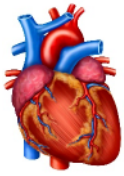
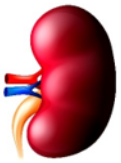


DEFEAT–AKI

Deferoxamine for the Prevention of Cardiac Surgery-Associated Acute Kidney Injury



Detailed Protocol
November 4, 2025

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ABBREVIATIONS

AEs = Adverse Events
AESI = Adverse Events of Special Interest
AKI = Acute Kidney Injury
ARDS = Acute Respiratory Distress Syndrome
ATN = Acute renal failure Trial Network
BIDMC = Beth Israel Deaconess Medical Center
BWH = Brigham and Women's Hospital
CABG = Coronary Artery Bypass Graft
CKD = Chronic Kidney Disease
CPB = Cardiopulmonary Bypass
CTCAE = Common Terminology Criteria for Adverse Events
DEFEAT-AKI = Deferoxamine for the Prevention of Cardiac Surgery-Associated Acute Kidney Injury
DFO = Deferoxamine
DFP = Deferiprone
DFX = Deferasirox
DSMB = Data and Safety Monitoring Board
ECMO = Extracorporeal Membrane Oxygenation
eGFR = Estimated Globular Filtration Rate
eIC = Electronic Informed Consent
FDA = Food and Drug Administration
FiO_2 = Fraction of inspired oxygen
GI = Gastrointestinal
Hi-DEF = High-Dose Deferoxamine in Intracerebral Hemorrhage
HFpEF = Heart failure with preserved ejection fraction
HFrEF = Heart failure with reduced ejection fraction
ICH = Intracerebral Hemorrhage
ICU = Intensive Care Unit
i-DEF = Intracerebral Hemorrhage Deferoxamine
IRB = Internal Review Board
IRI = Ischemia-Reperfusion Injury
IV = Intravenous
KDIGO = Kidney Disease: Improving Global Outcomes
KIM-1 = Kidney Injury Molecule-1
MGH = Massachusetts General Hospital
NGAL = Neutrophil Gelatinase-Associated Lipocalin
NIDDK = National Institute of Diabetes and Digestive Kidney Diseases
NIH = National Institutes of Health
PaO_2 = Partial pressure of arterial oxygen
PEEP = Positive end-expiratory pressure
PI = Principal Investigator
PMBCs = Peripheral Blood Mononuclear Cells
POD = Post-Operative Day
RBCs = Red Blood Cells
RCT = Randomized Clinical Trial
RRT = Renal Replacement Therapy
SOFA = Sequential Organ Failure Assessment
STEMI = ST-Elevation Myocardial Infarction
VAD = Ventricular Assist Device

SYNOPSIS

Title	Deferoxamine for the Prevention of Cardiac Surgery-Associated Acute Kidney Injury
Short Title	DEFEAT-AKI
Phase	2
Study Design	Randomized, double-blind, placebo-controlled, parallel assignment
IND Status#	Exempt
ClinicalTrials.gov #	NCT04633889
Funding	National Institutes of Health (NIH) / National Institute of Diabetes and Digestive Kidney Diseases (NIDDK) grant R01DK125786 (PI: Leaf)
Rationale	Multiple lines of evidence support a central role of iron in causing acute kidney injury (AKI), including the finding that prophylactic administration of iron chelators attenuates AKI in numerous animal models. Patients undergoing cardiac surgery may be particularly susceptible to iron-mediated kidney injury due to the profound hemolysis that often occurs from cardiopulmonary bypass (CPB), along with intraoperative transfusion of red blood cells.
Target Population	Adults undergoing cardiac surgery who are at high risk of postoperative AKI
Inclusion Criteria	1. Age ≥18 years 2. Undergoing coronary artery bypass graft (CABG) and/or valve surgery with CPB. 3. AKI risk score ≥6 at the time of screening (**Table 4**) 4. Written informed consent from the patient or surrogate
Exclusion Criteria	1. AKI, defined as any of the following: ▪ Increase in serum creatinine ≥0.3 mg/dl in 48h ▪ Increase in serum creatinine ≥50% in 7d (if no value available in last 7d, use most recent value in last 3 months) ▪ Urine output ≤0.5 ml/kg/h x 6 consecutive hours (only assessed in patients with hourly monitoring via Foley catheter) ▪ Receipt of renal replacement therapy (RRT) within 7d 2. Advanced chronic kidney disease (eGFR <15 ml/min/1.73m^2 or end-stage kidney disease receiving RRT) 3. Hemoglobin <8 g/dL (closest value in the prior 3 months) 4. Fever (temperature ≥38ºC) in the last 48h 5. Suspected or confirmed bacteremia, endocarditis, or pyelonephritis 6. Pneumonia, aspiration, or bilateral pulmonary infiltrates from an infectious etiology reported on chest x-ray or CT scan in the last 7d 7. Positive COVID-19 test in the last 10d 8. Previous iron chelation therapy (including prior participation in DEFEAT-AKI) 9. Known hypersensitivity to DFO 10. Taking prochlorperazine 11. Known severe hearing loss 12. Pregnant or breastfeeding 13. Prisoner 14. Concurrent participation in another interventional research study in which the intervention has potential interaction with DFO 15. Surgery to be performed under conditions of circulatory arrest 16. Receiving extracorporeal membrane oxygenation 17. Durable ventricular assist device (VAD) prior to surgery (does not include Impella device or intra-aortic balloon pump) 18. Any condition which, in the judgement of the investigator, might increase the risk to the patient 19. Conflict with other research studies
Number of Subjects	330
Length of Participation	Until hospital discharge

Interventions	Deferoxamine 30 mg/kg (max total dose, 6g) or an equal volume of normal saline placebo administered as a continuous intravenous infusion over 12h, beginning immediately prior to cardiac surgery
Primary Objectives and Primary Endpoint	**Primary objective:** To evaluate the potential efficacy of deferoxamine in reducing the risk of postoperative AKI in patients undergoing cardiac surgery. **Primary endpoint:** Incidence of postoperative AKI, defined as follows: urine output <0.5 ml/kg/h for 6 consecutive hours (assessed within the first 48h or until the Foley catheter is removed, whichever occurs first); an increase in serum creatinine ≥0.3 mg/dl within the first 48h; an increase in serum creatinine ≥50% in the first 7 days; or receipt of renal replacement therapy in the first 7 days.
Secondary Objectives and Corresponding Endpoints	**Secondary objectives:** ▪ To evaluate the potential efficacy of deferoxamine in reducing renal tubular injury in patients undergoing cardiac surgery. ▪ To evaluate the potential efficacy of deferoxamine in reducing the risk of postoperative myocardial injury, atrial fibrillation, prolonged mechanical ventilation, and sepsis in patients undergoing cardiac surgery. **Secondary endpoints:** ▪ Urinary markers of tubular injury, NGAL and KIM-1, assessed preoperatively, at the end of CPB, on arrival to the ICU, and daily for 2 days postoperatively. ▪ Postoperative myocardial injury, atrial fibrillation, prolonged mechanical ventilation, and sepsis within 7 days following cardiac surgery.
Exploratory Objectives and Corresponding Endpoints	**Exploratory objectives:** To test the effects of deferoxamine on circulating markers of iron, oxidative stress, and inflammation **Exploratory endpoints:** Circulating markers of iron status (catalytic iron, total iron, total iron binding capacity, ferritin, and hepcidin), oxidative stress (F[2]-isoprostane and myeloperoxidase), and inflammation (monocyte phenotype), each assessed pre- and postoperatively.
Sites	Brigham and Women's Hospital (BWH) Massachusetts General Hospital (MGH) Beth Israel Deaconess Medical Center (BIDMC)
Study Duration	Estimated total study duration is 4 years
Data and Safety Monitoring Committee (DSMB)	A DSMB will convene prior to study launch and after enrollment of 10, 25, 50, and 75% of the total enrollment has taken place to review safety data and any unanticipated problems. The DSMB will make recommendations as needed. The DSMB will not perform a futility analysis or consider early study completion for efficacy.

1. BACKGROUND AND SIGNIFICANCE

1.1 Overview

1.1.1 Burden of acute kidney injury

Acute kidney injury (AKI) is a sudden episode of kidney failure or kidney damage that occurs within a few hours or a few days. AKI complicates >500,000 hospitalizations annually in the US, at a cost of ~$10 billion, and its incidence is rising.[1, 2] Patients who develop AKI are at greatly increased risk of death acutely, and those who survive have an increased risk of chronic kidney disease (CKD), cardiovascular events, and reduced quality of life.[1, 3-5] Despite years of investigation, development of therapies to prevent and treat AKI has been largely unsuccessful. Investigation of novel therapeutic targets is urgently needed. **Deferoxamine for the Prevention of Acute Kidney Injury (DEFEAT-AKI) is a phase II randomized clinical trial to investigate the effects of iron chelation with deferoxamine (DFO) on cardiac surgery-associated AKI.**

1.1.2 Role of iron in animal models of AKI

Iron is essential for many biologic processes, including oxygen transport, DNA repair, and cell proliferation. When present in excess and in non-physiologic forms, however, iron is toxic to the kidneys and other organs due to its ability to catalyze the Fenton and Haber-Weiss reactions, thereby generating hydroxyl radicals and causing cellular oxidative injury.[6, 7] Multiple lines of evidence support a central role of iron in AKI: exogenous iron infusion exacerbates renal injury;[8-10] iron content is markedly increased in the kidneys of animals exposed to myriad nephrotoxic insults;[11-13] genetic manipulation of key proteins involved in iron metabolism, regulation, and transport affect AKI susceptibility;[14-16] and administration of hepcidin, the master regulator of iron homeostasis, is protective against AKI.[17, 18] **Most notably, iron chelators reliably attenuate AKI in numerous animal models (Table 1).**

1.1.3 Principles of iron chelation and potential mechanisms of protection

An important aspect of chelation therapy in preventing organ injury is the removal from the circulation of toxic forms of non-transferrin-bound iron, also known as “catalytic iron,” which is composed primarily of iron-citrate and iron-albumin complexes.[19] Catalytic iron is taken up by highly vascular organs, such as the kidneys and heart, leading to elevated concentrations of intracellular iron. Therapeutic iron chelators form a complex with catalytic iron and promote its excretion via the urinary and fecal routes. Underlying the protective effects of iron chelators in animal models of AKI are both iron-dependent and independent mechanisms. Iron-dependent mechanisms include the prevention of Fenton chemistry induced by catalytic iron. This protects against oxidative injury and ferroptosis, a form of iron-dependent cell death.[20] Iron chelators also act as electron donors to reduce high oxidation states of heme iron, such as ferryl (Fe^{4+}) hemoglobin and myoglobin.[21, 22] Thus, iron chelators may prevent heme-mediated injury independently of their ability to bind catalytic iron. Finally, iron chelators affect cell proliferation and survival independently of heme and iron by, for example, upregulating hypoxia-inducible factor 1-alpha[23-25] and direct scavenging of superoxide free radicals.[26]

1.1.4 Efficacy and safety of iron chelators in humans

Iron chelators have been used for over half a century to treat patients with genetic hemoglobin disorders complicated by iron overload, such as beta thalassemia. Iron chelators in general, and DFO specifically, have also been used in human studies to test their ability to prevent acute organ injury, including AKI, and have demonstrated promising initial results and an excellent safety profile (section 1.3).

