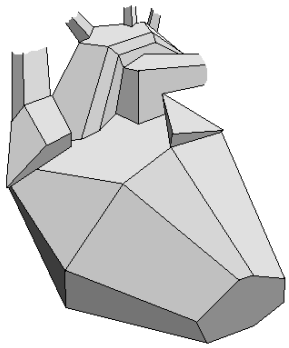
Intravenous Iron supplement for Iron deficiency in Cardiac transplant recipients: The IronIC trial



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PROTOCOL VERSION NO. 1.41

March 20tieth 2018

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Intravenous **Iron** supplement for **I**ron deficiency in **C**ardiac transplant recipients: The **IronIC** trial

I hereby declare that I will conduct the study in compliance with the Protocol, ICH GCP and the applicable regulatory requirements:

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Intravenous **Iron** supplement for **I**ron deficiency in **C**ardiac transplant recipients: The **IronIC** trial

Sponsor	Oslo University Hospital
Phase and study type	Phase II, interventional, randomised controlled trial
Investigational Medical Product (including active comparator and placebo) :	Intravenous iron isomaltoside or matching placebo (NaCl 0.9 %)
Centres:	Oslo University Hospital, Rikshospitalet, Oslo, Norway
Study Period:	Estimated date of first patient enrolled (study start): February 1st 2018 Anticipated recruitment period: February 1st 2018 – January 31st 2019 Estimated date of last patient completed (last patient, last visit: Study end): July 30tieth 2019
Treatment Duration:	1 day
Follow-up:	6 months
Objectives	The main goal of this study is to evaluate the effect of a single dose of intravenous iron isomaltoside on exercise capacity in cardiac allograft recipients with iron deficiency. Secondary objectives are to assess the impact of treatment on: (i) iron stores, (ii) muscle strength, (iii) body composition, (iv) cognitive function, (v) quality of life, (vi) markers of myocardial disease and inflammation, and (vii) safety and tolerability.
Endpoints:	Primary endpoint: The primary endpoint will be the between-group difference in the baseline-adjusted peak oxygen consumption measured 6 months after trial intervention. Secondary endpoints: • The number of patients with absolute or functional iron deficiency • Muscle strength as measured by a hand-grip (device) • Body composition • Quality of life as assessed by the SF-36 and EQ 5D 3L EuroQoL questionnaires • Cognitive function • N-terminal pro-B-type natriuretic peptide (NT-proBNP) • Cardiac troponin T (TnT) • C-reactive protein (CRP)

- Inflammatory and vasoactive peptides

Study Design:	This is a single site, dual arm, double blind, randomised, placebo controlled trial.
Main Inclusion Criteria:	Heart transplant recipient. Iron deficiency defined as serum ferritin < 100 µg/l or ferritin between 100 and 300 µg/l in combination with a transferrin saturation < 20 %. Age between 18 and 80 years. Signed informed consent and expected compliance with protocol.
Main Exclusion Criteria	Contraindications to study medication. Failure to obtain written informed consent
Sample Size:	100 patients
Efficacy Assessments:	Assessment of peak oxygen consumption; biomarkers reflecting iron stores and metabolism, myocardial stress, and inflammation; muscle strength, cognitive function and quality of life
Safety Assessments:	Physical examination. In-hospital observation for at least two hours after study drug administration. Safety visit at general practitioner 3 months after study drug administration. A 24-hour contact number will be provided. Repeat assessment after six months.

List of abbreviations and definition of terms

Abbreviation or special term	Explanation
AE	Adverse event
CANTAB	Cambridge Neuropsychological Test Automated Battery
CRF	Case report form (electronic/paper)
CRP	C-reactive protein
DMC	Data monitoring committee
GCP	Good clinical practice
ICH	International Conference on Harmonisation
NT-proBNP	N-terminal pro-B-type natriuretic peptide
SAE	Serious adverse event
SUSAR	Suspected unexpected serious adverse reaction
TnT	Cardiac troponin T

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1 Introduction

Heart transplantation is the treatment of choice for selected patients with end-stage heart failure.[1] World-wide, the median survival after transplantation is approximately 11 years.[2] Early survival is limited by early graft failure and acute rejections,[3] whereas complications to immunosuppressive therapy, such as infections and cancers, are among the most important causes of death in long-term survivors.[3] On the other hand, a major impediment to long-term survival is cardiac allograft vasculopathy caused by smouldering rejection.[4, 5] Long-term success in heart transplantation is therefore dependent on compromising between detrimental immunosuppression and low-grade inflammation.

Iron is an essential nutrient and co-factor not only in haemoglobin, but in several key respiratory reactions. It readily engages in one-electron oxidation-reduction reactions between its ferric (3+) and ferrous (2+) states. We exploit this property in oxidative phosphorylation, where iron acts as an intermediate in the electron transport chain. However, the same chemical quality explains why an excess of free, reactive iron is toxic. Ferrous iron can participate in redox chemistry known as the Fenton reaction, where it reacts with hydrogen peroxide or lipid peroxides to generate ferric iron, OH−, and highly reactive radicals. These compounds damage lipid membranes, proteins, and nucleic acids. Since cellular iron overload as well as iron deficiency are detrimental, the levels of reactive iron must be carefully controlled and limited.[6]

Exercise capacity and health-related quality of life are reduced in cardiac allograft recipients despite good over-all results regarding graft function.[7, 8] We have shown exercise capacity after heart transplantation depends mainly on peripheral factors rather than on allograft function.[9, 10] Numerous mechanisms unrelated to haemodynamic dysfunction may underlie the impaired exercise tolerance in heart transplant recipients, one of which may be iron deficiency. Iron plays a key role in oxygen uptake, transport, storage, and metabolism in skeletal muscle.[11] Heart transplant recipients have multiple reasons for having iron deficiency, including previous heart failure, perioperative blood loss, frequent blood sampling, renal failure, and polypharmacy. Among our heart transplant survivors, 48 % have iron deficiency (defined as serum ferritin < 100 µg/l or ferritin between 100 and 300 µg/l in combination with a transferrin saturation < 20 %) [Abstract submitted to ISHLT congress, Nice 2018]. We hypothesise that intravenous iron therapy will improve peak oxygen consumption, muscle strength, functional capacity and quality of life in these patients. The IronIC trial is designed to test this hypothesis.

1.1 Iron metabolism

Dietary iron is absorbed in the small intestine and transferred to the circulation. Before absorption, ferric iron in the diet must be reduced to ferrous iron at the apical surface of enterocytes. Iron is then transported into the cell by the divalent metal transporter-1, an energy-dependent symporter that is essential for intestinal uptake of inorganic sources of dietary iron. Heme, another source of dietary iron, is internalised through receptor-mediated endocytosis. This process seems to be regulated by iron stores.[12] Ferrous iron is exported through the basolateral membrane of the enterocyte to the interstitial space by the exporter ferroportin and oxidised by the ferroxidase hephaestin. Ferroportin is negatively regulated by the iron-regulatory hormone hepcidin.[13]

The major transport protein for circulating iron is transferrin. Once extracellular, iron is bound with high affinity by this protein. The cellular uptake of iron occurs through receptor-mediated endocytosis of the iron/transferrin complex through transferrin receptor-1. Iron is then exported from the endosomal vesicle by the divalent metal transporter-1, suggesting that a ferrireductase must exist within the endosomal vesicle. Ferrous iron is thought to enter a labile intracellular iron pool, from where it can be utilised for intracellular functions.[11]

The portion of intracellular iron that is not needed for immediate use is stored by ferritin. Twenty-four ferritin subunits assemble to form the apoferritin molecule, each of which can sequester up to approximately 4500 iron atoms. Ferritin also possesses enzymatic properties, and oxidises

ferrous iron to its ferric form.[14] Small amounts of iron-poor ferritin are present in the serum, where it can be measured and quantified. Serum ferritin is elevated in iron overload, but also in inflammatory conditions.

