases will be identified over the proposed 18-month period of enrollment (100 unique cases/month). For the pilot study presented above, this recruitment strategy allowed us to enroll eight subjects over 3 months. Accordingly, we believe that this clinical volume is sufficient to support our proposed enrollment target of 1-2 randomized subjects per month.

We will also recruit patients at NYU Langone Health with use of the MyChart patient portal of the EPIC electronic health record. A DATACORE query of the EPIC database will be conducted to identify potentially eligible subjects (based on inclusion criteria of age 21-75 years, ICD9 and ICD10 codes for acute, chronic, and acute on chronic systolic heart failure, and acute, chronic, and acute on chronic diastolic heart failure, and exclusion criteria of ICD codes for permanent pacemaker or implantable defibrillators). After approval from DATACORE, an invitation letter will be placed in MyChart (text of invitation letter is attached in Research Navigator).

We will also recruit patients using printed advertisements in the free morning newspaper, AM New York, and electronic advertisement on Facebook pages with content relevant to heart failure patient. The advertisement text is attached in Research Navigator.

A telephone script for respondents to MyChart invitation letters, and print and electronic advertisements is attached in Research Navigator. Personal identifiers will only be recorded for respondents who meet eligibility for a screening visit and express willingness to be scheduled for a screening visit.

There are no vulnerable populations enrolled in this study.

There are no collaborating sites for this study. All research data will be created and analyzed at NYULMC.

b) Sources of Materials

The research material generated in this protocol will consist of clinical assessments, laboratory measurements and physiological measurements obtained from the human subject participants.

Historical information related to demographics and the cause, severity, and treatment of heart failure will be recorded from the medical record or obtained by subject interview. Other research data will be obtained as a result of completion of the research procedures described above. These data include blood test results, 6-minute walking test results, and the results of the 31P-MRS and MRI analysis of skeletal muscle bioenergetics in response to plantar flexion exercise. There will be no data collection related to prospective clinical testing or clinical care. The research database will not retain any identifiers after completion of study procedures.

The Principal Investigator and other trained study personnel will have access to private health information only during active participation in the study. This information will be used only for scheduling participant visits, for confirmation of correct identity at study visits, and for review of medical records as described in study procedures. All identifying information will be kept separate from the other research records in a locked cabinet in a locked room or on a secure electronic database. All identifiers (except signed consent forms) will be destroyed after the subject has completed the last visit. No identifiers will be retained in the research database after completion of study procedures.

A random Study ID code will be assigned to each participant. This code will be used on all written and electronic research records. The Principal Investigator and study coordinator will be able to link the Study ID code back to the personal information during the interval of active participation in the study, but this link will be destroyed after the subject has completed the study procedures. Primary source documents with identifiers including signed consent forms and contact information will be kept separately in a locked cabinet in a locked room. All primary source documents with identifiers (except signed consent forms) will be destroyed after the subject has completed the last visit. Other primary source documents including case report forms, laboratory reports, and results of the 6-minute walking test, and the 31P-MRS and MRI analysis of skeletal muscle bioenergetics in response to plantar flexion exercise will be kept in a project notebook in a locked cabinet in a locked office. Research data without identifiers will be recorded on the NYULMC REDCap system. The REDCap system provides secure password protected storage of research information behind the NYU firewall and provides quality control and audit trail functions to validate the integrity and quality of the research data.

c. Potential Risks

The risks of the research procedures in this protocol are generally similar to the risks of the same procedures performed in the course of clinical care. The potential risks to study participants are as follows;