1.1.5 Rationale for investigation of iron chelation in patients undergoing cardiac surgery

Cardiac surgery is the ideal setting for investigation of iron chelation for AKI prevention. Cardiac surgery results in elevated circulating levels of catalytic iron[27] due to multiple factors: exposure of red blood cells (RBCs) to non-physiological surfaces and shear stress in extracorporeal circuits, resulting in

profound hemolysis; transfusion of RBCs, which become more fragile during storage, resulting in additional hemolysis;[28-30] ischemia-reperfusion injury (IRI) to the kidneys and other organs due to cross-clamping of the aorta and intraoperative hypotension;[31] and skeletal muscle injury, resulting in the release of iron-rich myoglobin into the circulation.[32] Consistent with these mechanisms, we found that longer duration of cardiopulmonary bypass (CPB) is associated with higher levels of catalytic iron postoperatively (section 1.2.2).[27] **Thus, cardiac surgery exposes patients to an acute iron load resulting from hemolysis, transfusion, rhabdomyolysis, and other factors.** There are also important logistical considerations that favor cardiac surgery. Unlike most clinical settings where AKI occurs, cardiac surgery is often planned, and thus allows iron chelation therapy to be initiated **prophylactically**. Accordingly, toxic iron species released into the circulation during cardiac surgery can be chelated in real time before AKI has occurred. This feature is critical, since nearly all animal models using iron chelators investigated their effect on AKI prevention rather than treatment.[33, 34]

Finally, **cardiac surgery-associated AKI is itself a major public health burden.** One multinational study conducted among ~30,000 critically ill patients identified cardiac surgery as the second most common cause of AKI.[35] Over 500,000 cardiac surgeries are performed in the U.S. annually,[36] ~80% of which are performed with CPB. Depending on the definition, AKI occurs in as many as 72% of patients undergoing cardiac surgery,[37, 38] with a 6- to 18-fold associated increase in acute mortality compared to those without AKI.[39-41] AKI requiring RRT occurs less frequently, but is associated with striking (>60%) in-hospital mortality.[39, 42, 43]

1.2 Preclinical and clinical studies supporting the proposed research

1.2.1 Preclinical studies

Multiple lines of evidence from preclinical studies support a central role of iron in AKI, most importantly the finding that iron chelators reliably attenuate AKI in numerous animal models. These studies are summarized in **Table 1** and are reviewed elsewhere in detail.[33]

Table 1. Animal models demonstrating protection from AKI with iron chelators[33]

Reference	Animal Model	Renal Injury	Iron chelation regimen	Demonstration of Renal Protection		Other / Notes
				BUN/Cr*	Hist	
Shah et al.,[44] 1988	Rats	Glycerol	DFO 30 mg/kg IV immediately prior to glycerol inj, then DFO 30 mg/day SC pump x 24h	Yes	Yes	
Paller et al.,[45] 1988	Rats	1. Glycerol 2. Hgb 3. IRI (Uni)	1. DFO 25 mg/kg/h IV x 1h concomitantly with glycerol inj, then 12 mg/kg/h x 3h, then 6 mg/kg/hr x 3h 2. DFO 200 mg/kg/h IV x 1h immediately after Hgb inj 3. DFO 200 mg/kg/h IV x 1h immediately prior to reperfusion	Yes Yes Yes	NR NR NR	
Paller et al.,[8] 1988	Rats	IRI (Uni)	DFO 50 or 200 mg/kg/h IV infusion x 60 min starting immediately prior to reperfusion	Yes	Yes	Iron-saturated DFO was not protective
Walker et al.,[46] 1988	Rats	Gent	DFO 20 mg IV immediately prior to gent inj, then 20 mg/day SC pump x 8d	Yes	Yes	Iron-saturated DFO was only partially protective
Zager et al.,[47] 1992	Rats	Glycerol	DFO 120 mg/kg IV infusion for 2h immediately after glycerol inj; Mannitol 12.5 ml/kg IV infusion for 2h immediately after glycerol inj	Yes	Yes	DFO+Mannitol- compared to Mannitol only-treated rats had better functional and histologic protection
Gonzalez-Fajardo et al.,[48] 1994	Rabbits	IRI (Bi)	DFO 25 mg/kg IV immediately prior to clamping and immediately prior to reperfusion	No	NR	
Ben Ismail et al.,[49] 1994	Rats	Gent	DFO 100 mg/kg IM concomitantly with gent inj	No	No	
Haraldsson et al.,[50] 1995	Rabbits	IRI (Uni)	DFO 30 mg/kg IV immediately prior to clamping and immediately prior to reperfusion; Mannitol 3 ml/kg IV immediately prior to clamping and immediately prior to reperfusion	Yes	NR	DFO+Mannitol- compared to Mannitol only-treated rats had a higher Cr clearance
Watanabe et al.,[51] 1998	Rats	Cisplatin	DFO 100 mg/kg IP 60 min prior to Cisplatin and continued QD x 10d	Yes	NR	
Baliga et al.,[52] 1998	Rats	Cisplatin	DFO 30 mg/day SC pump starting 24h prior to Cisplatin and continued daily x 4d	Yes	Yes	
Saad et al.,[53] 2001	Rats	DXR	DFO 25, 125, 250, 375, and 500 mg/kg IP x1 administered 30 min prior to DXR inj	Yes	Yes	Only rats treated with DFO at 375 mg/kg or 500 mg/kg had histologic protection
Chander et al.,[54] 2002	Rats	Glycerol	DFO 50 and 100 mg/kg SC 30 min prior and 12h after Glycerol inj	Yes	Yes	Higher dose provided better renal protection
Ozdemir et al.,[55] 2002	Mice	Cisplatin	DFO 100 and 200 mg/kg IP 60 min prior to Cisplatin and continued QD x 10d	Yes	Yes	GGT (marker of Cisplatin toxicity) reduced by DFO
De Vries et al.,[56] 2004	Mice	IRI (Uni)	Apotransferrin (0.1, 0.25, 0.5, and 5 mg) IP x1 just immediately prior to removal of clamps	Yes	NR	
Naghibi et al.,[57] 2004	Rats	Vanc	DHB 50 and 100 mg/kg SC inj starting 30 min prior to Vanc and continued QD x 7d	Yes	Yes	
Bulucu et al.,[58] 2008	Rats	Adriamycin (NS model)	DFO 20 mg/kg IV x1 immediately after Adriamycin inj	N/A	NR	DFO- compared to sham-treated rats had an attenuated rise in UPCR (Cr levels were not affected by Adriamycin)
Petronilho et al.,[59] 2009	Rats	Gent	DFO 20 mg/kg SC concomitantly with Gent inj and continued on days 1, 4 and 7; NAC 20 mg/kg SC concomitantly with Gent inj and continued q8h X 7d	Yes	NR	Rats treated with DFO+NAC compared with either DFO or NAC alone had an attenuated rise in BUN and Cr
Vlahokos et al.,[60] 2011	Pigs	Hepatic IRI	DFO 150 mg/kg IV infusion over 24h starting concomitantly with hepatic artery ligation	N/A	Yes	No significant effect of ischemia or DFO on BUN and Cr
Bernardi et al.,[61] 2012	Rats	IRI (Bi)	DFO 20 mg/kg intra-aortic inj immediately prior to induction of ischemia; NAC 20 mg/kg intra-aortic inj immediately prior to induction of ischemia	Yes	NR	DFO+NAC-treated rats, compared to rats treated with either DFO or NAC alone, had an attenuated rise in Cr
Milona- Jijon et al.,[62] 2012	Rats	Chromium	DFO 100, 200, and 400 mg/kg IP administered 30 min prior to potassium chromium inj	Yes	Yes	Dose-dependent renal protection with higher doses of DFO; DFO administration after chromium inj was unable to attenuate nephrotoxicity
Sivakumar et al.,[63] 2014	Mice	$AlCl_3$	DFP 0.72 mmol/kg PO vs. combo of DFP+DFO; dosing for the latter was 0.89 mmol/kg IP starting 30 min after $AlCl_3$ and continued QD x 5d	Yes	Yes	Protection was seen in both DFP groups compared to sham-treated mice; however, the DFP+DFO group had greater protection than DFP alone
Makhdoumi et al.,[64] 2018	Rats	Cisplatin	DFP 50, 100, and 200 mg/kg PO starting 5d prior to Cisplatin and continued QD x 10d	Yes	Yes	Only rats treated with DFP at 100 mg/kg, but not 50 or 200 mg/kg, were protected from nephrotoxicity

Table 1. Animal models demonstrating protective effects of iron chelators in AKI. *Refers to an attenuated rise in BUN and/or Cr compared to sham-treated animals. Abbreviations: AKI, acute kidney injury; $AlCl_3$, aluminum chloride; Bi, bilateral; BUN, blood urea nitrogen; Cr, creatinine (serum or plasma); DFO, deferoxamine; DFP, deferiprone; DHB, 2,3- dihydroxybenzoic acid; DXR, doxorubicin; Gent, gentamicin; GFR, glomerular filtration rate; GGT, gamma-glutamyl transferase; Hgb, hemoglobin; Hist, histologic; IM, Intramuscular; Inj, injection; IP, intraperitoneal; IRI, ischemia-reperfusion injury; IV, intravenous; NAC, n-acetylcysteine; N/A, Not applicable; NR, Not reported; NS, nephrotic syndrome; PO, per os; QD, daily; SC, subcutaneous; Uni, unilateral; UPCR, urine protein-creatinine ratio; Vanc, vancomycin.

1.2.2 Clinical studies

Clinical studies supporting the proposed research include the demonstration of elevated levels of catalytic iron in the circulation of patients at risk of AKI, including both cardiac surgery-associated AKI and AKI in the setting of critical illness.

Catalytic iron and cardiac surgery-associated AKI

We measured plasma catalytic iron pre-, intra-, and postoperatively in 250 adults undergoing cardiac surgery with CPB.[27] Catalytic iron levels peaked at the end of CPB, and were higher in patients who developed the composite endpoint of postoperative AKI requiring RRT or death (RRT/death) (**Figure 1**). In multivariable models, patients with catalytic iron levels in the highest versus lowest quartile on postoperative day 1 had a **3.99-fold higher odds of AKI** (95% CI, 1.60 to 9.93) and a **6.71-fold higher odds of RRT/death** (95% CI, 1.37 to 32.87). Finally, longer duration of CPB (P<0.001) and greater number of RBC transfusions (P<0.01) each associated with higher catalytic iron postoperatively, consistent with hemolysis as an important source of catalytic iron in this context.[27]

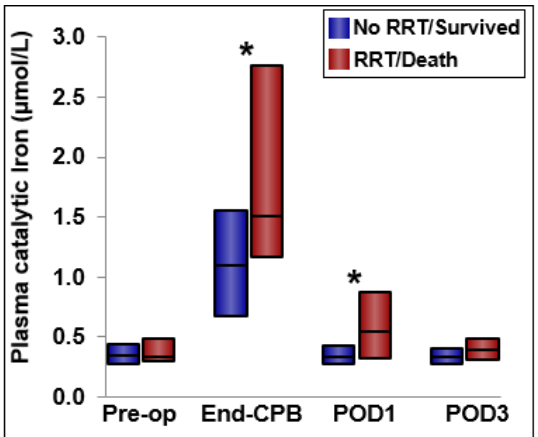

Figure 1. Plasma catalytic iron levels in adults undergoing cardiac surgery. Catalytic iron levels peak at the end of CPB and are higher in patients with vs. without AKI requiring RRT or death (*n*=22 and *n*=228, respectively). Abbreviations: CPB, cardiopulmonary bypass; POD, postoperative day. Data are shown as median (IQR). *P<0.01. Leaf et al., Kidney Int, 2015.