The content of cytoplasmic ferritin is regulated by the availability of intracellular free iron. This regulatory response is posttranscriptional. When intracellular iron stores are depleted, iron regulatory proteins 1 and 2 bind to the iron responsive element of the ferritin mRNA and prohibit translation. Conversely, when iron levels are high, ferritin synthesis increases.[14] On the contrary, the binding of iron regulatory proteins to an iron regulatory element in transferrin receptor 1 mRNA lengthens its half-life, ultimately leading to increased transferrin receptor display on the cell surface in situations of iron depletion.

Ferritin and transferrin, established markers of iron availability, are regulated not only by iron stores, but also by inflammation. Ferritin is an "acute phase" protein, the serum concentrations of which increase in inflammatory conditions.[14] In fact, ferritin can be viewed not only as an iron regulatory protein, but also as a protein that takes part in the cellular defence against stress and inflammation. Transferrin receptor-1 and transferrin, on the other hand, are down-regulated in inflammatory states. These responses contribute to low available iron in states of inflammation, presumably to deprive invading microorganisms of an essential nutrient.[15]

The major regulator of systemic iron metabolism is the peptide hormone hepcidin. Full iron stores induce hepcidin transcription through the bone morphogenetic protein signalling pathway. However, hepcidin expression is also up-regulated in inflammation.[16] Its mode of action is to bind to, and induce degradation of, ferroportin.[13] Hepcidin thus exerts its control over systemic iron metabolism by regulating the transfer of dietary, recycled, and stored iron from intracellular compartments to the extracellular fluid, regulating the amount of iron available for iron-demanding tissues.[15] This gate-keeping mechanism reduces the effect of oral iron in patients with inflammation, and can be partially circumvented by intravenous administration of iron.

1.2 Iron deficiency in heart failure

Iron deficiency is prevalent in patients with heart failure.[17, 18] Iron deficiency is associated with a worse prognosis, and randomised controlled trials have shown that correction of iron deficiency with intravenous iron therapy improves functional capacity, quality of life, and 6-minute walk distance.[19, 20] Current guidelines therefore recommend intravenous iron substitution in patients with heart failure with reduced ejection fraction and iron deficiency.[21] Intravenous iron is more effective, better tolerated, and improves quality of life to a greater extent than oral iron supplements.[22-24] In the IRONOUT HF trial, in which 225 patients with systolic heart failure were randomised to oral iron supplement or placebo, there was no effect on oxygen uptake, 6-minute walk distance, or quality of life. The authors attributed the negative results to the minimal effect on iron stores, suggesting that oral iron does not adequately replenish iron stores in patients with heart failure.[25]

Cardiac allograft recipients resemble patients with heart failure in many respects. Prior to transplantation, and in some instances after heart transplantation, they have had overt heart failure. Moreover, due to the immunologic challenge posed by the allograft, and their susceptibility to infection due to immunosuppressive treatment, cardiac allograft recipients have low-grade inflammation. This low-grade inflammation makes it difficult to interpret iron stores, and results in dysregulated iron metabolism.

To our knowledge, there have been no studies to assess the effect of intravenous iron therapy in heart transplant recipients who have iron deficiency. There is reason to believe that a liberal definition of iron deficiency should be used in cardiac allograft recipients, and we have elected to use the well-established definition used in patients with heart failure: serum ferritin < 100 µg/l or ferritin between 100 and 300 µg/l in combination with a transferrin saturation < 20 %. Because oral iron supplement is less effective then intravenous iron in general, and in patients with heart failure in particular,[25] we assume that oral iron supplement is inadequate in heart transplant recipients. We

have designed the IronIC trial to assess the effect of intravenous iron isomaltoside on exercise capacity, muscle strength, cognition and quality of life in iron-deficient heart transplant recipients.

1.3 Therapeutic information

Isomaltoside 1000 is an oligosaccharide with a mean molecular weight of 1,000 Da, which consists predominantly of unbranched chains corresponding to 3–5 glucose units.[26] The iron in Monofer® is tightly bound to isomaltoside in a matrix containing approximately 10 iron molecules per one isomaltoside pentamer. This structure releases iron to iron-binding proteins in a controlled manner with little risk of free iron toxicity.[27]

1.3.1 Pre-Clinical & Clinical Experience with iron isomaltoside

According to the Summary of Product Characteristics (SPC), available at www.medicines.org.uk, iron complexes have been reported to be teratogenic and embryocidal in non-anaemic pregnant animals at high single doses above 125 mg iron/kg body weight. The highest recommended dose in clinical use is 20 mg iron/kg body weight. In a murine fertility study with Monofer®, there were no effects on male reproductive performance and spermatogenic parameters. There have been no adequately sized and controlled trials to assess the safety of iron isomaltoside in pregnancy, and Monofer® should not be used in pregnancy unless clearly necessary. Iron does not transfer easily to human milk, and no adverse effects on breastfed infants are anticipated. In patients with chronic kidney disease, the plasma iron half-life is approximately 30 hours after intravenous administration, regardless of the infused dose.[28] After infusion, the iron in iron isomaltoside follows first-order kinetics.[29]

Clinical experience with Monofer® has been reviewed by Kalra and Bhandari.[30] In short, the efficacy and safety of intravenous iron isomaltoside has been investigated in patients with chronic kidney disease,[31-34] inflammatory bowel disease,[35, 36] and postpartum haemorrhage.[37] In general, the treatment has been efficacious and associated with few adverse events. In a double-blind trial, 60 patients undergoing coronary bypass surgery were randomised to receive 1000 mg iron isomaltoside (up to 20 mg/kg body weight) or saline. The fall in haemoglobin was significantly less pronounced in patients allocated to iron. No adverse events were observed. In 20 patients with congestive heart disease and iron deficiency anaemia who received on average 868 mg (range 650–1000 mg) of iron isomaltoside, there was a significant improvement in health-related quality of life and no serious adverse events. Specifically, there were no acute or delayed hypersensitivity reactions.[38]

1.3.2 Known and potential risks and benefits

Intravenous iron transfusion carries a very low,[30] but clinically significant risk of hypersensitivity reactions. This risk is increased in patients with known allergies, an allergic diathesis, or autoimmune diseases. Heart transplant recipients are treated with immunosuppressants and possibly less prone to experiencing these reactions. To prevent serious consequences of a hypersensitivity reaction, the patients will be monitored in-hospital during the study drug infusion and for at least two hours afterwards. The Monofer® SPC specifically recommends at least 30 minutes of observation. The infusions will be administered at the cardiology ward at Rikshospitalet. This ward is well-staffed and equipped to deal with acute hypotension/cardiac arrest/respiratory failure. Hypersensitivity-reactions should be treated with immediate stop in transfusion, volume resuscitation, and supportive care and cardiopulmonary resuscitation as required. The investigator and the responsible physician must decide whether reintroduction of the study drug infusion is safe after the episode has been resolved, depending on the severity of the episode. Nominal hypotension without symptoms and without threatening organ function is acceptable and should not result in study drug withdrawal.

Subcutaneous/extravenous infusions can lead to skin reactions and prolonged discolouration of the skin. Vigilant assessment of the infusion side is therefore necessary during drug infusion, and careful testing of the peripheral cannula with infusion of a test dose of isotonic saline should be performed prior to study drug infusion.

The benefit of intravenous iron supplement in iron-deficient heart transplant recipients is unknown. Experience from other populations,[23, 24] and from patients with heart failure in particular, suggests that intravenous iron can replete iron stores and improve exercise capacity and health-related quality of life in patients with iron deficiency. This trial is designed to assess if similar results can be obtained in iron-deficient heart transplant recipients.

1.4 Rationale for the study and purpose

Iron deficiency is associated with poor exercise capacity, lethargy and reduced quality of life. Our results show that iron deficiency is prevalent in heart transplant recipients. Cardiac allograft recipients have reduced exercise capacity and quality of life compared with the age and gender matched general population. We assume that some of these symptoms are due to iron deficiency, and hypothesise that intravenous iron supplement will improve peak oxygen consumption, muscle strength, functional capacity, cognition, and health-related quality of life in heart transplant recipients with iron deficiency.

