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High Dose Deferoxamine in Intracerebral Hemorrhage (Hi-Def) Trial: Rationale, Design, and Methods

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Abstract

Background—Hemoglobin degradation products, in particular iron, have been implicated in secondary neuronal injury following intracerebral hemorrhage (ICH). The iron chelator Deferoxamine Mesylate (DFO) exerts diverse neuroprotective effects, reduces perihematoma edema (PHE) and neuronal damage, and improves functional recovery after experimental ICH. We hypothesize that treatment with DFO could minimize neuronal injury and improve outcome in ICH patients. As a prelude to test this hypothesis, we conducted a Phase I, open-label study to determine the tolerability, safety, and maximum tolerated dose (MTD) of DFO in patients with ICH. Intravenous infusions of DFO in doses up to 62 mg/kg/day (up to a maximum of 6000 mg/day) were well-tolerated and did not seem to increase serious adverse events (SAEs) or mortality. We have initiated a multi-center, double-blind, randomized, placebo-controlled, Phase II clinical trial (High Dose Deferoxamine [HI-DEF] in Intracerebral Hemorrhage) to determine if it is futile to move DFO forward to Phase III efficacy evaluation.

Methods—We will randomize 324 subjects with spontaneous ICH to either DFO at 62 mg/kg/day (up to a maximum daily dose of 6000 mg/day) or saline placebo, given by intravenous infusion for 5 consecutive days. Treatment will be initiated within 24 hours after ICH symptom onset. All subjects will be followed for 3 months and will receive standard of care therapy while participating in the study. At 3 months, the proportion of DFO-treated subjects with a good clinical outcome, assessed by modified Rankin Scale, will be compared to the placebo proportion in a futility analysis.

Conclusions—The Hi-Def trial is expected to advance our understanding of the pathophysiology of secondary neuronal injury in ICH and will provide a crucial “Go/No Go” signal as to whether a Phase III trial to investigate the efficacy of DFO is warranted.

Keywords

Intracerebral hemorrhage; Deferoxamine; Clinical trial; Futility design

Introduction

Intracerebral hemorrhage (ICH) is a devastating disease with no specific treatment other than supportive medical care. Recent attention has been largely focused on medical, endoscopic, and surgical treatments aimed at limiting hematoma expansion or hematoma reduction as a therapeutic target. However, neuronal injury after ICH is not only related to direct tissue damage and hematoma expansion. Apoptosis, iron-mediated oxidative stress, inflammation, autophagy, and edema formation contribute to secondary neuronal injury and disability after ICH [1–4]. There is an unmet need for novel interventions to target the secondary effects of ICH.

The iron chelator deferoxamine mesylate (DFO) is a promising candidate [5]. It confers substantial neuroprotection after hemorrhage in various animal models; reduces brain edema and neuronal death in the perihematoma region; and improves performance on sensorimotor behavioral tests [5–8]. DFO has multiple and diverse neuroprotective properties. By forming a stable complex with ferric iron, it decreases free iron’s availability for the production of hydroxyl radicals. DFO also alters iron regulatory genes and protein binding activity, thereby reducing cellular vulnerability to iron; prevents apoptosis by activating a signal transduction pathway leading to expression of genes known to compensate for oxidative stress, and inhibiting prolyl 4-hydroxylase activity; induces transcription of heme oxygenase-1; suppresses upregulation of activated c-Jun N-terminus kinase seen after ICH; and exerts anti-inflammatory effects by stimulating cyclooxygenase, and anti-phagocytic effects [2, 5, 9–12]. DFO also appears to have a modest blood pressure lowering effect when administered by intravenous infusion, which might be beneficial in ICH [13].

To translate these findings into the clinical setting, we completed a Phase I dose-finding study to determine the maximum-tolerated dose of DFO in ICH patients [14]; DFO infusions up to 62 mg/kg/day were well tolerated with an acceptable safety profile. A Phase II clinical trial [high dose deferoxamine (Hi-Def) in ICH] is currently under way to assess the futility of DFO as a therapeutic intervention in ICH before embarking on a large Phase III trial. In this article, we describe the Hi-Def trial design and methods, and provide the rationale for the choice of its innovative two-arm futility design.