Catalytic iron and critical illness-associated AKI

We measured plasma catalytic iron levels in 121 patients within 24h of arrival to the intensive care unit (ICU), and found that higher concentrations associated with a greater risk of AKI in both univariate and multivariable models (adjusted odds ratio per 1-SD higher catalytic iron, 1.67; 95% CI, 1.04 to 2.67).[65] We also measured plasma catalytic iron and other iron parameters in 807 patients with AKI requiring RRT who enrolled in the Acute renal failure Trial Network (ATN) study. Higher quintiles of catalytic iron associated with a monotonic increase in the risk of 60-day mortality (**Figure 2A**).[66] Other iron markers were also associated with death, but the magnitude of association was greatest for catalytic iron (**Figure 2B**).[66]

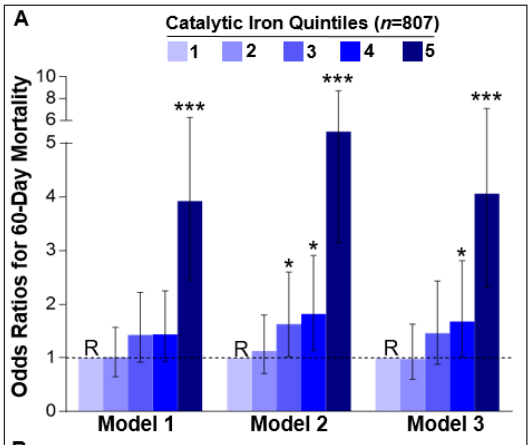

Figure 2. Higher plasma catalytic iron associates with death. Panel A shows odds ratios (ORs) for 60-day mortality according to quintiles of catalytic iron. Quintile 1 was the reference (R) group for all models. Model 1 is unadjusted; model 2 is adjusted for age, sex, race, baseline eGFR, diabetes mellitus, CHF, COPD, and chronic liver disease; model 3 is further adjusted for ICU type, mechanical ventilation, APACHE II score, oliguria, sepsis, shock, albumin, creatinine, and IL-6. Panel B shows adjusted ORs for death according to ln-transformed iron parameters standardized to 1 SD. *P<0.05, **P<0.01, ***P<0.001. Leaf et al., J Am Soc Nephrol, 2019.

Conclusions from these data

1. Catalytic iron levels are markedly elevated following CPB.
2. Catalytic iron levels are significantly higher among patients who develop AKI and RRT/death following CPB.
3. Catalytic iron levels rise acutely and early in the course of AKI following CPB surgery, a setting in which the timing of renal injury is known. This establishes CPB surgery as an important setting to test the efficacy of iron chelation in reducing the risk of AKI, as we now propose.
4. Higher catalytic iron levels are strongly, monotonically, and independently associated with AKI and death in settings beyond CPB surgery – namely critical illness – establishing a potential role for iron chelation in other AKI settings as well.

1.3 Rationale for investigation of DFO for AKI prevention in humans

1.3.1 Overview of iron chelators

Three iron chelators are approved by the Food and Drug Administration (FDA) for the treatment of iron overload: deferoxamine (DFO), deferiprone (DFP), and deferasirox (DFX). These agents have important differences in their routes of administration, pharmacokinetics, and potential adverse effects (**Table 2**). For multiple reasons described below and elsewhere,[33] **DFO is the agent best suited for use in randomized clinical trials (RCTs) investigating AKI prevention.**

1.3.2 Animal models using DFO

DFO is the agent used in the vast majority of animal models demonstrating efficacy of iron chelation in attenuating AKI (**Table 2**). Further, DFO attenuates **extrarenal acute organ injury** in numerous models, including cardiac ischemia-reperfusion injury (IRI) and reperfusion-induced arrythmias;[67-69] mechanical ventilation- and lipopolysaccharide-induced acute lung injury;[70, 71] endotoxemia-mediated acute liver injury;[72, 73] and intracerebral hemorrhage (**Figure 3**).[74] DFO also improves survival in animal models of sepsis.[75, 76]

Table 2. Comparison of FDA-approved iron chelators[33]

Chelator	Deferoxamine (DFO)	Deferasirox (DFX)	Deferiprone (DFP)
Brand name	Desferal	Exjade, Jadenu	Ferriprox
Year of FDA approval	1968	2005	2011
Route of administration	IV, IM, SC	PO	PO
Indication	Acute and chronic iron overload	Chronic iron overload	Chronic iron overload
Iron-binding affinity (pM)	26.6	22.5	19.9
Adverse effects	GI,* hypotension,** rash, anaphylaxis	GI,* cytopenias, AKI, liver toxicity	GI,* neutropenia, liver toxicity
Elimination half-life (hrs)	0.28	8-16	1.9
Dosing frequency	Continuous	Once per day	Three times per day
Metabolism	Plasma enzymes	Liver (Glucuronidation)	Liver (Glucuronidation)
Excretion	Urine and feces	Feces (84%), urine (8%)	Urine (75-90%)
Contraindications for renal impairment	eGFR<10: contraindicated	eGFR<40: contraindicated	None

*Gastrointestinal (GI) effects include abdominal discomfort, nausea, vomiting, and diarrhea; **Only observed at very high doses

1.3.3 Favorable biochemical properties of DFO

DFO has the highest iron-binding affinity among the chelators, and is the only chelator that is available parenterally (**Table 2**). The latter property is notable, since parenteral administration circumvents the limitations associated with enteral drug administration in acute illness, including concerns related to aspiration and impaired gastrointestinal absorption.

1.3.4 Preliminary data on DFO and prevention of acute organ injury in humans

DFO was the first iron chelator approved by the FDA, and thus has the longest track record of both efficacy and safety in humans for the treatment of **chronic** iron overload conditions. DFO is also the only iron chelator that is FDA-approved for the treatment of **acute** iron overload (**Table 2**), and has

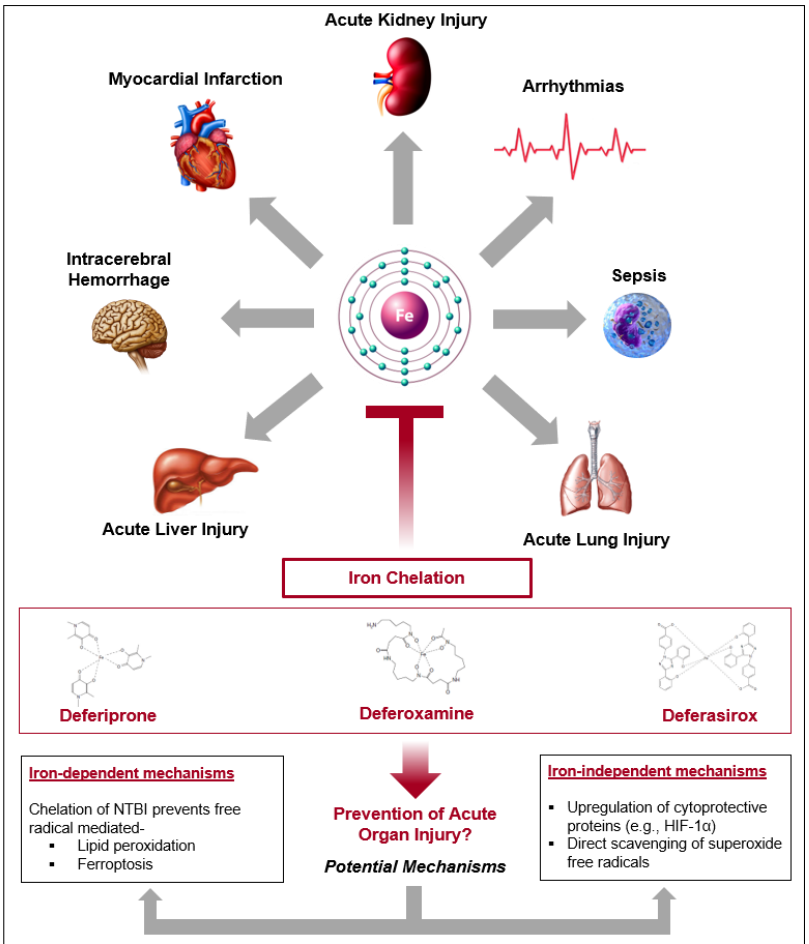

Figure 3. Renal and extrarenal acute organ injury models in which iron chelation is protective. Abbreviations: HIF-1α, hypoxia-inducible factor 1ᵅ; NTBI, nontransferrin-bound iron. Leaf et al., J Am Soc Nephrol, 2019.

been investigated as a therapeutic strategy for preventing acute organ injury in multiple clinical contexts (**Table 3**), including AKI, cardiopulmonary bypass surgery, and intracerebral hemorrhage.

Clinical studies of DFO for prevention of AKI

Fraga et al.[77] investigated the effects of DFO versus placebo on the incidence of AKI in 80 critically ill patients with prolonged hypotension. Although the overall incidence of AKI was similar between groups, DFO-treated patients had a lower incidence of severe AKI, as well as lower mean SCr on hospital discharge compared to placebo-treated patients.

Clinical studies of DFO in patients undergoing cardiopulmonary bypass (CPB) surgery and in patients with ST-elevation myocardial infarction (STEMI)

Several groups studied the effects of DFO in patients undergoing CPB surgery and in patients with STEMI.[78-81] DFO-treated patients had lower generation of superoxide radicals and plasma markers of oxidative stress compared to placebo-treated patients. One RCT found that DFO-treated patients had higher left ventricular ejection fraction following CPB surgery compared to placebo-treated patients.[80]

Clinical studies of DFO in patients with intracerebral hemorrhage (ICH)

Selim et al.[82] performed the largest RCT to date of DFO administration for acute organ injury prevention. They enrolled 294 patients with ICH into a phase II trial of DFO versus placebo. DFO-treated patients had similar neurological outcomes at 90 days compared to placebo-treated patients, but had a trend toward improved neurologic outcomes at 180 days.

Importantly, in each of these studies, DFO was well tolerated and did not increase the frequency of serious adverse events compared to placebo (Table 3).