1.5 Rationale for study endpoint

Measurement of peak oxygen capacity is the gold standard for assessing exercise capacity.[39] Exercise capacity is an established surrogate endpoint in patients who are evaluated for heart transplantation.[40] Exercise capacity is associated with mortality before[41] and after[42] heart transplantation, and is tightly associated with quality of life in these patients. We have extensive experience in measuring peak oxygen uptake in heart transplant recipients.[42, 43] Moreover, the accuracy of this measurement is good, resulting in low intra-individual variability in repeated measurements.

2 Study objectives and related endpoints

The main goal of this study is to evaluate the ability of a single administration of intravenous iron isomaltoside to increase peak oxygen consumption in cardiac allograft recipients who have iron deficiency defined as serum ferritin < 100 µg/l or ferritin between 100 and 300 µg/l in combination with a transferrin saturation < 20 %.

Secondary objectives are to assess the impact of treatment on: (i) iron stores, (ii) muscle strength, (iii) body composition, (iv) cognitive function, (v) quality of life, (vi) markers of myocardial disease and inflammation, and (vii) safety and tolerability.

2.1 Primary endpoint

The primary endpoint will be the baseline-adjusted between-group difference in peak oxygen consumption as measured on a treadmill exercise test 6 months after study drug administration.

2.2 Secondary endpoints

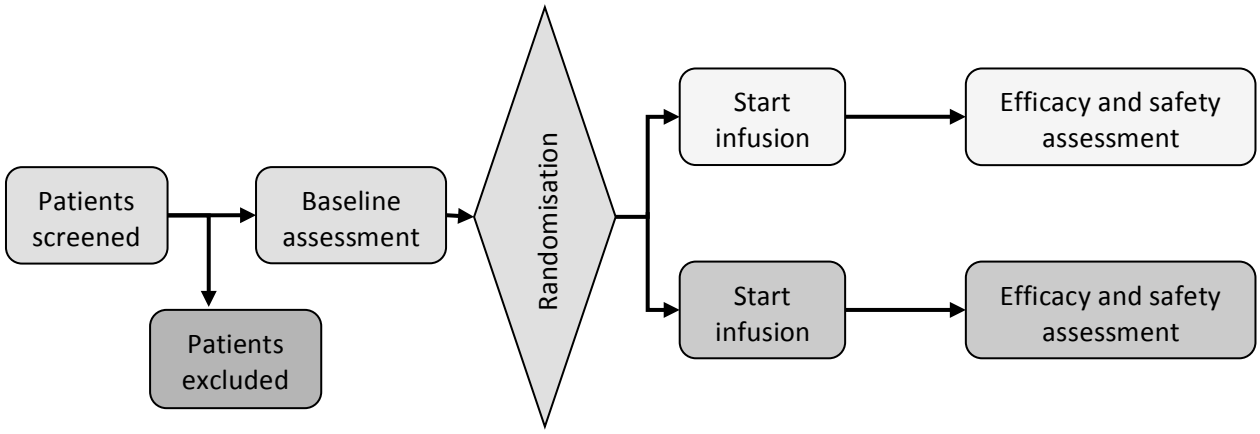
Secondary endpoints:

- The number of patients with absolute or functional iron deficiency
- Muscle strength as measured by a hand-grip dynamometer
- Body composition
- Cognitive function as assessed by the Cambridge Neuropsychological Test Automated Battery
- Quality of life as assessed by the SF-36 and 5D EuroQoL questionnaires
- N-terminal pro-B-type natriuretic peptide (NT-proBNP)
- Cardiac troponin T (TnT)
- C-reactive protein (CRP)

- Inflammatory and vasoactive peptides

3 Overall study design

This is a phase 2, double blind, randomised, placebo-controlled trial. Participants will be randomised in a 1:1 fashion to receive a single intravenous dose of iron isomaltoside or matching placebo. The study is designed to show superiority with regard to the primary endpoint in patients assigned to active treatment versus patients allocated to the placebo arm.



Study Period	Estimated date of first patient enrolled (study start): February 1st 2018. Anticipated recruitment period: February 1st 2018 – January 31th 2019. Estimated date of last patient completed (last patient, last visit: Study end): August 1st 2019.
Treatment Duration:	One day (single iv dose drug administration)
Follow-up:	6 months

4 Study population

4.1 Selection of study population

The IronIC trial will be conducted at Oslo University Hospital, Rikshospitalet, in Oslo, Norway. Oslo University Hospital Rikshospitalet is the only centre in Norway to perform transplantations. We perform approximately 35 heart transplantations per year. With a median survival of more than 12 years, we have a population of approximately 500 cardiac allograft recipients who attend regular follow-ups twice per year. Per routine, we measure ferritin and transferrin saturation in all of these patients. We plan to recruit patients who are admitted for their yearly control, and who have iron deficiency defined as serum ferritin < 100 µg/l or ferritin between 100 and 300 µg/l in combination with a transferrin saturation < 20 %. A dedicated study nurse will send a written invitation to participate in the trial prior to the patient's routine control at our centre.

4.2 Number of patients

We aim to enrol 100 patients in this trial.

4.3 Inclusion criteria

Patients will be screened for eligibility upon admittance for routine follow-up at least one year after heart transplantation.

All of the following conditions must apply prior to administering the investigational medicinal product:

- Cardiac allograft.
- Presentation at least one year after heart transplantation.
- Iron deficiency defined as serum ferritin < 100 µg/l or ferritin between 100 and 300 µg/l in combination with a transferrin saturation < 20 %.
- Age between 18 and 80 years.
- Informed consent obtained and documented according to Good Clinical Practice (GCP), and national/regional regulations.

4.4 Exclusion criteria

Patients will be excluded from the study if they meet any of the following criteria:

- Anaemia (Haemoglobin < 100 mg/l)
- Haemochromatosis
- Haemosiderosis
- Porphyria cutanea tarda
- Blood dyscrasias or any disorders causing haemolysis or unstable red blood cells
- Decompensated liver disease (Child-Pugh score 7 or higher)
- End-stage renal failure, i.e. eGFR < 15 ml/min or on renal replacement therapy
- Planned cardiac surgery or angioplasty within 6 months
- Planned major surgery within 6 months
- Medical history of unresolved cancer (except for basal cell carcinoma)
- Treatment with systemic steroids more than the equivalent of 10 mg Prednisone/day at the time of informed consent or change in dosage of thyroid hormones within 6 weeks prior to informed consent
- Any uncontrolled endocrine disorder except type 2 diabetes
- Pregnancy
- On erythropoietin analogues
- Known sensitivity or intolerance to iron isomaltoside or other parenteral iron preparations
- Intravenous iron supplement within 6 months prior to inclusion
- On oral iron substitution (unless the subject agrees to stop treatment prior to randomisation)
- Ongoing rejections or infections
- Alcohol or drug abuse within 3 months of informed consent that would interfere with trial participation or any ongoing condition leading to decreased compliance with study procedures or study drug intake
- Intake of an investigational drug in another trial within 30 days prior to intake of study medication in this trial or participating in another trial involving an investigational drug and/or follow-up

5 Treatment

For this study, intravenous iron isomaltoside (Monofer®) and matching placebo are defined as investigational medicinal products.

5.1 Drug identity, supply and storage

Medication and comparator

Active drug: Intravenous iron isomaltoside dissolved in 100 ml NaCl 0.9 %.
Placebo: Intravenous sodium chloride 0.9%; 100 ml.

Supply, packaging, labelling, handling, storage and accountability
The manufacturer supplies study medication to the investigators on site. It is labelled with information according to local regulation. The study medication will be stored in a locked refrigerator (2-8°C), protected from unintended use. All study medications supplied for this trial will be retained in a safe place at all times of the study. Only personnel authorised by the principal investigator should dispense the study medication, and the accountability is the responsibility of investigator. An up to date study medication inventory (dispensing records) will be maintained at all times.