Study Design and Methods

Design

Hi-Def is a prospective, multi-center, double-blind, randomized, placebo-controlled, Phase II clinical trial coordinated by Beth Israel Deaconess Medical Center and the Data Coordination Unit (DCU) at the Medical University of South Carolina. The primary objective of the trial is to assess whether it is futile to move DFO forward into Phase III efficacy testing. The primary outcome measure is the mRS score at 90 days, which is dichotomized to define good functional outcome as a score of 0–2. Under the futility hypothesis, if the difference in good outcome proportions is statistically less than absolute 12 % in DFO compared to the control, it would be futile to move DFO forward to Phase III.

Secondary objectives are to confirm the safety of this dose regimen of DFO infusions (62 mg/kg/day, up to a maximum daily dose of 6,000 mg), given for 5 consecutive days, in a large cohort of ICH patients; and to perform a set of pre-specified exploratory analyses to estimate: (1) the differences between early (< 12 h) and late (>12–24 h) time windows in DFO treatment effect on functional outcome; (2) the effect of DFO on neurological and cognitive functions, assessed by the National Institute of Health Stroke Scale (NIHSS), Stroke Impact Scale-16 (SIS-16), and Montreal Cognitive Assessment; and (3) the effects of DFO on relative perihematoma edema (PHE) progression, the incidence of symptomatic cerebral edema (defined as edema accompanied by an unexplained increase in NIHSS score >4 points, or a decrease in Glasgow Coma Scale score >2 points) during the first week after ICH, and brain atrophy as potential markers of DFO biological activity on brain tissue.

The Hi-Def trial is supported by the National Institute of Neurological Disorders and Stroke (U01 NS074425) and registered with clinicaltrials.gov (NCT01662895). The trial is approved by the US Food and Drug Administration (Investigational New Drug #77306) and Health Canada (Clinical Trial Application #160713).

Methods

An anticipated total of 324 subjects with spontaneous ICH will be enrolled from approximately 28 centers in the United States and Canada. In order to be eligible for the trial, the diagnosis of ICH must be confirmed by brain computed tomography (CT) scan, and treatment must be expected to be initiated within 24 h of ICH symptom onset. The full set of inclusion/exclusion criteria are detailed in Table 1. Eligible patients for whom written informed consent is obtained will be randomized to receive either DFO or a matching saline placebo. The randomization scheme, centrally implemented by the DCU, controls imbalances associated with baseline ICH score (0–2 vs. 3), ICH onset-to-treatment time (OTT) window (< 12 h vs. >12–24 h), and concurrent use of anticoagulants at ICH onset.

All study participants will be followed for 90 days and will receive standard of care therapy according to the guidelines from the Stroke Council of the American Heart Association. Subjects will undergo repeated clinical, imaging, and laboratory evaluations at regular intervals throughout their participation in the study according to the study protocol. All adverse events will be reported through day-7 or discharge (whichever is earlier), and SAEs will be collected through study completion. Study participants will be assessed for the clinical outcomes in person at 30 (± 7) and 90 (± 7) days; the subjects or their surrogates will be contacted by phone on day 60 (± 7) days.

Study Procedures

Randomized subjects will be reassessed immediately prior to the study drug administration to obtain a pre-treatment baseline neurological status (NIHSS and GCS “Glasgow Coma Scale”). Study drug should not be initiated in subjects who exhibit significant clinical and neurological deterioration (i.e., develop fixed and dilated pupils or GCS decreases to ≤ 6); these subjects will be deemed post-randomization screen failures, and their study participation will be terminated. Weight-adjusted DFO (62 mg/kg/day, up to a maximum daily dose of 6,000 mg/day) or saline placebo infusions (according to randomization assignment) will be initiated within 24 h of ICH symptom onset and given by IV infusion for 5 consecutive days in a blinded manner. Vital signs and neurological functions will be assessed at least once every 4 h during drug infusions. In addition, general physical and visual and auditory assessments will be performed daily up to 24 h following the last infusion. The study-specific assessments are provided in Table 2.

A plain CT scan of the brain will be performed at three protocol-specified time points: (1) pre-randomization to establish the diagnosis of ICH; (2) within 24 h of completion of the last infusion of the study drug to assess for PHE growth; and (3) at 90 days to assess for the size of the residual hematoma cavity and brain volume. Computerized volumetric measurements of the imaging data will be performed by blinded operators at the central imaging laboratory at Beth Israel Deaconess Medical Center. Additional brain imaging with magnetic resonance imaging or CT angiography at other time points will be performed at the discretion of the treating physicians as part of routine clinical care.