Table 3. Randomized, placebo-controlled trials of DFO for acute organ injury prevention[33]

REF	Clinical Setting	N	Iron Chelation Regimen	Efficacy Findings	Safety Concerns
Menasche et al.[78], 1988	Cardiac Surgery	24	DFO 30 mg/kg IV during CPB	DFO-treated patients had lower generation of superoxide radicals compared to placebo-treated patients	None
Menasche et al.,[79] 1990	Cardiac Surgery	20	DFO 30 mg/kg IV during CPB	DFO-treated patients had an attenuated rise in plasma TBARS postoperatively compared to placebo-treated patients	None
Paraskev et al.[80], 2005	Cardiac Surgery	45	DFO 57 mg/kg* IV during CPB	DFO-treated patients had higher LVEF postoperatively compared to placebo-treated patients	None
Chan et al.,[81] 2012	STEMI undergoing PCI	60	DFO 500 mg IV bolus followed by 50 mg/kg IV x 12h	DFO-treated patients had lower plasma iron and F(2)-isoprostane levels, but similar infarct size on cardiac MRI	None
Fraga et al.[83], 2012	Critical illness with hypotension	30	DFO 14 mg/kg† IV + NAC within the first 48h of hypotension	DFO+NAC-treated patients had lower markers of oxidative stress and lower SCr on hospital discharge compared to placebo-treated patients	None
Fraga et al.[77], 2016	Critical illness with hypotension	80	DFO 14 mg/kg† IV + NAC within the first 48h of hypotension	DFO+NAC-treated patients had a similar incidence of AKI, but had a lower incidence of severe AKI and a lower SCr at hospital discharge	None
Selim et al.[82], 2019	ICH	294	DFO 32 mg/kg per day IV for 3 days	Similar neuro outcomes at 3-months, but a trend toward improved outcomes at 6-months in DFO-treated patients	None

*Actual dose was 4 g, corresponding to 57 mg/kg in a 70-kg adult; †Actual dose was 1 g, corresponding to 14 mg/kg in a 70-kg adult. Abbreviations: BUN, blood urea nitrogen; CPB cardiopulmonary bypass; ICH, intracerebral hemorrhage; LVEF, left ventricular ejection fraction; NAC, N-acetylcysteine; neuro, neurological; PCI, percutaneous coronary intervention; SCr, serum creatinine; STEMI, ST-elevation-myocardial infarction; TBARS, thiobarbituric acid reactive substances.

1.4 Rationale for DFO dosing regimen

We will administer 30 mg/kg (max total dose, 6g) of DFO as a continuous IV infusion beginning immediately prior to cardiac surgery (after induction of anesthesia and placement of a central venous catheter) and continued intra- and postoperatively for 12h. The determination of this dosing regimen is based on several considerations designed to optimize both efficacy and safety. First, 30 mg/kg is consistent with the human equivalent doses of DFO that are effective in attenuating AKI in animal models (**Table 1**).[33] Second, 30 mg/kg is within the range that exerts anti-inflammatory/anti-oxidative effects in human studies of acute organ injury prevention, including patients undergoing CPB surgery (**Table 3**), and was demonstrated to be well tolerated in this patient population. Third, 30 mg/kg is within the FDA-recommended dosing range for DFO of 20-40 mg/kg/day for the treatment of chronic iron overload. The infusion will be administered over 12 hours based on our findings that catalytic iron levels are highest in the immediate perioperative period (**Figure 1**).

2. SPECIFIC AIMS

Overview of Study Design

In Aim 1, we will perform a phase 2, double-blind, randomized, placebo-controlled trial to investigate the effects of DFO administration on the incidence of AKI following cardiac surgery. We will randomly assign 330 patients undergoing cardiac surgery at three major academic hospitals in Boston to receive DFO (30 mg/kg; max total dose, 6g) or an equal volume of normal saline. DFO will be administered as a continuous 12h IV infusion starting immediately prior to cardiac surgery (after induction of anesthesia and placement of a central venous catheter). We will also collect pre- and postoperative blood, urine, and peripheral blood monocytes for exploration of secondary outcomes.

Aim 1

To test the effects of DFO on the incidence of AKI and extrarenal acute organ injury following cardiac surgery, as assessed by KDIGO-defined changes in serum creatinine and urine output over a 7-day period, urinary markers of tubular injury (NGAL and KIM-1), and the incidence of postoperative myocardial injury, atrial fibrillation, prolonged mechanical ventilation, and sepsis.

Aim 2

To test the effects of DFO on catalytic iron, oxidative stress, and inflammation following cardiac surgery.

3. SUBJECT SELECTION

3.1 Inclusion criteria (each of the following)

1. Age ≥18 years
2. Undergoing coronary artery bypass graft (CABG) and/or valve surgery with CPB. Includes combination surgeries that involve CABG and/or valve plus other procedures (e.g., Maze procedure, aortic aneurysm repair)
3. AKI risk score ≥6 at the time of screening (**Table 4**)
4. Written informed consent from the patient or surrogate

Table 4. Risk factors for cardiac surgery-associated AKI

Risk Factor	Points
Male sex	1
eGFR (ml/min/1.73m^2)*	
45-59	1
30-44	2
<30	3
Hemoglobin (g/dL)*	
10-11.9	2
<10	4
WBC count (per mm^3)*	
<4	1
>12	2
Platelet count (10^9/L)*	
<150	1
>400	2
Albumin (g/dL)*	
3.6-4.0	1
<3.6	2
Hypertension	1
Diabetes mellitus	1
CHF†	1
Chronic liver disease‖	1
Prior cardiac surgery	2
Combined CABG/valve	1

*Closest value in the last 3 months.
†Any of the following: CHF; HFrEF; HFpEF; NYHA class III or IV; diastolic dysfunction; or LVEF≤40%.
‖Any of the following: chronic liver disease; cirrhosis; NAFLD; AIH; chronic viral hepatitis.

Abbreviations: AIH, autoimmune hepatitis; CHF, congestive heart failure; eGFR, estimated glomerular filtration rate (assessed using CKD-EPI equation); HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; LVEF, left ventricular ejection fraction; NAFLD, non-alcoholic fatty liver disease.

3.2 Exclusion criteria (at the time of screening)

1. AKI, defined as any of the following:
   - Increase in serum creatinine ≥0.3 mg/dl in 48h
   - Increase in serum creatinine ≥50% in 7d (if no value available in last 7d, use most recent value in last 3 months)
   - Urine output ≤0.5 ml/kg/h x 6 consecutive hours (only assessed in patients with hourly monitoring via Foley catheter)
   - Receipt of renal replacement therapy (RRT) within 7d
2. Advanced chronic kidney disease (eGFR <15 ml/min/1.73m^2 or end-stage kidney disease receiving RRT)
3. Hemoglobin <8 g/dL (closest value in the prior 3 months)
4. Fever (temperature ≥38ºC) in the last 48h
5. Suspected or confirmed bacteremia, endocarditis, or pyelonephritis
6. Pneumonia, aspiration, or bilateral pulmonary infiltrates from an infectious etiology reported on chest x-ray or CT scan in the last 7d
7. Positive COVID-19 test in the last 10d
8. Chronic iron overload (including conditions such as hemochromatosis and beta thalassemia major) or previous iron chelation therapy (including prior participation in DEFEAT-AKI)
9. Known hypersensitivity to DFO
10. Taking prochlorperazine
11. Known severe hearing loss
12. Pregnant or breastfeeding. Women of childbearing potential will not be included without documentation of a negative pregnancy test, unless they have previously undergone hysterectomy or bilateral oophorectomy. Women of child bearing potential will be defined as any woman who has begun menstruation and not yet reached menopause. Menopause will be defined as any woman over the age of 45 who has not had a menstrual period for at least 12 consecutive months.
13. Prisoner
14. Concurrent participation in another interventional research study in which the intervention has potential interaction with DFO
15. Surgery to be performed under conditions of circulatory arrest
16. Receiving extracorporeal membrane oxygenation
17. Durable ventricular assist device (VAD) prior to surgery (does not include Impella device or intra-aortic balloon pump)
18. Any condition which, in the judgement of the investigator, might increase the risk to the patient
19. Conflict with other research studies

3.3 Source of subjects

Patients scheduled to undergo cardiac surgery at BWH, MGH, and BIDMC, all located in Boston, MA.

3.4 Recruitment methods

Patients will be enrolled prior to cardiac surgery, either at their in-person preoperative evaluation (typically 7-10 days prior to surgery), by phone/zoom if their preoperative evaluation is conducted virtually (due to COVID-19 or other factors), or as inpatients. Additional details on enrollment are provided in section 4. No remuneration will be provided for participating.

4. SUBJECT ENROLLMENT

4.1 Methods of enrollment and procedures for obtaining informed consent

Consecutive patients scheduled to undergo cardiac surgery at BWH, MGH, and BIDMC will be screened on a daily basis by research coordinators at each site using electronic medical records. A standardized screening checklist will be used to assess each patient's eligibility.

4.2. Procedures for obtaining informed consent

Overview

The chiefs of cardiac surgery at BWH, MGH, and BIDMC are supportive of this research study and have approved approaching the cardiac surgical patients for screening and consenting. All patients, regardless of method of consent, will receive a copy of their consent form signed by both the patient and the study staff obtaining consent. Recruitment will take place with the assistance of the preoperative evaluation centers at BWH, MGH, and BIDMC, as described further below.

Consent of outpatients at their preoperative visit

For patients who have an in-person preoperative appointment (typically 7-10 days prior to the date of surgery), research study staff will ascertain their willingness to discuss the study at the conclusion of their preoperative visit. A member of the patient's clinical team (e.g., the nurse, nurse practitioner, or physician caring for the patient) will initially introduce the study to the patient and obtain the patient's permission to be contacted by study staff before they are approached by study staff. For patients who express interest in hearing about the study, research staff will briefly introduce the study, including providing patients with a paper handout (attached) that includes an overview of the study. Research staff will be able to answer basic questions about the study but will not obtain consent. If the patient expresses interest in further discussing the study, a physician investigator will explain the study in detail, answer questions, and seek written consent.

Consent of outpatients by phone/zoom

The preoperative evaluation centers at BWH, MGH, and BIDMC will incorporate a question regarding research into their standardized list of preoperative assessment questions (already being done at BWH, and in process at MGH and BIDMC). During their remote preoperative appointment (conducted by phone or zoom), patients will be asked by their nurse or nurse practitioner if they would like to "opt out" of communication about research studies they may be eligible to participate in based on their upcoming procedure. The nurse or nurse practitioner will document in the electronic medical record whether the patient wishes to opt out of communication about research opportunities. Research study staff will check the patient's appointment notes to identify which patients may be contacted. In a minority of cases, if there is no documentation in the patient's chart regarding whether they would like to "opt out" of communication about research studies, eligible patients will be contacted by phone by a member of their clinical team (e.g., the nurse or nurse practitioner who saw the patient during their preoperative evaluation). The clinical team member will ask the patient if they are willing to be contacted by the research team to hear about the study.

Eligible patients who agree to be contacted will be called by a physician investigator to explain the study, answer questions, and seek consent. If the patient wishes to consent after the physician investigator has explained the study, the patient will be sent an email with an embedded REDCap survey link to the electronic Informed Consent (eIC) form, which is 21 CFR Part 11 compliant. On this survey page, patients will verify their information is accurate and will read the consent form. Patients will acknowledge that they have both read and understood the informed consent form, and that they agree to participate in the research study. Consent forms are stored as a PDF in the subjects' REDCap record, and subjects that have provided consent electronically will be notified automatically via email when their final consent form is available for download in REDCap. The Informed Consent Process is documented in REDCap for each subject with the addition of new fields to capture remote and

electronic consent. No medical content, diagnoses, study descriptors, or other PHI will be present in unsecured emails sent to subjects.

As an alternative to the REDCap eIC form, patients who wish to consent after the physician investigator has explained the study may receive the consent form by email or fax, and may send the signed copy of the consent form back to the research team by email or fax. In such cases, patients will be asked to bring in a signed copy of the original consent form on the day of surgery.

