FULL PROTOCOL TITLE

FUTILITY STUDY OF DEFEROXAMINE MESYLATE IN INTRACEREBRAL HEMORRHAGE

SHORT TITLE

INTRACEREBRAL HEMORRHAGE DEFEROXAMINE TRIAL (iDEF TRIAL)

Study Chair:

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Supported by:

The National Institute of Neurological
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Sponsor of IND:

Magdy H Selim, MD, PhD
IND# 77,306

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STUDY ADMINISTRATIVE ORGANIZATION

EXECUTIVE COMMITTEE

The Executive Committee consists of Magdy Selim, MD, PhD (the study Principal Investigator and chair); Sharon Yeatts, PhD (Biostatistician and Principal Investigator of the Statistics and Data Management Center (SDMC)); Yuko Palesch, PhD (Biostatistician); Aaron Perlmutter, MPH, MSW (Project Manager), Catherine Dillon, CCRP (the Supervisory Data Manager); Andre Thornhill (the Data Manager); and Claudia Moy, PhD (NINDS-appointed liaison).

The Advisory Committee members, Drs. Daniel Hanley, Steven Greenberg, Lewis Morgenstern, and Guohua Xi are also expected to participate in the Executive Committee teleconferences, as needed.

The Committee will be responsible for the development and amendment of the study documents (including the protocol, case report forms (CRF), and manual of operations); collection, review and oversight of the dissemination of serious adverse event (SAE) occurrences and other important events pertinent to the study; and communication among all components of the study administrative organizations.

THE STUDY CHAIR OFFICE

The Study Chair Office, housed in the Department of Neurology – Stroke Division at the Beth Israel Deaconess Medical Center (BIDMC) in Boston, provides overall scientific coordination and fiscal management of the trial and is responsible for preparing progress reports for the NINDS and FDA. The office is comprised of the trial's Principal Investigator (Magdy Selim, MD, PhD), his Study Coordinator, and a senior research administrator from BIDMC. The Principal Investigator provides overall leadership to the entire Trial to ensure its successful implementation. He will visit all clinical sites on a periodic basis and collaborate with the SDMC in organizing all necessary meetings and conference calls. As the Sponsor of the Investigational New Drug (IND) application, he ensures that the trial is conducted according to FDA's Good Clinical Practice (GCP) guidelines and regulations. The Study Coordinator assists the PI in day-to-day implementation of the trial and serves as a major contact person for investigators and study coordinators at the clinical sites. The senior research administrator, together with the PI, is responsible for the budgetary management of the grant. These responsibilities include preparation of consortium agreements and subcontracts, handling of invoices, and directing disbursement of funds.

THE STATISTICS AND DATA MANAGEMENT CENTER (SDMC)

The Data Coordination Unit (DCU), located in the Department of Public Health Sciences - College of Medicine at the Medical University of South Carolina, will serve as the SDMC for the trial. The SDMC is responsible for statistical design and analysis, database development and maintenance, data and project management activities, as well as interim safety monitoring and report generation.

DATA AND SAFETY MONITORING BOARD (DSMB)

The DSMB is appointed by the Director of NINDS and managed by the NINDS Clinical Trials group. It is comprised of Neurologists with special expertise in stroke and ICH, a hematologist, a statistician, and an ad hoc expert in pulmonary diseases and critical care medicine. Its responsibility will be the oversight of participant safety, review of the safety reports, requesting additional data/information (if necessary), and advising the NINDS regarding continuation/ discontinuation of the study. Peter Gilbert, Sc.M., serves as the NINDS-appointed liaison for the DSMB.

INDEPENDENT MEDICAL SAFETY MONITORS (MSM)

Two experts (a Neurocritical care specialist and a pulmonary critical care specialist with special expertise in Adult Respiratory Distress Syndrome) serve as the Independent Medical Safety Monitors (MSMs) for the study. They will be responsible for monitoring the study with regard to safety on an ongoing basis and reviewing all serious adverse events quickly to identify any safety concerns. The MSMs' task is to review all adverse events and to adjudicate all serious adverse events as recruitment progresses. The MSMs will communicate with the investigators for any questions or clarifications regarding an event, in order to determine whether events are unexpected and related to study drug administration. They will report to the Executive Committee their findings that any SAE is unexpected and suspected to be related to the study drug. They will also review and adjudicate all cases of respiratory compromise to ascertain the underlying cause and to determine whether the event is related to the study drug.

STUDY TEAM ROSTER

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STUDY PROTOCOL SYNOPSIS

Protocol Title	Intracerebral hemorrhage DEFeroxamine (iDEF) trial
Clinical Trial Phase	Phase II
Clinical Sites	>20 North American clinical sites
Statistics and Data Management Center	Data Coordination Unit, Department of Public Health Sciences, College of Medicine, Medical University of South Carolina, Charleston, SC
Sponsor Institution	Beth Israel Deaconess Medical Center, Boston, MA
Study Period	Planned enrollment duration – 36 to 40 months Planned duration of study for each participant – 180 ± 7 days Planned duration of the study – 36-48 months
Study Population	Patients with spontaneous intracerebral hemorrhage (ICH)
Study Design	A prospective, multi-center, double-blind, randomized, placebo-controlled, phase-II clinical trial. Subjects will be randomized to either deferoxamine mesylate (DFO) at 32 mg/kg/day (up to a maximum daily dose of 6000 mg/day), or saline placebo, given by IV infusion for 3 consecutive days. Treatment will be initiated within 24 hours after ICH symptom onset. Randomization will control baseline imbalances associated with baseline ICH score, ICH onset-to-treatment time (OTT), ICH volume, baseline NIHSS score, and warfarin use. All subjects will be followed for 6 months and will receive standard of care therapy while participating in the study. Throughout the study, we will continue to assess the safety of DFO. At the conclusion of the study, the proportion of DFO-treated subjects with a good clinical outcome at 3 months (defined as modified Rankin Scale (mRS) score of 0-2) will be compared to the placebo proportion in a futility analysis to determine if it is futile to move DFO forward to Phase III efficacy evaluation.
Primary Objectives	**1-** TO assess whether it is futile to move DFO into Phase III evaluation as a therapeutic intervention for ICH, by comparing the outcome of DFO-treated subjects to placebo-treated subjects with respect to good outcome (defined as mRS of 0-2 at 90 days), in a futility analysis. The futility hypothesis specifies that if the difference in good outcome proportions is less than 12% in favor of DFO, then it would be futile to move DFO forward to Phase III evaluation. 2- TO assess the safety of DFO infusions (at a dose of 32 mg/kg/day, up to a maximum daily dose of 6000 mg/day), given for 3 consecutive days, in a large cohort of ICH patients. We specifically wish to collect more data on treatment-related adverse events in order to ascertain that patients with ICH can complete this dose given over 3-day duration of infusion without experiencing unreasonable neurological complications, mortality, or other serious adverse events related to DFO use, in particular adult respiratory distress syndrome (ARDS).
Secondary Objectives	**1-** TO explore the differences between early (≤12h) and late (>12-24h) time windows in DFO treatment effect on functional outcome. **2-** TO determine the overall ordinal distribution of scores on mRS at 3 months in DFO- and placebo-treated subjects; and to perform a dichotomized analysis considering the proportion of DFO- and placebo-treated subjects with mRS 0-3 **3-** TO perform dichotomized analyses considering the proportion of DFO- and placebo-treated subjects with mRS 0-2 and 0-3; and to determine the overall ordinal distribution of scores on mRS at 6 months. **4-** TO determine mortality during the 90- and 180-day follow-up periods (all causes and

	ICH-related). **5**- TO obtain data on the changes in NIHSS between presentation and day-90, and Montreal Cognitive Assessment (MoCA) and Stroke Impact Scale (SIS)-16 scores at 3 months to explore the effects of DFO on neurological, functional, and cognitive functions.
Exploratory Objectives	**1-** TO explore the effects of treatment with DFO on relative perihematoma edema (PHE) volume progression between baseline and post-treatment CT scans in DFO-treated patients compared to placebo as potential markers of DFO's biological activity on brain tissue. **2-** TO explore whether the effect of DFO on outcome is dependent on initial ICH volume, after adjusting for other confounding variables that can affect outcome, to determine if specific limits for ICH volume should be specified as exclusion/inclusion criteria for future studies. **3-** TO explore the effects of DFO on the size of ventricular enlargement in patients with intraventricular extension of ICH, not requiring an external ventricular drain, as a potential marker of treatment utility in intraventricular hemorrhage. **4-** TO explore the effect of DFO on the incidence of symptomatic cerebral edema (unexplained increase in NIHSS >4 points or decrease in GCS >2 points) during hospitalization, up to day 7 or discharge, whichever is earlier. **5-** TO explore whether progression of PHE can be a radiological/biological marker of activity that can be correlated with clinical outcomes and treatment effect of DFO.
Sample Size	Approximately 294 subjects
Inclusion Criteria	1) Age ≥ 18 and ≤ 80 years; 2) The diagnosis of ICH is confirmed by brain CT scan; 3) NIHSS score ≥6 and GCS >6 upon presentation; 4) The first dose of the study drug is expected to be administered within 24h of ICH symptom onset; 5) Functional independence prior to ICH, defined as pre-ICH mRS ≤1; and 6) Signed and dated informed consent is obtained.
Exclusion Criteria	1) Previous chelation therapy or known hypersensitivity to DFO products; 2) Known severe iron deficiency anemia (defined as hemoglobin concentration < 7g/dL or requiring blood transfusions); 3) Abnormal renal function, defined as serum creatinine >2 mg/dL; 4) Planned surgical evacuation of ICH prior to administration of study drug (placement of a catheter for ventricular drainage is not a contraindication to enrollment); 5) SUSPECTED secondary ICH related to tumour, ruptured aneurysm or arteriovenous malformation, hemorrhagic transformation of an ischemic infarct, or venous sinus thrombosis; 6) Infratentorial hemorrhage; 7) Irreversibly impaired brainstem function (bilateral fixed and dilated pupils and extensor motor posturing); 8) Complete unconsciousness, defined as a score of 3 on item 1a of the NIHSS (Responds only with reflex motor or autonomic effects or totally unresponsive, and flaccid); 9) Pre-existing disability, defined as pre-ICH mRS ≥2; 10) Coagulopathy - defined as elevated aPTT or INR >1.3 upon presentation; concurrent use of direct thrombin inhibitors (such as dabigatran), direct factor Xa inhibitors (such as rivaroxaban or apixaban), or low-molecular-weight heparin; 11) Patients with confirmed aspiration, pneumonia, or evident bilateral pulmonary infiltrates on chest x-ray or CT scan prior to enrollment; 12) Patients with significant respiratory disease such as chronic obstructive pulmonary disease, pulmonary fibrosis, or any use (chronic or intermittent) of inhaled O_2 at home; 13) FiO_2 >0.35 (>4 L/min) prior to enrollment; 14) Sepsis (present source of infection ± lactic acidosis), Systemic Inflammatory Response Syndrome (Temp >100.4F or <96.8F; Heart rate >90; Respiratory rate >20 or $PaCo_2$ <32 mmHg; WBC >12, <4, or bands >10%), or shock (SBP <90 mmHg) at presentation; 15) The presence of 4 or more of the following risk modifiers for ARDS prior to enrollment: a) Tachypnea (respir-

	atory rate >30); b) SpO_2 <95%; c) Obesity (BMI >30); d) Acidosis (pH <7.35); e) Hypoalbuminemia (albumin <3.5 g/dL); or f) Concurrent use of chemotherapy; 16) Taking iron supplements containing ≥ 325 mg of ferrous iron, or prochlorperazine; 17) Patients with heart failure taking > 500 mg of vitamin C daily; 18) Known severe hearing loss; 19) Known pregnancy, or positive pregnancy test, or breastfeeding; 20) Positive drug screen for cocaine upon presentation; 21) Patients known or suspected of not being able to comply with the study protocol due to alcoholism, drug dependency, noncompliance, living in another state or any other cause; 22) Any condition which, in the judgement of the investigator, might increase the risk to the patient; 23) Life expectancy of less than 90 days due to comorbid conditions; 24) Concurrent participation in another research protocol for investigation of another experimental therapy; and 25) Indication that a new DNR or Comfort Measures Only (CMO) order will be implemented within the first 72 hours of hospitalization.
Study Intervention and Follow-up	Each subject will receive an IV infusion of DFO (32 mg/kg/day) or a matching saline vehicle (placebo) for 3 consecutive days. The maximum daily dose will not exceed 6000 mg per 24 hours, regardless of body weight, and the infusion rate will not exceed 7.5 mg/kg/hour. Subjects will be monitored closely and evaluated daily for the first 3 days (during study drug infusion while in hospital) and on day 7 or discharge from the hospital, whichever occurs first. Clinic visits will take place on days 30±7 and 90±7. A telephone interview will be conducted on days 60±7 and 180±7.
Randomization	A combination of minimization and biased coin methodologies will be used to randomize participants to either DFO or placebo in 1:1 ratio. Randomization will control baseline imbalances associated with baseline ICH score, ICH onset-to-treatment time (OTT), ICH volume, baseline NIHSS score, and concurrent use of anticoagulants at the time of ICH onset, as well as clinical site.
Efficacy Outcome Measures	The primary outcome measure is the mRS, dichotomized to define good functional outcome as mRS 0-2 at 90 days.
Safety Outcome Measures	All adverse events (serious and non-serious) will be assessed until day-7 or discharge (whichever is earlier), and serious adverse events (SAEs) until day-90. Safety endpoints will include all DFO-related adverse events until day-7 or discharge (whichever is earlier), and SAEs through day-90. Mortality (all cause and ICH-related) will be assessed through day 180. The following adverse events will be defined as **EVENTS OF SPECIAL INTEREST** for safety surveillance during this study: 1- Anaphylaxis (at any time point during study drug infusion); 2- Hypotension (defined as a decrease in blood pressure requiring medical intervention at any time point during drug infusion that cannot be explained by other causes); 3- Respiratory compromise of any cause during the in-hospital phase; and 4- Development of new and unexplained visual or auditory changes after initiating treatment with the study drug. Analyses of safety data will be carried out on an ongoing basis throughout the trial.

STUDY OBJECTIVES

1.1 PRIMARY OBJECTIVES

- To assess whether it is futile to move deferoxamine mesylate (DFO) into Phase III evaluation as a therapeutic intervention for spontaneous intracerebral hemorrhage (ICH), by comparing the outcome of DFO-treated subjects to placebo-treated subjects with respect to good outcome (defined as modified Rankin Scale [mRS] score of 0-2 at 90 days), in a futility analysis. The futility hypothesis specifies that if the difference in good outcome proportions is less than 12% in favor of DFO, then it would be futile to move DFO forward to Phase III evaluation.
- To assess the safety of DFO infusions (32 mg/kg/day, up to a maximum daily dose of 6000 mg/day), given for 3 consecutive days, in a large cohort of ICH patients. We specifically wish to collect more data on treatment-related adverse events in order to ascertain that patients with ICH can complete this dose given over 3-day duration of infusion without experiencing unreasonable neurological complications, increased mortality, or other serious adverse events related to DFO use, in particular adult respiratory distress syndrome (ARDS).

1.2 SECONDARY OBJECTIVES

As secondary analyses of the primary outcome, we also plan to:

- Explore the differences between early (≤12h) and late (>12-24h) time windows in DFO treatment effect on functional outcome.
- Determine the overall ordinal distribution of scores on mRS at 3 months in DFO- and placebo-treated subjects, and to perform a dichotomized analysis considering the proportion of DFO- and placebo-treated subjects with mRS 0-3. Although mRS 0-3 is less favorable than the primary outcome of mRS 0-2, it would still be a desirable effect in patients with ICH given that no treatments exist to reduce disability. The trial is adequately powered to assess the futility hypothesis using mRS 0-3 as the outcome based on an absolute difference in treatment effect <13% in favor of DFO.

In addition, we will:

- Perform dichotomized analyses considering the proportion of DFO- and placebo-treated subjects with mRS 0-2 and 0-3; and determine the overall ordinal distribution of scores on mRS at 6 months.
- Determine mortality during the 90- and 180-day follow-up periods (all causes and ICH-related).
- Obtain data on the changes in NIHSS between presentation and day-90, and Montreal Cognitive Assessment (MoCA) and Stroke Impact Scale (SIS)-16 scores at 3 months to explore the effects of DFO on neurological, functional, and cognitive functions.

1.3 EXPLORATORY OBJECTIVES

Additional planned exploratory analyses include assessments of:

- The effects of treatment with DFO on relative perihematoma edema (PHE) volume progression between baseline and post-treatment CT scans in DFO-treated patients compared to placebo as potential markers of DFO's biological activity on brain tissue.
- Whether the effect of DFO on outcome is dependent on initial ICH volume, after adjusting for other confounding variables that can affect outcome, to determine if specific limits for ICH volume should be specified as exclusion/inclusion criteria for future studies.

- The effects of DFO on the size of ventricular enlargement in patients with intraventricular extension of ICH, not requiring an external ventricular drain (EVD), as a potential marker of treatment utility in intraventricular hemorrhage (IVH).
- The effect of DFO on the incidence of symptomatic cerebral edema (unexplained increase in NIHSS >4 points or decrease in GCS >2 points) during hospitalization, up to day 7 or discharge, whichever is earlier.
- Whether progression of PHE can be a radiological/biological marker of activity that can be correlated with clinical outcomes and treatment effect of DFO.

2 BACKGROUND

2.1 RATIONALE

2.1.1 INTRACEREBRAL HEMORRHAGE IS A MAJOR PUBLIC HEALTH PROBLEM

Intracerebral hemorrhage (ICH) is a major public health problem. Approximately 70,000 patients are diagnosed with ICH in the United States (US), and up to 400,000 in the Far East, each year [Qureshi 2009; Zhang 2003], and the number of patients with ICH is rising with the aging of the population. ICH is a frequent cause of disability and mortality and confers a substantial burden on the healthcare system and society [Russell 2006]; approximately 40% of ICH patients die within one month, and more than 70% of the survivors are left with serious and permanent disability. The financial burden of ICH is enormous; it is estimated that the overall annual costs for ICH patients in the US alone exceed seven billion US dollars.