Risk of ferric carboxymaltose (Injectafer®): Ferric Carboxymaltose (Injectafer®, 750 mg iron/15 ml single use vial) has been demonstrated to be safe and effective in the treatment of iron deficiency anemia and is FDA-approved for treatment of iron deficiency in adult patients. The most common adverse reactions recorded in clinical trials (>2%) are nausea, hypertension, flushing, hypophosphatemia, and dizziness. Rare occurrences of anaphylactic or anaphylactoid reactions have also been reported. The dosing and route of administration used in this study are consistent with the FDA-approved prescribing information for this drug. In the FAIR-HF study, ferric carboxymaltose was administered repeatedly over 6 months to 304 subjects with chronic heart failure. Reported adverse events did not differ from placebo. There were no anaphylaxis events or other allergic reaction events reported. In the CONFIRM-HF study, 150 heart failure subjects received intravenous ferric carboxymaltose for 1 year. Overall adverse events did not differ from placebo. There were no anaphylaxis events, but there was one episode of rash, one episode of urticaria, and one episode of flushing reported in the ferric carboxymaltose group. Taken together, this reported experience of repeated dosing of ferric carboxymaltose over 6-12 months in a combined group of 454 patients with heart failure does not suggest a signal of increased risk of adverse effects in heart failure when compared to clinical trial population data included in the package label.

Risks of MRI:

Magnetic Field Risks: MRI uses a strong magnetic field to create images of the body. Because of the strong magnetic field, the metal objects within the body or within the MRI room could cause injury. Subjects with metal implants will be excluded from the study. Standard operating procedures in MRI rooms check for any possible external metal objects before the scan.

Risk of Anxiety/Claustrophobia: The MRI scans are obtained is a small space that may be stressful for some subjects with claustrophobia. We will exclude subjects with known claustrophobia and terminate the MRI scan upon subject request.

Risk of High Noise Levels: The MRI scanner produces loud noises that may increase anxiety in some subjects. Disposable earplugs to reduce the noise levels but will still allow voice communication with the scanner operator will be provided to all subjects.

Risk of MRI Systems Failure (Quench): This is a rare event caused by loss of the magnetic field. Subjects will be immediately brought out of the magnet room if system failure is detected.

Risk of Neurostimulation: Some subjects may experience muscle twitches or tingling sensations and a slight increase in body temperature during some types of scan activity. These are very unlikely under current MRI guidelines.

Risks in Pregnant Women: Although there are no known risks of MRI on pregnant women or a fetus, there is a possibility of yet undiscovered pregnancy related risks. Since there is no direct benefit from participating in this protocol for a pregnant woman, we will exclude pregnant women. If a woman becomes pregnant during the study, we will request to follow the health of the mother and fetus during pregnancy, and the health of the mother and child for 30 days after delivery.

Incidental MRI findings; The MRI scan is for research purposes only and is not directed toward or designed for clinical diagnosis. Per Department of Radiology procedures, all MRI scans will be reviewed by a clinical radiologist, and any abnormal findings will be reported to the subject, and the subject's physician.

Risk of phlebotomy: Phlebotomy is commonly associated with transient pain and minor bleeding at the needle site, and rarely major bleeding, infection or vasovagal reaction with transient hypotension. The risks of phlebotomy in this study do not differ from risks of phlebotomy for clinical blood tests.

Risk of intravenous line: Placement of an intravenous catheter is commonly associated with transient pain and minor bleeding at the needle site, and rarely major bleeding, infection or vasovagal reaction with transient hypotension. The risks of placement of an intravenous catheter in this study do not differ from risks of phlebotomy.

Other Study Risks:

Risk of exercise: The 6-minute walk test is a self-limited submaximal exercise test in which the study subject selects the walking pace and has the option to stop and rest as needed. Since the purpose of this test is to simulate daily walking activity, the small risks associated with submaximal exercise do not substantially differ from risk associated with exercise in daily activities. A study with over 2000 elderly subjects demonstrated no serious adverse events associated with the 6-minute walk test. The plantar flexion exercise during the 31P-MRS and MRI procedure consists of rhythmic plantar flexion against low resistance and is akin to stepping on a brake or gas pedal in a car. Accordingly, the small risk associated with this submaximal exercise does not substantially differ from risk associated with exercise in daily activities.

Risk of breach of confidentiality: There is a small risk of breach of confidentiality of protected health information related to participation in this protocol.