5.2 Dosage and administration

After the participant has provided informed consent, he or she will be randomised to receive a single intravenous administration of iron isomaltoside (Monofer®) or matching placebo. The active drug, iron isomaltoside, will be administered as a single, intravenous infusion of 20 mg/kg body weight (rounded off to the nearest 100 mg) dissolved in 100 ml NaCl as recommended by the drug manufacturer ("on-label" treatment). Patients allocated to placebo will receive an intravenous infusion of 100 ml NaCl 0.9% administered in the same manner. Because iron isomaltoside is a dark-brown solution that is easily distinguishable from the saline placebo, study personnel responsible for the preparation and administration of the study drug will be aware of the group assignments and will therefore not be involved in any study assessments. To ensure that patients are unaware of treatment allocation, we will cover the injection site, syringes and intravenous lines with sheets to shield the injection site from the patient's view

The active drug dilution/placebo will be prepared and administered, according to the randomisation code, by nurses who do not participate in the trial. The investigational medicinal products will only be administered when staff trained to evaluate and manage anaphylactic reactions is immediately available, in an environment where full resuscitation facilities can be assured. The patients will be observed for adverse effects for at least two hours following the injections.

5.3 Duration of therapy

The investigational medicinal products (iron isomaltoside or placebo) will be administered as a single intravenous infusion over 30 minutes only (1,67 ml/min). No other study-specific intervention will be provided. For intravenous iron, a single dose of 20 mg/kg is expected to provide adequate iron supplement in patients with haemoglobin above 100 mg/l.

5.4 Monitoring during drug administration

The study drug will be administered in a hospital setting, with frequent attendance by experienced nurses. Vital signs are observed and documented prior to study drug infusion. The patients will be hospitalised for at least two hours after the study drug infusion, and thereafter for as long as deemed necessary by the responsible physician.

5.5 Concomitant medication

All concomitant medication (incl. vitamins, herbal preparation and other "over-the-counter" drugs) used by the patient will be recorded in the patient's file and CRF. Study participation does not preclude administration of drugs that are provided on clinical indication. On the contrary, study participants will receive standard-of-care treatment as recommended in prevailing guidelines. Concurrent treatment with steroids other than low doses of corticosteroids (equivalent to 10 mg prednisone or less) at the time of randomisation is an exclusion criterion. New immunosuppressants should not be introduced within the first month after randomisation unless necessary as deemed by the treating physician.

5.5.1 Prohibited treatments during study period

The following treatments are not permitted during the study:

- parenteral iron supplement (other than the initial study drug infusion)
- oral iron substitution

5.6 Drug accountability

The responsible site personnel will confirm receipt of study drug and will use the study drug only within the framework of this clinical study and in accordance with this protocol. The manufacturer (Pharmacosmos®) provides the active study drug. The study drug or matching placebo will be administered by trained hospital personnel. The manufacturer supplies study medication to the investigators on site. It will be labelled with information according to local regulation. The study medication will be stored in a locked refrigerator (2-8°C), protected from unintended use. All study medications supplied for this study must be retained in a safe place at all times of the study. Only personnel authorised by the principal investigator should dispense the study medication, and the accountability is the responsibility of investigator. A study medication inventory (dispensing records) for all medication dispensed must be maintained at all times. A unique, three-digit number will be assigned to each study drug dose (iron isomaltoside or placebo) by an independent nurse. The study drug number and drug dose (sham dose if placebo) will be available in the patient file. A list linking study drug numbers and batch-numbers and expiry dates will be kept in a sealed envelope in a locked closet, but can be opened if unblinding is necessary (see paragraph 10.2.3). The study drug inventory must contain information on Monofer® vial batch numbers and expiration dates. A study nurse will be responsible for drug storage, including keeping a temperature log, and the study drug inventory.

5.7 Drug labelling

The investigational product will have a label permanently affixed to the outside and will be labelled according with ICH/GCP and national regulations, stating that the material is for clinical trial / investigational use only and should be kept out of reach of children.

The bag with the dissolved solution will have an affixed label stating (in Norwegian)

This bag contains … mg iron isomaltoside or placebo (Study drug number …) dissolved in 100 ml NaCl 0.9 % for the IronIC trial. The drug is meant for intravenous administration over 30 minutes (1.67 ml/min). The content of this bag was prepared by … .

Labels will also include blank lines (in Norwegian) for:

Patient's initials
Patient's study number
Date dispensed
Name of prescribing doctor

"Denne posen inneholder … mg jernisomaltosid eller placebo (studiemedikamentnummer …) i 100 ml 0,9 % NaCl til bruk i IronIC-studien. Posen skal gis intravenøst ila 30 minutter (1,67 ml/min). Denne posen ble tilberedt av _____.
Pasientens initialer:_____
Studienummer:_____
Dato:_____
Forskrevet av dr:_____
»

5.8 Subject identification

Each study participant is identified by a unique subject number that is assigned after the subject signs the informed consent form. This number is generated through the randomisation process. Once assigned, the subject number cannot be reused for any other subject. The same primary identifier will be used throughout the study.

6 Study procedures

6.1 Table

	Baseline		3 months	6 months
Informed consent	x	Study drug infusion		
Clinical examination	x		x	x
ECG	x			x
Echocardiography	x			
Safety samples[1]	x		x	x
Serum hCG[2]	x			
Randomisation	x			
Biobank samples[3]	x			x
6-minute walk test	x			x
Measurement of peak oxygen consumption	x			x
Hand grip strength	x			x
Body mass composition	x			x
Cognitive function	x			x
Quality of life	x			x
Adverse events		←x→		

[1]Haematological parameters, renal function, serum electrolytes, tests of liver damage, TnT, NT-proBNP and CRP are measured routinely in our transplant recipients. [2]In pre-menopausal women only [3]Secondary endpoints (i.e, NT-proBNP, markers of iron metabolism and inflammation).

6.2 By visit

Study participants will be recruited from the pool of heart transplant recipients who attend regular follow-up at Oslo University Hospital, Rikshospitalet. A dedicated study nurse not responsible for the routine treatment of the patients, will ask eligible patients to participate when clinical assessment, ferritin/transferrin and safety blood samples (routine blood samples in heart transplant recipients) have been performed and analysed.

6.2.1 Before treatment starts

Informed consent

Written informed consent (Appendix A) must have been provided voluntarily by each subject before any study specific procedure is initiated.

The following tests will be performed at baseline:

Physical examination

A physical examination (including examination of heart, lungs, abdomen, neck and assessment of peripheral circulation and oedema) must be performed; vital signs (blood pressure, and heart rate); and height and weight must be recorded.

Medical history

A medical history must be obtained, and age; gender; NYHA functional status; risk factors (hypertension, smoking, and diabetes); reason for and time since heart transplantation; and concomitant disease must be recorded.

Concomitant medication

All concomitant medication (incl. vitamins, herbal preparation and other "over-the-counter" drugs) used by the participant within 28 days of treatment start must be recorded in the CRF by generic name and dose.

Laboratory analyses

Blood samples will be obtained to determine: Haemoglobin; white blood cell count, platelet count; serum potassium; serum sodium; glucose, glycosylated haemoglobin (HbA1c); creatinine; ALT; bilirubin; albumin; INR; CRP; N-terminal pro-B-type natriuretic peptide (NT-proBNP); total cholesterol; ferritin; transferrin, serum iron and total iron binding capacity. Serum hCG must be evaluated to exclude pregnancy in pre-menopausal females, and repeated prior to study drug infusion if more than 72 hours have passed between screening and study drug administration. Blood for efficacy analyses must be drawn and appropriately labelled and stored for later analysis.

Treadmill test

A treadmill test for the assessment of peak oxygen consumption is performed at baseline. The results of this test will be used for adjustment of the test-result six months after study drug infusion. The latter result, with adjustment for the baseline result, constitutes the primary endpoint of the IronIC trial.

Hand grip strength

Right and left hand grip strengths will be measured by a hand-held dynamometer.