At present, there is no specific treatment for ICH beyond supportive general medical care. Attention has been focused on medical, endoscopic, and surgical treatments targeting hematoma and its expansion [Mendelow 2005; Mayer 2008]. However, the utility of these approaches alone is likely to be limited to only a selected subset of ICH patients, since ICH expansion often occurs early within the first few hours of onset [Brott 1997; Flaherty 2005]. Although the hematoma gradually resolves after ICH, restoration of function is usually incomplete, indicating that neuronal injury after ICH is not only related to direct tissue damage and hematoma expansion [Brown 2005]. Other processes including apoptosis, necrosis, iron-mediated oxidative stress, inflammation, autophagy, and edema formation contribute to secondary neuronal injury and disability after ICH [Regan 1993-1996; Castillo 2002; Goldstein 2003; Huang 2002; Wu 2003; Wagner 2003; Leira 2004; He 2008].

There is an unmet need for safe and effective neuroprotective strategies to target the secondary effects of ICH in order to limit brain injury, facilitate neuronal repair, and improve functional outcome. The iron chelator, deferoxamine mesylate (DFO), is potentially promising as a candidate therapeutic intervention to improve the overall outcome of ICH patients.

2.1.2 THE ROLE OF HEMOGLOBIN DEGRADATION PRODUCTS AND IRON IN SECONDARY NEURONAL INJURY AFTER ICH

Hemolysed red blood cells release their hemoglobin into the brain parenchyma after ICH. The time course for hemoglobin hemolysis is approximately 2-3 days [Macdonald 2004]. Hemoglobin is a potent neurotoxin, and its toxicity is largely iron-mediated [Regan 1993-2003; Hua 2002; Goldstein 2003; Xi 1998; Huang 2002]. Hemoglobin degradation products include the iron-containing heme, which is metabolized by heme oxygenase to yield ferric iron. The released iron is implicated in neuronal injury and delayed brain edema formation after ICH via several mechanisms including: activation of lipid peroxidation, exacerbation of excitotoxicity, inhibition of Na^+/K^+ ATPase activity, and catalysis of Haber-Weiss/Fenton reaction {$Fe^{++} + H_2O_2 \rightarrow Fe^{+++} + OH^{\circ} + OH^{0}$}, in which superoxide and hydrogen peroxide (H_2O_2) are

converted into highly reactive toxic hydroxyl radicals (OH◦) leading to oxidative stress and cell death [Regan 1993-1996; Winterboum 1995; Goldstein 2003; Huang 2002; Wu 2003; Wagner 2003; Winterbourn1995; Nakamura 2005].

Experimental studies examining the time course of iron's role in brain injury after ICH detected an increase in iron-positive cells, heme oxygenase protein, and markers of DNA damage in the peri-hematoma area within the first day after ICH, peaking by day 3 [Nakamura, 2003-2005; Wu 2003]. Emerging evidence also links iron to neuronal injury in ICH patients. Serum ferritin upon admission correlates with the relative PHE on day-3 (which coincides with the timing for hemoglobin hemolysis), but not at baseline [Mehdiratta 2008], and functional outcome at 3 months [Perez 2010]; and the iron content within the hematoma, estimated by MRI, correlates with the relative PHE volume [Lou 2009]. These studies indicate that iron accumulates in the brain after ICH, and that its toxicity contributes to neuronal injury over many days following ICH. This delayed time window can have important therapeutic implications.

2.1.3 DEFEROXAMINE MESYLATE

2.1.3.1 PHARMACOKINETICS –

Deferoxamine mesylate (DFO) [N-(5-C3-L (5 aminopentyl) hydroxycarbamoyl)-propionamido)pentyl)-3(5-(N-hydroxyacetoamido)-pentyl)carbamoyl)-proprionhydroxamic acid] is a hydrophilic, water-soluble, chelator. It complexes almost exclusively with ferric iron, at physiologic pH values, chelating iron from ferritin and hemosiderin; and forms a stable complex that prevents iron from entering into further chemical reactions. The drug's serum protein-binding rate is less than 10%, and it is distributed throughout all body fluids. It has a volume of distribution of 0.8-1.35 L/kg [Lee 1993]. The initial distribution half-life of DFO is approximately 5-10 minutes in humans [Perrone 2002]. Peak plasma concentrations between 80 and 130 µmol/L are measured 3 minutes after IV injection of DFO at a dose of 10 mg/kg [Allain 1987]. During IV infusion of 50 mg/kg/day, steady state concentrations are generally obtained between 6-12 hours with a mean concentration of 7.4 ± 2.73 µM. The drug has a biphasic elimination pattern; the half-life is 1h in the first rapid phase and 6h in the second slow phase [Allain 1987]. Despite a molecular weight of 560.7 daltons (656.8 as mesylate) and low lipid solubility, the drug can diffuse into the brain down a concentration gradient, and there appears to be specific mechanism(s) of uptake by neuronal cells. DFO accumulates in the brain at a significant concentration quickly after injection [Keberle 1964; Yokel 1991; Palmer 1994; Hershko 1988; Brancaccio 1991] and reduces cerebrospinal fluid free iron level in rats subjected to experimental ICH, indicating that it crosses the blood brain barrier after ICH [Wan 2006-2009].

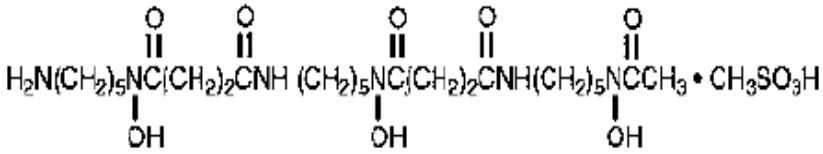

Figure 2.1.3.1: Chemical Structure of DFO

2.1.3.2 SAFETY CONSIDERATIONS –

Deferoxamine mesylate is approved by the Federal Drug Administration (FDA) for the treatment of acute iron intoxication and chronic iron overload and has been extensively used in clinical practice for more than 40 years. Safety studies indicate that it is relatively well tolerated. The following adverse reactions have been observed, but there are not

enough data to support an estimate of their frequency: At the injection site: localized irritation, pain, burning sensation, swelling, induration, infiltration, itching, erythema, or wheal formation. These injection site reactions may be associated with arthralgia, fever, headache, myalgia, nausea, or abdominal pain. Systemic allergic and hypersensitivity reactions: rash, urticaria, anaphylactic reaction with or without shock, or angioedema. Cardiovascular system: tachycardia, hypotension, and shock. Impairment of cardiac function has also been reported in patients with severe chronic iron overload following concomitant treatment with DFO and vitamin C in excess of 500 mg per day. Digestive system: abdominal discomfort, nausea, vomiting, or diarrhea. Hematologic system: thrombocytopenia and leucopenia. Musculoskeletal system: muscle cramps. Nervous system: dizziness, paresthesias, tinnitus, high-frequency sensorineural hearing loss, or visual disturbances, such as blurred vision, decreased visual acuity, pigmentary retinopathy, visual field defects, and cataracts. The ocular and auditory disturbances were reported when DFO was administered over prolonged periods of time, at high doses, in patients with low ferritin levels. These disturbances were reversible upon immediate cessation of treatment, in most cases. Respiratory system: acute respiratory distress syndrome has been reported following treatment with excessively high doses of intravenous DFO in patients with acute iron intoxication or thalassemia. Urinary system: dysuria and impaired renal functions. In addition, rare infections, such as Yersinia and Mucormycosis have been reported in patients with iron-overload. These are thought to be attributed to iron-induced increased susceptibility to infections particularly in patients with low ferritin levels, and are unlikely to occur in patients without iron overload. In a recent study, the most frequent adverse events of DFO (40-50 mg/kg/day, 5 days/week, for 48 weeks) in adult patients with transfusion related iron overload were abdominal pain (34%), diarrhea (26%), fever (26%), joint pain (13%), vertigo (13%), and dyspepsia (8.7%) [Piga 2006]. Other less frequent adverse effects of DFO were local reactions at the site of injection, such as pain, swelling, or erythema. There were no serious adverse events related to DFO use, and the gastrointestinal symptoms were mild and resolved spontaneously within a few days without drug interruption.

Serious adverse effects are uncommon and are seen primarily with IV doses higher than 125 mg/kg/day, daily doses exceeding 6000 mg, and chronic long-term use [Porter, 1989]. Hypotension and shock have been reported in up to 2% of patients using IV DFO [Westlin 1971]. They were mostly seen with rapid intravenous infusions, and rarely reported when the drug is given at a rate ≤1 mg/kg/minute, and a dose ≤125 mg/kg/day [Westlin 1971; Porter 1989]. We will use a slower infusion rate ≤7.5 mg/kg/hour and limit the maximal dose that any subject can receive to 6000 mg per day in the current study.

Small studies have investigated the use of DFO in patients without systemic iron overload who have similar comorbidity profile to that of stroke patients, such as patients with coronary artery disease, diabetes mellitus, and elderly subjects with Alzheimer's disease [Duffy, 2001; Hattori, 2002-2003; Crapper, 1991], and healthy volunteers [Allain; 1987]. The doses used varied from 10 to 80 mg/kg body-weight-adjusted or 250 to 500mg fixed-dose regimens, and the duration of treatment varied from a single treatment to repeated daily dosing up to one year. Previous studies in cardiopulmonary bypass patients and in thalassaemia patients with cardiac disease used IV DFO doses of 50 to 100 mg/kg [Tamary 1994; Davis, 2000; Ioannis, 2004]. Gordeuk et al. also used DFO infusions (100 mg/kg per day for 72 hours) in non-iron overloaded volunteers with asymptomatic Plasmodium falciparum parasitemia and patients with cerebral malaria [Gordeuk, 1992]. These various dose regimens resulted in no serious adverse events to treatment in these studies.

Because DFO is not approved by the FDA for the treatment of patients with ICH, the primary investigator was granted a research (non-commercial) Investigational New Drug (IND) by the FDA to investigate the use of DFO in this patient population (IND #77,306). The supporting data section below (section 2.2.4) details our experience with DFO use in ICH patients. Our Phase I study indicated that repeated IV infusions of DFO in doses up to 62 mg/kg/day with a maximum daily dose of 6000 mg/day in patients with ICH were largely safe and tolerable. The most observed adverse events were injection site reaction (irritation, pain, or erythema; 15%), IV infiltration (20%) and a modest decrease in blood pressure which did not require any medical intervention (40%). While hypotension may be an undesirable side effect in patients with ischemic stroke, a modest reduction in blood pressure may prove to be beneficial, and is often advocated, in

patients with ICH. One subject developed visual hallucination during IV infusion of DFO. Almost all of these adverse events were mild, self-limited, did not require specific treatment, and resolved spontaneously. There were no deaths or serious adverse events related to DFO use.

In the first part of phase II investigation of DFO in ICH (HI-DEF trial, where subjects were treated with a continuous infusion of DFO at 62 mg/kg/day for 5 consecutive days), however, we observed 7 cases of ARDS among the first 42 participants; 6 of these cases were in the DFO treatment arm, and 2 of these were fatal. An expert blinded to treatment assignment reviewed all these cases and concluded that a plausible cause for ARDS, other than the study drug or ICH itself, was identified in 4 cases, while no other explanation, other than the study drug or ICH, was apparent for the remaining 3 cases. HI-DEF was terminated due to safety concerns, given the imbalance in the frequency of ARDS between the DFO- and placebo-treated groups. This prompted further review of 2 cases of respiratory failure, reported as the result of aspiration pneumonia, in Phase I study; both in the 62 mg/kg/day dose-tier. An expert review concluded that one of these cases was an undiagnosed case of ARDS, and that aspiration was a plausible explanation for it. We have undertaken considerable precautions to minimize the potential pulmonary toxicity of DFO and ARDS risk and to enhance the safety of future participants in the current modified protocol by reducing the daily dose of DFO from 62 mg/kg/day to 32mg/kg/day, decreasing the duration of treatment from 5 to 3 days, and excluding subjects at high risk for ARDS.

The effects of DFO on the fetus are unknown. It is also not known if DFO is excreted in human milk. Therefore, women who are pregnant and those who are breast-feeding will be excluded from this study.

2.1.3.3 NEUROPROTECTIVE EFFECTS OF DFO –

By forming a stable complex with ferric iron, DFO decreases free iron's availability for the production of hydroxyl radicals. DFO also alters iron regulatory genes and proteins binding activity, thereby reducing cellular vulnerability to iron [Chen 2010; Messer 2010]. However, DFO has multiple and diverse neuroprotective properties, which may be only partly related to its iron chelating abilities. DFO also prevents apoptosis induced by glutathione depletion and oxidative stress in embryonic cortical neuronal cultures by activating a signal transduction pathway leading to activation transcription factor 1/cAMP response element-binding protein (ATF-1/CREB) and hypoxia inducible factor (HIF-1), and expression of genes known to compensate for oxidative stress [Zaman 1999]. It inhibits prolyl 4-hydroxylase activity, which may lead to protection from oxidative stress-induced cell death [Siddiq 2005; Ratan 2008]; induces transcription of heme oxygenase-1; suppresses the upregulation of activated c-Jun N-terminus kinase (JNK) seen after ICH [Wan 2009]; exerts anti-inflammatory effects by stimulating cyclooxygenase [Tanji 2001]; blocks the neurotoxic effects of hemoglobin via inhibition of glutamate-mediated excitotoxicity [Regan 1996]; and exerts anti-phagocytic effects in animal models of ICH [He 2008]. Our phase I study also suggests that DFO has a modest blood pressure lowering effect when administered by IV infusion, which might be beneficial in patients with ICH [Anderson 2008; Suri 2008].

2.2 SUPPORTING EVIDENCE

2.2.1 SUPPORTING EVIDENCE THAT DFO ATTENUATES NEURONAL INJURY AFTER ICH

2.2.1.1 IN VITRO STUDIES

Several studies have shown that DFO can reduce hemoglobin-induced neurotoxicity in experimental models of ICH. Regan and Rogers [Regan, 2003] showed that delayed treatment with DFO markedly attenuates the production of reactive oxygen species and neuronal death induced by adding hemoglobin to mixed neuronal/astrocyte cell cultures. In another study, Regan and Panter [Regan, 1993] showed that DFO completely blocked hemoglobin-induced neuronal death in neocortical cultures derived from fetal mice. Regan and Panter [Regan, 1996] also showed that hemoglobin potentiates the neurotoxicity of glutamate agonists in primary murine cortical cultures, and that this effect was

attenuated by DFO. Goldstein et al [Goldstein, 2003] showed that treatment with DFO significantly reduces the production of reactive oxygen species and cell death induced by hemin in human neuron-like cells. Similarly, Levy et al [Levy, 2002] showed that DFO diminishes hemin-induced cell death in pheochromocytoma (PC12) and neuroblastoma (SH-SY5Y) cell lines.

2.2.1.2 IN VIVO STUDIES

Bilgihan et al [Bilgihan, 1994] studied the effects of DFO on lipid peroxidation and Na-K ATPase activity after experimental ICH in guinea pigs, and found that DFO treatment reduces brain malondialdehyde content and induces recovery of Na-K ATPase activity, suggesting that DFO can exert potential neuroprotective effects to counteract the deleterious effects of ICH. Pelit et al [Pelit, 2003] examined the effects of systemic treatment with DFO on the pathological changes in the optic nerve after experimental retrobulbar hematoma in rabbits, and were able to detect ultrastructural changes and abundant iron pigment accumulation in the orbital fat tissue following induction of hematoma. These microscopic changes were significantly less pronounced in DFO-treated rabbits. Huang et al [Huang, 2002] examined the effects of intraperitoneal administration of DFO (500 mg/kg) on brain edema in rats pre-treated with hemoglobin degradation products via stereotactic infusion into the brain; they found that DFO significantly attenuated the brain edema induced by hemoglobin and its breakdown products. In similar studies, Nakamura et al [Nakamura, 2004] investigated the effects of repetitive administration of DFO (100 mg/kg intraperitoneally every 12 hours), starting 2, 6, and 24 hours after induction of ICH in rats, on markers of DNA oxidative damage and repair. They found that treatment with DFO ameliorated ICH-induced changes in 8-hydroxyl-2'-deoxyguanosine (8-OHdG), a marker of oxidative DNA damage, and increased levels of apurinic/apyrimidinic endonuclease/redox effector-factor 1 (APE/Ref-1), a protein involved in DNA repair following oxidative damage, indicating that DFO may be a potential therapeutic agent for ICH by reducing the oxidative stress caused by the release of iron from the hematoma.

Several experimental studies investigated whether DFO can reduce ICH-induced brain injury, by examining its effects on brain edema, severity of neurological deficits, and performance on sensorimotor behavioral tests [Nakamura, 2003-2004; Wan 2006-2009; Gu, 2009; Okauchi 2009]. In a piglet ICH model, ICH resulted in development of a reddish perihematoma zone, and iron accumulation, ferritin upregulation, and neuronal death within that zone, which were all reduced by intramuscular administration of DFO at 50 mg/kg bid for 3 or 7 days after ICH [Okauchi 2009] (Figure 2.2.1.2-A-C).

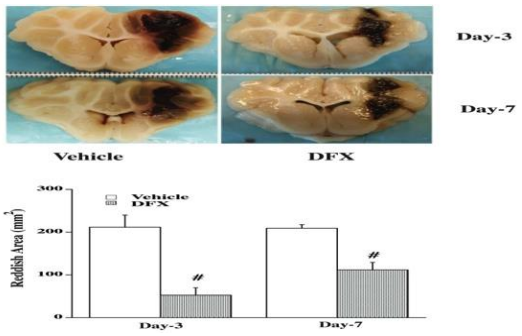

Figure 2.2.1.2- A: Deferoxamine (DFX) reduces the reddish zone around the hematoma after 3 and 7 days of treatment in a pig model of ICH.