Consent of inpatients

For patients already in the hospital, recruitment and written consent will take place at the bedside, typically 1-2 days prior to surgery, by a physician investigator. Potential subjects will be initially approached by a member of their clinical team (e.g., their nurse) who has first-hand knowledge of the potential subject's medical history to ascertain their willingness to discuss the study with the physician investigator. If the potential subject agrees to discuss the study further, he/she will be introduced to the physician investigator by the clinical team member. Patients will be provided a paper handout that includes an overview of the study, along with the consent form, and will be given an opportunity to read the consent form and ask questions. Patients will then decide if they wish to decline, enroll, or if they need additional time to think about it, in which case they will be provided with a business card and encouraged to follow-up with any questions. In a minority of cases due to scheduling issues or re-scheduling, patients may be approached on the day of surgery. Utmost care will be taken, and best clinical judgment will be used to determine if a patient is a candidate for same-day enrollment. This will include asking the patient's nurse for input on the suitability for same-day enrollment, talking with the patient about their level of anxiety, and discussing the general idea of research with the patient and their family.

Consent of inpatients using a hybrid phone/in-person approach

On rare occasions there may be eligible patients in the hospital, but the physician investigator(s) for that site may not be available to consent the patient in-person. Under such circumstances, similar consenting procedures will take place as described above under, "Consent of inpatients", except that potential subjects will be approached initially by a non-physician member of the study team (e.g., a clinical research coordinator). As above, this initial approach will only occur after a member of the potential subject's clinical team has ascertained their willingness to discuss the research with the study team. The non-physician member of the study team will briefly introduce the study. If the patient wishes to hear more about the research, a physician investigator will then describe the study further by phone, including the purpose of the study, the study activities that will take place, and the risks and benefits of participating. The physician-investigator will also answer any questions. Patients will then decide if they wish to decline, enroll, or if they need additional time to think about it. For patients who wish to enroll, they will sign a paper informed consent form (ICF). A sticker with the patient's name and MRN will be placed on each page of the ICF. The signature page of the ICF that includes a line for the physician-investigator to sign and date will be scanned and emailed to the physician-investigator, who will then sign it and email it back to a member of the study team. This variation of the consent process, when employed, will be documented by a note in the study file.

Consent of patients who lack decisional capacity

It is unusual for patients undergoing cardiac surgery to lack decisional capacity. However, in a minority of cases there may be eligible patients who lack decisional capacity due to acute severity of illness (e.g., intubation/sedation) or other factors. In these cases, study investigators will follow identical procedures as above with the patient's surrogate if the patient is deemed to lack decisional capacity by the clinical team. The Mass General Brigham preferred order of surrogates will be followed, and the investigator will document the relationship of the surrogate to the subject in the research record. The order of surrogates will be:

i) court appointed guardian with specific authority to consent to participation in research or authority to make health care decisions for a class of diagnostic and therapeutic decisions inclusive of the proposed research;
ii) health care proxy/person with durable power of attorney with specific authority for making health care decisions inclusive of the proposed research; or
iii) spouse, adult child, or other close family member who knows the subject well and has been involved in their care.

For patients deemed to lack decisional capacity who are enrolled by surrogate consent, assent of subjects will be required for participation in the research unless the subject is incapable of providing assent due to his/her medical condition (e.g., sedation). For patients deemed to lack decisional capacity who are enrolled by surrogate consent and who subsequently regain capacity during the study, investigators will explain the study (including updating them on what has happened so far as a result of being in the study), answer any questions, and ask for written informed consent to continue to participate. Alternatively, patients will have the option of withdrawing from the study.

Non-English speaking patients will be eligible for inclusion. We will use official hospital interpreters and the Mass General Brigham approved 'short form' consent document in the native language of the patient and/or their surrogate.

Using a checklist, we will document the following items of the informed consent process:

- If consent was obtained in person or remotely (if remotely, we will document the method: electronic Informed Consent, email, or fax)
- If consent was obtained from the subject or the surrogate
- If consent was obtained from a subject who was physically unable to talk or write, we will document the means by which consent was given by the subject (e.g., orally)
- If consent was obtained from the surrogate:
  -Reason why subject lacked decisional capacity
  -Relationship of surrogate to subject
  -Whether the patient was able to assent (if not, document the reason why)
  -Whether the subject regained capacity to consent a later time (if so, document whether the subject provided consent to continue to participate or not)
- Whether an interpreter was required and the short form used
- Confirmation that a copy of the consent form was provided to the subject or surrogate
- Confirmation that the patient's electronic medical record has been updated to reflect that the patient has consented to be enrolled in the research study

4.3 Treatment assignment and randomization

Participants will be randomly assigned in a 1:1 fashion to receive DFO or an equal volume of normal saline. Randomization will be stratified by site and by preoperative eGFR (<60 vs. ≥60 ml/min/1.73m^2) at the time of screening. Treatment assignment lists will be generated centrally by the study biostatistician, Wei Wang PhD, using permuted block sizes of 4 and sent to the research pharmacies at each site. Patients, physicians, nurses, and study investigators will be blinded to treatment assignment.

5. STUDY PROCEDURES

5.1 Drugs to be used

We will administer DFO or an equal volume of normal saline as an IV infusion over 12h. DFO will be administered at a total dose of 30 mg/kg (maximum total dose, 6g). The DFO (or normal saline) infusion will begin immediately prior to cardiac surgery (after induction of anesthesia and placement of a central venous catheter) and will be continued intra- and postoperatively for a total of 12h. The rationale for the dose of 30 mg/kg is provided in section 1.4. In preparing the DFO infusion, the research pharmacy at each site will follow similar methods used in prior DFO studies[80, 81] and according to the manufacturer recommendations: the prescribed amount of DFO powder, based on the patient's weight, will be dissolved in sterile water to achieve a concentration of 10%. The solution will then be further diluted in normal saline to achieve a final volume of 240mL to standardize the amount of fluid administered in both study arms. The DFO (or normal saline) will be delivered from the research pharmacy to the operating room by a member of the research pharmacy staff or a clinical research coordinator, and will be administered to the patient by the patient's nurse as a continuous 12h IV infusion through a central venous catheter. The DFO or normal saline infusion will begin immediately after induction of anesthesia and placement of a central venous catheter, which is typically inserted into an internal jugular or subclavian vein in the operating room 1 hour prior to the start of surgery for routine clinical care.

5.2 Data to be collected

Upon enrollment, the following variables will be recorded: age; sex; race; ethnicity; comorbidities; home medications; type of surgery; urgency of surgery (elective, urgent, or emergent); and date of surgery. The following data will be recorded postoperatively:

-Cardiopulmonary bypass (CPB) time
-Aortic cross clamp time
-Estimated blood loss intraoperatively
-Requirement for (and number of) red blood cell transfusions intraoperatively and for 7d
-Requirement for (and volume) of Cell Saver used intraoperatively
-Duration of invasive mechanical ventilation
-Myocardial injury, atrial fibrillation, ARDS, and infection/sepsis within 7d
-Physiologic and vital sign data, including heart rate, blood pressure, and urine output for 7d
-Daily lab values, including hemoglobin and serum creatinine, for 7d
-Receipt of renal replacement therapy within 7d
-Reoperation during hospitalization
-ICU and hospital length of stay
-ICU and hospital mortality

5.3 Methods for data collection

Each site will review electronic medical records and enter patient-level data into a central online database using REDCap, a secure, HIPAA-compliant, web-based application for building and managing databases. REDCap has many advanced features including multi-site access, audit trails, branching logic, and validation ranges for each variable to minimize keystroke errors. Clinical research coordinators will enter detailed data on demographics, comorbidities, vital signs, physiologic parameters (e.g., hourly urine output), laboratory measures, medications, and clinical outcomes.

5.4 Biological sample collections

Overview

We will collect blood and urine samples at five time points: preoperatively (after insertion of an arterial line [for blood] and after insertion of a Foley catheter [for urine]); at the end of CPB; on arrival to the ICU; and on postoperative days 1 and 2 (**Figure 4**). Blood will be obtained from arterial or central venous catheters, which are inserted preoperatively as part of routine clinical care. If, during the course of the study, neither an arterial nor central venous catheter remains in place (e.g., on postoperative day

2), blood will be obtained by peripheral venipuncture by a phlebotomist, in which case all efforts will be made to time the blood collection with a clinical blood draw to minimize discomfort. The total volume of blood collected will be 80mL. Blood will be collected in serum and heparinized vacutainers, since EDTA chelates iron. Urine (12cc) will be obtained from Foley catheters or from a clean catch voided sample if the Foley catheter is no longer in place.

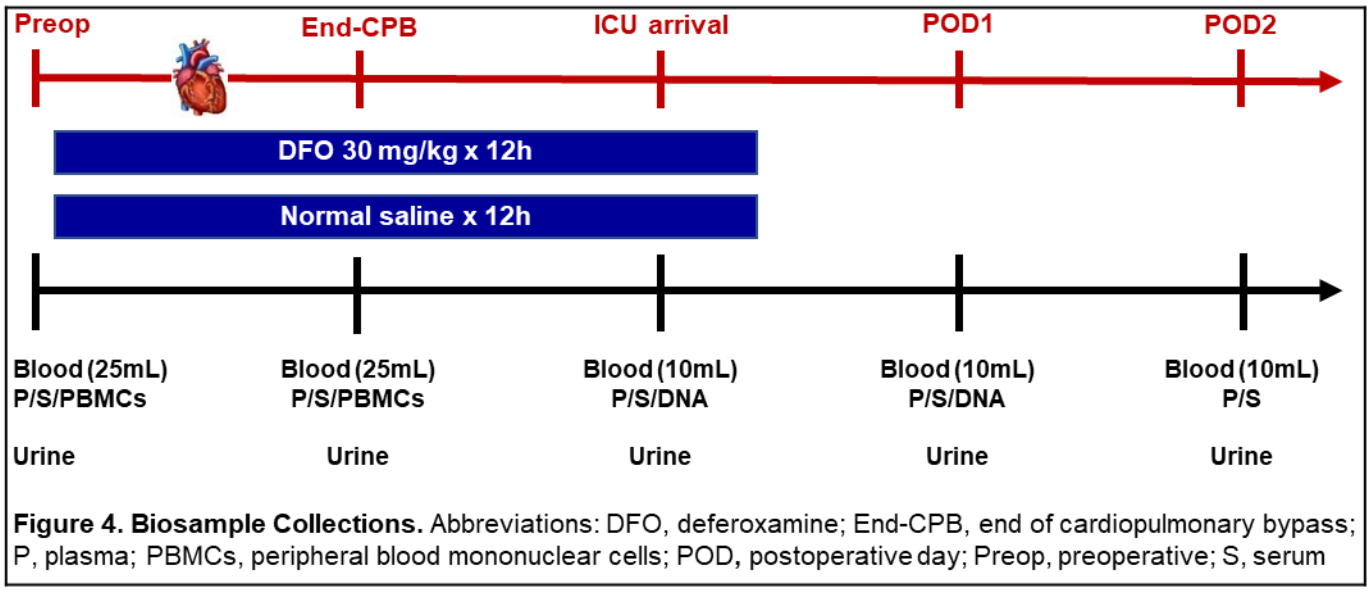

Figure 4. Biosample Collections. Abbreviations: DFO, deferoxamine; End-CPB, end of cardiopulmonary bypass; P, plasma; PBMCs, peripheral blood mononuclear cells; POD, postoperative day; Preop, preoperative; S, serum

Blood volume

The blood volumes shown in **Figure 4** represent the minimum volume required to perform the comprehensive assays for the study. Preoperatively and at the end of CPB we will collect 25mL of blood, which will allow us to isolate peripheral blood mononuclear cells (PBMCs) and store them in liquid nitrogen for immunophenotyping, including flow cytometry, RNA sequencing (RNA-Seq), and qPCR. Specifically, collection of 25mL of blood will be divided as 20mL of plasma and 5mL of serum. The 20mL of plasma will allow us to isolate approximately 20 x 10^6 PBMCs, since each mL of blood contains approximately 10^6 PBMCs. We will aliquot and store these 20 x 10^6 PBMCs into four aliquots of 5 x 10^6 PBMCs each. Aliquot #1 will be used for flow cytometry studies to assess monocyte expression of IL-6, TNFα, HLA-DR, TLR4, PD-L1, IL-1β, IL-10, CD163, heme oxygenase-1, ferroportin, transferrin receptor 1, and iron uptake transporters (DMT1, ZIP14). These markers will be used to assess the effect of DFO (versus normal saline) on the inflammatory phenotype of monocytes postoperatively. Further, they will be used to assess whether the effect of DFO versus normal saline on clinical parameters (AKI and extrarenal acute organ injury) differs according to preoperative differences in monocyte expression of these markers. Aliquot #2 will be used to quantify intracellular iron concentrations using the calcein-AM method.[84] Aliquot #3 will be used for RNA-Seq of monocytes to facilitate discovery of novel transcripts influenced by DFO. Aliquot #4 will be used for qPCR to validate the targets identified by RNA-Seq.