Laboratory assessments beyond those required for the study will be done whenever clinically indicated. The Hi-Def study incorporates a blood banking repository sub-study. Blood samples will be collected from Hi-Def participants who consent to participate in the sub-study before initiation and following completion of the study drug infusions. These samples will be stored for future, yet to be fully specified, research in ICH, including possible pharmacogenetic studies.

Statistical Considerations

Outcome Measures and Data Analysis

Efficacy—The primary 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 (π_{DFO}) will be compared to the placebo proportion (π_{placebo}) in a futility analysis. The primary futility hypothesis is tested via generalized linear model relating the probability of a good outcome to the treatment, adjusting for injury severity (ICH score stratum), OTT window, and anti-coagulant use as covariates. The binomial distribution is used with the identity link to derive an estimate of the adjusted risk difference for good outcome. Adjustment for OTT window, ICH score, and anti-coagulant use are included in the model to obtain proper significance due to the inclusion of these variables in the randomization scheme.

The primary futility hypothesis, $H_0: (\pi_{\text{DFO}} - \pi_{\text{placebo}}) \geq 0.12$, will be tested at one-sided alpha (the probability that an effective intervention will be called ineffective or futile) of 0.10. The corresponding alternative hypothesis, $H_A: (\pi_{\text{DFO}} - \pi_{\text{placebo}}) < 0.12$, defines futility as a treatment effect less than absolute 12 % in favor of DFO. This hypothesis will be tested 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 statistically significant result would suggest that it is futile to move DFO forward to Phase III testing.

As secondary analyses of the primary outcome, the presence of a differential treatment effect between 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 is underpowered to definitively address this question, the magnitude of the treatment effect, and corresponding confidence interval, will be estimated for each time window.

A secondary futility analysis, conducted as described above, will also be performed using mRS 0–3 versus 4–6 as the dichotomous outcome. Although mRS 0–3 is less favorable than the primary outcome (mRS 0–2), it would still be a desirable effect given that no treatment exists to reduce disability following ICH.

Safety—All adverse events will be assessed through day-7 or discharge (whichever is earlier), and SAEs through study completion (day-90). Safety endpoints will include all DFO-related adverse events until day-7 or discharge (whichever is earlier), and all SAEs and all deaths through day-90. The following events will be defined as safety events of special interest for safety surveillance during the 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); and (3) development of new and unexplained visual or auditory changes after initiating treatment with the study drug.

Study Samples

Classically, ITT analysis includes all subjects randomized, regardless of whether they receive the treatment or not, and is considered the gold standard in Phase III clinical trials. However, in order to evaluate the effect of DFO as administered, the futility analysis is conducted under a modified intention-to-treat (mITT) principle. Outcome data from all subjects who are randomized and in whom the study infusion begins, even if it is discontinued prematurely, will be analyzed in the treatment arm to which they were randomly assigned; randomized subjects in whom the study infusion is never initiated will be excluded from the primary analysis. Safety analysis will also involve data from the mITT sample.

As a sensitivity analysis, the futility hypothesis will also be tested according to the true ITT principle—all subjects randomized will be included and considered in the treatment group to which the subject was randomized, regardless of the treatment actually received. As study participation is terminated for subjects in whom the study infusion is never initiated, the missing primary outcome will be imputed based on available baseline data.

Sample Size

The total sample size of 324 subjects was calculated to achieve 80 % power for the futility analysis described above using a one-sided significance level of 0.10. It is anticipated that approximately 28 % of control subjects will have mRS 0–2 at 90 days; this is based on the weighted average of the good outcome proportions reported in the placebo-treated subjects of previous ICH trials [15–18]. If the true good outcome proportions in the DFO and placebo arms are identical ($\pi_{\text{DFO}} = \pi_{\text{placebo}} = 0.28$, 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 [19]. The final sample size is inflated by a factor of 1.23 [20] to account for dilution of the treatment effect associated with a conservative drop-out rate of 10 % (due to loss-to-follow-up and withdrawal of consent), as well as an anticipated <4 % of randomized subjects in whom the study drug is not initiated.

Lost-to-Follow-up and Missing Data

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).

Missing primary outcome data will be imputed via standard multiple imputation methods, wherein a logistic regression model predicting outcome based on pertinent baseline and treatment data is used to generate multiple data sets with complete primary outcome data.

