FERAHEME AS AN MRI CONTRAST AGENT FOR PEDIATRIC CONGENITAL HEART DISEASE

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PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Investigator, my duties include ensuring the safety of the study subjects enrolled under my supervision and providing the National Institutes of Health with complete and timely information, as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site. Furthermore, on behalf of the study staff and myself, I agree to maintain the procedures required to carry out the study in accordance with accepted GCP principles and to abide by the terms of this protocol.

Protocol Number: 0001

Protocol Title: FERAHEME AS AN MRI CONTRAST AGENT FOR PEDIATRIC CONGENITAL HEART DISEASE

Protocol Date: 1/13/2016

Investigator Signature	*Date*

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1 LIST OF ABBREVIATIONS

The following abbreviations and special terms are used in this study report.

Abbreviation or special term	Explanation
%	Percent
3D	Three Dimensional
4D	Four Dimensional
AE	Adverse event
bSSFP	Balanced steady state free precession
CE-MRA	Contrast-Enhanced Magnetic Resonance Angiography
CFR	Code of Federal Regulations
CHD	Congenital Heart Disease
CKD	Chronic Kidney Disease
CNR	Contrast to noise ratio
CO2	Carbon dioxide
CRF	Case report form
CT	Computed Tomography
CVMRI	Cardiovascular magnetic resonance imaging
DMC	Data Monitoring Committee
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
eCRF	Electronic case report form
eGFR	Estimated glomerular filtration rate
EMA	European Medicines Agency
FDA	Food and Drug Administration
FOV	Field of view
GBCA	Gadolinium Based Contrast Agents
GCP	Good Clinical Practice
Gd	Gadolinium
GFR	Glomerular filtration rate
HIPAA	Health Insurance Portability and Accountability Act of 1996
ICF	Informed consent form
ICH	International Conference on Harmonisation

Abbreviation or special term	Explanation
ICU	Intensive Care Unit
IDA	Iron deficiency anemia
IEC	Independent Ethics Committee
IND	Investigational New Drug
IRB	Institutional Review Board
IV	Intravenous
kg	Kilogram
LV	Left ventricular
M.D.	Medical doctor
mg	milligram
min	Minute
MR	Magnetic Resonance
MRA	Magnetic Resonance Angiography
MRI	Magnetic resonance imaging
MUSIC-MRI	Multiphase, Steady-State Imaging with Contrast Enhancement
NICU	Neonatal intensive care unit
NSF	Nephrogenic systemic fibrosis
PA	Pulmonary artery
pCO2	Partial pressure of carbon dioxide
PDB	Prospect of direct benefit
PI	Principal Investigator
RF	Radiofrequency
ROI	Region of interest
SAE	Serious adverse event
SAP	Statistical analysis plan
UCLA	University of California at Los Angeles
USPIO	Ultra-Small, Super-Paramagnetic Iron Oxide

2 PROTOCOL SYNOPSIS

TITLE	Feraheme as an MRI Contrast Agent in Pediatric Congenital Heart Disease
Principal Investigator	J. Paul Finn, M.D.
FUNDING ORGANIZATION	National Institutes of Health (Sponsor)
NUMBER OF SITES	1
OBJECTIVES	1. To compare the diagnostic effectiveness of Ablavar®-based ventilator-gated MUSIC-MRI with Feraheme®-based ventilator-gated MUSIC-MRI in pediatric patients with CHD. (Study Part I) 2. To determine the diagnostic effectiveness of an accelerated self-gated MUSIC-MRI cardiovascular MRI technique (henceforth referred to as 'self-gated MUSIC-MRI') for pediatric patients with CHD, which ultimately may not require sedation or general anesthesia. (Study Part II) 3. To summarize the safety of Feraheme® and Ablavar® as an MRI contrast agent in pediatric patients with CHD. MUSIC-MRI (MUltiphase Steady-state Imaging with Contrast enhancement is a high resolution 3D cardiac-gated cineangiographic imaging technique played out over multiple time phases of the cardiac cycle. The MUSIC-MRI sequence is described in Han et al. (1)
BRIEF RATIONALE	The standard clinical cardiovascular MRI practice for children with CHD frequently involves the use of gadolinium-based contrast agents (GBCA) to enhance tissue contrast. Most GBCAs are small molecules that quickly cross the capillary wall and access the interstitial space, a process which diminishes the signal contrast between blood vessels and surrounding tissue. Therefore, these types of GBCA are most useful for first-pass MR angiography, wherein the images are acquired quickly during the initial 15-30 seconds post-injection when the GBCA concentration is much higher in the arteries than in the interstitial space. For young children with complex CHD, the stringent requirements for high spatial resolution, and the need for cardiac gating and good blood-myocardium contrast in order to provide detailed evaluation of intracardiac structures are not compatible with conventional GBCA-based first-pass MR angiography. Even with Ablavar® (gadofosveset trisodium), an FDA approved GBCA with longer intravascular half-life than other GBCAs, cardiac-gated Ablavar®-enhanced MRI may be insufficient for young

	children with CHD based on our institutional experience and on data from the literature; there remains diminished blood-tissue contrast during the high-resolution cardiac-gated MRI. Furthermore, there have been safety concerns regarding gadolinium deposition in brain tissues after repeated GBCA exposure as well as concerns of nephrogenic systemic fibrosis (NSF) associated with GBCA injection in young children < 2 years old who may have immature renal function. The long-term health consequences of these effects in the pediatric population are unclear. For the above reasons, we seek to study the diagnostic imaging effectiveness of Feraheme (Feraheme®), an FDA-approved drug for parenteral iron supplementation, as an MRI contrast agent in children with CHD. Although Feraheme® has been approved for the treatment of iron deficiency anemia secondary to renal disease, Feraheme® has been used as an off-label MRI contrast agent at select medical centers, including a Stanford study of Feraheme®-enhanced pediatric tumor imaging under a separate IND.
STUDY DESIGN	The study has two parts: **Part I**: Open-label, exploratory, case-control, single-center diagnostic efficacy study in 80 children (age newborn-6 years old) to compare Feraheme®-enhanced and Ablavar®-enhanced MRI **Part II**: Open-label, exploratory, single-center diagnostic efficacy study in 40 children (age newborn-6 years old) to validate advanced MRI techniques to enable respiratory-motion self-gated image acquisitions. Such acquisition strategies may ultimately enable high-resolution 4D MRI of children with CHD who undergo MRI during free-breathing without sedation or anesthesia.
NUMBER OF SUBJECTS	Part I: 80 (40 control and 40 test group) Part II: 40
SUBJECT SELECTION CRITERIA	**Inclusion Criteria**: 1. Male or female pediatric patients of all ethnicities (age newborn to 6 years) with known or suspected CHD with inconclusive echocardiographic exams and are referred for cardiovascular MRI for further evaluation of cardiac anatomy and function. 2. Written informed consent obtained from subject's legal representative/guardian(s) and ability for subject to comply with the requirements of the study.