Body composition

Body composition (weight, total water, total fat, percent fat, the ratio of extracellular water to intracellular water [measuring oedema], and visceral fat) will be measured at baseline and after 6 months with the InBody 770 body composition analyser.

Quality of life

Self-reported, health-related quality of life will be gauged with the SF-36 and EQ 5D 3L questionnaires.

Cognitive function

Cognitive function will be assessed with the Cambridge Neuropsychological Test Automated Battery (CANTAB).

6.2.2 3-month safety visit

The study participants will be asked to visit their general practitioner for a clinical examination and blood sampling 3 months after study drug infusion. The blood samples will be sent to the laboratory at Oslo University Hospital, Rikshospitalet, for delayed (after end-of-study) assessment of haemoglobin, serum ferritin, serum iron, serum transferrin, and transferrin saturation.

6.2.3 End of study visit

This study visit six months after study drug administration is designed to assess efficacy and safety.

Medical history

A medical history must be repeated, and NYHA functional status; any change in risk factors (hypertension, smoking, diabetes mellitus), and concomitant disease must be recorded. Any medical events since inclusion in trial must be evaluated. Current well-being, symptoms, potential side effects and physical capacity must be assessed.

Physical examination

A physical examination must be performed, and results (including examination of heart, lungs, abdomen, neck and assessment of peripheral circulation and oedema); vital signs (blood pressure, and heart rate); and height and weight must be recorded.

Concomitant medication

All concomitant medication (incl. vitamins, herbal preparation and other "over-the-counter" drugs) used by the participant within 28 days of treatment start must be recorded in the CRF by generic name and dose.

Laboratory analyses

Blood samples will be obtained to determine: Haemoglobin; white blood cell count, platelet count; serum potassium; serum sodium; glucose, glycosylated haemoglobin (HbA1c); creatinine; ALT; bilirubin; albumin; INR; CRP; N-terminal pro-B-type natriuretic peptide (NT-proBNP); total cholesterol; ferritin; transferrin, serum iron and total iron binding capacity. Blood for efficacy analyses must be drawn and appropriately labelled and stored for later analysis.

Treadmill test

A treadmill test for the assessment of peak oxygen consumption is performed after six months. The results of this test, with adjustment for the baseline result, constitute the primary endpoint of the IronIC trial.

Hand grip strength

Right and left hand grip strengths will be measured by a hand-held dynamometer.

Body composition

Body composition (weight, total water, total fat, percent fat, the ratio of extracellular water to intracellular water [measuring oedema], and visceral fat) will be measured at baseline and after 6 months with the InBody 770 body composition analyser.

Quality of life

Self-reported, health-related quality of life will be gauged with the SF-36 and EQ 5D 3L questionnaires.

Cognitive function

Cognitive function will be assessed with CANTAB.

Safety assessment

Any untoward medical event (i.e. any AE, SAE or SUSAR) since the last visit must be recorded in the case report form (CRF) and the patient medical record.

6.2.4 After end of treatment

We do not plan to perform study visits after the six-month follow-up.

6.3 Criteria for patient discontinuation

Patients may be discontinued from study treatment and assessments at any time. Specific reasons for discontinuing a patient for this study are:

- Voluntary discontinuation: participating patients are free to discontinue his/her participation in the study at any point in time, without prejudice to further treatment.
- Major protocol deviation
- Incorrect randomisation, i.e. the patient does not meet the required inclusion/exclusion criteria for the study
- Patient lost to follow-up
- Patient's non-compliance to study treatment and/or procedures

6.4 Procedures for discontinuation

6.4.1 Patient discontinuation

Patient withdrawal must be documented in the CRF as well as in hospital records. If possible, a final assessment should be obtained (end of study visit). The reason for discontinuation is recorded. The investigator is obliged to follow up any significant adverse events until the outcome either is recovered or resolved, recovering/resolving, not recovered/not resolved, recovered/resolved with sequelae, fatal or unknown. Patients who withdraw will be included in the intention-to treat analysis.

6.4.2 Trial discontinuation

The whole trial may be discontinued at the discretion of the primary investigator or the sponsor in the event of any of the following:

- Occurrence of AEs unknown to date in respect of their nature, severity and duration
- Medical or ethical reasons affecting the continued performance of the trial
- Difficulties in the recruitment of patients
- Cancellation of drug development

The sponsor and principal investigator will inform all investigators, the relevant Competent Authorities and Ethics Committees of the termination of the trial along with the reasons for such action. If the study is terminated early on grounds of safety, the Competent Authorities and Ethics Committees will be informed within 15 days.

7 Assessments

7.1 Assessments of efficacy

Efficacy will be evaluated through a comprehensive assessment of peak oxygen uptake, hand-grip strength, self-assessed quality of life, assessment of cognitive function, and biochemistry. All efficacy assessments will be made prior to study drug infusion and at the end-of-study visit after six months. The results will be reported as baseline-adjusted between-group differences.

7.1.1 Peak oxygen consumption

Peak oxygen consumption will be measured on a treadmill according to a protocol that we have successfully employed in heart transplant recipients.[43] The protocol employs a modified treadmill walk test in compliance with the European Society of Cardiology recommendations for cardiopulmonary exercise testing in patients with chronic heart failure.[44] The test begins with warm-up period of 10 min on a Runrace Treadmill (Technogym, Cesena, Italy), during which the individual walk speed is determined. The inclination of the treadmill is then increased by 2% every 2 min until volitional fatigue. Test termination criteria are a respiratory exchange ratio (RER) >1.05 and/or a rated perceived exertion (Borg 6–20 scale) >18.[45] Lung function and breath gas exchange will be measured using the Sensormedics Vmax (Yorba Linda, CA, USA). ECG and HR will be monitored

before, during and after exercise. Peak oxygen consumption, carbon dioxide production, maximum ventilation and respiratory exchange rate will be continuously monitored. Blood pressure will be measured automatically (Tango; Sun Tech Medical Instruments, NC, USA) before exercise, every 2 min during exercise, and after exercise. After termination of the test, the treadmill will be stopped and the patients rest in an upright position for a recovery period of 2 min. VO2peak will be calculated as the mean of the three highest ten-second measurements before volitional fatigue prior to the end of exercise. A Borg score >18 and/or a respiratory exchange ratio > 1.05 will be used as criteria for an adequate maximal exercise test.

Summary of parameters to be recorded:

Before start: Heart rate, oxygen saturation and blood pressure. At peak exercise: treadmill inclination, heart rate, blood pressure, Borg score, oxygen saturation, peak oxygen consumption, expiratory exchange ratio, and peak ventilation volume.

7.1.2 Hand grip strength

As an estimate of muscle strength, we will measure peak hand grip strength using a hand grip strength dynamometer.

7.1.3 Body composition

We will use the InBody 770 Body Composition Analyzer to assess patients' body composition. The patients steps onto the apparatus and holds on to the hand grips. The InBody 770 scanner uses six different electromagnetic frequencies to obtain 30 impedance measurements and three frequencies to obtain 15 resistance measures. The results can be integrated to yield body total water content, total fat content, percent fat, the ratio of extracellular water to intracellular water [measuring oedema], and visceral fat.

7.1.4 Quality of life

Quality of life will be examined at baseline and at the end of the study using two validated questionnaires; the SF36[46] and the EQ 5D 3L EuroQoL questionnaire.[47] The SF-36v2 is a 36-item general health-related quality of life instrument that has 36 questions that contribute to 8 domains or scale scores of physical functioning, role-physical, bodily pain, general health, vitality, social functioning, and role-emotional and mental health.[46] The EQ 5D 3L EuroQoL questionnaire consists of 2 pages, the EQ-5D descriptive system and the EQ visual analogue scale. The quality of life questionnaires (in Norwegian) can be found in appendix B.

7.1.5 Cognitive function

Cognitive function will be assessed with the Cambridge Neuropsychological Test Automated Battery (CANTAB).[48, 49] The CANTAB test battery is performed electronically on a dedicated tablet. CANTAB is language-independent, culturally neutral, and requires no technical knowledge or prior familiarity with computers. It helps to determine an individual's cognitive health across five key domains and includes an integrated depression scale to assess the current mood of the person taking the test.