Interim Data Monitoring

There is no planned interim analysis for overwhelming efficacy or futility. An independent Data and Safety Monitoring Board (DSMB) has been appointed by the NINDS to monitor the progress of the trial. The DSMB will review accumulated safety data following the completion of the in-hospital phase for the first 20 subjects, and semi-annually thereafter. If any of these analyses reveal serious emerging concerns, the DSMB may recommend protocol modifications to assure safety. There is no limit on the number and timing of analyses aimed at guaranteeing the safety of the participants, and there are no formal boundaries or stopping rules. However, an apparent consistent and persistent evidence of net harm that tends to overwhelm any benefit may allow for premature termination of the study by the DSMB and NINDS.

Rationale for the Study Design

Rationale for the Choice of the Treatment Time Window

The decision to initiate DFO treatment up to 24 h after ICH symptom onset is based on data from animal studies. In aged rats, DFO administered within 2, 4, 12, 24, and 48 h after the induction of ICH improved forelimb placing and corner turn scores compared to vehicle treatment regardless of the time to treatment window. However, functional recovery occurred faster when the treatment with DFO was begun within 24 h as opposed to 48 h (28 vs. 56 days), suggesting greater efficacy for earlier treatment [8]. This finding might 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.

Despite suggesting that earlier treatment might accelerate recovery, animal data do not strongly support limiting treatment to an earlier time window, such as within 12 h. Although the optimal therapeutic window of DFO to reduce PHE formation was about 12 h, the correlation between PHE and deficits on sensorimotor behavioral tests conducted weeks-to-months after ICH was weak [8]. In humans, the influence of PHE on long-term recovery of ICH patients remains debatable [21–24]. A serial MRI study of the natural history of PHE in patients with ICH found that PHE correlated with worsening neurological status at 48 h, but not 3-month functional outcome [25]. Therefore, restricting the time-to-treatment to 12 h or even less, for the sole purpose of its potential effect on PHE, seems unnecessary. In addition, attempting to restrict the OTT window to <12 h could limit recruitment into the study as well as the generalizability of the results to the wider ICH population. Inclusion of OTT window in the randomization algorithm and analysis allows us to explore a potential differential treatment effect according to time window.

Rationale for the 5-Day Duration of Treatment

Animal studies suggest that treatment with DFO for any duration ≥ 2 days results in improved functional performance compared with vehicle treatment, and there is a dose response in regards to duration of treatment up to 7 days [8]. A minimum duration of treatment of 3 days was thought to be prudent from a pathophysiological and clinical standpoint. In ICH patients, although PHE volume continues to grow for approximately 2 weeks after ICH, the growth is fastest in the first 2–3 days, and the initial growth (and not the later one) is associated with neurological worsening [23–25]. In addition, the time course for hemoglobin hemolysis is approximately 2–3 days [26], and iron in the perihematoma tissue peaks at day 3 after experimental ICH [27, 28]. Although a 7-day duration of treatment was optimal in animal studies [8], the 5-day treatment duration was chosen in Hi-Def based on careful considerations of animal data and the practical aspects related to clinical trial conduct and care of ICH patients. Extending the treatment duration to >5 days

in ICH patients posed significant practical challenges. We surveyed participating sites and found that the average length of hospital stay (LOHS) for ICH patients was approximately 6.5 ± 1.2 days (median 6 days). Overall, the LOHS was 7 days in only 36 % of patients. These considerations raised the possibility that even if 7-day treatment with DFO were to be more efficacious, it might prove difficult to apply in real-world practice, limiting the agent's usefulness. Five days represented the best compromise between maximizing drug efficacy and successfully testing and applying the agent in practice; it provides the potential for improved drug efficacy without significantly jeopardizing recruitment into the trial.

Rationale for the Futility Design and Placebo Arm

The traditional concurrently controlled Phase II design, intended to estimate treatment effect and assess variability, is often criticized as an underpowered Phase III trial. The single-arm futility design [29] was proposed as an alternative, with the objective of weeding out ineffective therapies. A treatment effect less than the pre-specified futility threshold would be considered evidence that DFO is not of sufficient promise to consider a large-scale and costly Phase III trial. The futility design cannot by itself provide sufficient evidence to indicate that treatment with DFO is efficacious, since it is not designed to test the efficacy hypothesis. Furthermore, failure to declare futility does not guarantee a positive Phase III result, which is important in preserving clinical equipoise for future trials.