	Exclusion Criteria: 1. Standard clinical contraindications to MRI, including subjects with cochlear implants and implanted cardiac devices 2. Subjects with past or current diagnosis of iron overload due to hereditary hemochromatosis or other causes (for subjects receiving Feraheme injection only). 3. Subjects with known hypersensitivity or allergy to iron oxide particles. 4. Subjects with renal insufficiency defined as estimated glomerular filtration rate (eGFR) < 40 mL/min/1.73m^2 (for subjects receiving Ablavar injection only). 5. Subjects who are critically ill at the time of MRI and for whom the period of general anesthesia and separation from the critical care nursery or intensive care unit poses added risk as deemed by referring cardiologists, cardiac surgeons or the managing radiologist (for Part II only). 6. Other medical conditions, in the judgment of the clinician investigator, that would increase the risks to the child related to participation in the study.
TEST PRODUCT, DOSE, AND ROUTE OF ADMINISTRATION	**Ferumoxytol (Feraheme®):** Stock Feraheme® formulation will be diluted by a factor of ≤ 30 with normal saline and administered by slow intravenous infusion for a total dose of 4 mg of iron per kg of body weight. The total infusion time will be 10 minutes.
CONTROL PRODUCT, DOSE AND ROUTE OF ADMINISTRATION	**Gadofosveset (Ablavar®):** Manufacturer recommended dosing of 0.03mmol/kg (0.12mL/kg) will be diluted 4-8x (based on body size) with normal saline and administered intravenously over 15 seconds at a rate of 0.3-1 mL/sec, depending on patient size.
STUDY DURATION	**Screening and imaging:** 1 day **Total duration of the study:** 5 years.
CONCOMMITANT MEDICATIONS	**Allowed**: The subjects are allowed to take concomitant medications as necessary or directed by the patient's physician. **Prohibited**: None.
EFFICACY EVALUATIONS	In Part I, the Feraheme®-enhanced ventilator-gated MUSIC-MRI image quality will be scored and compared with Ablavar®-enhanced ventilator-gated MUSIC-MRI. In Part II, the ventilator-gated MUSIC-MRI image quality will be scored

	and compared with respiratory motion self-gated MUSIC-MRI using the contrast agent determined in Part I. The image quality scoring for both Part I and Part II will be performed for 7 anatomical structures that are important for pediatric CHD patients, (i.e. aortic root, main pulmonary artery, coronary arteries, ventricular out-flow tracts, valves, ventricular chambers, and atria) using a 1-4 point scale. Image sharpness across lumen-wall interfaces will be quantified using established methods at the interventricular septum and the ascending aorta. The blood-myocardium contrast-to-noise ratio will be quantified using established methods.
PRIMARY EFFICACY ENDPOINT	Composite image quality score among the 7 anatomical structures (aortic root, main pulmonary artery, coronary arteries, ventricular outflow tracts, valves, ventricular chambers, atria).
SECONDARY EFFICACY ENDPOINT	1. Image quality score at the aortic root. 2. Image quality score at the main pulmonary artery. 3. Image quality score at the coronary arteries. 4. Image quality score at the out-flow tracts. 5. Image quality score at the valves. 6. Image quality score at the ventricular chambers. 7. Image quality score at the atria. 8. Sharpness value at the interventricular septum. 9. Sharpness value at the ascending aorta. 10. Blood-myocardium contrast-to-noise ratio (CNR).
SAFETY EVALUATIONS	**Safety Evaluations**: 1. Incidence of acute AE will be monitored. 2. Continuous patient monitoring and recording of vital signs (pre-, during, and at least 30 min-post contrast agent injection) will be provided by expert and specialized staff, including pediatric anesthesiologists and /or staff from the UCLA Neonatal or Pediatric Intensive Care Units. 3. Incidence of other AE up to 60 days post-MRI via a medical record review. **Procedures to Reduce Risks of Feraheme Infusion**: 1. All patients will be screened for contraindications to Feraheme® administration, including a history of allergy to intravenous iron products and iron overload. 2. Performing MRI in newborns to 6 years of age under sedation and/or general anesthesia with airway protection is considered the standard of care at most institutions performing pediatric cardiac MRI for this age group. In the case of a clinically urgent or life-threatening AE during the procedure, there will already be in place full airway protection and support of respiration.

	3. Patients will undergo continuous monitoring of heart rate, blood pressure, oxygen saturation and ECG tracing for the duration of the MRI procedure and, at a minimum, 30 min after the injection. In the case of a clinically important AE, changes in vital signs will be evident immediately and appropriate treatment initiated. Protocols are in place to deal with acute AEs related to hypersensitivity and/or anaphylaxis. 4. The dose of Feraheme® will be up to 4 mg /kg (approximately half the single therapy dose), diluted with saline and infused slowly per FDA guidelines.
PLANNED INTERIM ANALYSES	**Part I** After 50% enrollment is achieved in both the test and control groups, the Investigators will convene the DMC and request an interim analysis. The Investigators will not be blinded to the treatment assignment or to the images, but only to the image quality scores, which will be provided by independent experienced readers. If statistical significance is achieved for the primary efficacy endpoint in the interim analyses, the Investigators will close enrollment for Part I and will begin enrollment for Part II. If statistical significance is not achieved during the interim analyses, we will continue recruiting patients into the two groups (Feraheme® and Ablavar®) and the final statistical analyses will require a 0.025 level of confidence to claim statistical significance. **Part II** There will be no interim analyses in Part II of our study. **Safety Evaluations** Adverse events (AEs) will reviewed and monitored by the DMC on a quarterly basis. SAEs will be reported within 48 hours and the DMC will be convened within one week to evaluate the protocol.
STATISTICS	**PRIMARY EFFICACY ENDPOINTS**: ***<u>Part I</u>: Diagnostic efficacy study in 80 children (age newborn-6 years old) to compare Feraheme®-enhanced and Ablavar®-enhanced MRI*** Control Group= Ablavar® + MUSIC-MRI Test Group= Feraheme® + MUSIC-MRI

	The primary efficacy endpoint is the sum of diagnostic image quality scores (i.e. the composite score) from the 7 pre-identified anatomical structures. A parametric two-sided t-test will be used to compare the Feraheme® group and the Ablavar® group where both groups will use ventilator-gated MUSIC-MRI. This comparison will be a two-sided test at the 0.05 level of significance. A formal interim analysis for the image quality will be performed when 20 CHD subjects in each group have completed the image acquisition. The interim analysis will be prepared by an independent statistician and presented only to the independent DMC who will make recommendations about the ongoing conduct of the study. Two hypotheses will be used to preserve the overall type 1 error rate of 0.05 between this single interim analysis and the final analysis of the mean differences in the composite image quality scores. A two-sample t-test will be used to compare the Feraheme®-enhanced and the Ablavar®-enhanced groups for the primary analysis at the 0.025 level of significance. ***Part II: Diagnostic efficacy study to compare self-gated MUSIC-MRI with ventilator-gated MUSIC-MRI in 40 children*** Control = Contrast agent from Part I + ventilator-gated MUSIC-MRI Test = Contrast agent from Part I + self-gated MUSIC-MRI Part II will start after results from Part I are available. The superior contrast agent as determined by Part I of the study or in the case that statistical significance is not achieved in Part I, the appropriate contrast agent in consultation with the DMC will be used for Part II. The primary efficacy endpoint is the composite image quality score from the 7 pre-identified anatomical structures. A parametric two-sided paired t-test will be used to compare the ventilator-gated MUSIC-MRI and self-gated MUSIC-MRI groups. The quality of the self-gated MUSIC-MRI images is expected to be not much worse than those from ventilator-gated MUSIC-MRI images. This comparison of non-inferiority will be a one-sided test at the 0.025 level of significance with a -1.75 margin in the composite image quality score. **SECONDARY EFFICACY ENDPOINTS**:

	Part I: The image quality score for each individual anatomical structure will be compared between the two contrast agents. 1. Image quality score at the aortic root. 2. Image quality score at the main pulmonary artery. 3. Image quality score at the coronary arteries. 4. Image quality score at the out-flow tracts. 5. Image quality score at the valves. 6. Image quality score at the ventricular chambers. 7. Image quality score at the atria. 8. Sharpness value at the interventricular septum. 9. Sharpness value at the ascending aorta. A parametric two-sided t-test will be performed after an appropriate transformation of individual score if needed. Similarly, the sharpness values and the CNR will be compared between the two groups using a two-sided t-test. *Part II*: The image quality score for each individual anatomical structure will be compared between the two techniques (ventilator-gated MUSIC-MRI vs. self-gated MUSIC-MRI). A paired t-test will be used to determine the difference in image quality scores using appropriate transformation if needed. Similarly, the sharpness values and the CNR will be compared between the two groups using a two-sided t-test. **SAFETY EVALUATIONS:** Safety will be assessed through summaries of adverse events, vital signs recordings, and any relevant clinical laboratory test data (including change from baseline). Safety analyses will include all patients who receive any amount of study drug (safety population). All adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Descriptive statistics will be used rather than inferential statistics. An independent DMC will monitor the safety data on an ongoing basis in this trial and will review data from a pre-specified interim analysis.
ABBREVIATED STUDY FLOW	**Part I**: 1. Patient is referred for cardiovascular MRI after inconclusive echocardiography exam. 2. The Investigators discuss with the patient's legal guardian(s) the study design and risks/benefits for Feraheme and Ablavar administration.

	3. The patient's legal guardian(s) decide(s) to participate in one of the study groups. 4. Patient undergoes general anesthesia per our institutional standard protocol or is transferred from the neonatal ICU already sedated and intubated. 5. Patient undergoes cardiovascular MRI (ventilator-gated MUSIC-MRI) using either Feraheme or Ablavar and with continuous monitoring of vital signs and any AEs. AE treatments are delivered as needed. 6. Patient recovers from anesthesia or sedation. 7. Patient is followed up at 60 days post-MRI via a medical record review to capture any additional AE. **Part II**: 1. Patient is referred for cardiovascular MRI. 2. The Investigators discuss with the patient's legal guardian(s) the study design and risks/benefits for either Feraheme or Ablavar administration, depending on the outcome of Part I. 3. The patient's guardian(s) provide written informed consent form. 4. Patient undergoes general anesthesia per our institutional standard protocol or is transferred from the neonatal ICU already sedated and intubated. 5. Patient undergoes cardiovascular MRI (ventilator-gated MUSIC-MRI and self-gated MUSIC-MRI) using the contrast agent determined in Part I and with continuous monitoring of vital signs. AEs are recognized immediately and treatment delivered as appropriate. 6. Patient recovers from anesthesia or sedation. 7. Patient is followed up at 60 days post-MRI via a medical record review to capture any additional AE.

3 STUDY HYPOTHESES

1. Feraheme®-enhanced MUSIC-MRI, which incorporates cardiac and respiratory gating, provides superior dynamic evaluation of anatomical structures that are important for pediatric CHD patients compared to Ablavar®.
2. The self-gated MUSIC-MRI is non-inferior to ventilator-gated MUSIC-MRI.

4 BACKGROUND

Contrast agents are used in cardiovascular MRI to augment tissue contrast. These agents typically exert strong T1 effects, which enhance MRI signal and tissue contrast. The large majority of

compounds used clinically are gadolinium-based contrast agents (GBCA), which contain gadolinium (Gd), a paramagnetic rare earth metal not found naturally in biological systems.

4.1 Overview of Cardiovascular MRI

Cardiovascular MRI (CVMRI) refers to a group of MRI techniques that address anatomical, structural and functional assessment of the cardiovascular system. CVMRI has a number of advantages compared to other non-invasive imaging modalities, including lack of ionizing radiation, inherent 3D capabilities, unrestricted field of view (FOV) and flexibility in combining spatial and temporal resolution. Uniquely, MRI can generate a wide range of soft tissue contrast to image specific aspects of the magnetic state of the tissues. In order to make an MR image, radiowave pulses and magnetic field gradient pulses are applied in a specific and customized order, called a 'pulse sequence', to highlight the properties of tissue magnetization most relevant to the clinical question. The administration of pharmaceutical contrast agents, such as GBCA and ferumoxytol, result in dramatic changes in the sensitivity of MRI to these tissue properties and the biodistribution of the agent is reflected in the image. In many situations, both contrast-enhanced and non-contrast enhanced images are acquired and may provide complementary information. For vascular anatomical assessment, techniques such as contrast-enhanced MR angiography (CE-MRA) and non-contrast balanced steady state free precession (bSSFP) are often used to define vascular and cardiac anatomy. For functional assessment, sequential 2-dimensional cardiac cine imaging is the most widely used MRI technique for quantifying functional parameters of the heart, such as ventricular volumes and ejection fraction.

Most MRI techniques require several seconds to several minutes to acquire and if an imaged organ moves during this time, the image becomes degraded by artifact. In all cases, gross body movement must be avoided and this is achieved in cooperative patients by requesting that they make no voluntary movements during the scan. However, some physiological movements, such as cardiac, respiratory and bowel peristalsis are partially or wholly involuntary and, for these, specific corrective or preventative maneuvers must be implemented. Therefore, cardiovascular MRI generally requires physiological motion compensation techniques for both cardiac and respiratory motion. The ECG signal is commonly used as a cardiac gating signal, although its reliability at higher field strengths (>= 3 Tesla) is more limited due to artifact caused by blood flowing in a stronger magnetic field (magnetohydrodynamic effect) (2). Respiratory motion artifact can be mitigated by breath holding, as and when appropriate, or by pausing mechanical ventilation in children (3). However, the maximum duration of a breath hold (on the order of 30 seconds) sets an upper limit on the amount of spatial and temporal detail that can be acquired in that time with MRI. Whereas modern scanners can acquire sufficient detail in this time for a 3-dimensional image without cardiac gating (such as for the first pass of a GBCA bolus), cardiac gated, multi-phase 3-dimensional imaging takes several minutes to acquire and is therefore incompatible with breath-holding. Techniques exist to compensate for breathing motion artifact when image acquisition takes several minutes. The motion of the diaphragm can be monitored by a so called 'navigator echo', sampled regularly at the lung-liver interface such that the image acquisition can be restricted to a specific time window within the breathing cycle. Such a navigator-gating strategy reduces respiratory motion related image blurring; however, the navigator sampling process takes time and interrupts the continuous radiowave pulsing necessary, and is therefore not well suited to techniques that sample the full cardiac cycle in steady state and which may undergo retrospective data sorting, such as MUSIC-MRI in our protocol. The current study will utilize two alternative respiratory motion compensation strategies which do not interrupt the steady state: 1) ventilator

gating and 2) respiratory motion self-gating. With both methods, the radiofrequency and gradient activity continues without interruption throughout the entire respiratory and cardiac cycles. The ventilator gating strategy uses the air pressure signal from the endotracheal tube in patients undergoing positive pressure ventilation and directly reflects the ventilator driving pressure. Hence, this technique is not applicable to patients breathing spontaneously. For these cases, a promising alternative strategy is respiratory motion self-gating (4), whereby respiratory motion is detected by its periodic effect on the amplitude of the continuously acquired MRI signal, without the need for a navigator preparation. Respiratory self-gated cardiovascular MRI has the following benefits compared to navigators: 1) it does not disrupt the steady state MRI signal and allows us to capture the entire cardiac cycle during our MUSIC-MRI cardiac-phase resolved scans; 2) it provides a direct account of the effect of respiratory motion on the organ of interest.