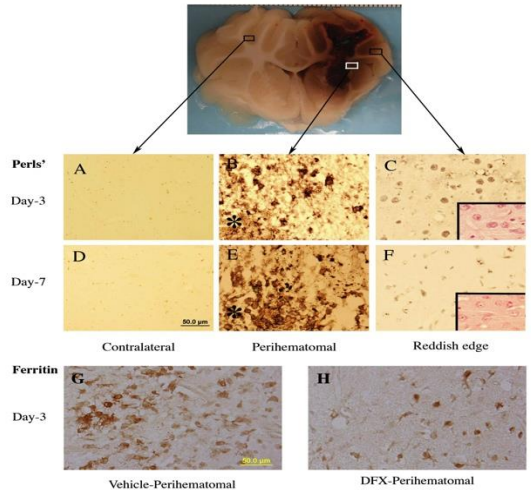

Figure 2.2.1.2- B

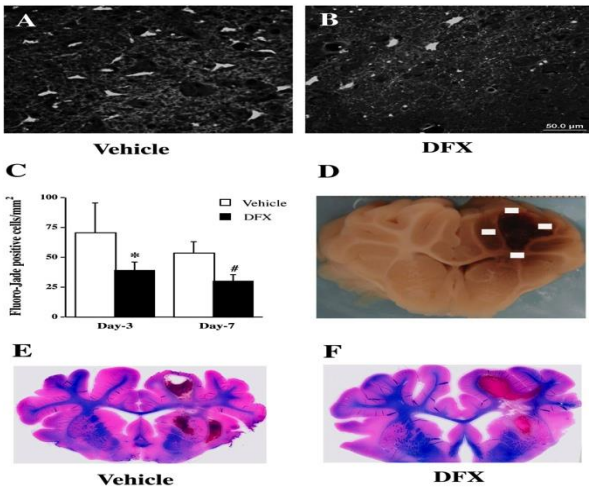

Figure 2.2.1.2- C

Figures 2.2.1.2- (B-C): B shows iron histochemistry (Perls staining) and ferritin immunoreactivity in the brain after ICH (* in D & E indicates the hematoma; inserts in C & F indicates hematoxylin and eosin staining). C shows Fluoro-Jade C-positive cells, which indicates neuronal degeneration, in the perihematoma area [A-C]; D shows 4 sampled field for Fluoro-Jade C cell counting); and Luxol fast blue staining of the white matter [E & F]. Courtesy of Dr. Guohua Xi.

2.2.2 SAFETY AND TOLERABILITY OF DEFEROXAMINE IN PATIENTS WITH ACUTE CEREBRAL HEMORRHAGE (R01 NS 057127)

OVERVIEW AND OBJECTIVES - This was a prospective, open-label, multiple-tier, dose-finding, multi-center, preliminary clinical study to evaluate the safety and tolerability of repeated treatments with DFO in patients with ICH. The study was planned to evaluate DFO dose-tiers ranging from 7 to 125 mg/kg/day (up to a maximum dose of 6000 mg/day) using the Continual Reassessment Method (CRM). The initial dose of the drug was administered within 18h after stroke symptom onset by IV infusion at a rate of 7.5 mg/kg/hour and repeated daily for 3 consecutive days. Four clinical sites participated in the study (Beth Israel Deaconess Medical Center, Massachusetts General Hospital, Medical College of Wisconsin, and Hartford Hospital). The Division of Biostatistics and Epidemiology at the Medical University of South Carolina served as the Statistics and Data Management Center for the study. Subject recruitment began in July 2008.

THE PRIMARY OBJECTIVES were: 1) To evaluate the safety and tolerability of repeated IV infusions of DFO in patients with spontaneous ICH; and 2) To determine the maximum tolerated dose (MTD) of DFO (highest dose regimen ≤ 125 mg/kg that can be safely administered and tolerated) in patients with ICH to be adopted in subsequent studies to test the efficacy of DFO in ICH.

Enrollment into every dose tier, starting with 7 mg/kg daily for 3 days, was completed before enrollment into the next tier began. Each subsequent dose level was determined using the Piantadosi-modified CRM (Piantadosi 1998). The dose-toxicity curve was re-estimated based on safety data generated throughout the study. At least 3 patients were enrolled in each dose-tier. The safety information, guiding transition from one dose tier to the next, was based on the number of subjects in a cohort who experienced pre-defined dose-limiting toxicities (DLT) during the first 7-day period or until discharge, whichever occurred first, following initiation of DFO treatment. The acceptable probability of DLT

was set at 0.40. The dose-limiting toxicity (DLT) was pre-defined as any of the following adverse events, fatal or non-fatal, occurring within 7 days or until discharge, whichever occurs first, of treatment initiation: 1) Anaphylaxis (at any time point during DFO infusion); 2) Hypotension (defined as a decrease in SBP > 20 mm Hg or DBP > 10 mm Hg, or a SBP ≤ 85 mm Hg, confirmed by 3 consecutive readings, requiring medical treatment at any time point during DFO infusion that cannot be explained by other causes); 3) Worsening neurological status (defined as an increase ≥ 4 points on NIHSS, or a decrease of ≥ 2 points on GCS, that cannot be explained by other causes, compared to baseline values occurring at any point during DFO infusions); 4) Mortality within 7 days of hospitalization; and 5) Any adverse event prolonging hospital stay, resulting in emergent medical therapy, or resulting in death. All of these events were considered SAEs for the purpose of this study. Patients underwent repeated clinical (neurological, visual and auditory, and general physical examinations) and laboratory evaluations at regular intervals throughout their participation in the study, from the day of enrollment until day 90, for adverse events. The 30-day neurological and functional status was determined by NIHSS, extended Glasgow outcome scale (eGOS), Barthel index (BI), and mRS. The patients or their surrogates were contacted by phone on day 90±7 days to compute mRS, eGOS, and BI, and to assess 3-month mortality.

STUDY STATUS AND RESULTS: Enrollment was completed in January 2010; the last subject completed the 90-day assessment in April 2010. A total of 20 subjects were enrolled into 5 DFO dose tiers, ranging from 7 mg/kg/day to 62 mg/kg/day. Because the recommended subsequent dose was within 5% (a pre-specified convergence criterion) of the 62 mg/kg/day dose, the study was concluded with the 62 mg/kg/day dose as the recommended MTD.

Number of subjects enrolled by dose-tier**:** 7 mg/kg = 4; 32 mg/kg = 3; 47 mg/kg = 3; 57 mg/kg = 4; 62 mg/kg = 6. An additional cohort of 3 subjects was treated at 62 mg/kg once it was determined to be the MTD to ascertain the safety and tolerability of this dose before proposing its use in the current study). The detailed results are included in Appendix I. The main results are summarized below.

SAFETY DATA: Sixteen non-serious adverse events were possibly or probably related to the study drug. The most common were injection site irritation and IV infiltration. Six subjects experienced 12 SAEs and 3 subjects had 4 DLTs. Three subjects (15%) died during the 90-day follow-up period: one subject died in hospital within 7 days of ICH onset and two between 7-30 days after ICH onset. None of the SAEs, DLTs, or mortalities was adjudicated to be related to the study drug. There were 2 cases of respiratory failure; both in the 62 mg/kg/day dose-tier and were thought to be related to aspiration pneumonia and adjudicated as being unrelated to the study drug. However, a recent expert review found that one of these cases was an undiagnosed ARDS, and agreed that aspiration was a plausible explanation for it.

PHYSIOLOGICAL DATA: Laboratory data - There were no differences in routine laboratory values and in the incidence of abnormalities and change from baseline in EKG parameters. Overall, daily DFO infusions at a rate of 7.5 mg/kg/hour up to 62 mg/kg/day (up to a maximum of 6000 mg/day) were well tolerated without significant alterations of hemoglobin or hematocrit, hematological parameters, and renal or hepatic functions. Treatment with DFO for 3 consecutive days at all 5 dose-tiers did not result in iron deficiency. Overall, the median values of the change in iron parameters from baseline to after the 3rd DFO infusion were as follows: serum iron (3.5 ug/dl; 95% CI -23, 45); transferrin (-18 ng/ml; 95% CI -43, 5); total iron binding capacity (8.5 ug/dl; 95% CI -47, 30); and ferritin (32.5 ng/ml; 95% CI 6, 74). We found no relationship between DFO dose and changes in serum iron studies. Vital signs (heart rate; respiratory rate; oxygen saturation; and temperature) - Overall, DFO administration did not result in important alterations in these variables. Blood pressure: DFO had a moderate BP-lowering effect. Overall, the median mean BP at baseline was 95.8 mmHg (95% CI 84, 100.3) vs. 93.2 mmHg (95% CI 87.6, 98) during the infusions. A total of 8 patients (40%) experienced a maximal drop in mean BP >20% (median 0.29; 95%CI 0.27, 0.39) at some point during the infusions compared to baseline values. One subject required vasopressors; his hypotension was thought to be related to intubation and anesthesia. None of the remaining 7 subjects required any medical treatment.

RADIOLOGICAL DATA: We found no radiological evidence to suggest that treatment with DFO exacerbates the natural enlargement of ICH or PHE over 72h after treatment. The overall median change in relative PHE volume from screening to post-3rd DFO infusion was 0.48 (95% CI 0.10, 0.76) and from screening to day-7 or discharge (whichever

occurred first) was 0.87 (95% CI 0.51, 1.28). These exploratory results contrast with previous studies of the natural history of relative PHE evolution [Mehdiratta 2008; Gebel 2002], which showed that the relative PHE volume almost doubles by 72h.

FUNCTIONAL OUTCOME DATA: Two subjects withdrew consent, one after the 7-day visit (mRS =5), and one after the 30-day visit (mRS =1). Among the remaining 18 subjects, 9 subjects (50%) had mRS scores of 0-2; 2 (11%) had a score of 3; and 7 (39%) had scores of 4-6.The 90-day mortality rate was 15%.

CONCLUSIONS: Repeated daily infusions of DFO at doses up to 62 mg/kg/day (up to a maximum of 6000 mg/day) in patients with acute spontaneous ICH for 3 consecutive days after ICH onset are feasible, well tolerated and do not increase serious adverse events or mortality.

2.2.3 HIGH DOSE DEFEROXAMINE IN INTRACEREBRAL HEMORRHAGE (HI-DEF IN ICH) (U01 NS074425)

OVERVIEW AND OBJECTIVES - Part 1 of the current study was a prospective, multi-center, double-blind, randomized, placebo-controlled, phase-II clinical trial. Subjects were randomized to either DFO at 62 mg/kg/day (up to a maximum daily dose of 6000 mg/day), or saline placebo, given by continuous IV infusion for 5 consecutive days. Treatment was initiated within 24 hours after ICH symptom onset. Randomization controlled for baseline imbalances associated with baseline ICH score (0-2 vs. ≥3), ICH onset-to-treatment time (OTT) window (≤12h vs. >12-24h), and concurrent warfarin use. All subjects were followed for 3 months and received standard of care therapy while participating in the study.
THE MAIN OBJECTIVES WERE: **1-** TO assess whether it is futile to move DFO forward as a therapeutic intervention for ICH into Phase III evaluation, by comparing the outcome of DFO-treated subjects to placebo-treated subjects with respect to good outcome (defined as mRS of 0-2 at 90 days), in a futility analysis; and **2**- TO assess the safety of DFO infusions (at a dose of 62 mg/kg/day, up to a maximum daily dose of 6000 mg/day), given for 5 consecutive days, in a large cohort of ICH patients. We specifically wished to collect more data on treatment-related adverse events in order to ascertain that patients with ICH could complete this dose given over 5-day duration of infusion without experiencing unreasonable neurological complications, mortality, or other serious adverse events related to DFO use.

STUDY STATUS AND SAFETY RESULTS - Throughout the study, we continued to assess the safety of DFO. Enrollment was placed on hold after enrollment of 42 subjects to investigate a potential safety concern – Adult Respiratory Distress Syndrome (ARDS). ARDS is reported as a potential side effect of prolonged intravenous infusions of high dose DFO in the product's package insert. Five cases of ARDS were reported. An expert in ARDS, blinded to treatment assignments, reviewed all 5 cases as well as 3 cases reported as respiratory failure and 3 cases reported as pulmonary edema. The expert concluded that 2/3 cases reported as pulmonary edema were possibly/probably ARDS, suggesting a total of 7 ARDS cases. The expert review identified a plausible cause for ARDS, other than the study drug or ICH itself, in 4 cases, while no other explanation was apparent for the remaining 3 cases. After careful review of the data, the DSMB unblinded the investigators to the treatment assignment. Six of the 7 cases of ARDS occurred in the DFO-treated group. Three of the 42 enrolled subjects died (7%); all had ARDS, and the cause of death was attributed to ARDS in 2 subjects. No other safety concerns emerged. A total of 36 SAEs were reported in 15 subjects; 22 of which occurred in 9 DFO-treated subjects. One MedWatch report was submitted for hypophosphatemia as it was judged to be severe, unexpected, and possibly related to the study drug. Overall, hypophosphatemia was reported as an AE in 3 subjects (2 in the DFO- and 1 in the placebo group). Other analyses are yet to be performed.

CONCLUSIONS - The NINDS-appointed DSMB concluded that: "although the ARDS cases in the treatment group were in the ballpark frequency of at least one paper in the literature on ARDS in patients with ICH [Elmer 2013], the imbalance in the frequency of ARDS cases between the treatment and placebo groups suggested that pulmonary toxicity of the

drug was highly likely". HI-DEF was therefore terminated due to safety concerns, and the initial protocol has been modified to minimize the potential for pulmonary toxicity of DFO and to enhance the safety of future participants.

2.3 SUMMARY OF BACKGROUND AND RATIONALE

Collectively, the above data indicate that: 1) extensive preclinical investigations, in vitro and in vivo, by different investigators and in different species, show that treatment with DFO confers neuroprotection after ICH; 2) although DFO may work directly by chelating iron, it also has several other neuroprotective properties which can be beneficial after ICH; 3) repeated daily administrations of IV infusions of DFO to ICH patients is feasible and largely well-tolerated; and 4) the potential for pulmonary toxicity and ARDS is higher in ICH patients treated with continuous IV infusions of DFO at 62 mg/kg/day (up to a maximum total dose of 6000 mg/day), for 5 consecutive days; a lower dose and shorter duration of treatment might help to ameliorate this toxicity to improve the benefit/risk ratio.

These findings indicate that DFO is a potentially promising candidate intervention to target the secondary effects of ICH, and provide a rationale for its therapeutic use to improve the overall outcome in patients with ICH, particularly if the safety concerns encountered in part 1 (HI-DEF) of this study can be ameliorated by lowering the daily and total dosage of DFO and improved selection of subjects to exclude those at high risk for ARDS. Because hemoglobin degradation starts hours-days after ICH onset, the potentially delayed and slow pace of hemoglobin- and iron-mediated injury may facilitate treatment at a delayed time window after ICH onset. This could extend the potential utility of DFO as a therapeutic intervention to a large proportion of ICH patients. DFO is relatively inexpensive and is likely to be a highly cost-effective therapy for ICH and complementary to ongoing efforts targeting hematoma and its expansion. We generally hypothesize that treatment with DFO would minimize ongoing neuronal injury after ICH, via several diverse mechanisms, and would improve the outcome in these patients. As a prelude to test this hypothesis, this Phase II study will assess the futility of DFO as a therapeutic intervention in ICH before embarking on a large Phase III trial. In addition to allowing us to assess the futility of moving DFO forward to phase III efficacy testing as a potential therapeutic intervention in ICH, this study will allow us to gain experience with logistical aspects of trial conduct, and the data and results from this study will be used to guide our planning for a possible future Phase III study.

2.4 SIGNIFICANCE

This study will provide a crucial "go/no-go" signal to determine if embarking on a large-scale, costly phase III trial to investigate the efficacy of DFO as a treatment for ICH is worthwhile. This study will also provide important information to guide the planning and conduct of a future phase-III trial, if it is determined that the treatment with DFO is not futile. It will allow us to gain experience with logistical aspects of trial conduct, such as blinding and randomization, prior to phase-III testing. Furthermore, results from this study can provide valuable information regarding the dichotomized mRS outcome rate among control subjects, the potential for a differential treatment effect in the ≤12 vs. >12-24 hour time windows, and appropriate inclusion and exclusion criteria (HI-DEF has been already informative in this regard); information which can guide the design of a potential future Phase III trial. In addition, we plan to collect data on various outcome scales (mRS, NIHSS, SIS-16 [Duncan 2003], and MoCA [Pendlebury 2010]), which will allow us to explore the utility of these various outcome measures in phase-III.

If successful, this study can potentially result in new means to improve the outcome of patients with ICH. A successful study demonstrating the efficacy of DFO in ICH would be of considerable significance to the field and the society. A wealth of data, generated from various planned exploratory analyses in this study, can still help to advance our knowledge and understanding of the pathophysiology of ICH, such as the relationship between PHE volumes and outcome and the relationship between ICH and cognitive function, even if DFO is found to be futile. These additional analyses can generate novel hypotheses for future investigations in ICH. The potential advantage of novel treatments for ICH and better understanding of its pathophysiology and relationship to outcome with regard to patients' welfare, public health and cost containment is noteworthy.

3 STUDY DESIGN

3.1 STUDY OBJECTIVES

THE STUDY HAS TWO PRIMARY AIMS: 1- To determine if it is futile to move DFO forward to Phase III evaluation, using mRS 0-2 at 90 days as the outcome based on an absolute difference in treatment effect ≥12% in favor of DFO; and 2- To further assess the safety of DFO infusions, at a dose of 32 mg/kg/day, given over a consecutive 3-day period, in particular serious adverse events including ARDS.

THE SECONDARY AIMS are to: 1- Explore the differences between early (≤12h) and late (>12-24h) time windows in DFO treatment effect on functional outcome; 2- Determine the overall distribution of ordinal scores on mRS at 3 months in DFO- and placebo-treated subjects, and to perform a dichotomized analysis considering the proportion of DFO- and placebo-treated subjects with mRS 0-3; 3- Perform dichotomized analyses considering the proportion of DFO- and placebo-treated subjects with mRS 0-2 and 0-3; and to determine the overall ordinal distribution of scores on mRS at 6 months; 4- Determine mortality during the 180-day follow-up period (all causes and ICH-related); and 5- Obtain data on the changes in NIHSS between presentation and day-90, and MoCA and SIS-16 scores at 3 months to explore the effects of DFO on neurological, functional, and cognitive functions.