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 patient management, variations in data quality and protocol adherence, and differences in the eligibility criteria and specification of primary outcome measure among the various studies). These confounding variables can distort estimates of the historical reference proportion, are difficult to account for in a single-arm study, and can dramatically alter the relevance of the statistical hypothesis being tested. The inclusion of a concurrent placebo arm addresses these concerns and allows for secondary exploratory analyses to examine the effects of DFO on PHE progression and brain atrophy as surrogate markers of its biological activity on brain tissue.

Rationale for the 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, in the absence of evidence that smaller differences will change practice patterns, previous and ongoing Phase III trials in ICH, such as ATACH 2, used MCID of 10 % or greater. For example, in the GAIN International and GAIN America studies of the glycine antagonist, Gavestinel, the rate of mRS 0–2 was approximately 4–5 % higher in Gavestinel-treated patients compared with placebo, but this difference was not considered sufficiently promising to warrant further development as a therapy for ICH [16]. Given the lack of previous positive Phase III trials in ICH, the specified threshold for futility was based on data from previous ICH studies, and the NINDS rt-PA trials in ischemic stroke. In the Phase II study of factor VIIa in ICH (the only positive study in ICH), the proportion of treated patients with mRS scores 0–2 at 3 months was 12.8–17 % (depending on the dose) greater than the placebo patients [18]. 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 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) [30], the futility threshold was set at absolute 12 %. In other words, a treatment effect which is less than 12 % in favor of DFO will be considered evidence of futility. A lower futility threshold would require smaller effect sizes in order to declare futility, and thus larger effect sizes could be considered non-futile, but at the expense of a much larger sample size. The specified futility threshold of 12 % 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 therapy with its associated expense and resources, which could hinder the development of truly effective treatments for ICH).

The Rationale for the 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 [31]. 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, we will also evaluate the proportion of DFO- and placebo-treated subjects with mRS 0–3 at 90 days. 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 less than 13 % in favor of DFO.

Discussion

ICH confers a substantial global burden on the healthcare system and society [32]. Effective and innovative therapies for ICH are desperately needed. There are several conceptual innovative and translational aspects to the Hi-Def trial, its design, and candidate intervention, DFO.

On a translational level, the trial builds on a large body of pre-clinical and clinical research [5–9] and represents an ideal example for a roadmap to bring new therapies from the bench to the bedside following the stroke therapy academic industry roundtable recommendations [33]. Unlike previous and ongoing clinical investigations in ICH which mostly focus on targeting hematoma size, the Hi-Def trial targets the secondary late effects of ICH, in particular those mediated by hemoglobin degradation products and iron, which continue for days after ictus. This provides the opportunity to deliver therapy within a relatively long time window, which enhances the utility of this potential therapy and expands its applicability to a large number of ICH patients. Furthermore, DFO is relatively inexpensive, simple to administer, and does not require highly specialized skills or facilities. If DFO proves to be efficacious, it may provide a complimentary therapy to ongoing endeavors to reduce hematoma size and its expansion.

The design of Hi-Def is innovative in the area of ICH and stroke research. We chose a Phase II concurrently controlled futility design, which has important feasibility, cost, and time saving implications. The concurrently controlled futility design allows us to avoid the pitfalls associated with the use of historical data and to directly compare the treatment arms, while maintaining the objective of establishing futility rather than demonstrating efficacy of

the intervention. The information obtained from such designs can help guide the allocation of resources in future stroke research, as it allows us to rapidly discard ineffective therapies.

Analysis of the treatment effect in most trials in ICH to date has relied on post-stratification adjustment for various variables known to influence outcome. In Hi-Def, we elected to use covariate-adjusted randomization based on ICH score, OTT, and concurrent use of anticoagulants to prevent imbalances between the treatment groups for these variables. The ICH score and anticoagulation-associated ICH are known to influence overall prognosis after ICH and OTT could potentially influence the effectiveness of our therapeutic intervention.