The cognitive domains assessed are:

- Executive function - Central control, planning, strategy, and flexible thinking
- Processing speed - The ability to perform mental tasks quickly and efficiently
- Attention - The ability to concentrate and actively process information
- Working memory - How we hold information while processing or acting on it
- Episodic memory - Memory of events and experiences: what happened, where and when?

Benefits of using the CANTAB platform includes dedicated courses for the staff administering the test, the highly accurate and validated evaluation of cognitive performance, the good discriminatory

ability, even in high-functioning individuals, and the dedicated technical support. CANTAB is already accepted for use in research at Oslo University Hospital, and was used in the world-wide Ebbinghausen study[49] that assessed cognitive function in patients treated with evolocumab vs placebo.

7.1.6 Blood samples

We will draw blood samples for biobanking at baseline, prior to study drug infusion, and at 6 months. For biobanking, 18 ml blood should be drawn into tubes containing EDTA as the anticoagulant, 6 ml into tubes with citrate as the anticoagulant and 6 ml into tubes without additives. The tubes with EDTA and citrate should be put on ice immediately and be centrifuged at 3700 rotations/min for 20 minutes. The tubes without additives should be left at room temperature for 1-2 hours prior to centrifugation at 3500 rotations/min for 15 minutes. The tubes containing citrate as the anticoagulant should be put on ice immediately. The resulting plasma and serum, respectively, should be stored in multiple aliquots (at least four aliquots of serum and six aliquots of plasma per patient per visit), and labelled with the unique trial subject number, and the letter A signifying baseline, and the letter B signifying the end-of-treatment visit. The samples should be stored at -80 degrees Celsius until analysis.

Blood samples for safety will be collected at baseline, and 6 months after randomisation (end-of-study). These blood samples will be drawn and analysed at the study sites as per clinical routine. Troponin values will be used for endpoint analyses.

NT-proBNP will be assayed on a MODULAR platform (Roche Diagnostics, Basel, Switzerland). Plasma levels of CRP will be determined by a high-sensitivity particle-enhanced immunoturbidimetric assay (Tina-quant CRP [Latex] HS, Roche Diagnostic, Basel, Switzerland). TnT will be assessed using a high-sensitive immunoassay (Roche hs-TnT).

7.2 Safety and tolerability

Safety will be monitored by the assessments described below as well as the collection of AEs at every visit. Significant findings that are present prior to the signing of informed consent must be included in the relevant medical history/ current medical condition page of the CRF. For details on AE collection and reporting, refer to Section 8.

For the assessment schedule, refer to Flow chart in Section 6.1.

Physical examinations will be performed at baseline and after 6 months. Vital signs including heart rate and blood pressure will be recorded before, during, and after study drug infusion. Monitoring must be especially vigilant with regard to signs of acute hypersensitivity reactions/abrupt hypotension.

Blood samples for safety analyses will be drawn at baseline and after 3 and 6 months. Local laboratory cut-points for normal values will be used for safety analyses. Haemoglobin; white blood cell count, platelet count; serum potassium; serum sodium; glucose, glycosylated haemoglobin (HbA1c); creatinine; ALT; bilirubin; albumin; INR; CRP; N-terminal pro-B-type natriuretic peptide (NT-proBNP); total cholesterol; ferritin; transferrin, serum iron and total iron binding capacity will be evaluated at each visit. In addition, blood samples for the measurement of iron stores will be drawn 3 months after study drug administration.

8 Safety monitoring and reporting

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an adverse event (AE) or serious adverse event (SAE). Each patient will be

instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious.

The methods for collection of safety data are described below.

8.1 Definitions

8.1.1 Adverse event (AE)

An AE is any untoward medical occurrence in a patient administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment.

An adverse event (AE) can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

The term AE is used to include both serious and non-serious AEs.

If an abnormal laboratory value/vital sign are associated with clinical signs and symptoms, the sign/symptom should be reported as an AE and the associated laboratory result/vital sign should be considered additional information that must be collected on the relevant CRF.

8.1.2 Adverse events of special interest (AESI)

In general, AESIs are AEs that occur in categories of special interest with regard to determining the benefit/risk profile and overall safety of a drug. Two kinds of AEs are of particular concern regarding intravenous iron infusions:

- Hypersensitivity/anaphylactic reactions: Infusion with Monofer® can result in acute hypersensitivity reactions characterised by hypotension and signs of anaphylaxis. These reactions may occur even in patients who have had previous, uneventful iron infusions, and are more common in patients with allergic diatheses or autoimmune diseases.
- Skin discolouration: Paravenous leakage may cause skin irritation and long-lasting discolouration.

8.1.3 Serious adverse event (SAE)

Any untoward medical occurrence that at any dose:

- Results in death
- Is immediately life-threatening
- Requires in-patient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability or incapacity
- Is a congenital abnormality or birth defect
- Is an important medical event that may jeopardise the subject or may require medical intervention to prevent one of the outcomes listed above.

Medical and scientific judgment is to be exercised in deciding on the seriousness of a case. Important medical events may not be immediately life-threatening or result in death or hospitalisation, but may jeopardise the subject or may require intervention to prevent one of the listed outcomes in the definitions above. In such situations, or in doubtful cases, the case should be considered as serious. Hospitalisation for administrative reason (for observation or social reasons) is allowed at the investigator's discretion and will not qualify as serious unless there is an associated adverse event warranting hospitalisation.

8.1.4 Suspected unexpected serious adverse reaction (SUSAR)

Adverse Reaction: all untoward and unintended responses to an investigational medicinal product related to any dose administered;

Unexpected Adverse Reaction: an adverse reaction, the nature or severity of which is not consistent with the applicable product information as provided in the Monofer® SPC.

Suspected Unexpected Serious Adverse Reaction: SAE (see section 8.1.3) that is unexpected and possibly related to the investigational medicinal products. Any SAE not mentioned in the investigational medicinal product SPC, paragraph 4.8, is to be considered a suspected unexpected serious adverse reaction (SUSAR).

8.2 Time period for reporting adverse events and serious adverse events

For each patient the he standard time period for collecting and recording AE and SAEs will begin at the start of study treatment and will continue for half a year subsequent to study drug administration (until the end-of-study visit). We will proactively follow up all AEs and SAEs for each patient during the course of the study; events will be followed up to resolution, unless the event is considered to be unlikely to resolve due to the underlying disease. Every effort should be made to obtain a resolution for all events, even if the events continue after discontinuation/study completion. Mandatory reporting of new AEs ends after the end-of-study visit 6 months after study drug infusion.

8.3 Recording of adverse events

If the patient has experienced adverse event(s), the investigator will record the following information in the CRF:

The **nature of the event**(s) will be described by the investigator in precise standard medical terminology (i.e. not necessarily the exact words used by the patient).

The **duration of the event** will be described in terms of event onset date and event ended data.

The **intensity** of the adverse event will be categorised as mild / moderate / severe / life-threatening / death according to Common Terminology Criteria for Adverse Events version 4.0:

- Mild: asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated;
- Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily life;
- Severe: Severe or medically significant but not immediately life-threatening; hospitalisation or prolongation of hospitalisation indicated; disabling; limiting self-care activities of daily life;
- Life-threatening consequences: urgent intervention indicated.

The **causal relationship** of the event to the study medication will be assessed as one of the following:

- Unrelated: There is not a temporal relationship to investigational product administration (too early, or late, or investigational product not taken), or there is a reasonable causal relationship between non-investigational product, concurrent disease, or circumstance and the AE.
- Unlikely: There is a temporal relationship to investigational product administration, but there is not a reasonable causal relationship between the investigational product and the AE.
- Possible: There is reasonable causal relationship between the investigational product and the AE. Dechallenge information is lacking or unclear.
- Probable: There is a reasonable causal relationship between the investigational product and the AE. The event responds to dechallenge. Rechallenge is not required.
- Definite: There is a reasonable causal relationship between the investigational product and the AE.