ADDITIONAL PLANNED EXPLORATORY ANALYSES include assessments of: 1- The effects of treatment with DFO on relative PHE volume progression between admission (i.e. screening) and post-treatment CT scans in DFO-treated patients as potential markers of DFO's biological activity on brain tissue [Gebel 2002; Mehdiratta 2008; Leira 2004; Okauchi 2009-2010]; 2- Whether the effect of DFO on outcome is dependent on initial ICH volume, after adjusting for other prognostic variables, to determine if specific limits for ICH volume should be specified as exclusion/inclusion criteria for future studies; 3- The effects of DFO on the size of ventricular enlargement in patients with intraventricular extension of ICH , not requiring EVD, as a potential marker of treatment utility in IVH [Chen 2011]; 4- The effect of treatment with DFO on incidence of symptomatic cerebral edema (unexplained increase in NIHSS >4 points or decrease in GCS >2 points) during hospitalization, up to day 7 or discharge whichever is earlier; and 5- Whether progression of PHE can be a radiological/biological marker of activity which can be correlated with clinical outcomes and treatment effect of DFO.

3.2 OVERVIEW OF STUDY DESIGN

This is a prospective, multi-center, double-blinded, randomized, placebo-controlled, phase-II clinical trial. The total sample size required is 294 subjects with ICH. Subjects will be randomized in a 1:1 ratio to receive either DFO at 32 mg/kg/day (up to a maximum daily dose of 6000 mg/day), or a matching saline vehicle (placebo), given by IV infusion for 3 consecutive days, in a blinded manner. Treatment will be initiated within 24 hours after ICH symptom onset. Subjects will undergo repeated clinical, imaging, and laboratory evaluations at regular intervals throughout their participation in the study, from the day of randomization until day 180. All adverse events will be assessed until day-7 or discharge (whichever is earlier), and new SAEs until day-90. Mortality and continuing SAEs will be assessed until day 180. Functional status will be determined by mRS scores, in person at 30±7 and 90±7 days; the patients or their surrogates will be contacted by phone on days 60±7 and 180±7 to assess mRS [Merino, 2005].

3.3 RATIONALE FOR STUDY DESIGN AND PROTOCOL MODIFICATIONS

The rationale for the proposed dose regimen, therapeutic time window, duration of treatment, study design, inclusion of a placebo group, and surrogate measures of DFO efficacy is as follows:

Dose Reduction and New Dose Selection: The 62 mg/kg/day dose was identified in our Phase I study as the MTD in ICH subjects and was the dose used for the 42 participants enrolled in HI-DEF. This was based on safety data from the phase I study, where DFO did not appear to increase the overall rate of SAEs or mortality compared with placebo-treated patients in previous ICH trials [Mayer 2005; Mayer 2008; Lyden 2007; Haley 2005], and the notion that the efficacy of a drug often has a monotonically non-decreasing dose-response relationship. However, the observation of 6 cases of ARDS among DFO-treated participants in HI-DEF, and the reported association of prolonged high-dose intravenous infusions of DFO with the development of ARDS [Tenenbein 1992] prompted us to reduce the dose to 32 mg/kg/day (up to a maximum daily dose of 6000 mg per day) in the current protocol to maximize safety and minimize harm. Pre-clinical studies in aged rats compared 3 dose regimens of DFO; 10 mg/kg, 50 mg/kg, and 100 mg/kg every 12 hours [Okauchi 2009]. Based on mass constant conversion factors and the FDA guidance for the industry for estimating the human equivalent dose (HED) in clinical trials, the calculated HEDs are approximately 3, 16 and 32 mg/kg/day, respectively [http://www.fda.gov/downloads/Drugs/GuidanceCompliance RegulatoryInfmation/Guidances/ ucm078932.pdf]. The latter dose regimens (HEDs 16 and 32 mg/kg/day) were superior to the first dose regimen in improving performance on sensorimotor behavioral tests at days 28 and 58 following induction of experimental hemorrhage. We now opt for a dose of 32 mg/kg/day based on the following considerations:

I. This dose, while assuring increased tolerability and safety of the drug, represents the upper limit of effective doses in animal models - Data from rat models of ICH indicate that HEDs ranging from 16 to 32 mg/kg/day are effective in reducing brain edema formation and brain tissue atrophy, and improving neurological and functional recovery [Okauchi 2009].
II. This dose provides a better safety margin - No cases of respiratory failure or distress were reported in any of the 10 participants who received DFO in doses ranging from 32 to 57 mg/kg/day in our Phase I study (32 mg/kg/day, n=3; 47 mg/kg/day, n=3; 57 mg/kg/day, n=4).
III. Equivalent dose in piglet model of ICH exerts biological activity on the brain tissue. It reduced the perihematomal reddish zone, white matter injury, iron accumulation, ferritin upregulation, and neuronal death within that zone [Gu 2009].

The 6000 mg/day limit, irrespective of body weight, is based on FDA recommendations and the manufacturer's brochure to avoid the risk of serious adverse effects of higher dose-regimens.

TIME-TO-TREATMENT WINDOW: The decision to initiate DFO treatment up to 24h after ICH symptom onset and not beyond, and to balance randomization based on OTT, is based on data from animal studies. Okauchi et al [Okauchi 2010] examined the effects of DFO when administered within 2h, 4h, 12h, 24h, and 48h after the induction of ICH on brain edema formation, forelimb placing and corner turn scores, and brain atrophy in aged rats. They found that: 1) Regardless of the time-to-treatment window, DFO improved forelimb placing and corner turn scores compared to vehicle treatment, and the differences in rats' performance of these tests between these various time points were not significant; 2) DFO effects on functional recovery, however, were seen faster when the treatment was begun within 24h as

opposed to 48h (28 days vs. 56 days); and 3) DFO reduced brain tissue loss and atrophy when administered within 24h after induction of ICH compared to 48h delayed treatment. The faster improvement of function noted in these studies is suggestive of greater efficacy for earlier treatment and might also translate into potentially significant cost savings from reduction in healthcare related expenses and earlier ability of the patients or their caregivers to return to work and avoid lost wages. In addition, very few patients in the phase I study presented more than 24h after ICH onset, indicating that only few additional patients are likely to be enrolled by extending the OTT beyond 24h at this stage.

Conversely, animal data do not strongly support limiting treatment to an earlier time window, such as within 12h. Although Okauchi et al [Okauchi 2010] reported that the optimal therapeutic window of DFO to reduce PHE formation was about 12h, the correlation between PHE and deficits on sensorimotor behavioral tests weeks-to-months after ICH was weak, which they attributed to the sensitivity of their assays. Previous studies have shown that PHE formation correlates with forelimb placing during the acute phase of ICH [Hua 2002]. A serial MRI study of the natural history of PHE in patients with ICH reported similar results; PHE correlated with worsening neurological status at 48h, but not 3-month functional outcome [Venkatasubramanian 2011]. Indeed, the influence of PHE on long-term recovery of ICH patients is debatable [Inaji 2003; McCarron 1999; Sansing 2011; Sykora 2009; Zazulia 1999]. Therefore, restricting the time-to-treatment to 12h or even less, for the sole purpose of its potential effect on PHE, seems unnecessary. In addition, very few patients presented within 12h in the phase-I study. Attempting to restrict the OTT window to less than 12h could limit recruitment into the study as well as the generalizability of the results to the wider ICH population. Therefore, OTT time is incorporated into the randomization algorithm in order to achieve an acceptable treatment balance. Similar to animal studies, this would also allow for further exploratory analysis to assess the effect of early vs. delayed treatment with DFO on PHE and outcome.

DURATION OF TREATMENT: Pre-clinical studies that examined the optimal duration of treatment with DFO following ICH produced variable results. In one study, treatment with DFO (HED ~ 16 mg/kg/day) for 2, 5, 7, or 14 days improved performance compared with placebo on forelimb placing test at days 28 and 56 [Okauchi 2010]. However, residual neurological deficit was present on corner turn test in animals treated for less than 7 days, and the 7-day treatment was associated with less adverse reactions than the 14 days, leading the investigators to conclude that the optimal duration of treatment in this model was 7 days. In other studies, treatment with DFO at HEDs of 16 and 32 mg/kg/day for 3 days resulted in significant improvement in rats' performance on both forelimb placing and corner turn tests and reduced brain atrophy at 56 weeks compared with placebo [Okauchi 2009]. In piglets, treatment with DFO for 3 or 7 days had similar protective effects on perihematomal reddish zone, white matter injury, iron accumulation, ferritin upregulation, and neuronal death within that zone [Gu 2009]. In other experiments, treatment with DFO at 200 mg/kg/day for 3 or 7 days after ICH also improved forelimb placing and corner turn scores and reduced brain edema [Nakamura 2003-2004]. Collectively, these results suggest that treatment with DFO for any duration ≥2 days results in improved functional performance, compared with vehicle treatment.

The phase I study assessed varying doses of DFO administered over a 3-day infusion period. The 3-day infusion duration was chosen for the following reasons: 1- Edema growth is fastest in the first 2-3 days after ICH onset and this initial growth (and not the later one) is associated with neurological deterioration [Venkatasubramanian 2011; Inaji 2003; Zazulia1999]; 2- The time course for hemoglobin hemolysis and release of its degradation products including iron is approximately 2-3 days [Macdonald 2004]; and 3- Iron in the perihematoma tissue peaks at day 3 after experimental ICH [Wu 2003]. Extending the duration of treatment to 5 days in HI-DEF was based on the above data in aged rats [Okauchi 2010]. The pulmonary toxicity of DFO is believed to be related to prolonged intravenous infusions of high doses of the drug, and a review of the 6 cases of ARDS in the DFO arm in HI-DEF revealed that the mean time from treatment to ARDS onset was ~ 78 hours, median ~ 76 hours. The longer duration of treatment might have contributed to increased pulmonary toxicity of the drug observed in HI-DEF, where 6/21 (28.6%) subjects in the treatment arm developed ARDS compared with 1/6 (16.7%) of patients who received the 62 mg/kg/day dose in phase I. Lowering the duration of treatment would decrease the total dose of the drug and might help to minimize the pulmonary toxicity of the drug without compromising the potential effectiveness of the drug. Therefore, we chose to decrease the duration of treatment to 3 days in this revised protocol.

Overall, a 3-day treatment provides the potential for improved drug safety and tolerability without significantly jeopardizing its efficacy. The 3-day treatment with DFO was superior to saline vehicle in improving functional outcome in rat models of ICH [Okauchi 2010]; although iron release from the hematoma continues for days-to-weeks, the time course of iron release from the hematoma may not directly correlate with the timing of iron toxicity. With time, there is an up-regulation in endogenous iron chelators after ICH which may limit iron toxicity and the need for more extended treatment with DFO; and the 32 mg/kg/day dose proposed in part 2 of this study exceeds the animal dose (100 mg/kg/day; HED 16 mg/kg/day) used in studies examining the optimal duration of DFO therapy after experimental ICH, and it is possible that the higher dosage and route of administration (IV as opposed to intramuscular in animal studies) might be as effective within a shorter treatment period.

STUDY DESIGN AND PLACEBO GROUP: The futility design directly addresses the question of whether or not DFO at a dose shown to be effective in animal models of ICH given over a clinically feasible duration of 3 days is of sufficient promise in improving functional outcome after ICH to consider a large-scale and costly phase-III trial. The proposed futility design is logically consistent with the aims of Phase II trials, which are to weed out ineffective interventions. It is not a substitute for phase-III and cannot by itself provide sufficient evidence to indicate that treatment with DFO is efficacious, since it is not designed or intended to test the efficacy hypothesis. Furthermore, non-futility in this phase-II study does not guarantee a positive phase-III result, which is important in preserving clinical equipoise for Phase III. Lastly, the futility hypothesis differs from hypotheses specified in traditional phase III efficacy trials and the probabilities of type-I and -II errors are interpreted differently. In futility analysis, we are less concerned about falsely concluding an ineffective treatment is possibly effective.

The advantage of the proposed two-arm design over a single-arm phase-II futility design is that it avoids the pitfalls associated with the use of historical data (temporal changes in other aspects of patient management, variations in data quality and protocol adherence, and differences in the eligibility criteria and specification of primary outcome measure between the various studies). These confounding variables can distort estimates of the historical reference proportion and are difficult to account for in a single arm study. Besides addressing these concerns, the inclusion of a placebo arm allows us to conduct secondary exploratory imaging analyses to determine the natural history of PHE progression and the effects of DFO on PHE as a surrogate marker of its biological activity on brain tissue. Furthermore, the proposed two-arm design allows us to configure and evaluate the logistics of randomization and blinding processes.

CHOICE OF FUTILITY THRESHOLD: The Minimum Clinically Important Difference (MCID) in ICH is to some extent arbitrary and is largely derived from Traumatic Brain Injury literature, where demonstration of effectiveness was set at a 10% increase in the percentage of patients with favorable outcome in many clinical trials of head injury. Indeed, previous and ongoing Phase III trials in ICH, such as ATACH-2, used MCID of 10% or greater in the absence of data supporting that lower magnitude of difference will change practice patterns. For example, in the GAIN International and GAIN America Studies of the glycine antagonist, Gavestinel, the rate of mRS 0-2 was approximately 4%-to-5% higher in Gavestinel-treated patients, compared with placebo, but this difference was not considered encouraging to further develop the drug as a therapy for ICH [Haley 2005]. We relied on data from previous ICH studies, the NINDS rt-PA trials in ischemic stroke, and outcome data from the phase-I study to derive a threshold for futility, based on mRS score of 0-2 at 3 months, in the current study. In phase-II study of factor VIIa in ICH, the proportion of treated patients with mRS 0-2 at 3 months was 12.8% to 17% (depending on the dose) greater than the placebo patients [Mayer 2005]. In the NINDS rt-PA trials, the proportion of rt-PA-treated patients with mRS scores of 0-2 at 3 months was 12% greater than their placebo-treated counterparts. Taking these numbers into consideration, the fact that no specific treatments exist to prevent disability after ICH, and the frequent observation of "winner's curse" in previous stroke trials (a phenomenon by which effect size tends to be overestimated in pre-phase III trials, only to become smaller in larger Phase III trials which involve more sites and greater heterogeneity), a 12% difference in the proportion of DFO-treated subjects who achieve

favorable outcome compared with placebo was chosen as the threshold for futility in the current study. Although setting the bar lower in this futility study may result in a more positive outcome favoring moving deferoxamine forward for Phase III evaluation, at the expense of a much larger sample size, the general consensus was that an effect size of 12% for the current proposal provides a balance between setting the bar too high (leading to rejection of a potentially effective therapy) or too low (leading to further testing of a marginally-effective mediocre therapy with its associated expense and resources, which could hinder the development of truly effective treatments for ICH).

CHOICE OF EFFICACY OUTCOME MEASURE: The mRS was chosen for the primary outcome assessment because of its high inter-rater reliability and to be consistent with previous ICH trials. The decision to use a dichotomous outcome aims to increase the sensitivity of detecting meaningful differences by reducing the rate of misclassification or score assignment. Favorable outcome, as opposed to poor outcome, was specified based on careful review of the putative beneficial mechanism(s) of DFO and expected lack of an effect from treatment on reducing hematoma growth, a major predictor of mortality and significant disability after ICH [Davis 2006], as well as the phase-I outcome data. However, it is possible that DFO might result in an increase in the proportion of patients with mRS 0-3 (and hence a decrease in the proportion of patients with mRS 4-6) instead of mRS 0-2. Although mRS 0-3 is less favorable than the primary outcome of mRS 0-2, it would still be a desirable effect in patients with ICH given that no treatments exist to reduce disability. Therefore, the proportion of DFO- and placebo-treated subjects with mRS 0-3 at 90 days will also be evaluated. The trial is adequately powered to assess the alternative futility hypothesis using mRS 0-3 as the outcome based on an absolute difference in treatment effect ≥13% in favor of DFO. Given increased acceptance of ordinal analysis of mRS in the stroke field and recognition that ICH patients may take longer to recover, we added ordinal analysis across all mRS scores and extended the follow up period to include assessment of mRS by phone at 6 months as secondary outcome measures.

SURROGATE MEASURES OF DFO BIOLOGICAL ACTIVITY: We plan to examine the effect of treatment with DFO on relative PHE volume and progression at days 3-4 as a surrogate marker of biological activity on brain tissue. Delayed brain edema formation following the first 24h after ICH is related to red blood cells' lysis and hemoglobin- and iron-mediated toxicity [Xi 1998/2002; Lee 1996; Qing 2009]. Treatment with DFO reduces brain edema, CSF free iron levels, iron deposition in the perihematoma region, and residual cavity volume after experimental ICH [Nakamura 2003-2004; Wan 2006-2009; Gu 2009; Okauchi 2009]. Human studies suggest that PHE continues to grow for weeks after ICH, and that edema progression is fastest during the first few days [Venkatasubramanian 2011]. Although the influence of PHE on long-term recovery after ICH is debatable [Hua 2002; Inaji 2003; Zazulia1999; McCarron 1999; Sykora 2009; Sansing 2011], there is evidence that early edema progression correlates with early neurological deterioration [Venkatasubramanian 2011; Inaji 2003]. Therefore, secondary analyses will examine the effects of treatment on PHE immediately following completion of the infusion and not weeks later, since PHE growth during the early time period seems to be more clinically relevant. Regardless of the clinical impact of PHE, demonstrating an effect of DFO on this variable would at least provide secondary evidence of the agent's biological activity under the chosen dosing regimen. The relative PHE volume (the absolute PHE volume divided by the hematoma volume) was chosen as it has been used previously to detect serial changes in edema volume while adjusting for subsequent hematoma expansion or retraction [Gebel 2002; Mehdiratta 2008].

4 SELECTION AND ENROLLMENT OF SUBJECTS

Study subjects will be recruited from the emergency departments or inpatient services at > 20 hospitals in North America under the responsibility of the site investigators. Patients with diagnosis of ICH, as confirmed by CT scan, in whom the first dose of the study drug can be administered within 24h from symptom-onset (determined from the time the patient was last known to be without presenting deficits), will be prospectively enrolled, according to the eligibility criteria. To minimize selection bias, consecutive patients who meet all eligibility criteria will be considered for enrollment.