The volume of blood drawn at the other time points will be 10mL, as shown in **Figure 4**. This will include 5mL of blood drawn into a serum vacutainer and 5mL drawn into a heparinized plasma vacutainer. The serum and plasma will be centrifuged, subaliquoted, and stored at –80C. 5mL of blood contains approximately 2.5mL of plasma/serum (approximately 50% of whole blood is plasma/serum), which will allow us to archive eight 300uL plasma and serum aliquots at each time point. These aliquots will be used to measure the following parameters, and for storage for future hypotheses and mechanistic pathways: catalytic iron, total iron, total iron binding capacity, ferritin, hepcidin, troponin I, and oxidative stress parameters (F[2]-isoprostane and myeloperoxidase). Finally, we will collect the buffy coat (for DNA isolation) on ICU arrival and on post-operative day (POD) 1 (**Figure 4**).

Patients with a hemoglobin level <8 g/dl are excluded (section 3.2). Additionally, if during the course of the study the patient's clinical team determines that collection of blood is unsafe for the patient (e.g., if the patient is experiencing major bleeding postoperatively) then the blood sample will not be collected until the clinical team determines that it is safe. Urine will be collected for assessment of markers of tubular injury, including NGAL and KIM-1.

Sample processing, cataloging, storing, and monitoring

We will centrifuge samples as soon as possible and no more than 6h after collection and store the plasma, serum, and urine at –80C. At two time points (preoperatively and at the end of CPB) we will also isolate PBMCs and store them in liquid nitrogen. We will use screw cap micro tubes for plasma, serum, and urine samples, each pre-labeled with a unique barcode and a printed label. Each site will catalogue the date and time of collection, processing, and storage at –80C (or in liquid nitrogen, for PBMCs) for each sample. Samples will be transported every 3-6 months from MGH and BIDMC to BWH, which will serve as the central biorepository for the study. The location of each sample will be cataloged using Freezerworks, a sophisticated biorepository management and inventory program.

5.5 Sending biosamples and data outside MGB

Sending biosamples to research collaborators outside Mass General Brigham

Pending execution of a Material Transfer Agreement, we will send blood and urine samples to the following collaborators: Dr. Mohan Rajapurkar, at the Muljibhai Patel Society for Research in Nephrology (Nadiad, Gurajat, India), and Dr. Rajesh Gupta, at the University of Toledo (Toledo, OH). The purpose of shipping these samples is for measurement of catalytic iron and other iron parameters, such as hemopexin, haptoglobin, and free hemoglobin. These measurements are quite specialized, and the collaborators listed above have unique expertise in performing these measurements. Samples will be labeled with a study ID only. No patient-level data will be shared with collaborators at the Muljibhai Patel Society for Research in Nephrology.

Sending coded data to research collaborators outside Mass General Brigham

Coded data from RNA-Sequencing of peripheral blood mononuclear cells will be shared with Drs. Matthew Sampson and Dongwon Lee at Boston Children's Hospital, who have expertise in bioinformatics and analysis of omics data, including transcriptomics. No protected health information will be shared with collaborators at Boston Children's Hospital. We will also send a limited dataset of randomized patients to the bioinformatics company, Telperian, a clinical innovation company that integrates machine learning with clinical trial data to identify subgroups of patients who may benefit from the intervention.

6. BIOSTATISTICAL ANALYSIS

6.1 Primary endpoint definition

The primary endpoint is the **incidence of postoperative AKI**, defined according to the KDIGO criteria as any of the following: urine output <0.5 ml/kg/h for ≥6 consecutive hours (assessed within the first 48h or until the Foley catheter is removed, whichever occurs first; an increase in SCr ≥0.3 mg/dl within the first 48h; an increase in SCr ≥50% within the first 7 days; or receipt of RRT within the first 7 days.[85] The immediate preoperative SCr will be used as the baseline value for all analyses. SCr is measured at least once daily for routine clinical care postoperatively. Assessment of urine output monitoring will begin at the start of surgery.

6.2 Statistical analysis for the primary endpoint

Hypothesis 1: DFO will reduce the incidence of postoperative AKI. We will use the χ^2 test to compare the proportion of patients in the DFO versus normal saline (placebo) group who develop the primary endpoint. We will perform sensitivity analyses to assess the composite outcome of AKI or death, since death is a competing risk for AKI. We will use logistic regression to assess for heterogeneity of therapeutic response to DFO across the following subgroups: age; sex; race (White vs. non-White); preoperative eGFR (<60 vs. ≥60 ml/min/1.73m^2); AKI risk score (≤8 vs. >8); duration of CPB (<120 vs. ≥120 min; <120 vs. 120-180 vs. >180 min)); intraoperative RBC transfusion; and type of surgery (elective vs. urgent/emergent).

6.3 Sample size and power

Based on our preliminary data and prior studies,[37, 38] the anticipated AKI event rate in the placebo group is **50%**. We hypothesize the AKI event rate in the DFO group will be **34%**, an effect size that has been proposed to be reasonable for studies in AKI.[86, 87] A total of 296 patients (*n*=148 per group) will provide 80% power to detect the above treatment effect, with a two-sided α of 0.05. Enrollment of 330 patients provides a small buffer for potential early withdrawal from the study, which we anticipate will be uncommon due to the nature of the study (inpatient and short duration).

6.4 Secondary endpoints

Other renal endpoints (clinical)

- Effect of DFO across all stages of AKI, defined by changes in SCr according to the KDIGO criteria[85] within 7 days of cardiac surgery.
- Requirement for loop or thiazide diuretics in the setting of ≥2 hours of oliguria (urine output <0.5 ml/kg/h for ≥2 consecutive hours) within 48h of cardiac surgery.
- Incidence of Major Adverse Kidney Events,[88] defined as any of the following within 7 days following cardiac surgery: ≥100% increase in SCr, receipt of RRT, or death.

Other renal endpoints (urinary tubular injury markers)

We will assess the effect of DFO on renal tubular injury using longitudinal measures of urine NGAL and KIM-1, each assessed pre- and postoperatively (**Figure 4**). These injury markers provide complementary data to functional markers (e.g., SCr),[89] and have been used in multiple RCTs.[90-93] Urine NGAL and KIM-1 will be normalized to the urine creatinine to account for the influence of dilution.

Extrarenal acute organ injury

We will assess the incidence of postoperative myocardial injury, atrial fibrillation, prolonged mechanical ventilation, and sepsis. The consensus definition and anticipated incidence for each endpoint is described in **Table 5**, and each was chosen based on a strong biologic rationale (**Table 5**). Assessment of atrial fibrillation will be limited to patients who do not have it at baseline. For sepsis, we will document the suspected or confirmed source of infection: pneumonia, genitourinary, biliary, bacteremia, sternal wound infection, or other. We will also document the presence/absence of septic shock, defined as sepsis with persistent hypotension requiring vasopressors to maintain mean arterial pressure ≥65 mm Hg and having a serum lactate level >2 mmol/L despite adequate volume resuscitation.[94]

Table 5. Secondary extrarenal endpoints			
Endpoint	**Definition**	**Incidence***	**Biologic Rationale**
Myocardial injury	Peak postop troponin I† elevation >10 times the 99th percentile URL, assessed on POD1 and 2.[95]	15%	DFO attenuates cardiac IRI[67, 68] and improves myocardial contractility in animals.[96] In a RCT in 45 adults undergoing cardiac surgery, patients treated with DFO had a higher LVEF postop compared to placebo.[80]
AF	‡Confirmed on a 12-lead ECG or a rhythm strip, confirmed by a healthcare provider, or treated (either medically [e.g., amiodarone] or with electrical cardioversion). Patients with AF at baseline will be excluded.	40%	In animal models, DFO prevents CPB-associated arrhythmias.[69] In patients with chronic iron overload from beta-thalassemia major, arrhythmias are an indication for DFO treatment intensification.[97]
Prolonged ventilation	‡Requirement for mechanical ventilation >24h postop.	20%	In animal models, DFO attenuates the severity of mechanical ventilation- and lipopolysaccharide-induced acute lung injury.[70, 71]
Sepsis	‡Life-threatening organ dysfunction caused by a dysregulated host response to infection (assessed within 7d postop). Organ dysfunction is defined as an acute increase in the total SOFA score ≥2 points consequent to the infection.	10%	DFO improves monocyte phagolysosome function in vitro.[98] Moreover, DFO improves survival in animal models of sepsis.[75, 76, 99]
*Anticipated incidence based on our preliminary data and published literature. †Troponin I is less affected by skeletal muscle breakdown than troponin T.[100] ‡Defined as per the Third International Consensus Definition (Sepsis-3).[94] Abbreviations: AF, atrial fibrillation/flutter; DFO, deferoxamine; ICH, intracerebral hemorrhage; LVEF, left ventricular ejection fraction; POD, postoperative day; URL, upper reference limit.			

We will assess time to liberation from IV vasoactive medications, as well as the Vasoactive-Inotropic Score during the first 24 hours. Finally, we will assess the number of ventilator-, hospital-, and ICU-free days to day 28, as well as all-cause hospital mortality. Patients who die prior to 28 days will be assigned 0 ventilator-, hospital-, and ICU-free days.

Iron and oxidative stress markers

We will assess the effect of DFO on iron and oxidative stress markers, including catalytic iron, total iron, total iron binding capacity, ferritin, hepcidin, F(2)-isoprostane, and myeloperoxidase, each assessed pre- and postoperatively (**Figure 4**).

6.5 Statistical analysis for secondary endpoints

Hypothesis 2: DFO will attenuate peak postoperative increases in urinary markers of tubular injury (NGAL and KIM-1) and iron and oxidative stress, and will reduce the incidence of myocardial injury, atrial fibrillation, prolonged mechanical ventilation, and sepsis. For binary variables we will use the χ^2 test to compare the proportion of patients in each group who develop the endpoint. For continuous variables we will compare baseline (preoperative)-adjusted peak postoperative levels of each marker in DFO- versus placebo-treated patients using the two sample t-test or Wilcoxon rank-sum test depending on the distribution. We will also use a Generalized Estimating Equation for repeated measures to analyze longitudinal assessments of each marker in DFO- versus placebo-treated patients. For analyses of urinary tubular injury markers, we will perform sensitivity analyses using a global rank endpoint to account for anuria as a competing risk.[101, 102] Finally, we will evaluate a composite endpoint of any renal or extrarenal acute organ injury or hospital mortality.