We also carefully drafted our inclusion and exclusion criteria to minimize a high rate of early death during the time period of our intervention, which could substantially limit our ability to evaluate the effects of DFO treatment in our study population; and to avoid inclusion of patients with minimal deficits and high chance for complete recovery, which could make it difficult to discern a potential beneficial effect. Therefore, we limited inclusion to only those with GCS score >5 and measurable neurological deficits, defined via NIHSS score ≥ 6 . We also excluded patients with infratentorial ICH given the high rate of surgical intervention in patients with cerebellar hemorrhages and dismal prognosis in most patients with brainstem hemorrhages.

In addition to providing a crucial “Go/No Go” signal as to whether a Phase III trial to investigate the efficacy of DFO is worthwhile, the Hi-Def trial is expected to generate a wealth of data that will help to advance our understanding and knowledge of the pathophysiology of secondary neuronal injury in ICH. For example, pre-planned exploratory analyses will allow us to probe the relationship between ICH, brain atrophy, and cognitive function, as well as the relationship between dichotomized PHE volumes and outcome. These additional analyses, together with the blood banking repository, can generate novel hypotheses and opportunities for future investigations in ICH.

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Table 1

Inclusion and exclusion criteria for the Hi-Def trial

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 24 h 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 are obtained
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 tumor, 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 low-molecular-weight heparin
11	Taking iron supplements containing 325 mg of ferrous iron, or prochlorperazine
12	Patients with heart failure taking >500 mg of vitamin C daily
13	Known severe hearing loss
14	Known pregnancy, or positive pregnancy test, or breastfeeding
15	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
16	Any condition which, in the judgement of the investigator, might increase the risk to the patient
17	Life expectancy of less than 90 days due to co-morbid conditions
18	Concurrent participation in another research protocol for investigation of another experimental therapy
19	Indication that a new DNR or comfort measures only (CMO) order will be implemented within the first 72 h of hospitalization

Table 2

Schedule of assessments in the Hi-Def trial

	Screening	Baseline	0 h	24 h	48 h	72 h	96 h	120 h	Day 7 or discharge ^b	As needed	Day 30	Day 60 (phone)	Day 90	End of study
		Prior to study drug start	±6 h	±6 h	±6 h	±6 h	±6 h	±6 h	±6 h		±7 days	±7 days	±7 days	
Study procedures														
Inclusion/exclusion criteria	X													
Demographics and medical history	X													
Informed consent	X													
Subject enrolment/randomization		X												
Study drug administration			X	X	X	X	X	X						
Clinical assessments														
Physical examination	X		X	X	X	X	X	X		X	X		X	
Visual and auditory assessment ^a	X		X	X	X	X	X	X	X	X	X		X	
NIHSS	X	X	X	X	X	X	X	X	X	X	X			
GCS	X	X	X	X	X	X	X	X	X	X				
ICH score	X													
mRS	X								X		X	X	X	
MoCA									X		X		X	
SIS-16											X		X	
Vital signs (BP and pulse) ^c	X		X	X	X	X	X	X	X	X				
Safety monitoring		X	X	X	X	X	X							
Urinary output			X	X	X	X	X	X						
Laboratory assessments														
Hematology	X							X		X				
Serum chemistry	X							X		X				
Coagulation parameters	X							X		X				
Urine analysis	X							X		X				
Liver function tests	X							X		X				
Kidney function tests	X							X		X				

Screening	Baseline	0 h	24 h	48 h	72 h	96 h	120 h	Day 7 or discharge ^b	As needed	Day 30	Day 60 (phone)	Day 90	End of study
	Prior to study drug start	±6 h	±6 h	±6 h	±6 h	±6 h	±6 h	±6 h	±7 days	±7 days	±7 days	±7 days	
Serum iron studies (iron, ferritin, transferrin, and iron binding capacity)	X						X		X				
Pregnancy test (women of childbearing potential)	X												
Blood sample for future research ^d	X						X						
Brain imaging													
CT scan	X						X ^e		X			X	
Miscellaneous													
Prior medications	X												
Concomitant medications		X	X	X	X	X	X	X		X	X	X	
Concomitant non-drug therapies		X	X	X	X	X	X	X		X	X	X	
Adverse events		X	X	X	X	X	X	X	X	X	X	X	
End of study													X

^a Assessments for cataracts, visual loss or field cut, disturbed color of tinnitus—should be performed, whenever possible in awake patients

^b Day 7 or discharge—whichever comes first

^c Vital signs (blood pressure and pulse) and neurological functions will be assessed at least once every 4 h during intravenous drug infusion

^d 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

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