4.2 Challenge of Cardiovascular MRI in Children

The clinical value of MR is well established for a variety of cardiovascular disorders (5–9). Children with congenital heart disease (CHD) typically have both intra-cardiac and extra-cardiac vascular anomalies. Therefore, the ability to visualize both types of morphologic abnormalities is crucial. Although echocardiography is readily available, it may leave several morphologic and hemodynamic questions unresolved due to issues such as inadequate acoustic window. Cardiac CT with either single or dual source technology can yield high spatial resolution images with increasingly small radiation doses. However, sub-milliSievert doses are achievable only in the most ideal situations (low body mass index, sinus rhythm with excellent beta blockade). Exposure to radiation, however low the dose, should be considered only when alternative methods are not available. Furthermore, neonates and infants may have heart rates that challenge the temporal resolution of even dual source CT scanners. With MRI, the temporal resolution of cine imaging can be adjusted to cope with even the fastest heart rates (10). MRI has been shown to be a versatile, non-invasive, and non-radiating technique for the evaluation of neonates, infants, and children, including those who are critically ill (9,11,12).

In young children, the small caliber of the heart and blood vessels requires higher spatial resolution than in adults (5,7) for comparable detail. For adults and larger children, spatial resolution as previously reported (13–15) may be entirely adequate; for small children with diminutive central vessels, the requirements for high spatial resolution are much more stringent. Further, children (particularly newborns to age 6) also often require the MRI exam to be done under general anesthesia (9) as they are unable to obey commands and may be critically-ill.

4.3 MRI clinical standard of care in children with CHD

In children with CHD, echocardiography is universally regarded as the primary diagnostic imaging modality. When echocardiography is inconclusive, MRI is the preferred second line imaging modality due to the absence of ionizing radiation and the ability to incorporate functional as well as anatomic information. MRI for pediatric CHD routinely involves the use of GBCAs to evaluate extra-cardiac vascular anatomy, which may be complex and anomalous, but knowledge of which is essential to inform patient management. Gd is a rare earth metal not found naturally in biological systems, but with paramagnetic properties that greatly enhance the MRI signal. Because free Gd ions are toxic, GBCAs are stable chelates wherein the Gd ions are tightly bound with very high association constants. The chelating molecule in turn modulates the biological distribution,

potency and pharmacokinetics of the contrast agent. Most GBCAs are small diffusible molecules that, following bolus intravenous injection, sustain a short intravascular peak and quickly access the interstitial space. Therefore, these extracellular GBCA are useful mainly for first-pass MR angiography, wherein the images are acquired during the initial 15-30 seconds post-injection before the GBCA leaks from the intravascular space. In children with CHD, this limited time window will generally preclude simultaneous high-resolution cardiac and vascular image acquisition, due to the requirement for gating to freeze cardiac motion. However, high resolution imaging of the intracardiac structures, including the great arteries, the cardiac chambers, the valves, and the coronary arteries and the outflow tracts, is essential for planning surgery or other interventions for these patients.

Ablavar (Ablavar®, Lantheus Medical) is a GBCA, which binds reversibly to plasma proteins and therefore has a longer intravascular residence time and higher potency (r1 relaxivity) than the purely extracellular gadolinium agents. At any time, 70% - 80% of the Ablavar in the blood is reversibly bound to serum albumin and the remainder is distributed in the extracellular space. The binding to plasma proteins slows the initial distribution of Ablavar from the blood pool to the extracellular fluid space such that, whereas for the purely extracellular GBCAs the distribution half life is < 5 minutes, for Ablavar the distribution half life is on the order of 25 minutes. Because of its longer intravascular residence time, Ablavar extends the useful time window for vascular imaging to 20-30 minutes beyond the first pass. Once the distribution phase is complete, Ablavar and the other GBCAs are diluted by the extracellular fluid space (~15 liters in an average adult or ~30% of body weight in a child under 6 years) and their effectiveness in enhancing the vascular signal is correspondingly diminished. Therefore, from the perspective of vascular MRI, the duration of the initial distribution phase is the most relevant because the strength of the vascular signal is determined by the concentration of the agent in the blood when the scan is being performed. Once distributed, the elimination half-life (due to renal excretion) of Ablavar is on the order of 19 hours.

Ablavar is approved as a contrast agent for MR angiography in adult patients with peripheral vascular disease and has become the agent of choice at many institutions for pediatric CHD. Compared to the purely extracellular GBCAs, Ablavar has higher potency per unit dose of Gd (r1 relaxivity), provides a longer time window for vascular imaging and leaks into the interstitial fluid space more slowly. However, like the extracellular GBCAs, its primary mode of elimination is by renal filtration of the unbound plasma fraction. Although this is an improvement over the purely extracellular agents, an even longer vascular residence time would be desirable in children for several reasons. Firstly, for combined and simultaneous high-resolution cardiac and vascular imaging in CHD patients, the concentration of contrast agent in the blood should remain constant over the several minutes of the imaging scan. Secondly, in patients under anesthesia or sedation it is sometimes necessary to interrupt or repeat a scan if the anesthesiologist needs to adjust the depth or type of sedation on a patient specific basis. Inadequate sedation may result in patient motion artifact which can render the study non-diagnostic. Time spent adjusting the sedation parameters and confirming physiologic stability may push the image acquisition window beyond the optimum time window for Ablavar, resulting in diminished image quality even if motion artifact is absent.

Therefore, the overall effect of Ablavar® is qualitatively similar to the GBCAs with similar limitations, but on an expanded time scale which may be adequate if the study goes smoothly but which may result in compromised image quality if scans need to be paused or repeated. The hypothesized advantages of Feraheme over Ablavar are the purer and much longer intravascular residence time (intravascular half life of 15 hours vs 0.5 hours), such that high-resolution steady-state cardiac and vascular imaging can be completed successfully, whether or not scans need to be paused or repeated.

4.4 Safety Concerns with GBCAs

GBCAs have been used for over a quarter of a century and have a highly favorable safety profile, which is predominantly based on their stability in vivo. GBCAs are chelates designed to tightly bind gadolinium ions because free gadolinium ions are toxic (16). In recent years, some safety concerns have been raised relating to the association of nephrogenic systemic fibrosis (NSF) with GBCA exposure for patients with renal impairment, and observation of gadolinium deposition in brain tissue, even in patients with normal renal function (17–22). The gadolinium deposition in tissue may be particularly worrying in children because of their projected life-expectancy and in whom Gd may potentially persist indefinitely. Furthermore, there are concerns of hypersensitivity to GBCA injections (23,24) and association of Ablavar with prolongation of the QT interval on the ECG, which may pose more concern for cardiac arrhythmias in children with CHD than those with normal hearts.