6.6 Modified intention-to-treat analysis

The final analysis will be limited to patients who are initiated on the study drug, irrespective of whether they are able to complete the entire 12h infusion, consistent with a modified intention-to-treat analysis. Patients who consent but are subsequently found to be ineligible prior to initiation of the study drug infusion (e.g., if a patient develops a new fever several hours prior to surgery) will be withdrawn. In such cases, the reason for the withdrawal will be documented and reported, but these patients will not be included in the final analysis, nor will they count toward the total enrollment target of 300 analyzable patients. Accordingly, we anticipate enrolling up to 330 patients (10% buffer) to obtain 300 analyzable patients, accounting for withdrawal prior to receipt of study drug.

7. RISKS AND DISCOMFORTS

Overview of DFO safety profile
DFO was the first iron chelator approved by the FDA (in 1968), and thus has a long track record of safety. DFO is generally very well tolerated, as patients with genetic hemoglobin disorders complicated by iron overload often receive DFO on a daily basis for their entire lives.[103] Rare but serious potential adverse effects of DFO include hypersensitivity reactions, hypotension, acute respiratory distress syndrome (ARDS), infection, vision changes, and hearing loss.

7.1 Hypersensitivity reactions
Hypersensitivity reactions, including anaphylaxis, have very rarely been reported with DFO (less than 1 in 10,000). In a phase 2 RCT of DFO at 32 mg/kg per day for 3 days in patients with ICH, none of the 147 DFO-treated patients experienced anaphylaxis.[82]

7.2 Hypotension
DFO may cause hypotension if infused very rapidly (>15 mg/kg/h). For example, DFO-induced hypotension was initially observed in two of three children with acute iron poisoning who received large doses of IV DFO (ranging from 800-1500mg) administered over 15 minutes.[104] Accordingly, the FDA-recommended infusion rate for IV DFO for acute iron intoxication is to not exceed 15 mg/kg/h. Thus, the DFO dosing regimen in the current study (30 mg/kg over 12h hours, which equates to 2.5 mg/kg/h) is 6-fold lower than the maximum FDA-recommended infusion rate.

Regarding dose, the maximum FDA-approved dose for DFO for acute iron intoxication is 6g/day, or ~86 mg/kg in a 70-kg adult, and doses as high as 150 mg/kg/day for 3 months have been used without toxicity.[105] Thus, the dosing of 30 mg/kg in the current study is far below the maximum FDA-approved dose, and is unlikely to cause hypotension. Supporting this, one study administered DFO as an IV bolus of 500mg over 10 minutes in patients with STEMI.[81] This infusion rate of 50mg/min, despite being more than 15-times higher than the infusion rate in the current study (30 mg/kg over 12h, or ~3 mg/min in a 70-kg individual), did not result in any hypotension. Importantly, hypotension was also not observed in the studies of IV DFO for acute organ injury prevention in humans, summarized in **Table 3**. The largest such study, a phase 2 RCT of DFO at 32 mg/kg per day for 3 days in patients with ICH, found the incidence of hypotension was similar in the DFO-treated patients (1 of 144 [1%]) compared to placebo-treated patients (2 of 147 [1%]).[82]

7.3 ARDS
DFO may rarely cause ARDS when administered in very high doses. Tenenbein et al. described four adult patients with acute iron overdose who developed ARDS after receiving high doses of IV DFO (15 mg/kg/h) for prolonged periods of time.[106] DFO doses received by the four patients ranged from 24 to 120g delivered over 32 to 72 hours, corresponding with doses 10 to 50-times higher than those that will be used in the current study. The High-Dose Deferoxamine in Intracerebral Hemorrhage (HI-DEF) study (clinicaltrials.gov NCT01662895), which investigated a continuous IV infusion of DFO at 62 mg/kg/day for 5 consecutive days versus normal saline placebo in patients with ICH, was stopped early due to the observation of 6 cases of ARDS among the DFO-treated patients after enrollment of 42 subjects. Three modifications to the DFO dosing regimen were subsequently implemented: 1) the 62 mg/kg/day dose was reduced to 32 mg/kg/day; 2) the 5-day infusion was reduced to 3 days; and 3) the continuous infusion was changed to daily interrupted infusions of 12h/day, based on data that DFO-associated ARDS is more likely to occur in patients treated for longer than 24h.[106] These changes to the DFO dosing regimen were implemented in a phase 2 RCT of DFO in patients with ICH, and the incidence of ARDS was similar in the DFO- (2 of 144 [1%]) compared to placebo-treated patients (1 of 147 [1%]).[82]

7.4 Infection

In patients with chronic iron overload it has been reported that infections, especially gastroenteritis with *Yersinia* species, may be promoted by DFO. Among dialysis patients receiving DFO, rare cases of mucormycosis have also been reported. An increased risk of infection has not been reported with short-term use of DFO for acute organ injury prevention (**Table 3**). The largest such study, a phase 2 RCT of DFO at 32 mg/kg per day for 3 days in patients with ICH, found the incidence of infection was similar in DFO- and placebo-treated patients (35 of 144 [25.0%] and 36 of 147 [24.5%], respectively).[82]

7.5 Vision changes

DFO administered in high doses and over long periods of time has rarely been linked to vision changes. Effects on the eye were first noted when very high doses (>100 mg/kg/day) were given.[107] Symptoms may include night-blindness, impaired color vision, impaired visual fields, and reduced visual acuity. Severe cases may show signs of retinitis pigmentosa on fundoscopy, whereas milder cases are only demonstrable with electroretinography. The main risk factor appears to be high doses of DFO.[108]

The prevalence of DFO-associated ocular toxicity was evaluated in 84 children who were receiving DFO for an average of 6.6 years for transfusion-related iron overload due to thalassemia.[109] The DFO dosage was 25-50 mg/kg/day. Ophthalmic screening was performed at baseline and on an annual basis. Only one patient had abnormal findings, with central blurring, retinal pigmentary changes, and a reduced central response on electroretinography. This patient had a 3-year history of poor adherence and was temporarily receiving IV DFO at 50 mg/kg/day; all symptoms promptly recovered after changing to subcutaneous administration. Notably, vision changes have not been described in any of the short-term studies of DFO use for acute organ injury prevention (**Table 3**). The largest such study, a phase 2 RCT of DFO at 32 mg/kg per day for 3 days in patients with ICH, found that none of the 147 DFO-treated patients experienced vision changes.[82]

7.6 Hearing loss

Sensorineural hearing loss has rarely been reported in patients receiving high doses of DFO long-term. One study assessed the frequency of hearing loss cross-sectionally in 67 patients receiving regular DFO via subcutaneous pump infusion for β-thalassemia.[110] Patients received DFO at a mean dose of 50 mg/kg/day. In five patients, sensorineural hearing loss was detected. The mean exposure time to DFO in these five patients was 15 years. Importantly, hearing loss has not been reported with short-term use of DFO for acute organ injury prevention (**Table 3**). The largest such study, a phase 2 RCT of DFO at 32 mg/kg per day for 3 days in patients with ICH, found the incidence of hearing loss was similar in DFO- and placebo-treated patients (1 of 144 [1%] and 1 of 147 [1%], respectively).[82]

7.7 Summary of all potential adverse effects of DFO, and their frequency

Very common side effects (affecting more than 1/10 patients):
-Injection site reaction such as pain, swelling, reddening, itching of the skin
-Joint or muscle pain

Common side effects (affecting between 1/10 to 1/100 patients):
-Nausea
-Headache
-Fever

Uncommon side effects (affecting between 1/100 to 1/1,000 patients)
-Vomiting
-Abdominal pain
-Disturbances of hearing such as ringing, hearing loss

Rare side effects (affecting between 1/1,000 to 1/10,000 patients):

-Disturbances of vision such as blurred eyesight, abnormal color vision, night blindness, black spots in the vision, loss of vision, clouding of the lens of the eye, visual field defects, or decreased sharpness of vision
-Fungal or bacterial infections leading to high fever, shortness of breath, acute diarrhea, abdominal pain, general discomfort, or sore throat
-Dizziness, light-headedness (signs of low blood pressure that can occur when the drug is given too rapidly)

Very rare side effects (affecting less than 1/10,000 patients):
-Diarrhea
-Sensation of numbness or tingling in fingers and toes
-Breathlessness due to lung disorders
-Hives, difficulty breathing or swallowing, feeling of tightness in the chest with wheezing or coughing, dizziness, swelling mainly of the face and throat (signs of a severe allergic reaction or asthma)
-Disturbances of the nervous systems

Unknown frequency:
-Muscle spasms
-Abnormal kidney function test results or decreased urine output

Additional information on DFO safety can be found in the attached FDA product label.

7.8 Risks of blood draw

The total volume of blood collected for this study (assuming that a subject is hospitalized for at least 2 days postoperatively) is 80mL (about 5 tablespoons). Blood will be obtained from arterial or central venous catheters, which are inserted preoperatively as part of routine clinical care. If, during the course of the study, neither an arterial nor central venous catheter remains in place, blood will be obtained by peripheral venipuncture by a phlebotomist, in which case all efforts will be made to time the blood collection with a clinical blood draw to minimize discomfort. There is a very small risk of infection, lightheadedness, and/or fainting during blood draws.

7.9 Loss of confidential patient information

Loss of confidential patient information is a potential risk in any clinical study. To minimize this risk we will collect our primary database using REDCap, a secure, HIPAA-compliant, web-based application for building and managing databases. REDCap has many advanced features including multi-site access, audit trails, and the ability to export data with removal of identifiers. Electronic study documents containing protected health information will only be kept on password-protected hospital computers. Paper documents containing protected health information will be kept in binders stored in a secure location at each hospital site. All research staff are CITI-certified and will receive regular training at lab meetings regarding the importance of confidentiality of data.

Biological specimen tubes containing plasma, serum, DNA, and urine will be tagged with study ID#'s only and will not include any protected health information. Specimens will be stored in locked freezers at each of the three sites, and will be transported every 3-6 months from MGH and BIDMC to BWH, which will serve as the central biorepository for the study. No protected health information will be shared with collaborators outside of Mass General Brigham. Genetic data will not be placed onto publicly available repositories such as the National Institutes of Health (NIH) central repository.

8. POTENTIAL BENEFITS

8.1 Potential benefits to participating individuals

Individuals assigned to receive DFO may experience lower rates of postoperative AKI following cardiac surgery. Our hypothesis that iron chelation with DFO may prevent/attenuate AKI in patients undergoing cardiac surgery is supported by ample data from preclinical studies, as well as preliminary data in humans (discussed in detail in section 1).

8.2 Potential benefits to society

This study will help answer a critically important question about the potential pathologic role of iron in AKI. If we demonstrate a therapeutic effect of DFO on prevention of AKI or other acute organ injury, the findings from this study will lay the groundwork for conducting a larger, phase 3, RCT of iron chelation in the setting of cardiac surgery. Even null findings will yield important scientific insights into AKI. We will generate novel data on circulating measures of inflammation and oxidative stress in the perioperative setting, which we will explore in relation to clinical endpoints such as AKI. Using sophisticated translational immunology techniques, including flow cytometry and RNA-Seq, we will generate novel data on monocyte immunophenotype in the setting of AKI.