Action taken: **Which investigations/medical procedures/treatments that are initiated as a result of the adverse event.**

The **outcome** of the adverse event – whether the event is resolved or still ongoing.

It is important to distinguish between seriousness and severity of AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria in Section 8.1. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but is not an SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke, but would be an SAE.

8.4 Reporting procedure

8.4.1 Adverse events, adverse events of special interest and serious adverse events

All adverse events and serious adverse events that should be reported as defined in section 8.1.1 will be recorded in the patient's CRF. SAEs must be reported by the investigator to the sponsor, Oslo University Hospital, within 24 hours after the site has gained knowledge of the SAE. The Serious Adverse Event Report Form must be completed, documented in the CRF, signed and sent to Lars Gullestad. The initial report shall promptly be followed by detailed, written reports if necessary. The initial and follow-up reports shall identify the trial subjects by unique trial code numbers assigned to the latter. The sponsor keeps detailed records of all SAEs reported by the investigators and performs an evaluation with respect to seriousness, causality and expectedness. Adverse events of specific interest and any SAEs as defined above must be reported to the study drug manufacturer (Pharmacosmos®) within 24 hours after the site has gained knowledge of the event in question.

8.4.2 Suspected unexpected serious adverse reactions

SUSARs will be reported to the Norwegian Medicines Agency and the Ethics Committee according to national regulation. The following timelines should be followed:

The sponsor will ensure that all relevant information about suspected serious unexpected adverse reactions that are fatal or life-threatening is recorded and reported as soon as possible to the Norwegian Medicines Agency and the Regional Committee for Medical and Health Research Ethics in any case no later than seven (7) days after knowledge by the sponsor of such a case, and that relevant follow-up information is subsequently communicated within an additional eight (8) days. The sponsor shall ensure that suspected adverse reactions that are serious and unexpected are reported to the Norwegian Medicines Agency and to the Regional Committee for Medical and Health Research Ethics within 15 days of the sponsor after knowledge of the event. The sponsor shall inform all investigators of the trial substance in question of suspected adverse reactions that are serious and unexpected. An account of any interruption in treatment or any breaking of the treatment code, the investigator's assessment of the causal relationship, and consequences for further testing shall accompany the notification of suspected adverse reactions pursuant to the first and second paragraphs.

The Norwegian Medicines Agency may require that individual reports of adverse events described in collective reports should also be submitted. The sponsor shall keep detailed records of all adverse events that are reported to him by the investigator. The records shall be submitted to the Norwegian Medicines Agency on request.

8.4.3 Annual safety report

Once a year throughout the clinical trial, the sponsor will provide the Norwegian Medicines Agency with an annual safety report. The format will comply with national requirements.

8.4.4 Clinical study report

The adverse events and serious adverse events occurring during the study will be discussed in the safety evaluation part of the Clinical Study Report.

8.5 Procedures in case of emergency

The investigator is responsible for assuring that there are procedures and expertise available to cope with emergencies during the study.

Provide information regarding code break, if applicable (see also section 10.2.3).

8.6 Safety monitoring

An experienced specialist in internal medicine will be appointed to assess trial safety. This clinician will receive reports summarising patient recruitment and the number of AEs / SAEs after the first 20 patients have been randomised, and again after 50 % and 100 % of patient enrolment.. The clinician responsible for safety monitoring will receive additional information on demand, and can advise temporary or permanent stop in patient enrolment. He or she will have access to the randomisation code. The clinician responsible for safety monitoring is independent from the sponsor and will have no competing interest with regard to the study investigational products or study outcome.

9 Data management and monitoring

9.1 Case report forms (CRFs)

The designated investigator staff will enter the data required by the protocol into the case report form (CRF). The Principal Investigator is responsible for assuring that data entered into the CRF are complete, accurate, and that entry is performed in a timely manner. If any assessments are omitted, the reason for such omissions will be noted in the CRFs. Corrections, with the reason for the corrections will also be recorded. After database lock, the investigator will receive a digital copy of the subject data for archiving at the investigational site.

9.2 Source data

Data will be recorded directly into the CRF, which, along with the patients' medical record is to be considered the source data. Data important for patient safety and continued care must be duplicated in the patient's medical record as described below. Study-specific imaging data and blood analyses are independent source data.

The medical records of each patient should clearly describe at least:

- That the patient is participating in the study
- Date when the informed consent was obtained from the patient;
- Results of all assessments confirming a patient's eligibility for the study;
- Diseases (past and current; both the disease studied and others, as relevant);
- Surgical history, as relevant;
- Treatments withdrawn/withheld due to participation in the study;
- Results of assessments performed during the study;
- Treatments provided, changes in treatments during the study and the time points for the changes;
- Visits to the clinic / telephone contacts during the study, including those for study purposes only;
- Non-Serious Adverse Events and Serious Adverse Events (if any) including causality assessments;
- Date of, and reason for, discontinuation from study treatment;
- Date of, and reason for, withdrawal from study;
- Date of death and cause of death, if available.

9.3 Study monitoring

The investigator will be visited on a regular basis by the Clinical Study Monitor, who will check that the study is conducted as approved by the Ethics committee and adheres to GCP guidelines.

Sponsor's representatives (e.g. monitors, auditors) and/or competent authorities will be allowed access to source data for source data verification in which case a review of those parts of the hospital records relevant to the study may be required.

9.4 Confidentiality

The investigator shall arrange for the secure retention of the patient identification and the code list. Patient files shall be kept for the maximum period of time permitted by each hospital. The study documentation (CRFs, Site File etc.) shall be retained and stored during the study and for 15 years after study closure. All information concerning the study will be stored in a safe place inaccessible to unauthorised personnel.

9.5 Database management

Data will be entered into the CRF without delay and stored in a dedicated and secured area at OUS. Data will be stored in a de-identified manner, where each study participant is recognisable by his/her unique trial subject number. The data will be stored until Dec 31st 2034, or until the patient requires that his/her data are deleted. Data in the CRF will be handled according to GCP. Only the personnel authorised to enter and/or analyse data (i.e. investigators) will have access to the database.

9.6 Biobanking

A biobank will be established at the Dept. of Cardiology, Oslo University Hospital, Rikshospitalet for the analysis of serum and plasma. The samples will be stored in multiple aliquots and labelled with the unique trial subject number, and the letter A signifying baseline, and the letter B signifying the end-of-study visit after 6 months. The samples will be stored at -80 degrees Celsius in a dedicated research freezer. The material is scheduled for destruction by Dec 31st 2034. Professor Lars Gullestad at Oslo University Hospital, Rikshospitalet will be responsible for the biobank.

10 Statistical methods and data analysis

10.1 Determination of sample size

This trial is designed to assess the effect of intravenous iron on peak oxygen consumption in heart transplant recipients with iron deficiency. We consider an increase in peak oxygen consumption of 1.5 ml/kg/min, equivalent to approximately half a Metabolic Equivalent of Task (MET), to represent a clinically meaningful improvement. An increase in O2 consumption of 1.5 ml/kg/min is approximately 6 % of the baseline maximal oxygen consumption in our heart transplant recipients.[43] Data from the ACTION HF trial showed that a 6 % increase in peak oxygen consumption translated to an improved outcome in patients with heart failure.[50] With a mean difference between the groups of 1.5 ml/kg/min and an expected repeat-measurement standard deviation of 2.5 ml/kg/min,[43] a power of 80% and an α of 5 %, we will need at least 44 patients in each group.

To allow for drop-out and to improve the chances of obtaining significance for secondary endpoints, we aim to include 100 patients.

10.2 Randomisation

10.2.1 Allocation- sequence generation

Balanced, permuted block randomisation (in a 1:1: ratio for the two study arms) using random block sizes will be generated by the Research Support Unit at Oslo University Hospital using a computerised procedure. A complete, sealed randomisation list containing details of all patient

numbers and study group will be stored as essential documentation with the Trial Master File in a locked office.