[Nephrogenic systemic fibrosis (NSF)] In 2006, associations between NSF and GBCA exposure (25,26) led to a boxed warning alert by the US FDA (27) and the EMA (28) to restrict the use of GBCAs in patients with renal insufficiency. Autopsy studies have reported the presence of gadolinium in skin, heart, blood vessels, lungs, lymph nodes, spleen, liver, kidney, and dura of patients with NSF (16). Almost a decade later however, the pathophysiology of NSF remains unclear. Nevertheless, a consistent body of literature does suggest that slow clearance of GBCAs in patients with impaired renal function predisposes to transmetalation with release of free gadolinium ions, which have fibrogenic effects (29). The diagnosis of NSF is complex (30) and should only be made when clinical and histopathological criteria set forth by the Yale NSF Registry are met (31). NSF is described as skin discoloration and swelling progressing to erythematous papules, brawny lesions, and subcutaneous sclerosis (32,33) and occurs within 2-3 months of exposure to GBCAs, but late onset cases have been reported (34). Recently, a new entity, termed gadolinium-associated plaques, was reported in two patients by Gathings et al. (35), whereby neither patient had NSF while only one patient had renal failure. Both patients, however, had erythematous plaques associated with sclerotic bodies thought to be pathognomonic for NSF. This occurrence was related to gadodiamide.

The risk of GBCA-associated NSF is highest in those with an estimated glomerular filtration rate (eGFR) < 30mL/min/1.73m^2 (1-7%) and those with acute renal failure (12-20%). The risk is lower for those with moderately decreased renal function (30-59mL/min/1.73m^2) and even less for those with mild renal impairment (60-89mL/min/1.73m^2). While there are a multitude of publications on GBCA-associated NSF, the most interesting reports are cases in which NSF did not develop in those with severe chronic kidney disease (CKD) (15-29 mL/min/1.73m^2 eGFR), who were exposed to high doses of GBCAs (36,37). It is thought that other confounding risk factors beyond

impaired renal function such as concomitant acidosis, infection, acute pro-inflammatory events, immunosuppression, high-dose erythropoietin therapy, elevated iron /calcium /phosphate levels, and vasculopathy may play a role in the initiation of the NSF disease cascade.

Zou et al. (33) in their review of 370 biopsy confirmed cases of NSF, suggest that eliminating risk factors may substantially reduce the risk of NSF without having to shift to other imaging modalities, which may incur risk of radiation and iodinated contrast-induced nephropathy. These preventable risk factors include using the lowest dose possible for diagnostic result (limiting GBCA dosage to <0.1mmol/kg), avoiding non-ionic linear GBCAs in those on dialysis or having eGFR < 30mL/min/1.73m^2, dialyzing dialysis-dependent patients promptly after exposure, and delaying GBCA administration in the setting of acute renal failure particularly when proinflammatory conditions may exacerbate oxidative stress. The Contrast Media Safety Committee of the ESUR (29) also recently published recommendations for NSF-risk mitigation where they outlined the level of evidence and identified classes of recommendations for clinical practice. For the pediatric population, no specific evidence based-guidelines exist. Guidelines for adults are typically used. There have been 10 biopsy-proven pediatric cases of NSF, but since the implementation of guidelines, no new cases in children have been reported (38). To date, no cases of NSF have been reported in very young children (38). However, it is known that children younger than 2 years have immature renal function (39). The glomerular filtration rate (GFR) of pre-term and term newborns can be as low as 40 ml/min/1.73m^2, and it gradually increases to 66 ml/min/1.73m^2 at 2 weeks after birth (39). Due to concerns about nephrogenic systemic fibrosis (NSF), The European Society of Urogenital Radiology warns against the use of GBCA, including gadofosveset, in children less than 1 year old (40). **Therefore, a significant portion of the children in our study will have immature renal function with an unknown, but likely very low risk of developing NSF.**

[Possible retention in brain and other tissues] In July 2015, the US FDA issued a safety announcement regarding deposits of GBCAs in the globus pallidus and dentate nuclei of patients who have undergone multiple GBCA-enhanced MRI (41). The announcement was in response to reports of residual gadolinium deposits on non-contrast MRI of patients who had undergone multiple GBCA-enhanced MRI exams (42) -- some of whom had normal renal function (19,20), and in those exposed to ionic linear GBCA (gadopentetate dimeglumine) rather than macrocyclic GBCA (gadoteridol) (21). In a pictorial essay, Caruso et al. (17) first alluded to this phenomenon in 2001, but it was not until recently that the findings were publicized by Kanda et al. (18,20,21), confirmed by Errante et al. (19), and expanded by McDonald et al. (22). Their work is limited to patients with intracranial T1 shortening whereas work by others (43–45) suggests that insoluble gadolinium (in the form of gadolinium phosphate in bone tissue) may make up another portion of residual gadolinium deposit in the body. However, it remains unknown whether these deposits have any adverse clinical implications. Their findings need confirmatory work, particularly to better understand whether certain types of GBCAs are more prone to residual deposition. **If future research points to significant toxicity of these gadolinium deposits, young children exposed to GBCA, including the pediatric CHD population in the Ablavar group of our study, may be subject to higher risk due to their longer life expectancy compared to adults.**

[Hypersensitivity] Although the rate of reported cases of NSF has declined, issues relating to

hypersensitivity remain. The overall rate of AEs for all GBCAs given at clinical doses (0.1-0.2mmol/kg) is estimated to be between 0.07 to 2.4% (46). As a comparison, the rate of AEs for low-osmolality iodinated contrast agents is 0.15% (47) to 0.2% (46). While hypersensitivity-like reactions associated with GBCAs vary from 0.004% to 0.7%, the frequency of serious life-threatening anaphylactic reactions associated with these agents is exceedingly uncommon (0.001 to 0.01% (46) vs. 0.04% for nonionic low-osmolality iodinated contrast (46)). According to analysis of data reported in FDA MedWatch by Prince et al. (24), the risk of death associated with GBCA administration is less than 1 in a million ('equivalent to the risk of dying from a chest X-ray, consuming 0.5L of wine, smoking 1.4 cigarettes, or traveling 86 miles by car'). In contrast, the fatality rate for iodinated contrast is 0.9 per 100,000 injections or 2.1 fatalities per one million studies using low-osmolality contrast agents based on FDA data from 1990-1994 (46). The death rate for ionic linear GBCAs is seven times higher than from nonionic linear agents (24) whereas the frequency of acute adverse reactions to GBCAs is eight times higher in those with prior reactions to GBCAs. For those with prior reactions to GBCAs, the administration of gadobenate dimeglumine is contraindicated (46). Other risk factors for acute hypersensitivity reactions include a history of asthma and multiple drug or food allergies. Although there is no cross-reactivity between GBCA and iodinated contrast agents, the frequency of GBCA-related adverse event is 2.3-3.7 times higher in those with prior reaction to iodinated contrast (48).

4.5 What is Feraheme and what is its potential as an alternative MRI contrast agent?

Feraheme® (AMAG Pharmaceuticals, MA) is a proprietary formulation of the iron supplement ferumoxytol. Initially designed as an intravascular MRI contrast agent, Feraheme® was approved as a bolus intravenous (IV) iron supplement by the FDA in 2009, specifically for the treatment of iron deficiency anemia (IDA) secondary to chronic kidney disease (CKD). Each manufacturer-provided 17-mL vial contains 510mg elemental iron (30 mg Fe /mL) and was administered as an IV bolus in as little as 17 seconds (1 mL/sec), a practice recently revised in favor of slow infusion over 15 minutes (49). The therapeutic dose for the treatment of IDA calls for two 510-mg injections given 3-8 days apart. Compared to other IV iron supplements, Feraheme was developed to have lower free iron release (50), decreased immunologic allergic reaction, and improved safety profile (50–52). As an ultra-small superparamagnetic iron oxide (USPIO) nanoparticle, Feraheme has a long intravascular half-life of 14 hours, high r_1 relaxivity, and is incorporated into the hematopoietic pathway as supplemental iron once the outer carbohydrate shell is degraded (53). These properties along with its approved therapeutic use in patients with kidney dysfunction and lack of gadolinium make Feraheme an attractive alternative to GBCAs for first-pass and steady state MRI (53,54). To date, imagers have explored Feraheme for cardiovascular applications (55–57), renal transplant imaging (58), inflammation imaging (59–61), and cancer imaging (62–65).