4.1 Inclusion Criteria

1) Age ≥ 18 and ≤ 80 years
2) The diagnosis of ICH is confirmed by brain CT scan
3) NIHSS score ≥ 6 and GCS >6 upon presentation.
4) The first dose of the study drug is expected to be administered within 24h of ICH symptom onset (as described above).
5) Functional independence prior to ICH, defined as pre-ICH mRS ≤1.
6) Signed and dated informed consent is obtained.

4.2 Exclusion Criteria

1) Previous chelation therapy or known hypersensitivity to DFO products
2) Known severe iron deficiency anemia (defined as hemoglobin concentration < 7 g/dL or requiring blood transfusions
3) Abnormal renal functions, defined as serum creatinine greater than 2 mg/dl
4) Planned surgical evacuation of ICH prior to administration of study drug (placement of a catheter for ventricular drainage is not a contraindication to enrollment)
5) Suspected secondary ICH related to tumour, ruptured aneurysm or arteriovenous malformation, hemorrhagic transformation of an ischemic infarct, or venous sinus thrombosis
6) Infratentorial hemorrhage
7) Irreversibly impaired brainstem function (bilateral fixed and dilated pupils and extensor motor posturing)
8) Complete unconsciousness, defined as a score of 3 on item 1a of the NIHSS (Responds only with reflex motor or autonomic effects or totally unresponsive, and flaccid)
9) Pre-existing disability, defined as pre-ICH mRS ≥2
10) Coagulopathy - defined as elevated aPTT or INR >1.3 upon presentation; concurrent use of direct thrombin inhibitors (such as dabigatran), direct factor Xa inhibitors (such as rivaroxaban or abixapan), or low-molecular-weight heparin
11) Patients with confirmed aspiration, pneumonia, or evident bilateral pulmonary infiltrates on chest x-ray or CT scan prior to enrollment
12) Patients with significant respiratory disease such as chronic obstructive pulmonary disease, pulmonary fibrosis, or any use (chronic or intermittent) of inhaled O_2 at home
13) FiO_2 >0.35 (>4 L/min) prior to enrollment
14) Sepsis (present source of infection ± lactic acidosis defined as serum lactate >5 mmol/L and pH <7.35); Systemic Inflammatory Response Syndrome (defined as Temp >100.4F or <96.8F; Heart rate >90; Respiratory rate >20 or $PaCo_2$ <32 mmHg; WBC >12, <4 K/uL, or >10% bands); or shock (SBP <90 mmHg) at presentation
15) The presence of 4 or more of the following risk modifiers for ARDS prior to enrollment:
    a) Tachypnea (respiratory rate >30)
    b) SpO_2 <95%
    c) Obesity, defined as Body Mass Index (BMI) >30
    d) Acidosis (pH <7.35)
    e) Hypoalbuminemia (albumin <3.5 g/dL)
    f) Concurrent use of chemotherapy
16) Taking iron supplements containing ≥ 325 mg of ferrous iron, or prochlorperazine

17) Patients with heart failure taking > 500 mg of vitamin C daily
18) Known severe hearing loss
19) Known pregnancy, or positive pregnancy test, or breastfeeding
20) Positive drug screen for cocaine upon presentation
21) Patients known or suspected of not being able to comply with the study protocol due to alcoholism, drug dependency, noncompliance, living in another state or any other cause
22) Any condition which, in the judgement of the investigator, might increase the risk to the patient
23) Life expectancy of less than 90 days due to co-morbid conditions
24) Concurrent participation in another research protocol for investigation of another experimental therapy
25) Indication that a new DNR or Comfort Measures Only (CMO) order will be implemented within the first 72 hours of hospitalization.

4.3 STUDY ENROLLMENT PROCEDURES

4.3.1 SCREENING FOR POTENTIAL SUBJECTS

The majority of patients is expected to be recruited upon initial evaluation in the Emergency Department (ED). Therefore, the ED staff, members of the stroke team, and Neurology/Neurosurgery residents should be made aware of this study and in-serviced about its protocol to facilitate recruitment. It is expected that the stroke team and study coordinator (or other designated members of the study staff) will be informed about all patients who present to the ED with ICH, as confirmed by CT; and, that these patients will be reviewed by appropriate members of the study staff as quickly as possible to determine eligibility for participation in the study. All participating sites have an acute stroke response team, and it is expected that potential eligible candidates will be examined and interviewed within 60-90 minutes of their arrival to the ED.

The investigators at each site will be required to maintain a screening log for ICH patients who are found ineligible to participate in the study or those who are enrolled into another research study, documenting the patients' age, demographics, and the reason(s) for exclusion from the current study. The study coordinator at each site is required to enter the screening log data into the WebDCU™ study database on a monthly basis.

4.3.2 SCREENING EVALUATIONS

The following clinical, laboratory, and radiological assessments will be carried out upon screening of potential study subjects: 1) Demographic data; 2) Medical history; 3) Review of medications; 4) General physical examination and vital signs; 5) Neurological examination, including visual auditory assessment, NIHSS, and GCS; 6) Review of head CT scans and chest x-rays; 7) Review of all inclusion/exclusion criteria; 8) Determination of ICH score and pre-ICH mRS score; 9) Laboratory tests (hematology, serum chemistries, serum albumin, coagulation parameters, renal and hepatic function tests and urine analysis).

A baseline pre-treatment plain (non-enhanced) brain CT confirming the presence of ICH is required to establish the diagnosis of ICH. The head CT should be reviewed by a Radiologist or a stroke-trained Neurologist experienced in the interpretation of CT scans. The need for additional diagnostic tests, such as CTA, conventional angiogram, or MRI/MRA to rule out secondary causes of ICH in suspected cases should be based on the judgment of the investigators according to the standards of clinical practice at each participating institution and the guidelines from the Stroke Council of the American Heart Association [Morgenstern, 2010; please refer to appendix II].

A baseline chest x-ray is required before enrollment to exclude the presence of bilateral pulmonary infiltrates or pulmonary edema.

A negative serum pregnancy test is required for all women of childbearing potential prior to enrollment into the study.

4.3.3 INFORMED CONSENT

Upon confirmation of patient's eligibility based on the initial screening evaluation above, an informed consent will be obtained. In accordance with US FDA regulations (21 CFR 50) and ICH-GCP Consolidated Guidelines, a witnessed, IRB-approved, informed consent is required from all subjects, their legal representative, or family member (as defined in 21CFR50.3(m)) (legal representative and family members are also referred to as surrogates in this protocol) prior to participating in this study. At the initial contact with a potential candidate, the investigator(s) should provide a comprehensive explanation of the purpose, procedures, possible risks/benefits of the study in language that is understandable to a non-medically trained person; as well as participant responsibilities and the fact that his/her participation is voluntary, that he/she may withdraw from the study at any time, and that the decision not to participate or to withdraw will not affect subject's care in any way. Potential participants or their surrogates should be given ample opportunity to ask questions and to consider their decision. If the subject or the surrogate on behalf of the subject expresses a sustained interest, a signed and dated written informed consent will be obtained. Patients with a known history of dementia should be excluded from self-consent, thereby minimizing the possibility of invalid informed consent. A copy of the consent form must be given to the participant or the surrogate, and another copy placed in the subject's medical record. The informed consent must be obtained by either the clinical site PI or other members of the study team who are qualified to perform this task and whose names are listed on the Delegation of Authority Log.

4.3.4 ENROLLMENT

Clinically and radiologically eligible patients for whom informed consent is obtained (from the subject or a legal representative or family member) will be randomized. Calculation of the ICH score [Hemphill 2001; please refer to appendix III] is required prior to randomization. Administration of the study drug to eligible candidates will only be permitted after completion of liver function tests and urine analysis, if not done upon presentation.

Participants should be reassessed immediately prior to the study drug administration to obtain a pre-treatment GCS and baseline NIHSS. Patients who exhibit significant clinical and neurological deterioration (i.e. develop fixed and dilated pupils or GCS decreases to ≤ 6) prior to administration of the first dose of the study drug should not receive the study drug, and their participation in the study should be terminated.

To ensure that all eligible subjects presenting within 12h of ICH onset are enrolled in a timely fashion without delay: 1) enrollment and time from door (arrival-to-emergency room)-to-infusion will be monitored on an ongoing basis, and every attempt will be made to ensure that subjects are treated as soon as possible from symptom-onset; and 2) if enrollment into the early (≤12h) window is disproportionately low, we will implement a strategy of forced recruitment whereby each site will be required to enroll one subject into the early time window for each subject enrolled into the later (>12-24h) window (or maintain its overall ratio of ≤12h to >12-24h to at least 1:1) before subsequent enrollment into the later time window is permitted at this site.

4.3.5 DETERMINATION OF SUBJECT'S BODY WEIGHT

The subject's body weight will be obtained by self-report from the subject or his/her accompanying person to the ED, or estimated by the treating physician, if actual body weight cannot be determined during the acute phase of evaluation in order to prevent delays in administering the study drug. However, it is expected that all subjects will have their actual

body weight determined and recorded within 24 hours of admission, and that subsequent dosing will be based on actual body weight.

4.3.6 TREATMENT ALLOCATION AND RANDOMIZATION

Once it is determined that a patient meets all the eligibility criteria, the investigator(s) will log on to the secure, study-dedicated electronic database (WebDCU™) to enter demographics and required randomization data. The database will generate a randomization code. The investigator will then provide the site's pharmacy with the randomization code and subject weight. The pharmacist will retrieve the label with the corresponding randomization code from the Study Randomization Binder maintained separately in the pharmacy. The pharmacist will prepare the study drug for blinded administration. The pharmacists will be specifically instructed not to reveal treatment assignment to the investigators and not to break the blind. In the pilot phase I study, the reconstituted solution of DFO was colorless, and there were no specific treatment-related changes in laboratory tests including hematology, urine color, or adverse events to suggest that the active drug can be identified from placebo. The following additional measures will be taken to assure that the integrity of the blinding will remain intact: 1) An independent, unblinded statistician from the DCU, not involved in the operations of the study, will create the randomization algorithm; 2) Randomization codes will be numeric and will not contain any reference to the type or dose of treatment to be administered; and 3) The randomization binders will be kept in secured and locked cabinets in each pharmacy. There is no specific antidote to DFO. Therefore, unblinding is unnecessary in most cases. In cases of extreme emergency when the treating physicians request unblinding of treatment assignments for therapeutic purposes, the unblinding will only be revealed to the treating physicians but not the investigators. The treating physicians must be instructed not to reveal the blind to the subjects or study investigators. The study personnel (pharmacist) will be required to inform the Principal Investigator and the Project Manager within 24h in the event of unblinding. In cases where the treating physician is one of the study investigators, he/she also will be required not to reveal the identity of the study drug to other members of the study team, and not to perform subsequent study-related outcome assessments.

5 STUDY INTERVENTIONS

5.1 INTERVENTIONS, ADMINISTRATION, AND DURATION

The active study drug (DFO) will be supplied in vials containing 2 gm of sterile, lyophilized, powdered deferoxamine mesylate. The drug will be reconstituted for injection, by dissolving it in 20 ml of sterile water, then the volume of the reconstituted drug required for a dosing of 32mg/kg/day (up to a maximum of 6000 mg/day) will be calculated based on the subject's body weight and added to normal saline (0.9% sodium chloride) for administration by IV infusion. The drug will be reconstituted immediately prior to use to ensure microbiological safety. The matching placebo will be an isotonic saline solution (0.9% sodium chloride). Subjects will receive weight-adjusted intravenous infusions of the study drug. The maximum daily dose will not exceed 6000 mg per day regardless of subject's weight. The study drug will be administered using an intravenous cannula, dedicated to drug infusion, inserted into an antecubital vein preferably in a non-paralyzed limb, and a variable speed infusion pump. Alternatively, a central line, with a port dedicated to the drug infusion, may be used. It is important to maintain a dedicated line for the study drug infusion because it may be incompatible with other drugs. Applying warm compresses to the site of IV injection throughout each infusion period is strongly encouraged as a precaution to minimize the potential for local injection site reactions. The initial dose of the study drug must be administered within 12-24 hours (as appropriate) of ICH symptom onset. Every effort should be made to initiate drug administration in consenting subjects as soon as possible after their arrival to the ED. The infusion of the study drug must be administered daily during hospitalization for 3 consecutive days by IV infusion at a rate not to exceed 7.5 mg/kg/hour. The date and time of drug preparation and administration, as well as rate of infusion, will be recorded. Ideally, the IV infusions of the study drug should occur without any interruptions. However, it is possible that

interruptions will occur, for example to replace the IV access site. In such cases, the occurrence and duration of these interruptions will be documented with an explanation as to why they occurred.

5.2 SAFETY MONITORING DURING STUDY DRUG ADMINISTRATION

All patients will be closely monitored for safety and neurological function during the administration of the study drug, and for at least 24 hours after completion of the infusions, in Neurological Intensive or Stroke Care Unit(s), staffed by stroke-trained neurologists and nursing staff. Patients will be monitored for all adverse events from the time of study drug initiation until day-7 or discharge, whichever occurs first; and, all new SAEs will be monitored until day-90 or resolution of the SAE. Continuing SAEs beyond day-90 will be monitored until day-180.
The patients will be closely observed during the initial 30 minutes of the infusion for possible adverse effects, such as an allergic/anaphylactic reaction, symptomatic bradycardia or hypotension. If there are no adverse effects, the infusion will continue. If a patient's neurological status deteriorates during the infusion, the NIHSS score and GCS will be reassessed by a member of the stroke team/study staff. Significant neurological worsening will be defined as an increase in NIHSS score of ≥ 4 points or a decrease of ≥ 2 points in GCS, compared to pre-treatment baseline values, that cannot be explained by other causes. A change of this nature lasting >4 hours could lead to premature discontinuation of the drug infusion based on the judgment of the local primary investigator(s). Vital signs (blood pressure and pulse) and neurological functions will be assessed at least once every 4h during intravenous drug infusion. Daily urinary output checks will be carried out every day until the day following the last infusion. An unexplained drop of >20% in mean arterial pressure will trigger review by the treating physician to determine its cause and if medical intervention is required. Fluid support will be used to prevent or treat hypotension. In more severe cases, use of inotropic agents may be required.

The skin at the infusion site will be examined at the same time points to assess for the presence and extent of local site reactions/irritation. If evidence of mild erythema/irritation or swelling emerges during the infusion, more frequent monitoring will be instituted depending on the severity and observed rate of change to ensure that the reaction does not progress further. The use of local anti-inflammatory agents may also be considered. In the event that local irritation continues to progress along with worsening edema, and development of pain or papular/vesicular eruption, the infusion will be terminated at that site and restarted in a different location. Close monitoring of the new infusion site, as well as the original site, will continue. If irritation develops at the new site or progresses at the original site, the infusion will be terminated and will not be re-started in this patient. In cases of an allergic reaction or anaphylaxis, the use of antihistamines and steroids is often sufficient; ACLS airway management may be required in severe cases. Appropriate treatment and monitoring will proceed according to the standard clinical practice at each institution until the reaction has resolved. Routine physical and neurological examinations will be carried out on a daily basis during the infusions, and the NIHSS and GCS scores will be determined. Safety laboratories (Hematology; Coagulation; Serum chemistry; Kidney and liver functions; and urine analysis) will be performed on the day following the last infusion of the study drug. Laboratory studies that are abnormal or worsen from baseline will be repeated. The frequency of subsequent evaluation of safety laboratories will be determined by the treating physician on a patient-by-patient case.

All patients must be closely monitored for any signs or symptoms of respiratory compromise, including ARDS (based on Berlin criteria – see appendix VII). A diagnosis of ARDS will lead to premature discontinuation of the drug infusion. Any evidence of respiratory compromise should trigger the investigators to complete the AE CRF on Web-DCU™ within 24 hours of onset for immediate safety review by the Independent MSMs. In addition, the following data must be checked at least once daily for all intubated patients (or more frequently in patients with changes in respiratory status requiring changes in ventilator settings and increased oxygen requirement) throughout day-7 or discharge and entered into Web-DCU™: 1) PaO_2/FiO_2 ratio; 2) Plateau and peak pressures; and 3) Chest X-ray results (when available). A CHEST X-RAY IS REQUIRED IF THE PAO_2/FIO_2 RATIO IS <300.

In order to minimize the potential pulmonary complications of the study drug, the investigators MUST follow the guidelines by ARDSNet for management of intubated patients [http://www.ardsnet.org/system/files/Ventilator%20Protocol%20Card.pdf] – please refer to appendix VI and section 7.3 for more details.

5.3 HANDLING OF STUDY INTERVENTIONS

5.3.1 SUPPLY, STORAGE, PREPARATION, AND DISPOSITION OF STUDY DRUG

The active drug (DFO) will be purchased by the Department of Health and Human Services (DHHS) from the manufacturer and shipped to the Program Support Center, Supply Service Center, Department of Health and Human Services (DHHS-SSC), in Perry Point, MD. The DHHS-SSC will serve as the drug distribution center, purchase the study drug periodically during the trial, and resupply the sites as recruitment progresses. The research pharmacist at each clinical site will receive the study drug in shipments.

The active study drug (DFO) will be supplied in vials containing 2 gm of sterile, lyophilized, powdered deferoxamine mesylate. The drug will be reconstituted for injection by dissolving it in 20 ml of sterile water, then the volume of the reconstituted drug required for a dosing of 32 mg/kg/day (up to a maximum of 6000 mg/day) will be calculated based on the subject's body weight and added to normal saline (0.9% sodium chloride) in an IV bag to achieve a final concentration of 7.5 mg per ml for IV administration. The infusion rate will not exceed 7.5 mg/kg/hour. The matching placebo will be an isotonic saline solution (0.9% sodium chloride). The drug will be reconstituted immediately prior to use to ensure microbiological safety. However, the reconstituted solution may be stored at room temperature for approximately 24h before use. In the phase I study, the reconstituted solution of DFO was colorless, and there were no specific treatment-related changes in laboratory tests including iron, hematology, urine color, or adverse events to suggest that the active drug can be identified from placebo.