10.2.2 Allocation- procedure to randomise a patient

We will use a computerised randomisation procedure for treatment allocation. Patient randomisation will be performed by random draw of sealed, opaque envelopes containing cards with the words “iron, 20 mg/kg” or “placebo (study drug no. …)” once eligibility has been confirmed and the informed consent form has been signed. The study drug numbers will be assigned to each drug vial (or placebo card) by a nurse who does not participate in the trial or have competing interest regarding study outcome. This nurse will have exclusive access to the list linking study drug numbers, and batch numbers and expiry dates. According to the randomisation list, unique cards marked “iron, 20 mg/kg” or “placebo (study drug no. …)” will be placed in sealed, opaque envelopes by a nurse not participating in the trial. A copy of the randomisation list will be stored in a sealed, opaque envelope in a locked cupboard along with the study drugs, a mechanism employed to minimise the possibility that those enrolling and assigning patients will obtain access to the list.

10.2.3 Blinding and emergency un-blinding

The study participants (patients) and all study personnel, including investigators, personnel assessing outcomes, study nurses, data analysts and treating physicians and nurses, will be blinded to allocation to study drug. Only the non-investigating nurse(s) preparing and administering the study drug at randomisation will be aware of treatment allocation.

Un-blinding will be performed in the event of AEs where knowledge of the type of drug might be of importance to the personnel treating the patients, and in the event of SUSARS. Un-blinding will be performed by non-investigating personnel without competing interest, and the result will immediately be reported to the primary investigator, who will then take the required action(s). Unless unavoidable, other investigators and personnel assessing outcomes should not be made aware of treatment allocation.

10.3 Population for Analysis

The following populations will be considered for the analyses:

- Intention to treat (ITT) population: All randomised participants, regardless of protocol adherence.
- Per-protocol population (PP): Includes all subjects who have received study drug

10.4 Planned analyses

The main statistical analysis is planned when the last patient has completed the end-of-study visit 6 months after randomisation. Safety analyses will be performed biannually.

Deviation from the original statistical plan will be described and justified in the Clinical Study Report. Amendments to plan can be done until day of database lock.

10.5 Statistical analysis

All statistical tests will be performed using a two-sided 5 % level of significance. Continuous efficacy variables will be analysed using independent T-tests for comparisons between the treatment arms and baseline-adjusted ANCOVA for difference in changes. If necessary, values will be log-transformed to meet the assumptions of the tests. All analyses will primarily be analysed according to the intention-to-treat principle. Between-group differences in ordinal categorical variables, such as NYHA class, will be analysed using ordinal logistic regression, whereas the count variables will be assessed by Poisson regression. Demographic, efficacy and safety data will be summarised by treatment group using means, minimums, medians, maximums, interquartile ranges and standard deviations for continuous variables and frequency counts and percentages for categorical variables.

Per protocol analyses will be performed using the same methods as for the intention-to-treat analyses.

The primary endpoint, the baseline-adjusted between-group difference in peak oxygen consumption, will be calculated by ANCOVA according to the intention-to-treat principle, the statistical null-hypothesis being that the baseline-adjusted oxygen uptake does not differ between the two treatment arms. Secondary analyses will be made according to the per-protocol-principle.

Key, secondary endpoints:

The baseline-adjusted between-group difference in the change in i) hand grip strength, ii) normally distributed body composition data, and iii) other normally distributed data will be calculated by ANCOVA. The statistical null-hypotheses are that the changes in these characteristics do not differ between patients allocated to iron isomaltoside and patients allocated to placebo.

NT-proBNP, TnT and CRP at six months will be analysed using Mann Whitney U-tests, the statistical null-hypothesis being that the levels of these biomarkers do not differ between the treatment arms. Regarding quality of life, we specifically plan to assess the between-group number of patients with a minimal clinical difference in the SF36 score.

Secondary per protocol analyses will be performed using the same methods as for the intention-to-treat analyses. Exploratory analyses will be made for efficacy variables stratified by centre.

Safety analyses will include tabulation of type and frequency of all adverse events. Any serious adverse events will be reported with comprehensive narratives. Any value of safety laboratory parameters outside normal ranges will be identified.

Missing data will be omitted from analyses, i.e. there will be no imputation or estimation of missing values. Statistical analyses will be performed in IBM SPSS Statistics version 21 or later.

11 Study management

11.1 Investigator delegation procedure

The principal investigator is responsible for making and updating a “delegation of tasks” listing all the involved co-workers and their role in the project. He will ensure that appropriate training relevant to the study is given to all of these staff, and that any new information of relevance to the performance of this study is forwarded to the staff involved.

11.2 Protocol adherence

Investigators ascertain they will apply due diligence to avoid protocol deviations. All significant protocol deviations will be recorded and reported in the Clinical Study Report (CSR).

11.3 Study amendments

If it is necessary for the study protocol to be amended, the amendment and/or a new version of the study protocol (Amended Protocol) must be notified to and approved by the Competent Authority and the Ethics Committee according to EU and national regulations.

11.4 Audit and inspections

Authorised representatives of a Competent Authority and Ethics Committee may visit the centre to perform inspections, including source data verification. Likewise the representatives from sponsor may visit the centre to perform an audit. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analysed, and accurately reported according to the protocol, Good Clinical Practice (ICH/GCP), and any applicable regulatory requirements. The

principal investigator will ensure that the inspectors and auditors will be provided with access to source data/documents.

12 Ethical and regulatory requirements

The study will be conducted in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/GCP and applicable regulatory requirements. Registration of patient data will be carried out in accordance with national personal data laws.

12.1 Ethics committee approval

The study protocol, including the patient information and informed consent form to be used, must be approved by the regional ethics committee before enrolment of any patients.

The investigator is responsible for informing the ethics committee of any serious and unexpected adverse events and/or major amendments to the protocol as per national requirements.

12.2 Other regulatory approvals

The protocol will be submitted and approved by The Norwegian Medicines Agency and the Data Protection Officer (Personvernombudet) at Oslo University Hospital before commencement of the study. The protocol will also be registered in www.clinicaltrials.gov before the inclusion of the first patient.

12.3 Informed consent procedure

The investigator is responsible for giving the patients full and adequate verbal and written information about the nature, purpose, and potential risks and benefits of the study. Study participants will be informed as to the strict confidentiality of their patient data, but that their medical records may be reviewed for trial purposes by authorised individuals other than their treating physician.

It will be emphasised that study participation is voluntary and that the patient is allowed to refuse further participation in the protocol whenever she/he wants. This will not prejudice the patient's subsequent care. Written informed consent must be obtained for all study participants before enrolment in the study. This will be done in accordance with the national and local regulatory requirements. The investigator is responsible for obtaining signed informed consent.

A copy of the patient information and consent form will be given to the patients. The signed and dated consent forms will be filed in the Investigator Site File binder.

12.4 Subject identification

The investigator is responsible for keeping a list of all patients (who have received study treatment or undergone any study specific procedure) including patient's date of birth and personal number, full names and last known addresses.

The patients will be identified in the CRFs by a study-specific, unique identification number.

13 Trial sponsorship and financing

The IronIC trial is an investigator-initiated study supported by an unrestricted grant provided by Pharmacosmos®, who have also provided the study drug. The funding sources have had no role in the design of the study; neither will they participate in the implementation of the trial, in the analyses of the results, or in the decision to publish. The investigators take sole responsibility for the integrity of the data, the writing of the manuscript and the dissemination of the results.

14 Trial insurance

The Principal investigator has insurance coverage for this study through membership of the Drug Liability Association.

15 Publication policy

Upon study completion and finalisation of the study report the results of this study will either be submitted for publication and/or posted in a publicly assessable database of clinical study results.

The results of this study will also be submitted to the Competent Authority and the Ethics Committee according to EU and national regulations. All personnel who have contributed significantly with the planning and performance of the study (Vancouver convention 1988) may be included in the list of authors. The funding sources have had no role in the design of the study; neither will they participate in the implementation of the trial, in the analyses of the results, or in the decision to publish.

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