By exploiting the imaging properties of Feraheme® appropriately in small children, it is possible to acquire images with unprecedented detail, without the need for breath holding. Our group recently developed a technique termed 4-dimensional MUSIC-MRI, which produces 4 dimensional contrast-enhanced images of the beating heart in children, without breath holding (1). Additionally, Feraheme® can overcome issues related to both short-term and long-term gadolinium deposition in soft tissues. In our practice at UCLA, Feraheme®-enhanced MRI has informed clinical decision-making in children with complex and life threatening diseases in ways that would otherwise not have been possible. For this reason, we feel that we have sufficient

preliminary data to support the prospect of direct benefit (PDB) to patients who undergo MRI with Feraheme® in our investigator-initiated, single center, exploratory, open label clinical trial.

4.6 Overview of Non-Clinical Studies

A review of non-clinical studies is not relevant because Feraheme® is a medication approved by the FDA for IV treatment of iron deficiency anemia in adults with chronic renal disease.

4.7 Overview of Clinical Studies

Feraheme® has been FDA approved since 2009 for the treatment of iron deficiency anemia in adults with chronic kidney disease (CKD). Based on the manufacturer's estimate, there have been more than 1.2 million administrations since FDA approval in 2009 (Personal Communication, AMAG). The following sections summarize the safety profile of Feraheme® as well as Ablavar®, the control agent in this study, for adult/pediatric patients and for therapeutic/diagnostic use.

4.7.1 Feraheme as a Therapeutic Agent

The safety and efficacy of Feraheme® as an I.V. iron replacement therapy agent has been widely studied in adult chronic kidney disease (CKD) patients with iron deficiency anemia. Table 1 summarizes findings with regard to safety profile of Feraheme from major pre- and post-marketing studies (>100 patients) in the literature, which point to an anaphylaxis rate ranging from 0 to 0.2% at the approved therapeutic dose.

***Table** 1: Literature summary of AE/SAE rate of Feraheme injection (therapeutic dose @ approximately 7 mg iron/kg injected at up to 30 mg /sec) in CKD/Iron Deficiency Anemia patients (both pre- and post-marketing studies)*

Author/Year	Patient Population	# of Patients	AE Rate	SAE Rate	Anaphylaxis Rate
Spinowitz 2008 (66)	CKD	304	10.6%	None	None
Singh 2008 (51)	CKD stages 1-5	750	5.2%	1/750 (0.1%)	1/750 (0.1%)
Provenzano 2009 (52)	CKD stage 5D on hemodialysis	110	8.2%	1/110 (0.9%)	None
Vadhan-Raj 2014 (67)	Iron Deficiency Anemia	609	14.6%	4/609 (0.7%)	1/609 (0.2%)
Schiller 2014 (68)	Dialysis-dependent CKD	8666	1.25%	18/8666 (0.2%)	2/8666 (0.02%)
Hetzel 2014 (69)	Iron Deficiency Anemia	406	14.3%	2/406 (0.5%)	1/406 (0.2%)

AE: Adverse Event; SAE: serious AE

SAE includes hypotension, hypersensitivity/anaphylactoid reactions, syncope, dyspnea, loss of consciousness

Since the approval of Feraheme in 2009, there have been a number of large patient cohort studies (at least 10425 patients in total) (67–72). The patient cohorts included CKD patients and iron deficiency anemia patients without CKD but with a prior history of unsatisfactory oral iron therapy. The AE data reported in these post-marketing studies are summarized in Table 2. It should be noted that the anaphylaxis rate of post-marketing data so far is 0.03% based on pooled data.

Table 2. *Aggregate adverse events reported in post-marketing safety trials of Feraheme®*

Event type	n (total n=10425)	Total Percent	Percent Range
Gastrointestinal	174	1.74%	0.6% - 12.5%
Headache	57	4.21%	1.8% - 13.3%
Muscle spasm /arthralgias	40	2.96%	1.5% - 23.3%
Cough /sneezing	21	0.22%	0.1% - 5%
Pruritus /rash /flushing	68	0.68%	0.4% - 10%
Dizziness	56	0.56%	0.2% - 5%
Dyspnea /chest pain	48	0.48%	0.2% - 5%
Hypersensitivity	12	0.14%	0.1% - 0.1%
Hypotension	51	0.55%	0.4% - 2.5%
Peripheral edema	25	3.36%	2.5% - 3.5%
Anaphylaxis	3	0.03%	0% - 1.3%
CCAEE	9	0.89%	0.8% - 1%
Urinary tract infections, nasopharyngitis	39	5.67%	5.4% - 7.5%
**Hetzel et al (n=406), Vadhan-Raj et al (n=608), MacDougall et al (n=80), Schiller et al (n=8666), Auerbach et al (n=60), Lu et al (n=605); **CCAEE=Composite Cardiovascular Adverse Event Endpoint*			

4.7.2 Feraheme as a Diagnostic MRI Contrast Agent

Data in Literature: There is less published safety information related to use of Feraheme as a diagnostic agent. Table 3 summarizes the AE and SAE rates associated with diagnostic Feraheme administration in adults and children based on our literature search. To the knowledge of the Investigators, no clinically significant hypotension, hypersensitivity/anaphylactoid reactions, syncopy, dyspnea, loss of consciousness or fatalities have been reported so far that is associated with Feraheme administration as an MRI contrast agent.

Table *3: Literature summary of AE/SAE rate of Feraheme injection for MRI.*

Author/Year	Patient Age Group	# of Patients	AE Rate	SAE Rate	Anaphylaxis Rate

Ning 2015 (73)	Children & Young Adults	86	Not reported	None	None
Walker 2015 (74)	Adult	10	None	None	None
Nayak 2015 (58)	Children	10	None	None	None
Muehe et al. 2015 (75)	Children & Adult	49 Children & 19 Adults	4/85 (5%)	None	None
Ruangwattanapaisarn et al. 2015 (76)	Children	23	None	None	None
Klenk et al. 2014 (77)	Children & Young Adults	22	None	None	None
Bashir et al., 2013 (78)	Adult	16	None	None	None
D'Arceui et al. 2013 (79)	Adult	8	None	None	None
Alam et al. 2012 (80)	Adult	16	None	None	None
Thompson et al. 2012 (81)	Children	7	None	None	None
Hassan et al., 2011 (82)	Children	6	1/6 (16%)	None	None
Li et al. 2005 (83)	Adult	12	1/12 (8%)	None	None

AE: Adverse Event; SAE: serious AE

The Feraheme® doses used in the literature as a diagnostic contrast agent ranged from 3 mg /kg to 7 mg/kg (58,73–83). For imaging purposes at our institution, we have used 4 mg/kg in 208 patient studies, which is in line with the dose used in the majority of Feraheme®-enhanced MRI publications shown in **Table 3**. Therefore, we will continue using 4mg/kg in the current study, for both Part I and Part II studies, when applicable.