The shelf half-life of the drug is 2 years, when stored in a cool place (15°C-25°C) away from direct light and heat. The drug should be stored in a secured and locked location. The storage room temperature should be recorded at least daily, and a temperature log should be maintained. Deviations from the recommended storage conditions should be immediately communicated to the Project Manager and the PI. The study drug affected by the deviations should not be administered to subjects until the monitoring team has assessed the impact of the deviation and determined whether the study drug can be used. Any materials remaining after each infusion should be discarded by the site in accordance with standard clinical practices.

5.3.2 STUDY DRUG TRACKING AND ACCOUNTABILITY

The PI at each site has the overall responsibility for drug accountability at his/her site, which will be carried out in accordance with ICH/GCP and individual clinical site's Standard Operating Procedures. Upon receipt of the study drug, the research pharmacist will inspect and count the study drug supply and confirm receipt of the study drug in the Web-DCU™ study database. The pharmacy at each clinical site also must maintain records of the amount of study drug received, dispensed to study subjects, and destroyed or returned at the end of the study. Drug accountability records and storage temperature logs will be inspected by the study monitor and may be subjected to inspection by relevant authorities as well. Study drug supplies will be counted and reconciled at each site during monitoring visits and at the end of the study.

5.4 CONCOMITANT MEDICATIONS AND PRECAUTIONS

Concomitant medications will be recorded during the study period from the time of study drug initiation until day-7 or discharge (whichever is earlier). There are few restrictions on the use of concomitant medications for study

participants. The use of prochloroperazine (compazine), a phenothiazine derivative, is not allowed before treatment, during treatment, or up to 72 hours after completion of the study drug infusion, since the combination of DFO and compazine can lead to impairment of consciousness [Blake, 1985]. Concurrent use of other experimental therapy is not allowed. Vitamin C supplements will not be allowed in patients with heart failure during treatment with DFO.

All standard therapies used in the management of ICH patients will be allowed under close monitoring and supervision. These include volume expansion with normal saline or crystalloids, vasopressors, antiedema agents such as steroids or mannitol, anti-hypertensives including diuretics, anti-convulsants, anti-arrhythmic agents and anti-emetics, except for phenothiazine derivatives.

In order to minimize the variability in care and to assure consistency in management across sites, the general care of ICH patients should conform to the guidelines from the Stroke Council of the American Heart Association [Morgenstern, 2010], and the European Stroke Initiative Guidelines [Steiner, 2006]. Pertinent issues are detailed in Appendix II and III.

6 CLINICAL AND LABORATORY EVALUATIONS

6.1 PATIENT EVALUATION AND DATA COLLECTION SCHEDULE

All study data will be collected using Case Report Forms (CRFs) designed specifically for the study. Patient confidentiality will be maintained throughout, as participants will only be identified on the CRFs by a unique study ID number. Patients' identifying information and source documents for the CRF data should be kept by the primary investigator at each participating site. The data obtained will include: 1) Demographics; 2) Previous medical history; 3) Concomitant medications; 4) Vital signs; 5) Results of laboratory tests; 6) NIHSS and GCS scores; 7) ICH score; 8) mRS, SIS-16, and MoCA scores; 9) Adverse events; 10) Surgical and non-surgical treatments; and 11) Imaging findings, including chest x-rays, and CT and MRI scans identifying the location of ICH, presence of IVH or hydrocephalus, and etiology of ICH (after completing diagnostic work-up).

6.1.1 CLINICAL AND LABORATORY ASSESSMENTS

The following assessments will be carried out throughout the 90-day study period:

Upon <u>screening</u> of potential study subjects: 1) Review of head CT and determination of the time of ICH onset or the time the patient was last known to be without presenting deficits; 2) Demographic data; 3) Medical history; 4) Review of medications; 5) General physical examination; 6) Neurological examination, including NIHSS and GCS; 7) Visual and auditory assessment (which includes assessments for cataracts, visual loss or field cut, disturbed color vision, hearing loss, and presence of tinnitus), whenever possible in awake patients; 8) Determination of pre-morbid mRS; 9); Review of admission laboratory tests - Hematology [hemoglobin, hematocrit, red blood cell count and platelet count]; Coagulation [partial thromboplastin time and international normalized ratio]; and serum albumin; and 10) Review of all inclusion/exclusion criteria. A baseline chest x-ray is required to rule out the presence of bilateral pulmonary infiltrates or pulmonary edema. A pregnancy test is required for all women of childbearing potential prior to enrollment into the study. Subjects in whom all eligibility criteria are met should be considered for enrollment, and those who agree to participate via signed and dated informed consent should be enrolled.

Participants should be reassessed immediately prior to the study drug administration to obtain a pre-treatment baseline. **<u>The Pre-treatment baseline reassessments include</u>:** 1) Vital signs, including blood pressure and heart rate; 2)

Neurological examination, including NIHSS and GCS to confirm stability of their neurological status and to obtain pre-treatment baseline scores; and 3) Assessment for new adverse events since screening. Patients who exhibit significant clinical or neurological deterioration (i.e. develop fixed dilated pupils or GCS decreases to ≤6) prior to administration of the first dose of the study drug should not receive the study drug, and their participation will be terminated.

Administration of the study drug should only be permitted after obtaining pre-treatment blood sample for serum chemistry and glucose, hepatic function tests, and urine analysis (if not already done upon arrival to the ED).

Subjects who receive the study drug(s) will be evaluated daily for the first 3 days (during study drug infusion while in hospital) and on day-7 or discharge from the hospital, whichever occurs first. Clinic visits will take place on days 30±7 and 90±7. Telephone interviews will be conducted on day 60±7 and 180±7.

The following assessments should be performed **after each daily infusion** (every 24±6h) and through the day following completion of the last infusion: 1) General physical examination; 2) Visual and auditory assessments; 3) Neurological examination, including NIHSS and GCS; and 4) Vital signs, including blood pressure and heart rate. A repeat head CT scan and blood samples for hematology, serum chemistry, renal and hepatic function tests, coagulation studies, and urine analysis should be obtained within 24±6h following the last infusion of the study drug. Vital signs (blood pressure and pulse) and neurological functions should be assessed at least once every 4h during intravenous drug infusions. Daily urinary output checks should be carried out every day until the day following the last infusion. In addition, the following data must be checked at least once daily for all intubated patients (or more frequently in patients with changes in respiratory status requiring changes in ventilator settings and increased oxygen requirement) throughout day-7 or discharge and entered into Web-DCU™: 1) PaO_2/FiO_2 ratio; 2) Plateau and peak pressures; and 3) Chest X-ray results (when available). ***A chest x-ray is required if the PaO_2/FiO_2 ratio is <300.***

On day-7 or Discharge (whichever comes first), the following assessments are done: mRS, NIHSS, GCS, MoCA and visual and auditory assessments.

During hospitalization, the NIHSS and GCS should be done any time a subject's neurological status deteriorates, or whenever the investigator believes it is prudent to do an assessment. Laboratory assessments beyond those required for the study should be done whenever clinically indicated.

The **Day-30 (±7)** will be an in-person visit comprised of a general physical examination, visual and auditory assessments, review of SAEs, and assessments of NIHSS, SIS-16, MoCA and mRS.

The **Day-60 (±7)** assessments, which will be completed via telephone, will be comprised of a functional assessment to obtain a score on mRS and query for SAEs.

The **Day-90 (±7)** visit will be an in-person visit comprised of a general physical examination, visual and auditory assessments, and assessments of NIHSS, MoCA, SIS-16, and mRS. However, a telephone interview to obtain a score on mRS (the primary outcome measure) may be allowed on a case-by-case basis, after consultation with the PI, if the subject is unable to return for an in-person evaluation.

The **Day-180 (±7)** assessments, which will be completed via telephone, will be comprised of a functional assessment to obtain a score on mRS.

A study staff member certified in NIHSS and mRS and experienced in administering GCS, SIS-16, and MoCA should perform the assessments at all time points. We require that all assessments at all time points be performed by the same blinded

investigator, whenever possible, to minimize inter-rater variability. Data on the subject's functional status (mRS and SIS-16) can be obtained from a proxy if the subject is unable to provide the information. Please refer to Appendix V for details of NIHSS, GCS, SIS-16, and MoCA scales.

Concomitant medications and non-drug therapies should be assessed at all points of contact with the subject after study drug initiation. All adverse events (serious and non-serious) should be assessed until day-7 or discharge, whichever occurs first. All new SAEs will be monitored until day-90, resolution of the SAE, or withdrawal of consent, whichever is earlier. Continuing SAEs beyond day-90 will be monitored until day-180.

6.1.2 RADIOLOGICAL ASSESSMENTS

A baseline pre-treatment non-enhanced brain CT confirming the presence of ICH is required to establish the diagnosis of ICH. The need for additional diagnostic tests, such as CTA, MRI/MRA, or a conventional angiogram to rule out secondary causes of ICH in suspected cases should be based on the judgment of the investigators according to the standards of clinical practice at each participating institution and the guidelines from the ASA [Morgenstern 2010; refer to Appendix II]. The volume of ICH on admission scan must be determined by the local investigators using the ABC/2 method (*please refer to Appendix IV for instructions)* before randomizing the subject in WebDCU™. A repeat non-enhanced brain CT scan should be obtained within 24±6 hours after completion of the last infusion, with the same standard imaging protocols and scanner at a given hospital. *Additional CT scans, beyond those required for the study, should be done whenever clinically indicated.*

A baseline chest x-ray confirming the absence of bilateral pulmonary infiltrates or pulmonary edema is required before considering the subject for enrollment into the study. A repeat chest x-ray is required in intubated patients whenever the PaO_2/FiO_2 ratio is <300.

6.2 Schedule of Evaluations

Table 6.2 below summarizes the timing and type of assessments/evaluations throughout the study.

Schedule of Assessments and Data Collection

	Screening	Baseline	oh	24h	48 h	72h	24h post-last infusion (± 6h) and as needed	Day 7 or Discharge*	Day 30	Day 60 - Phone	Day 90	Day 180 - Phone	End of Study
		Prior to study drug start		± 6h	± 6h	± 6h		± 6h	± 7 days	± 7 days	± 7 days	± 7 days	
Screen Failure Log	X												
Inclusion/Exclusion Criteria	X												
Demographics & Medical History	X												
Informed Consent	X												
Subject Enrolment / Randomization		X											
Study Drug Administration			X	X	X	X							
Physical Examination	X			X	X	X	XX		X		X		
Visual & Auditory Assessment€	X			X	X	X	XX	X	X				
NIHSS	X	X		X	X	X	XX	X	X		X		

GCS	X	X		X	X	X	XX	X					
ICH Score	X												
mRS	X							X	X	X	X¥	X	
MoCA								X	X		X		
SIS-16									X		X		
Vital Signs (BP & pulse)$		X		X	X	X	XX	X					
Safety Monitoring			X	X	X	X							
Urinary Output				X	X	X							
Hematology	X						XX						
Serum Chemistry	X						XX						
Coagulation Parameters	X						XX						
Urine Analysis	X						XX						
Liver Function Tests	X						XX						
Kidney Function Tests	X						XX						
Serum albumin	X												
Pregnancy Test (women of childbearing potential)	X												
Blood sample for future research×		X					XX						
CT scan	X						XX						
Chest x-ray%	X						X						
Prior Medications	X												
Concomitant Medications			X	X	X	X		X	X	X	X		
Concomitant Non-Drug Therapies			X	X	X	X		X	X	X	X		
Adverse Events			X	X	X	X	X	X	X	X	X		
End of Study													X

€ Includes assessments for cataracts, visual loss or field cut, disturbed color of tinnitus – Should be performed, whenever possible in awake patients

* Day 7 or Discharge– whichever comes first

$ Vital signs (blood pressure and pulse) and neurological functions will be assessed at least once every 4h during intravenous drug infusion Laboratory tests may be performed outside the ±6h window, if it is a standard site procedure to perform laboratory tests[6] at a specific time each day.

XX These assessments must be performed within 24±6h following completion of the last infusion of the study drug

× We plan to collect (bank) additional blood samples at baseline and after the last infusion to be stored and analyzed in the future from subjects providing informed consent.

● Post-treatment scan should be performed within 24±6 hours of the last infusion even if it is terminated before day-5 of treatment

¥ A telephone interview to obtain a score on mRS (the primary outcome measure) may be allowed on a case-by-case basis if the subject is unable to return for an in-person evaluation on day 90.

% **Chest x-ray must be performed in any intubated patient if PaO_2/FiO_2 ratio is <300.**

6.3 Blood Banking Repository Sub-Study

The main study provides an opportunity to "bank" blood samples from the participants for future innovative ICH research, particularly if the efforts to develop DFO as a therapy for ICH are successful. Future pharmacogenetic studies may be considered to help define other therapeutic targets and responders vs. non-responders to DFO therapy. We, therefore, plan to collect additional blood samples at baseline and after the last DFO infusion (approximately 30 ml) to be stored and analyzed in the future. Subjects' participation in this blood-banking repository will be optional; they do not have to participate in the repository sub-study in order to participate initially or to continue their participation in the main study. The blood samples from all participating sites will be sent to the Beth Israel Deaconess Medical Center (The Coordinating Center) by overnight mail. All samples will be stripped of identifiers and randomly assigned a unique code number. The key linking code numbers and identifying information will be kept in a secure location accessible only to the principal investigator of the study or his designee, on a password protected network drive. All samples will be

stored at -80 degrees Celsius low-temperature, locked, freezer until final planned analyses are formulated and carried out. The samples will be stored indefinitely or until no more remains for genomic and protein research that can be performed at future dates.

The exact questions to be asked and tests to be done in the future are not fully identified at this stage. We tentatively plan to investigate the relationship between polymorphisms from a panel of genes encoding iron-handling proteins (which includes genes involved in both intra- and extra-cellular iron metabolism, such as ceruloplasmin, haptoglobin, hemopexin, transferrin receptor, ferritin heavy- and light-chain, and heme-oxygenase 1 and 2 genes) and PHE, outcome, and response to DFO therapy. Genotyping will be performed using high-throughput genome-wide SNP genotyping methods. Genotype results from DFO- and placebo-treated subjects will be examined for differences in allele frequency that may be associated with risk of malignant PHE progression or response to DFO therapy. Extracted DNA samples will be genotyped without knowledge of the treatment arm by chip-based methods utilizing mass spectrometry. The exact location of genotyping is undetermined at this point in time, although it is likely to be performed at the Broad Institute, given our ongoing collaboration in ICH Genome Wide Association Studies.

We plan to seek ancillary funding to support these additional analyses. Once funding is secured and the final analyses are formulated, we plan to seek an IRB approval for the specified analyses and use of the blood banking repository data.

7 ADVERSE EVENTS

7.1 DEFINITION OF ADVERSE EVENTS

Adverse events are any *untoward* medical occurrence whether or not they are considered related to the study drug. An adverse event can be any unfavorable and unintended symptom, sign, disease or clinically significant abnormal test result occurring during the study which were either not present at baseline, or if present, worsened during the study in terms of either severity or frequency. When evaluating possible adverse events, an assessment should be made regarding its seriousness, severity and temporal relationship to the administration of the study drug. An adverse event is considered serious if it is life-threatening, prolongs hospitalization, requires re-hospitalization, results in significant disability, leads to death, or requires medical or surgical intervention to prevent one of the other listed outcomes. An assessment should be made regarding the seriousness, severity and relationship to the administration of the study medication as well as to the ICH. The following factors should be considered when evaluating possible adverse events: 1) the temporal sequence from drug administration; 2) patient's response after drug discontinuation or re-introduction; and 3) severity of the event. The investigators, on the basis of their clinical judgment and guided by the following definitions, should determine the relationship of an adverse event to the study drug(s) as: definitely related, i.e. following in a reasonable temporal sequence, known to be a complication of DFO, and having no other explanation; probably related, i.e. following in a reasonable temporal sequence and not reasonably explained by the patient's clinical state or other therapies; possibly related, i.e. could have been explained by other therapies or patient's clinical state; or unlikely or not related. The severity of adverse events should be graded as mild, moderate, severe, life-threatening, or fatal.

All adverse events or complications will be entered into WebDCU™ and assigned to a system-organ class and preferred term using the MedDRA coding dictionary. Each subject must be monitored closely throughout his/her hospitalization for serious adverse events (SAEs). All adverse events (serious and non-serious) should be assessed until day-7 or discharge, whichever occurs earlier, and serious adverse events until day-90 or withdrawal of consent, whichever is earlier.

7.2 POTENTIAL COMPLICATIONS OF THE STUDY INTERVENTION

Aspects of the study that have potential for risks are blood withdrawals, CT scan imaging studies, and injections of the study medication(s). There are no additional risks for any of these procedures other than those present in any routine clinical situation, especially for blood draws and CT scans. Patients may experience some temporary discomfort, bruising, or, rarely, infection or the formation of a small clot or swelling at the site of the needle puncture in the process of drawing blood or starting the intravenous drip. The condition of ICH, in itself, carries a high risk for secondary complications including serious life-threatening adverse events, and even death, whether or not the active study drug (DFO) is used. Recent studies indicate that approximately 40% of patients with ICH have a serious adverse event during hospitalization, of which approximately 26% can be fatal [Haley, 2005; Mayer 2005; Mayer 2006; Lyden 2007].

DFO has been extensively used in clinical practice for more than 40 years, and is approved by the Federal Drug Administration for the treatment of acute iron intoxication and of chronic iron overload due to transfusion-dependent anemia. Safety studies indicate that it is relatively well tolerated. The adverse reactions observed with DFO have been previously detailed in section 2.2.2.

The most observed adverse events in our Phase I study in ICH patients were injection site reaction (irritation, pain, erythema, infiltration) and a modest decrease in blood pressure, which did not require any medical intervention. One subject developed visual hallucination during IV infusion of DFO. Almost all of these adverse events were mild, self-limited, did not require specific treatment, and resolved spontaneously. There were no deaths or serious adverse events related to DFO use. There were 2 cases of respiratory failure, both of which occurred in the 62mg/kg/day dose-tier. They were thought to be related to aspiration pneumonia, and unrelated to the study drug. However, a recent expert review found that one of these cases was an undiagnosed case of ARDS, and concurred that aspiration was a plausible explanation for it.