9. MONITORING AND QUALITY ASSURANCE

9.1 Independent monitoring of source data

Study investigators collecting the data will be blinded to the treatment group. The key linking the study ID with the treatment group will be maintained securely by the study's biostatistician and by the research pharmacies of each of the three participating sites.

9.2 Study team composition, oversight, and governance

Study team composition

David E. Leaf, MD, MMSc is the PI, and will be responsible for recruitment of patients at BWH, as well as the overall execution of the trial, including all communications with regulatory agencies. Aranya Bagchi, MBBS is the site principal investigator (PI) at MGH, and will be responsible for overseeing recruitment and all study activities at MGH. Shahzad Shaefi, MD, MPH is the site PI at BIDMC, and will be responsible for overseeing recruitment and all study activities at BIDMC. Jochen Muehlschlegel, MD, MMSc is a co-investigator and will assist with troubleshooting any enrollment or logistical issues that arise over the course of the study. Edy Kim, MD, PhD is a co-investigator and will lead the monocyte studies in Aim 2. Wei Wang, PhD is a biostatistician and will conduct the primary analyses for the trial. She will also generate randomized treatment assignment lists for the research pharmacies at each site. Matthew Sampson, MD, MSCE and Dongwon Lee, PhD are co-investigators and will lead the analyses of the monocyte transcriptomics in Aim 2.

Oversight

Dr. David Leaf is responsible for overseeing the overall conduct of the study across all three sites. Site PIs (Dr. David Leaf for BWH; Dr. Aranya Bagchi for MGH; Dr. Shahzad Shaefi for BIDMC) will be responsible for oversight of data and sample collection at each site. Drs. Leaf, Bagchi, and Shaefi will have regular conference calls and will meet in person at least 4 times per year. Additionally, Dr. Leaf will conduct in-person site visits at a minimum of every 6 months to ensure that all study procedures are being performed and documented in accordance with the protocol, with corrective actions taken as needed to ensure compliance. All data entry into REDCap is expected to be completed no later than 30 days from the date of hospital discharge or death.

Governance

Dr. Leaf will lead the study and have responsibility for all study decisions. Major study decisions will be made in close consultation with co-investigators, regulatory agencies, and content experts. Study decisions will be communicated to co-investigators in writing (by email). Study progress will be reported to the single Institutional Review Board through an online portal (Insight), and will be communicated to the Food and Drug Administration through 1571 forms, which will be sent via overnight mail. The primary mode of communication with members of the DSMB will be via email.

9.3 Safety monitoring and reporting

Overview

The patients in this study will be undergoing high risk surgery and will be critically ill postoperatively. It is therefore expected that they will have a number of unrelated adverse health events during the course of their hospital stay. Therefore, we will limit the scope of our adverse events (AEs) monitoring and reporting to the following AEs of special interest (AESI), which will be assessed by review of electronic medical records: hypersensitivity reactions (including anaphylaxis); hypotension; ARDS; infection/sepsis; and any AE that, in the view of the site PI, is definitely or possibly related to the study drug.

Hypersensitivity reaction

During the 12h study drug infusion, patients will be in the highly monitored setting of the operating room or the cardiothoracic ICU. If a patient develops anaphylaxis or any other serious AE during the study drug infusion that is related or possibly related to the study drug, the infusion will be discontinued

immediately. If a patient develops anaphylaxis, the patient will be treated as per routine clinical care, including IM epinephrine, methylprednisolone, antihistamines, normal saline, albuterol, and other therapies as determined by the clinical team.

Hypotension

Although hypotension has not been reported with the DFO dosing regimen that will be used in the current study, we will monitor the frequency and severity of hypotension in the perioperative period by calculating the vasoactive-inotropic score during the first 24 hours of the study, beginning with the start of the study drug infusion. The vasoactive-inotropic score is a validated method for integrating all vasoactive medications and their doses on an hourly basis into a single measure, and has been used in multiple settings,[111-113] including CPB surgery.[114]

ARDS

The incidence of ARDS will be assessed daily during the hospital stay for a maximum of 7 days. ARDS will be defined and its severity categorized according to the Berlin criteria[115] as follows:

Berlin Definition of ARDS	
Timing	Within 1 week of a known clinical insult
Chest imaging (chest radiograph or computed tomography)	Bilateral opacities – not fully explained by effusions, lobar/lung collapse, or nodules
Origin of edema	Respiratory failure not fully explained by cardiac failure or fluid overload Need objective assessment (e.g., echocardiography) to excluded hydrostatic edema if no risk factor present
Oxygenation	
Mild	PaO_2/FiO_2 201-300 mm Hg with PEEP≥5 cm H_2O
Moderate	PaO_2/FiO_2 101-200 mm Hg with PEEP≥5 cm H_2O
Severe	PaO_2/FiO_2 ≤100 mm Hg with PEEP≥5 cm H_2O

Abbreviations: FiO_2, fraction of inspired oxygen; PaO_2, partial pressure of arterial oxygen; PEEP, positive end-expiratory pressure

In addition to monitoring the incidence and severity of ARDS, we will also record the frequency of the following respiratory outcomes to further monitor for pulmonary toxicity that may not meet the strict criteria for ARDS:
-Requirement for mechanical ventilation >24h postoperatively
-Ventilator-free days to day 28

Infection/sepsis

We will document all suspected or confirmed infections in the first 7 days or until hospital discharge. Infections will be categorized according to the source (pneumonia, genitourinary, biliary, bacteremia, sternal wound infection, or other) and severity (infection alone, sepsis, or septic shock). Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. Organ dysfunction is defined as an acute increase in the total SOFA score ≥2 points consequent to the infection.[94] Septic shock is defined as sepsis with persistent hypotension requiring vasopressors to maintain mean arterial pressure ≥65 mm Hg and having a serum lactate level >2 mmol/L despite adequate volume resuscitation.[94]

Classifying AESI according to severity and relatedness to the study intervention

For each AESI, site PIs will document the severity and relatedness to the study intervention. Severity of AESIs will be assessed using the Common Terminology Criteria for Adverse Events (CTCAE) v5.0. Relatedness will be categorized as definitely, possibly, or not related to the study intervention, according to the following definitions:

- Definitely Related: The adverse event is clearly related to the investigational agent – i.e., an event that follows a reasonable temporal sequence from administration of the study intervention, follows a known or expected response pattern to the suspected intervention, or that is confirmed

by improvement on stopping the exposure and that could not be reasonably explained by the known characteristics of the subject's clinical state.
- Possibly Related: An adverse event that follows a reasonable temporal sequence from administration of the study intervention and follows a known or expected response pattern to the suspected intervention, but that could readily have been produced by a number of other factors.
- Not Related: The adverse event is clearly not related to the investigational agent – i.e., another cause of the event is most plausible; and/or a clinically plausible temporal sequence is inconsistent with the onset of the event and the study intervention and/or a causal relationship is considered biologically implausible.

Reporting of unanticipated problems, including unanticipated AEs

Site PIs will report all unanticipated problems involving risks to subjects or others to the overall study PI, Dr. David Leaf, within 24h of the date they first become aware of the problem. Dr. Leaf will report all unanticipated problems involving risks to subjects or others to the Mass General Brigham Institutional Review Board (IRB), which is serving as the single IRB for this study, and to the Chair of the Data and Safety Monitoring Board (DSMB), within 5 working days/7 calendar days of the date he first becomes aware of the problem. Other AEs and minor protocol deviations will be reported to the IRB during continuing review.

Unblinding

Physicians caring for study subjects in an emergency situation may determine that unblinding is necessary for clinical care. In such a scenario, physicians may be unblinded. Whenever possible, the physician caring for the patient should contact the site PI to notify them of the need to be unblinded to a particular subject and communicate any planned intervention so that such interventions can be documented. Physicians can be unblinded by contacting the research pharmacy at their site (available by phone/pager 24h/day, 7d/week) and requesting to be unblinded for provision of emergency care.

9.4 DSMB

A multidisciplinary panel of three physician-scientists with expertise in clinical trials, cardiac surgery, acute kidney injury, and iron chelation will meet in person or through videoconference prior to study launch and after enrollment of 10, 25, 50, and 75% of the total enrollment target. Additional meetings will be held more frequently as needed, as determined by the members of the DSMB. Enrollment rates, unanticipated problems, and data on AEs will be reviewed at each meeting. The members of the committee will have the option to review data on AEs in an unblinded manner. If they choose to exercise this option, an honest broker who is not involved in the direct conduct of the research will be available to unblind the committee members (study investigators will remain blinded).

The DSMB will submit a written report to the overall study PI (Dr. Leaf) within 3 days of each meeting. This report will be submitted to the IRB during Continuing Review, unless the DSMB recommends changes to the protocol or other study documents, in which case the report will be submitted to the IRB, along with an amendment incorporating the suggested changes, within 14 calendar days from the date the PI receives the report. The DSMB will be responsible for determining if the research should be altered or stopped. As this is a phase 2 RCT, no interim analyses are planned for potential early stopping of the trial for either futility or efficacy. The DSMB consists of the following three individuals, none of whom are affiliated with the trial:

1. Lorenzo Berra, MD (Chair)

Medical Director, Respiratory Care, Dept of Anesthesia, Critical Care and Pain Medicine, MGH
Reginald Jenney Associate Professor of Anaesthesia, HMS

2. Magdy Selim, MD, PhD

Chief, Division of Stroke and Cerebrovascular Diseases, BIDMC

Vice Chair, Committee on Clinical Investigations, BIDMC
Professor of Neurology, HMS

3. Maureen M. Achebe, MD
Clinical Director, Non-Malignant Hematologic Clinic, Dana-Farber Cancer Institute
Director, BWH Sickle Cell Program
Assistant Professor of Medicine, HMS

A minimum of two DSMB members (the Chair plus at least one other member) will be required for quorum. If there is a disagreement among members of the committee regarding recommendations for changes to the protocol, recommendations will be made according to the majority vote. If there are only two members present and there is a disagreement, the Chair will make the final decision.

Qualifications of DSMB members
Dr. Berra is the Medical Director of Respiratory Care and a staff anesthesiologist and intensivist in the Department of Anesthesia, Critical Care and Pain Medicine at MGH. He is an NIH-funded physician-scientist with expertise in RCTs in the setting of cardiac surgery.

Dr. Selim is the Chief of the Division of Stroke and Cerebrovascular Diseases at BIDMC. He is the PI of the Intracerebral Hemorrhage Deferoxamine (i-DEF) study, the largest RCT (n=294) of DFO for acute organ injury prevention performed to date.[82] He has been studying DFO in the context of ICH since 2009. He is also the Vice Chair for the Committee on Clinical Investigations (the IRB) at BIDMC.

Dr. Achebe is a hematologist with expertise in the clinical use of iron chelators, including DFO, through her experience in the management of iron overload in patients with hemoglobinopathies. She also has expertise with RCTs as the site PI for several multicenter trials through the Sickle Cell Disease Clinical Trials Network and several investigator-initiated and industry-funded trials in sickle cell disease and other red cell disorders.

9.5 Single IRB
In accordance with NIH policy, protection of human subjects will be monitored by a single IRB (the Mass General Brigham IRB).

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