2. Adequacy of Protection Against Risks

a. Recruitment and Informed Consent

Recruitment will be conducted in accordance of the policies of the New York University IRB and FDA regulation 21CFR, Part 50. Potential subjects will be identified by the primary cardiologist supervising patient care during periods of clinical stability in an ambulatory care setting. The primary cardiologist will determine whether the potential participant is willing to consider discussion of the study with research staff. If the primary cardiologist determines that the potential participant is willing to discuss the study, research staff will provide the potential participant with additional information about the study and offer the opportunity to read the IRB-approved consent form. The rationale, procedures and potential risks of the procedures in the study will be explained to each participant by the Principal Investigator or his appointed designee. Each subject will be told that participation in the studies described in this proposal is strictly voluntary, that refusal to participate will not alter the patient's relationship with her physician, that the studies constitute research and that the information obtained may increase understanding of the role of body iron and exercise in general but will not be specifically helpful to the individual patient's care. The subject’s comprehension of the major aspects of the study procedures, study risks, and study benefits will be determined by asking the subject to respond to questions about the study. A signed, IRB-approved informed consent form will be obtained from each subject by the Principal Investigator or his designee on the day of the enrollment. A copy of the most current version of the IRB-approved Patient Informed Consent Form, along with a copy of each subject’s signed and dated consent form will be kept in a designated research administrative file in a locked cabinet in a locked room. A signed copy of the consent form will be given to each subject. Due to the complexity of the study procedures, only subjects able to speak either English or Spanish will be enrolled. For Spanish speaking subjects, the entire consent process will be conducted in Spanish by a fluent native Spanish-speaking member of the research staff. A Spanish consent form will be created and submitted to the IRB after final IRB-approval of the English language consent form.

b. Protections Against Risk

The study is deemed to be greater than minimal risk, primarily due to the potential side effects of ferric carboxymaltose. To minimize risk, all procedures will be performed in clinically stable subjects who meet eligibility criteria under the direct supervision of the principal investigator, and/or other trained study personnel. All study procedures are performed within the CTSI Clinical Research Center or MRI Center with nearby access to emergency procedures in the event of unanticipated deterioration in clinical status.

Participant risks are also minimized by the following protections described in the study protocol:

- Study entry criteria to exclude potential participants at greater risk of adverse events
- Administration of the study agent, ferric carboxymaltose, according to the FDA-approved prescribing information
- Active surveillance for potential study drug adverse effects and other adverse events
- Pre-specified criteria for participant withdrawal to protect participant safety
- Pre-specified criteria for study termination or suspension to protect participant safety
- Use of the MRI imaging center standard clinical protocol to prevent transport of ferromagnetic materials into the imaging environment
- Safety monitoring by the Principal Investigator

No vulnerable subject populations will be enrolled in this study.

All research subject data will be kept strictly confidential, except when published for purposes of reporting data. In that case, the subjects are never identified. All electronic data will be de-identified and transmitted and stored with secure systems that meet or exceed Federal guidelines (REDCap database behind NYU firewall). The Principal Investigator will maintain files with identifying information in a locked cabinet in a locked room.

c. Potential Benefits of the Proposed Research to Human Subjects and Others

There is no expected benefit for participant in this study. The feasibility data acquired in this study may eventually help future heart failure patients, or patients with other diseases associated with functional iron deficiency.

d. Importance of the Knowledge to be Gained

Anemia of chronic disease associated with inflammation and functional iron deficiency is the second most common cause of anemia worldwide. Functional iron deficiency is common in patients with heart failure and may be associated with impaired functional capacity and increased risk for hospitalization and death. Intravenous iron therapy has been shown to improve various measures of functional capacity, but the mechanism of action and target population with greatest likelihood has not been identified. This study is designed to provide proof-of-concept data to support future investigations of the role of iron in the pathogenesis of exercise intolerance. This line of investigation may eventually have impact on therapeutic strategies for maintenance of iron homeostasis in healthy populations, and heart failure and other chronic inflammatory disease populations.

e. Data and Safety Monitoring Plan

The research procedures described in this protocol are deemed to be greater than minimal risk.