In the first part of phase II investigation of DFO in ICH (HI-DEF trial, where subjects were treated with a continuous infusion of DFO at 62 mg/kg/day for 5 consecutive days), 5 cases of ARDS were reported among the first 42 participants. A detailed expert review of reported cases of respiratory failure and pulmonary edema revealed 2 more cases of undiagnosed ARDS. Overall, 6/7 of the ARDS cases in HI-DEF were in the DFO treatment arm; 3 were fatal and the cause of death was ARDS-related in 2/3 cases. The expert who was blinded to treatment assignment identified a plausible cause for ARDS, other than the study drug or ICH itself, in 4/7 ARDS cases, while no other explanation, other than the study drug or ICH, was apparent for the remaining 3 cases. Although the overall rate of ARDS was in line with published reports in ICH patients [Elmer 2013], the imbalance in the frequency of ARDS between the DFO- and placebo-treated groups raised concerns that the pulmonary toxicity of the drug was likely. There were no other safety concerns. A MedWatch report was filed with the FDA and Health Canada for an AE of hypophosphatemia that was judged to be serious (potentially life-threatening), unexpected, and possibly related to the study drug. Overall, hypophosphatemia of varying severity was reported in 3 subjects in HI-DEF; 2 in DFO- and 1 in placebo-treated patients.

The effects of DFO on the fetus are unknown. It is also not known if DFO is excreted in human milk. Therefore, women who are pregnant and those who are breast-feeding will be excluded from this study. Those with child-bearing potential must have a negative pregnancy test before participating in the study.

7.3 PREVENTION AND MANAGEMENT OF ADVERSE EVENTS

Aseptic and sterile techniques should be used during blood draws, which should be performed by experienced phlebotomists. Application of warm compresses to the site of IV infusion throughout the infusion period is advised to minimize the potential for injection site irritation. In order to maximize safety during this study, we are using a slow infusion rate of < 7.5 mg/kg/hour and are limiting the maximal dose that any subject can receive to 6000 mg per day. The subjects must be carefully monitored throughout their participation in the study as detailed above. Any medical complications

should be managed as appropriate, according to the standard clinical practice at participating institutions. There is no specific antidote to DFO. General symptomatic and supportive measures should be applied.

Subjects should be admitted to the Neurological Intensive Care or Stroke Unit, undergo repeated clinical (neurological and general physical examinations) and laboratory evaluations at regular intervals throughout their participation in the study for safety monitoring, and receive standard medical management for ICH based on the American Stroke Association Guidelines [Morgenstern 2010]. Pertinent issues, including blood pressure management, are detailed in the appendix II and III. Laboratory studies that are abnormal or worsen from baseline should be repeated.

It is important that investigators ensure maintenance of intravascular volume prior to administration of the drug infusions, especially in hypovolemic patients, to minimize DFO-induced hypotension; and to carefully observe the subjects during the initial 30 minutes of the intravenous infusion for possible adverse effects, such as an allergic/anaphylactic reaction, arrhythmias, or hypotension. If there are no adverse effects, the infusion should continue. All patients should be kept hydrated and, if necessary, fluid support should be used to prevent or treat hypotension. In more severe cases, use of inotropic agents may be required.

The skin at the site of IV infusion must be carefully inspected periodically for signs of irritation, induration, or inflammation during physical examinations, coincident with the times of all assessments of vital signs, during the infusion of the drug and for 24 hours afterwards. Section 5.2 above details appropriate measures for management of local injection site reactions.

In order to minimize the potential pulmonary complications of the study drug, the investigators MUST follow the following guidelines by ARDSNet for management of intubated patients [http://www.ardsnet.org/system/files/Ventilator%20Protocol%20Card.pdf]– please refer to appendix VI: **Ventilator settings –** 1) Tidal volume (V_T)= ≤8 cc/kg PBW (predicted body weight); 2) Plateau Pressure (P_{plat}) <30 cm H_2O; 3) Peak Pressure (P_{peak}) <50 cm H_2O; and 4) Positive End-Expiratory Pressure (PEEP): at least 5 cm H2O. Investigators MUST also implement a **Ventilator Associated Pneumonia prevention protocol** – 1) Head of bed elevation at least 30 degrees or greater; and 2) Regular clorhexidine mouth wash and oral care. In addition, a conservative fluid management strategy that aims to minimize or eliminate positive fluid balance and cautions against indiscriminate use of blood transfusion unless hemoglobin concentration drops below 7 g/dL is recommended. All patients must be closely monitored for any signs or symptoms of respiratory compromise, including ARDS (based on Berlin criteria – see appendix VII). A confirmed diagnosis of ARDS should lead to premature discontinuation of the drug infusion. Appropriate treatment and monitoring, including the use of invasive mechanical ventilation, low tidal volume, permissive hypercapnea, or high PEEP, should proceed according to the standard clinical practice at each institution. In patients with respiratory failure, strategies that decrease oxygen utilization, such as antipyretics to control fever and sedatives to control agitation; and appropriate use of neuromuscular blockade when asynchrony with the ventilator persists despite adequate sedation are recommended.

Infections with Yersinia enterocolitica and Y. pseudotubeculosis have been reported in DFO-treated patients. Although these are unlikely to occur in patients without significant systemic iron overload, appropriate bacteriological tests should be performed, and suitable antibiotic coverage for Yersinia should be instituted if a patient in the study develops fever accompanied by acute enteritis, abdominal pain, or pharyngitis. Appropriate treatment and monitoring should proceed according to the standard clinical practice at each institution.

7.4 CRITERIA FOR INTERVENTION DISCONTINUATION

The investigators may terminate the study drug infusion if, in their judgment, its continued administration poses harm to the patient's medical condition. The only pre-established criteria for premature discontinuation of the study drug are: 1) severe allergic reaction or anaphylaxis; 2) worsening of renal functions, defined as serum creatinine >2 mg/dl on 2 repeated measures 8 hours apart; 3) development of ARDS based on Berlin criteria/definition (see appendix VII); or 4) if the patient or his proxy voluntarily withdraws consent. Subjects whose study drug is discontinued but who do not withdraw consent should still be followed for 180 days.

7.5 REPORTING OF ADVERSE EVENTS

In order to ensure prompt reporting of adverse events, all adverse events (as well as all related study data) must be entered into the WebDCU™ within five working days following the completion of the Baseline, Treatment, and Day-7 or discharge (whichever occurs first) trial phases. All serious adverse events (SAEs) must be reported on the WebDCU™ within 24 hours of the study site staff first being made aware of its occurrence. The 24-hour reporting requirement for SAEs applies to all study phases. The investigators are required to provide relevant information, including description of the adverse event, date/time of onset and resolution, severity and seriousness, action taken, and suspected relationship to the study drug. Similarly, all adverse events of respiratory compromise, whether serious or not, must be reported in WebDCU™ and the relevant CRF completed within 24 hours of the study site staff first being made aware of its occurrence.

Reporting of respiratory compromise, as well as any serious or life-threatening adverse event, will trigger notification of the event to the Project Manager (PM), the independent MSMs, and appropriate members of the Executive Committee (EC). The MSMs will conduct an independent review of each of these events to determine its relatedness to the study drug along with other elements, and to confirm or exclude the diagnosis of ARDS in cases of respiratory compromise. Within 72 hours of receipt of these events for review, the MSMs will enter into the trial's database their opinion regarding whether the adverse event is, in fact, serious, and if it is unexpected and related to the study drug. If the MSMs believe all three elements are present in the SAE, an expedited safety report (MedWatch) will be filed with the FDA within the stipulated regulatory guidelines: for fatal or life-threatening SAEs, no later than 7 calendar days after the sponsor's initial receipt of information, and for SAEs outside those categories, no later than 15 calendar days after the sponsor determines that the event requires expedited reporting (21 CFR 312.32(c)(1)). If the study site investigator(s) and the MSMs are not in agreement on these issues and fail to resolve their disagreement after direct communication, the opinion of the MSMs will be considered as the final adjudication. When a MedWatch Report is filed with the FDA, a copy of the MedWatch Report (or a link to a secure file in the trial database) will be sent to the DSMB through the NINDS liaison.

Each clinical site PI and primary Study Coordinator will receive an email notification that a MedWatch Report has been filed with FDA. The email notification will contain instructions for accessing the report. It is the responsibility of each clinical site PI to file these reports with his/her IRB in compliance with their institution's local requirements. After the submission of the initial MedWatch Report, the principal investigator at the corresponding clinical site will be responsible for obtaining follow-up information about the event and reporting it in the WebDCU™.

7.6 MONITORING OF ADVERSE EVENTS

Two independent Medical Safety Monitor (MSMs), appointed in consultation with the NINDS program director, will monitor the study with regard to safety on an ongoing basis to identify any safety concerns. The MSMs will review all cases of respiratory compromise and all SAEs to determine whether they are related to study drug administration, and to ascertain the diagnosis of ARDS in cases of respiratory compromise. They will communicate with the investigators

for any questions or clarifications regarding an event. Periodically throughout the study, the EC and the MSMs will review reports on the incidence rates of all reported adverse events, whether serious or not, with particular attention to SAEs, respiratory compromise including ARDS, and mortality during the first 7 days of hospitalization (or until discharge, whichever is earlier). Should such monitoring uncover issues that may threaten subject safety (e.g. unexpectedly high rate of adverse events), the study statistician and principal investigator will prepare a report to be submitted to the DSMB for their review and further actions to be taken, if any.

Two statistical reports will be generated semiannually (unless requested at a more frequent interval by the MSMs or DSMB) – an open report to be distributed to the Executive Committee and MSMs, and a closed report to be distributed only to the NINDS-appointed DSMB. Each semi-annual report will provide cumulative summary statistics on enrollment, subject status in the study, baseline characteristics, protocol violations, safety data (including a summary of the most frequent and most serious adverse events, a summary of all MedWatch Reports, and a listing of all subjects who were terminated from the study and the reason for termination), and data management/quality information. The statistics will be provided for the overall study. For the closed report only, the statistics will also be provided by partially blinded treatment group (A vs. B). If the DSMB wishes to be completely unblinded for these reports, a sealed identification envelope will be provided to the NINDS DSMB liaison; this envelope can be opened at the discretion of the DSMB. An annual report will be submitted to the FDA and Health Canada.

7.7 STOPPING RULE

The occurrence of ARDS will be continuously monitored during the course of the trial, in order to facilitate thorough review of the data by the DSMB and to stop enrollment into the trial if sufficient evidence of imbalance in the rate of ARDS exists. The NINDS-appointed DSMB recommended that recruitment be stopped if the difference in the number of confirmed ARDS cases between the groups is 5 at any time during the recruitment of the first 40 subjects; 10 at any time during the recruitment of subjects 41-80; 12 at any time during the recruitment of patients 81-120; or if the difference in the number of confirmed ARDS cases between the groups is statistically significant after 40, 80, or 120 subjects have completed the in-hospital phase based on a Pocock-adjusted, one-sided, 0.05 alpha level. This one-sided hypothesis is consistent with our concern for patient safety, wherein early termination is considered only if ARDS incidence is higher on the DFO arm than on the control arm.

These analyses will be based on the occurrence of confirmed ARDS cases (according to the Berlin criteria) thought to be at least possibly related to study drug. The ARDS cases will be reviewed on an ongoing basis throughout the trial by the MSMs (who will adjudicate all cases together to provide a consensus opinion about the diagnosis, severity, and possible plausible causes other than the study drug). . The DSMB will be notified of each individual ARDS occurrence upon confirmation by the MSMs, per their request. Given that a common definition for the diagnosis of ARDS will be used, we do not anticipate much discrepancy between the site's assessment and the MSM's assessment. However, in the event of disagreement, the MSM's assessment will be used.

8 STATISTICAL CONSIDERATIONS

8.1 RANDOMIZATION PROCEDURES

A combination of minimization and biased coin methodologies will be used to randomize participants to either DFO or placebo [Pocock 1975; Taves 1974]. The minimization method aims to control imbalance in the treatment groups with respect to pre-specified baseline characteristics; the biased coin approach is adopted to avoid deterministic assignment. Throughout the trial, the probability of treatment assignment will take into account the imbalance associated

with clinical site, baseline ICH score, OTT, baseline ICH volume, baseline NIHSS score, and concurrent use of anticoagulants at the time of ICH onset.

8.2 STUDY ENDPOINTS AND DATA ANALYSIS

8.2.1 CLINICAL ENDPOINTS

The primary efficacy outcome measure is the mRS, dichotomized to define good functional outcome as mRS score of 0-2 at 90 days. At the conclusion of the study, the proportion of DFO-treated subjects with a good outcome will be compared to the placebo proportion in a futility analysis. The primary futility hypothesis is tested via generalized linear model relating the probability of a good outcome to the treatment. Adjustment for OTT, ICH score, baseline ICH volume, NIHSS score, and anticoagulant use are included in the model to obtain proper significance due to the inclusion of these variables in the randomization scheme. The binomial distribution for Y, with the identity link, is used to derive an estimate of the adjusted risk difference for good outcome. The primary futility hypothesis, H_0: (π_{DFO}-$\pi_{placebo}$) ≥0.12, will be tested at one-sided alpha (the probability that an effective intervention will be called ineffective, or futile) 0.10. The corresponding alternative hypothesis, H_A: (π_{DFO}-$\pi_{placebo}$) <0.12, defines futility as a treatment effect less than absolute 12% in favor of DFO. The futility analysis will be conducted using a one-sided 90% upper confidence bound on the risk difference, which is consistent with the one-sided alternative hypothesis and stated level of significance. To declare futility, the entire interval must lie below the value 0.12, indicating that the true difference in risk of good outcome is less than 0.12 with 90% confidence. Under this design, a significant result would suggest that it would be futile to move DFO forward to Phase III testing.

As secondary analyses of the primary outcome, the presence of a differential treatment effect in the OTT windows will be explored. The generalized linear model described above for the primary analysis will be expanded to include an interaction between treatment and OTT window. While the Trial will be underpowered to definitively address this question, the magnitude of the treatment effect, and corresponding confidence interval, will be estimated for each time window. A dichotomized analysis considering the proportion of DFO- and placebo-treated subjects with mRS 0-3 will also be performed. Although mRS 0-3 is less favorable than the primary outcome of mRS 0-2, it would still be a desirable effect in patients with ICH given that no treatments exist to reduce disability. The trial is adequately powered to assess the futility hypothesis using mRS 0-3 as the outcome based on an absolute difference in treatment effect <13% in favor of DFO. We will also perform similar analyses at 180 days and ordinal analysis across all mRS scores as secondary outcome measures.

8.2.2 SAFETY ENDPOINTS:

All adverse events will be assessed until day-7 or discharge (whichever is earlier), and SAEs until day-90. Mortality (all causes and ICH-related) will be assessed until the end of the study (day-180). Safety endpoints of particular interest include all DFO-related adverse events until day-7 or discharge (whichever is earlier), all SAEs through day-90, and deaths (all causes and ICH-related) through day-180.

The following events will be defined as **ADVERSE EVENTS OF SPECIAL INTEREST** (AEOSI) for safety surveillance during this study: 1) Anaphylaxis (at any time point during study drug infusion); 2) Hypotension (defined as a decrease in blood pressure requiring medical intervention at any time point during drug infusion that cannot be explained by other causes); 3) Respiratory compromise of any cause during the in-hospital phase (day-7 or discharge, whichever is earlier); and 4) Development of new and unexplained visual or auditory changes after initiating treatment with the study drug.

All adverse events or complications will be submitted electronically via the WebDCU™ system and assigned to a system-organ class and preferred term using MedDRA coding dictionary. The number and percent of all adverse events will be summarized for each treatment arm by event type. Tables will be generated to detect any clinically significant laboratory abnormalities in change of values from baseline to end of the last infusion. We anticipate no missing safety data regarding adverse events, since patients will remain hospitalized and closely monitored until day-7 or discharge (whichever is earlier). The cumulative incidences of each AEOSI, as well as mortality, will be compared via 95% confidence intervals. Mortality will also be assessed via the log rank test for comparing survival curves.

Analysis of the safety data regarding the occurrence of ARDS will be carried out continuously throughout the trial by the unblinded study statistician, who will notify the EC and DSMB if recruitment must be stopped. The DSMB will be notified of each individual ARDS occurrence upon confirmation by the MSMs, per their request. Analysis of other safety data by the DSMB will occur semiannually (or more frequently if requested by the DSMB). If any of these analyses reveal serious emerging concerns, the EC, in consultation with the DSMB, may implement modifications to assure safety as necessary. Apparent, consistent and persistent evidence of net harm that tends to overwhelm any benefit may allow for premature termination of the study.

8.3 STUDY SAMPLES

ALL analyses will involve data from two samples:

1- Modified Intent-to-Treat (mITT): Outcome data from all subjects who are randomized and in whom the study infusion begins, even if it is discontinued prematurely, will be analyzed. Classically, ITT analysis is used in Phase III trials and includes all subjects randomized, regardless of whether they receive the treatment or not. However, in order to evaluate the efficacy of DFO as administered, randomized subjects in whom the study infusion is never initiated will be excluded from the primary analysis. As a sensitivity analysis, the futility hypothesis will also be assessed according to the ITT principle. All subjects randomized will be included and considered in the treatment group to which he/she was randomized, regardless of the treatment actually received. The outcomes for subjects in whom treatment is never initiated will be imputed based on available baseline data.

Safety analysis will involve data from all patients in whom study infusion begins, even if it is discontinued prematurely (i.e. the mITT sample).

2- Per-protocol sample, i.e. all subjects who have at least one post-treatment assessment and no major protocol violations that affect the analysis. The Executive Committee (EC) will adjudicate major protocol violations and patients' inclusion in the per-protocol population before unblinding. Analyses based on the per-protocol sample will be supportive in nature.