UCLA Institutional Experience in Children: Based on data in the literature and communications with other centers in the U.S. and Europe, including UCLA, there have been approximately 2,000 studies (both clinical and research) using Feraheme as a contrast agent. To our best knowledge, so far there have been no reports of serious or life threatening AEs associated with using Feraheme® as an imaging agent in children. In the therapeutic use of Feraheme, the two life-threatening safety concerns with Feraheme® in the overall experience with the product have been hypotension and hypersensitivity reactions. To date, 79 unique children with CKD or CHD have undergone Feraheme® enhanced MRI under anesthesia at UCLA, all without any AEs. We also retrospectively identified 34 pediatric patients who underwent clinically-indicated Ablavar®-enhanced MRI under general anesthesia and compared the hemodynamic parameters (heart rate,

systolic and diastolic blood pressure), oxygenation (pulse oximetry) and ventilation (end-tidal CO_2) between a subset of the Feraheme group (50 consecutive children with complete anesthesia records) and the Ablavar group (34 children with complete anesthesia records), as well as between different time points (pre-injection, immediately post-injection, 30 min post-injection and >1 hr post-injection) within each group. No statistically significant change in intra-group temporal variations of mean heart rate, blood pressure, oxygenation, or ventilation was observed immediately pre-injection, immediately post-, 30 minutes post, and >1 hour post-injection for both the Feraheme® group and the Ablavar® group (Figures 1&2, one-way ANOVA, $p>0.05$). There was no statistical difference in these parameters between the two groups using t-test, Wilcoxon, or ANOVA methods.

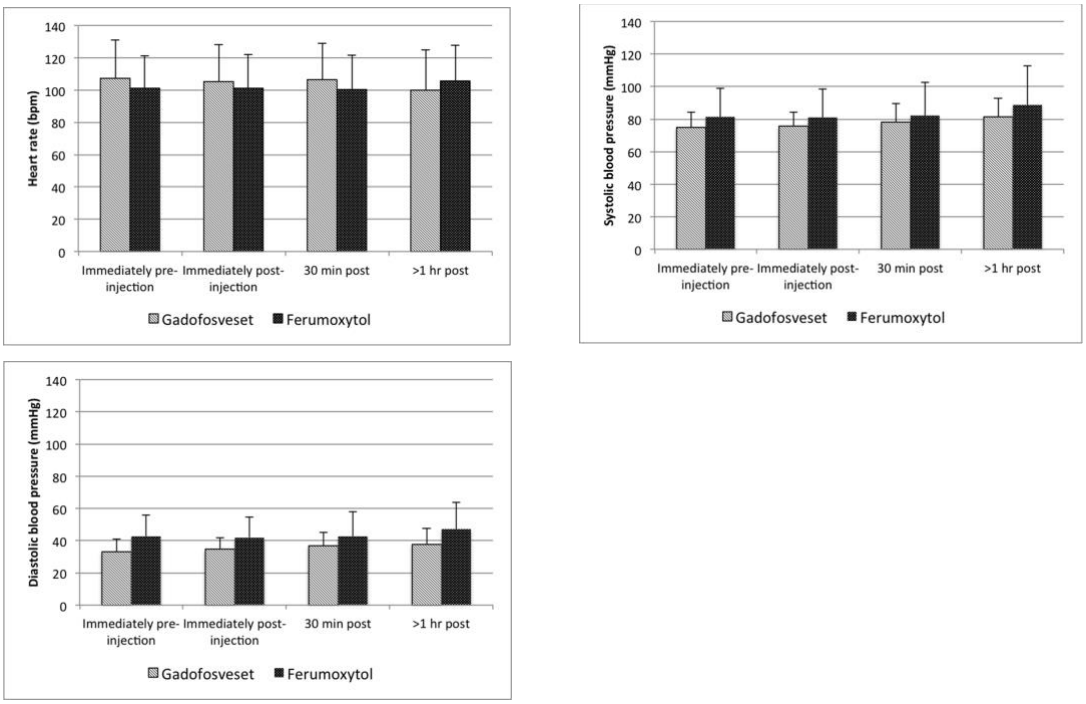

Figure 1. *Comparative measurements of hemodynamic variability in children who underwent Ablavar vs Feraheme-enhanced MRI exams under general anesthesia. The intra-group hemodynamic variations seen in mean heart rate (A), systolic blood pressure (B), diastolic blood pressure (C), immediately pre-injection, immediately post-injection, 30 minutes post-injection, and >1 hour post-injection were not statistically significant for both Feraheme and Ablavar groups ($p>0.05$). One-way ANOVA was used to test for statistical significance between measurements at different time points within each group. n=34 (age range 2 days to 12.5 years) for Ablavar enhanced MRI; n=50 (age 3 days to 19 years) for Feraheme-enhanced MRI. bpm: beats per minute*

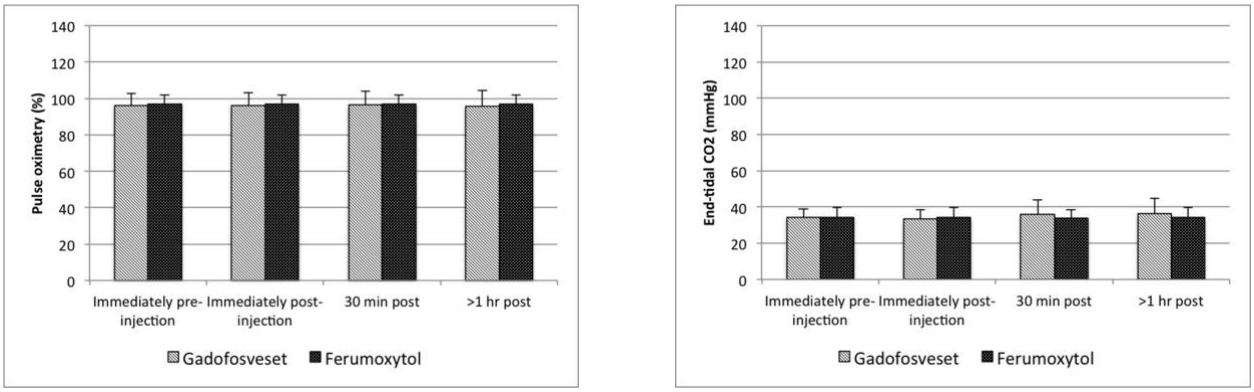

Figure 2. *Comparative measurements of oxygenation and ventilation in children who underwent Ablavar vs Feraheme-enhanced MRI exams under general anesthesia. With the exception of mean pulse oximetry at 30 minutes post-Ablavar injection (p=0.05), the intra-group mean pulse oximetry (A), and mean end-tidal CO2 (B) measurements immediately pre-injection, immediately post-injection, 30 minutes post-injection, and >1 hour post-injection were not statistically significant for both Ablavar and Feraheme groups (p>0.05). One-way ANOVA was used to test for statistical significance between measurements at different time points within each group. n=34 (age range 2 days to 12.5 years) Ablavar enhanced MRI; n=50 (age 3 days to 19 years) for Feraheme-enhanced MRI.*

Table 4 summarizes our safety data of Feraheme® in pediatric CHD patients at UCLA. Continuous monitoring of vital signs and symptoms of adverse reaction was performed up to 1 hour post-Feraheme® injection. Thus far, in 35 pediatric patients with CHD, we have not identified any adverse reactions in these patients.