The Principal Investigator will serve as the medical monitor for the study and will be responsible for monitoring and reporting of adverse events to the NYU IRB according to the following definitions and procedures:

Adverse Event Definitions

An Adverse Event (AE) is any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product and does not necessarily require a causal relationship with the study treatment. An AE can therefore be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporarily associated with the use of a medicinal product, whether or not related to the study procedures. Diseases, signs, symptoms, or laboratory abnormalities already existing at the time of enrollment are not considered AEs unless they worsen (i.e., increase in intensity or frequency). Surgical procedures themselves are not adverse events; they are therapeutic measures for conditions that require surgery. The condition for which the surgery is required may be an AE. Surgical procedures planned prior to randomization and the conditions leading to these measures are not AEs unless the event exacerbates or worsens after randomization.

A Serious Adverse Event (SAE) is any untoward event that:

1. Is fatal
2. Is life-threatening
3. Results in persistent or significant disability or incapacity
4. Is a congenital abnormality or birth defect
5. Requires inpatient hospitalization or prolongation of existing hospitalization with the following exceptions:
    - Preplanned (prior to the study) hospital admissions unless the hospitalization is prolonged.
    - Planned admissions (as part of a study—e.g., routine biopsies)
    - 23 hour hospitalization or re-hospitalizations
    - Hospitalization for elective procedure (e.g. generator change for ICD or pacemaker)
    - Emergency department visits

Important medical events that may not result in death, be life-threatening, or require inpatient hospitalization may be considered an SAE when, based on appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

Life-threatening indicates that the patient was, in the view of the investigator, at immediate risk of death from the adverse event as it occurred. It does not include an adverse event that, had it occurred in a more severe form, might have caused death.

Persistent or significant disability/incapacity indicates that the event resulted in permanent or significant and substantial disruption of the patients' ability to carry out normal life functions.

Classification of Adverse Events

The Principal Investigator will assess all AEs to determine the severity of the event, the possible causal relationship with study drug or study procedures, and whether the event is anticipated or unanticipated according to the following definitions:

1) Severity. The severity (intensity) of each AE will be determined by the Principle Investigator according to the following definitions:

Mild: Symptom(s) or sign(s) barely noticeable to the subject or does not make the subject uncomfortable. The AE does not influence performance or functioning. Treatments are not ordinarily needed for relief of symptom(s).

Moderate: Symptom(s) or sign(s) that cause the subject to be uncomfortable or may require downtitration of study drug. Treatment of symptom(s) may be needed.

Severe: Symptom(s) or sign(s) that cause the subject severe discomfort or may cause cessation of treatment with study drug. Treatment for symptom(s) are often needed.

Life-threatening: Symptom(s) or sign(s) of a sufficient severity/intensity to cause the subject to be at immediate risk of death. These cases will be classified as a Serious Adverse Event.

2) Relationship to study procedures. The temporal/causal relationship between the study procedures and the AE will be determined by the Principle Investigator according to the following definitions:

Definite: Clearly related to the study procedures.

Probable: Likely (high suspicion) related to the study procedures.

Possible: Possibly but not likely (low suspicion) related to the study procedures.

Unrelated: Clearly not related to the study procedures.

3) Anticipated or unanticipated. The relationship of the AE to the known side effect profile of the study drug and the known clinical manifestations of heart failure and other pre-existing co-morbid conditions will be determined by the Principal Investigator according to the following definitions:

Anticipated: Consistent with the known side effect profile of study drug or other study procedures and/or known clinical manifestations of heart failure or other pre-existing co-morbid conditions.

Unanticipated: Not known to be associated with study drug, other study procedures, heart failure, or other pre-existing co-morbid conditions.

IRB Reporting

Federal regulations require timely reporting by investigators to their local IRB of unanticipated problems posing risks to subjects or others. The following describes the NYULMC IRB reporting requirements, though Investigators at participating sites are responsible for meeting the specific requirements of their IRB of record.