8.4 SAMPLE SIZE CALCULATION

The sample size is calculated to achieve 80% power for the futility analysis described above using a one-sided alpha level of 0.10. It is anticipated that approximately 28% of control subjects will have mRS 0-2 at 3 months; this is based on the weighted average of the good outcome proportions reported in the placebo-treated subjects of the Factor VII, FAST, GAIN, and CHANT trials [Haley 2005; Lyden 2007; Mayer 2005-2008]. If the true good outcome proportions in the DFO and placebo arms are identical (π_{tx}=0.28, π_{ctrl}=0.28, a truly futile situation), 254 subjects (127 in each arm) are required to test the futility hypothesis with 80% power. This calculation takes into account the 0.12 null value specified in the futility hypothesis [Chow, 2003]. The primary analysis will be conducted according to a modified Intent-to-Treat (ITT) principle, wherein subjects in whom the study drug infusion is not initiated will be excluded from the analysis. Therefore, the final sample size was inflated by a factor of 1.11 (Friedman, Furberg, and DeMets) to account for dilution of the treatment effect associated with a conservative drop-out rate of 5% (due to loss-to-follow-up (LTFU) and

withdrawal of consent), and an additional factor of 1.04 to account for randomized subjects in whom the study drug is not initiated. Therefore, the final sample size is 294 subjects.

8.5 LOSS-TO-FOLLOW-UP AND MISSING DATA

Extensive efforts will be made to keep missing data, particularly the primary outcome (mRS at 90 days), to a minimum, and to ensure near complete follow-up. Missing primary outcome data will be imputed via standard multiple imputation (MI) methods (i.e. via logistic regression model predicting outcome based on pertinent baseline and treatment data). As a sensitivity assessment, we will use the Last Observation Carried Forward (LOCF) approach, wherein the last available score will be carried forward for missing day 90 assessments. If the treatment effect is robust, we expect to reach similar conclusions via these imputation methods, particularly for minimal missing data (<5%).

9 STUDY MONITORING

9.1 MONITORING FOR STUDY PROGRESS AND RECRUITMENT

The Executive Committee will monitor recruitment progress at each site on a monthly basis to address individual site issues and concerns. To minimize unnecessary delays in enrolling eligible subjects into the early time window (≤12h from ICH onset), we will monitor the time-from-door-to-infusion on an ongoing basis and make every effort to ensure eligible subjects are enrolled as quickly as possible after ED arrival. If we observe that enrollment into the early (≤12h) time window is disproportionately low compared to the >12-24h window, we will implement a strategy of forced recruitment whereby each site will be required to enroll one subject into the early time window for each subject enrolled into the later (>12-24h) window (or maintain its overall ratio of ≤12h to >12-24h to at least 1:1) before subsequent enrollment into the later time window is permitted by the site. If frequent delays are observed at a particular site, the Principal Investigator will be asked to take the necessary corrective steps. The Executive Committee additionally may require that the infusion of the study drug must be started within a certain time window following the subject's arrival to the emergency room, at a particular site or across all sites, if necessary.

The Executive Committee will also monitor accrual of subjects on an ongoing basis throughout the study, and will conduct formal reviews of the overall recruitment across all sites and site-by-site on a yearly basis (or at more frequent intervals, if needed). For sites whose recruitment is less than 75% of their expected rate (approximately 4-6 subjects per year), the local principal investigator will be asked to submit his/her own analysis of the barriers to recruitment at his/her site and corrective actions and plans. The "low" enrolling sites will be monitored closely at 3-month intervals to determine if further corrective actions or dismissal from the trial are warranted.

9.2 QUALITY MANAGEMENT AND DATA CONTROL

The Data Coordination Unit (DCU) in the Department of Public Health Sciences at the Medical University of South Carolina (MUSC) serves as the Statistics and Data Management Center (SDMC) for the trial, coordinating all statistical, data and project management responsibilities. The study data will be managed (including data queries) by the SDMC using the WebDCU™ system. This user-friendly web-based database system, developed and validated by the SDMC, will be used for regulatory document management, subject randomization, data entry, data validation, project progress monitoring, subject tracking, user customizable report generation and secure data transfer. In addition to the study database, the SDMC will provide the clinical site staff access (via password) to a standard set of web-enabled tools, including subject visit calendar, subject accrual status, case report form completion status, and outstanding DCR status pertaining to their respective clinical sites. Furthermore, all approved study materials, such as the protocol, informed consent template and manual of procedures, will be housed on the website to ensure that the clinical sites always have access to the most current trial documents.

Data should be independently entered by the designated personnel at each clinical site into WebDCU within five working days following the completion of any trial phase – Baseline, Treatment, day-7 or discharge (whichever occurs first), Month 1, Month 2, Month 3, and Month 6. Enrollment data, however, must be entered into the WebDCU database before the subject can be randomized. The SAE and adverse events of respiratory compromise data must be entered into the WebDCU within 24 hours of the site staff's first awareness of the event. It is critically important to the effective and efficient conduct of the study that ALL data be entered in a timely manner. An electronic copy of the CRFs will be made available to the clinical sites prior to initiation of the study to be used as worksheets to capture the required data for the study. The SDMC staff will perform range verification, consistency checks, and quality assurance on the data. The staff at the SDMC will contact the sites regarding missing data or queries on CRF data. They will maintain direct contact with the staff at the participating sites to ensure the study is conducted according to the Good Clinical Practice Guidelines and FDA and all applicable regulations.

Experienced clinical research monitors will be contracted through the SDMC to perform on site source data verification (SDV) during the study. The first visit will take place after enrollment of the first subject by the site and will involve source document verification of 100% of the data. For subsequent subjects, a checklist of key outcome and safety data variables requiring SDV will be developed based on the trial's endpoints. The checklist ensures that a target of no less than 40% of the clinical data submitted to the Hi-Def database are verified against source documents at the performance sites prior to finalization of the database. Of the data on the checklist, the safety and efficacy variables represent approximately half of the data to be verified. The remaining half of source monitored data include: 100% of deaths and 100% of serious adverse events and source data reviews based on the per-patient evaluation of safety parameters defined in the protocol. All informed consent and HIPAA documents will be verified by the clinical research monitor. Subsequent visits to each site will depend on the number of patients recruited at the site as well as other issues. Monitoring will also involve, as appropriate, correspondence and telephone contacts. In addition to data verification, the monitor will evaluate drug accountability, site facilities, and regulatory documents. The CRFs and corresponding source documents should be made available to the study monitor at each site visit. It is also expected that the PI, or a designated member of the research staff, will be available during the monitoring visit to review the data and resolve any queries. The close out monitoring visit will take place after the study is completed.

The SDMC will prepare, at selected intervals, a summary report of screened and enrolled patients, completeness and quality of CRF data, status of enrolled patients, a listing of SAEs, and a table of event-specific cumulative rates. The Executive Committee and the independent Medical Safety Monitors will review these reports and monitor the study performance (recruitment, compliance, protocol violations, and follow up) and safety data on an ongoing basis. The study statistician will also generate a comprehensive statistical report bi-annually to the DSMB, unless requested at more frequent intervals.

9.3 ONGOING SAFETY MONITORING DURING THE STUDY

Two independent Medical Safety Monitors (MSMs), appointed in consultation with the NINDS program director, will monitor the study with regard to safety on an ongoing basis to identify any safety concerns. As mentioned above in section 7.6, the MSMs will review all SAEs and cases of respiratory compromise and determine whether they are related to study drug administration, and will communicate with the investigators for any questions or clarifications regarding an event. Periodically throughout the study, the EC and the MSMs will review reports on the incidence rates of all reported adverse events, whether serious or not, with particular attention to SAEs, ARDS, and mortality during the first 7 days of hospitalization (or until discharge, whichever is earlier). Should such monitoring uncover issues that may threaten subject safety (e.g. an unexpectedly high rate of adverse events), the study statistician and principal investigator will prepare a report to be submitted to the DSMB for their review and further actions to be taken, if any.

Analysis of the safety data regarding the occurrence of ARDS will carried out continuously throughout the trial by the unblinded study statistician, who will notify the EC and DSMB if recruitment must be stopped (please refer to section 7.7 for the stopping rule). The DSMB will be notified of each individual ARDS occurrence upon confirmation by the MSMs, per their request.Analysis of the overall safety data by the DSMB will take place periodically throughout the trial. The first analysis is planned after the first 40 subjects have completed the in-hospital phase of the study. The number and timing of subsequent analyses will be determined in consultation with the DSMB as the study progresses.

Safety analyses will be carried out at least semiannually (unless requested at more frequent intervals by the DSMB or MSMs). Two statistical reports will be generated – an open report to be distributed to the Executive Committee and MSM, and a closed report to be distributed only to the NINDS-appointed DSMB. Each semi-annual report will provide cumulative summary statistics on enrollment, subject status in the study, baseline characteristics, protocol violations, safety data (including a summary of the most frequent and most serious adverse events, a summary of all MedWatch reports, and a listing of all subjects who were terminated from the study due to adverse events, whether or not study drug-related), and data management/quality information. The statistics will be provided for the overall study. For the closed report only, the statistics will also be provided by partially blinded treatment group (A vs. B). If the DSMB wishes to be completely unblinded for these reports, a sealed identification envelope will be provided to the NINDS DSMB liaison; this envelope can be opened at the discretion of the DSMB. An annual report will be submitted to the FDA.

10 HUMAN SUBJECTS

10.1 ETHICAL CONDUCT OF THE STUDY

This study will be conducted in accordance with Declaration of Helsinki, and in compliance with the protocol, applicable regulatory requirements, and GCP/ICH guidelines (please refer to appendix VIII). The PI at each site is responsible for the care and medical follow-up of the patients throughout their participation in the study. If the PI is not present in the clinical site, he/she will leave instructions for other members of the study staff and a telephone number where he/she can be reached, if needed.

10.2 INSTITUTIONAL REVIEW BOARD (IRB) REVIEW AND INFORMED CONSENT

This protocol, the ICF, and any subsequent modifications must be reviewed and approved by the local IRB at each of the participating institutions. A signed and dated ICF must be obtained from the subject, his/her legal representative, or family member as defined in 21CFR50.3(m). The ICF must also be signed and dated by a member of the study staff qualified to be delegated the authority to obtain informed consent, and a witness (if required by the local IRB). A copy of the ICF must be given to the subject, his/her legal representative or family member and the consent process must be documented in the subject's medical record. The PI or delegated sub-Investigator is responsible for ensuring that informed consent is obtained from each patient, his/her legal representative, or family member prior to conducting any study-related activities.

No deviations from or changes to the study protocol should be initiated except when necessary to eliminate immediate hazard to the patient. However, the IRB and PI must be informed of this as soon as possible thereafter. It is the study site PI's responsibility to report SAEs occurring during the study and MedWatch/Safety Report to their IRB, as required and as soon as possible.

10.3 SUBJECT CONFIDENTIALITY

All participating study investigators must ensure that the confidentiality of personal identity and all personal medical information of study participants will be maintained at all times. Additionally, the clinical sites are to follow privacy obligations to study participants under the Health Insurance Portability and Accountability Act (HIPAA). All records re

garding this study must be securely stored in a locked cabinet. Subject confidentiality will be strictly maintained by the use of a subject ID number. The study database and any study documents submitted to the SDMC will only identify study subjects by this unique study identification code. All data will be stored in a manner that is HIPAA compliant, without the ability to track the information back to a specific subject except through a password protected system. Personal medical information may be reviewed for the purpose of verifying data recorded in the CRF by site monitors. Other properly authorized persons, such as the regulatory authorities, also may have access to these records. Personal medical information, however, always will be treated as strictly confidential. All SDMC personnel are certified by the NIH Office of Human Subjects Research in the Protection (OHRP) of Human Research Subjects course, or other training courses acceptable to the NIH OHRP.

In order to maintain the confidentiality of subjects who elect to participate in the blood bank repository, as well: 1) the results of any genetic analyses performed on samples collected from this repository will not appear in the medical records and will not be released to the subjects or their healthcare providers; 2) the samples will be coded and the key will be kept in a separate locked file; and 3) in the event of future second or third party use of the samples, all codes will be removed so that there will be no method by which the sample can be tracked back to the subject.

10.4 DATA AND SAFETY MONITORING PLAN

The DSMB, appointed by the NINDS and managed by the NINDS Clinical Trials group, will be responsible for the oversight of safety of trial participants, review of the safety reports, requesting additional data/information (if necessary), and advising the NINDS regarding continuation/ discontinuation of the study. The study may be modified or discontinued at any time by the NINDS, the FDA, or the Executive Committee to ensure that research subjects are protected. All ARDS cases will monitored continuously throughout the trial by the unblinded study statistician, who will notify the EC and DSMB if recruitment must be stopped (please refer to section 7.7 for the stopping rule). The DSMB will be notified of each individual ARDS occurrence upon confirmation by the MSMs, per their request. Analysis of the overall safety data by the DSMB will take place periodically throughout the trial. The first analysis is planned after the first 40 subjects have completed the in-hospital phase of the study (day-7 or discharge, whichever is earlier). The DSMB will meet to review the accumulated safety data, with particular attention to mortality, SAEs, and adverse events of special interest (AEOSI) including ARDS, from initiation of treatment to day-7 or discharge (whichever occurs first) to ensure that there are no emerging safety concerns. Unblinding of treatment assignments will only be allowed by the Chair of the DSMB, if felt necessary, but the unblinded data will be confidentially maintained within the DSMB. Enrollment will continue with the recommendation of the DSMB, including necessary modifications, if needed, to address any safety concerns early on.

After this meeting, the DSMB will regularly meet at least once approximately every 6 months or at more frequent intervals if needed to review recruitment and safety data. Analyses of the safety data will be carried out on an ongoing basis throughout the trial, as mentioned above. All AEOSIs, all SAEs, deaths, and adverse events will be reviewed by the DSMB during their meetings. There will be no limit on the number and timing of analyses aimed at guaranteeing the safety of the patients. An apparent, consistent and persistent evidence of net harm that tends to overwhelm any benefit may allow for premature termination of the study. Please refer to section 7.7 from the pre-specified stopping rule based on the frequency of ARDS cases.

10.5 STUDY MODIFICATION/DISCONTINUATION

The study may be modified or discontinued at any time by the NINDS, the OHRP, the FDA, or a study site IRB (but only for that individual study site) as part of their duties to ensure that research subjects are protected.

11 PUBLICATION OF RESEARCH FINDINGS

Publication and presentations of the results of this trial will be governed by the policies and procedures developed by the Executive Committee in conjunction with the NINDS. The Publication Policy will be fully compliant with the voluntary NIH Public Access Policy mandated by the Consolidated Appropriations Act of 2008 (Division G, Title II, Section 218 of PL 110-161). The Executive Committee will follow NIH policies on data sharing (as described at the site: [http://grants2.nih.gov/grants/policy/data_sharing/data_sharing_ guidance.htm] and any updates thereto). Any manuscript(s) will be made available for review by the sponsor and the NINDS prior to submission.

A Publications Subcommittee of the Executive Committee, consisting of Drs. Magdy Selim, Sharon Yeatts, Yuko Palesch, and Claudia Moy will establish a writing group headed by the Study Chair to oversee the writing of the main study-related manuscripts. Any of the Trial's investigators (clinicians as well as study coordinators) wishing to write a manuscript must first submit to the Publications Subcommittee a brief description/proposal of the manuscript content. The Subcommittee will review the proposal in a timely manner, and if the proposal is approved, it will be disseminated to all study investigators to encourage those who have similar interests in the topic to participate in the writing group. The Publications Subcommittee will appoint the writing group members. The Publication Subcommittee must review and approve all writing and presentation activities of the study prior to submission to a journal or a meeting for scientific presentation.

12 ANCILLARY STUDIES POLICY

Any of the study investigator(s) wishing to conduct ancillary studies must submit in writing to the Executive Committee a 2-3 page outline of the proposal. The Executive Committee will discuss the proposal, and each member of the Executive Committee will vote to approve or disapprove the proposal. The key criteria for evaluation are scientific merit, relevance to the major goals of the main study, and the ancillary study's impact on the conduct and feasibility of the main trial. If the proposal is approved by the Executive Committee, it will be forwarded to the DSMB members and the NINDS Administrative Officer who will also vote for approval or disapproval. Upon approval by the Executive Committee, the DSMB and the NINDS, the investigator(s) may then submit the proposal to potential funding source(s), if needed.

13 RESOURCE- AND DATA-SHARING PLAN

The Executive Committee will follow the NIH policies on data sharing [http://grants2.nih.gov/grants/policy/ data_sharing/data_sharing_guidance.htm], and any updates thereto. Sharing of the data generated by this study will be carried out in several different ways to make our results available to the scientific community interested in stroke and ICH and to avoid unintentional duplication of research. Our plan for data sharing will include: 1) Presentations at national and international scientific meetings, such as the International Stroke Conference, the Annual meeting of the American Academy of Neurology, the European Stroke Conference, and the International Cerebral Hemorrhage conference; 2) Posting information about stroke and ICH written primarily for a general audience on the study's website; 3) Making a description of this clinical trial and summary of the results available on www.ClinicalTrials.gov.

We would also welcome collaboration with others who could make use of the data generated by the study. Upon completion of the study, the public use database will be prepared by stripping any and all personal identifiers. The public use database, consisting of several data files, will contain: (1) baseline and demographic characteristics; (2) outcomes assessments; (3) CT data; (4) concomitant medications and procedures; and (5) adverse events. Each data file will be made available as a formatted SAS dataset or other electronic format. The data files will be distributed along with the data dictionary and a brief instruction file. Anyone wishing to access the data may do so by completing a data-sharing agreement and data request form and submitting those forms to the SDMC or subsequently to an external data archiving unit chosen by the NINDS.

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15 APPENDIX

I. DFO in ICH (Phase I) Manuscript
II. RECOMMENDED PROTOCOL FOR MANAGEMENT OF PATIENTS WITH ICH
III. ASA/AHA ICH MANAGEMENT GUIDELINES
IV. DETERMINATION OF ICH SCORE AND HEMATOMA VOLUME (ABC METHOD)
V. ASSESSMENT SCALES (NIHSS – GCS - mRS – MoCA – SIS-16) AND INSTRUCTIONS
VI. ARDSNet RECOMMENDATIONS FOR MANAGEMENT OF INTUBATED PATIENTS
VII BERLIN CRITERIA FOR DIAGNOSIS OF ACUTE RESPIRATORY DISTRESS SYNDROME
VIII. DECLARATION OF HELSINKI