Report Promptly, but no later than 5 working days:

Researchers are required to submit reports of the following problems promptly but no later than 5 working days from the time the investigator becomes aware of the event:

- *Unanticipated problems including adverse events* that are unexpected and related

- *Unexpected: An event is "unexpected" when its specificity and severity are not accurately reflected in the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document and other relevant sources of information, such as product labeling and package inserts.*
- *Related to the research procedures: An event is related to the research procedures if in the opinion of the principal investigator, the event was more likely than not to be caused by the research procedures.*
- *Harmful: either caused harm to subjects or others, or placed them at increased risk*

Other Reportable events:

The following events also require prompt reporting to the IRB, though *no later than 5 working days*:

- *Complaint of a research subject* when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- *Protocol deviations or violations* (includes intentional and accidental/unintentional deviations from the IRB approved protocol) for any of the following situations:
  - *one or more participants were placed at increased risk of harm*
  - *the event has the potential to occur again*
  - *the deviation was necessary to protect a subject from immediate harm*
- *Breach of confidentiality*
- *Incarceration of a participant* when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- *New Information indicating a change to the risks or potential benefits* of the research, in terms of severity or frequency. (e.g. analysis indicates lower-than-expected response rate or a more severe or frequent side effect; Other research finds arm of study has no therapeutic value; FDA labeling change or withdrawal from market)

Other Reportable events:

- **Deviations from the study protocol**

Deviations from the protocol must receive IRB approval before they are initiated. Any protocol deviations initiated without IRB approval that may affect the scientific soundness of the study, or affect the rights, safety, or welfare of study subjects, must be reported to the IRB as soon as a possible, but ***no later than 5 working days*** of the protocol deviation.

- ***Withdrawal of IRB approval***

An investigator shall report to the American Regent Inc. (funding entity) a withdrawal of approval by the investigator's reviewing IRB as soon as a possible, but ***no later than 5 working days*** of the IRB notification of withdrawal of approval.

Data Handling and Record Keeping

Confidentiality: A random Study ID code will be assigned to each participant. This code will be used on all written and electronic research records. The Principal Investigator and study coordinator will be able to link the Study ID code back to the personal information during the interval of active participation in the study, but this link will be destroyed after the subject has completed the study procedures. Consent forms, links between Study ID and personal information, case report forms, laboratory reports, and results of the 6 minute walking test, and the 31P-MRS and MRI analysis of skeletal muscle bioenergetics in response to plantar flexion exercise will be kept in a project notebook in a locked cabinet in a locked office. Research data without identifiers will be maintained

on the NYULMC REDCap system. The REDCap system provides secure password protected storage of research information behind the NYU firewall and provides quality control and audit trail functions to validate the integrity and quality of the research data.

Confidentiality and HIPAA: Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

Source Documents: Source data for the current proposal will include nursing records from the CTSI, case report forms, laboratory reports, and MRI reports. These data will be kept in a project notebook in a locked cabinet in a locked room.

Case Report Forms: The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. The data on paper CRF will be transferred to the electronic database and then will be destroyed. The electronic database has quality features to ensure that all fields have entered values, and that entered values are of the correct data entry type, and that numbers fall within pre-specified ranges.

Records Retention: Source documents will be retained for at least 2 years after completion of the study. Electronic data will be retained for at least 5 years.

Study Monitoring, Auditing, and Inspecting

Study Monitoring Plan. This study will be monitored according to the monitoring plan as stated in the protocol. The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

Auditing and Inspecting. The investigator will permit study-related monitoring, audits, and inspections by the IRB/EC, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

Ethical Considerations

This study is to be conducted accordance with applicable US government regulations and international standards of Good Clinical Practice, and applicable institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted Institutional Review Board (IRB) or independent Ethics Committee (EC) in agreement with local legal prescriptions, for formal approval of the study conduct.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB/EC for the study. The formal consent of

a subject, using the IRB/EC-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject, and the investigator-designated research professional obtaining the consent.

Study Financial Information

Source of funding: This is an investigator-initiated proposal funded by American Regent, Inc. American Regent Inc. will provide study drug and funds to support study personnel and study procedures.

Conflict of Interests: All faculty investigators have completed their annual conflict of interest submission. Individual investigator conflict of interest forms are submitted with this application.

Human subject costs/payments: There are no costs to the subjects enrolled in this proposal. All procedures are done strictly for research purposes and there will be no billing to subject or their insurance. Subjects will receive a stipend in the form of a check as compensation for time, travel, and inconvenience as follows: Visit 1 (1 hour): $75; Visit 2 (3 hours with study drug infusion) $200; and Visit 3 (2 hours) $100. Total payment for subjects who complete all three visits is $375.

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