Table 4*: UCLA Patient Safety Data Summary after Feraheme Infusion as an MRI Contrast Agent in Pediatric CHD Patients*

	CHD
Total number	35
Dose (mg/kg)	3.9 ± 0.7
Infusion time ()	15s to 5min*
SAE	0
Hypotension (>10mmHg BP drop)	0
Edema	0
Cough	0
Muscle spasms	0
Rash	0
Dyspnea	0
Pyrexia	0

Vomiting	0

CHD: congenital heart disease; **s:** seconds; **min:** minute; **AE**: adverse event; **SAE**: Severe AE, including acute anaphylaxis and hypersensitivity reactions and clinically significant hypotension

*: After the recent FDA Boxed Warning (March 2015), the Investigators modified our administration protocol to infuse the dilute Feraheme over 5-10 minutes in the pediatric CHD patients.

4.7.3 Comparison of Feraheme® vs. Ablavar® Safety

According to the Feraheme® package label from the FDA, its anaphylactic/hypersensitivity rate is approximately 0.2% (3 out of 1726 administrations of the therapy dose in pre-marketing clinical trials) and hypotension was reported in 33 out of 1726 subjects, including 3 severe hypotensive reactions. According to the ferumoxytol (Rienso®) package from the European Medicines Agency (EMA), the rate of serious hypersensitivity and hypotension reactions combined is 0.2% (3 out of 1562 subjects), and out of the 3 serious reactions, 1 (<0.1%) was characterized as an anaphylactoid reaction. In a more recent study of 8666 CKD patients who underwent Feraheme® administration, two patients (0.02%) experienced anaphylactoid reactions (68). The aggregate rate of anaphylaxis in post-marketing trials is 0.03% (See Table 2), lower than that reported on package inserts, which was based on pre-marketing clinical trials. As a comparison, Ablavar (Ablavar®), which is a widely used gadolinium based MRI contrast agent, has an anaphylactoid/anaphylactic reaction rate of 0.1% (2 out of 1676 subjects according to its label from the FDA). However, Ablavar®, because it is a gadolinium based contrast agent, is included in the group of agents listed by the FDA as having a possible association with NSF in patients with insufficient renal function. Also, Ablavar is associated with cardiac electrical conduction effects, most specifically prolongation of the QT interval. This has caused some institutions to shy away from including it in their drug formulary. Furthermore, gadolinium is a foreign element and is not a natural constituent of biological systems. This concern is underscored by the recent discovery of gadolinium deposits in patients' brains after repeated exposure to GBCA (17–22), even those with normal renal function. Iron, on the other hand, is an essential element for life and iron deficiency is associated with a plethora of potentially serious health consequences. Despite these objective facts, Feraheme®, perhaps by inferential association with larger iron particles such as ferumoxides (Feridex®) which have been commonly associated with hypersensitivity and back pain, may be perceived as higher risk by the general community than gadolinium-based agents, which are generally deemed to have a very good safety profile. Table 5 summarizes the adverse reaction rates of Feraheme® (full therapeutic dose) and Ablavar® (0.03 mmol/kg) from their respective most recent FDA/EMA product labeling.

Table 5*: A comparison of AE/SAE incidence rates for Feraheme vs. Ablavar based on FDA/EMA product labels.*

AE Type	Feraheme	Ablavar
Hypersensitivity/anaphylactic	0.1%-0.2%	0.1%-0.2%
Risks of NSF for patients with renal impairment?	No	Yes
Risks of arrhythmia due to prolongation of ECG QT interval	No	Yes

Pruritus	1.2%	4%
Hypertension	1.0%	1%
Dizziness	2.6%	1%
Headache	1.8%	4%
Nausea	3.1%	4%
Hypotension	2.5%	(<1% and not shown on the Prescribing Information
Edema	1.5%	
Vomiting	1.5%	
Abdominal pain	1.3%	
Chest pain	1.3%	
Cough	1.3%	
Pyrexia	1.0%	
Back pain	1.0%	
Muscle spasms	1.0%	
Dyspnea	1.0%	
Rash	1.0%	
Vasodilatation	(<1% and not shown on the Prescribing Information)	3%
Paresthesia		3%
Injection site bruising		2%
Dysgeusia		2%
Burning sensation		2%
Venipuncture site bruise		2%
Feeling cold		1%

5 STUDY RATIONALE

In children with congenital heart disease (CHD), echocardiography is universally regarded as the primary diagnostic imaging modality. When echocardiography is inconclusive, MRI is the preferred second line imaging modality due to the absence of ionizing radiation and the ability to incorporate functional as well as anatomic information. The current MRI contrast agents are based on chelates of the rare earth element, gadolinium (Gd). Feraheme® (AMAG Pharmaceuticals) is a proprietary formulation of Feraheme, an ultra-small, super-paramagnetic iron oxide (USPIO) nanoparticle, approved by the FDA as an intravenous iron therapy for anemia in adult chronic kidney disease. Compared to GBCAs, Feraheme exhibits a unique combination of high signal potency (r_1 relaxivity) and a long intravascular residence time, which make it very powerful for

MR imaging of pediatric CHD. By exploiting these unique imaging properties of Feraheme appropriately, it is possible to acquire images with unprecedented detail, without the need for breath holding, both of which are crucial in tiny babies. Our group recently developed a technique termed 4-dimensional MUSIC-MRI (MUltiphase Steady state Imaging with Contrast) (1), which leverages the specific imaging properties of Feraheme to produce 4 dimensional images of the beating heart in children, during uninterrupted ventilation. By lifting the requirement for breath holding, it becomes possible to prolong the image acquisition time sufficient to capture multiple phases of the cardiac cycle with very high spatial resolution and with high contrast to noise ratio (CNR). By capturing a true, isotropic 3-dimensional set of images over multiple phases of the cardiac cycle, it becomes trivial to reconstruct 2-dimensional slices of the beating heart in any arbitrary plane. Effectively, this is equivalent to acquiring an infinite set of individual slices in multiple orientations, analogous to 2-dimensional cine MRI slices or 2-dimensional planes on echocardiography. However, in the case of 2-dimensional cardiac cine and echocardiography, the individual planes must be specified at the time of the study. This would be prohibitively time consuming, if not geometrically impossible and if not acquired during the original scan, such slices cannot be reconstructed after the fact even if they would help guide surgical decision-making. With MUSIC-MRI, any arbitrary slice can be interrogated after the fact and forever, providing a permanent repository for interrogation of any cardiac structure should this become clinically relevant in a way that was not originally anticipated. Already in our practice at UCLA, Feraheme MRI has informed clinical decision-making in complex diseases and critically ill children in ways that would not otherwise have been possible, even invasively. For example, decisions about whether to perform bi-ventricular vs. single ventricle repair in complex CHD and about the extent and nature of thrombosis and infection have been based soundly on information provided by the unique combination of Feraheme® and techniques developed at UCLA to exploit its properties. Feraheme® has already proved to be invaluable in these patients at our institution. We have accumulated sufficient preliminary evidence in our daily clinical practice (see Appendix) to support the prospect of direct benefit (PDB) in children undergoing Feraheme®-enhanced MRI.

Our current MUSIC-MRI technique uses the ventilator circuit air-pressure signal for respiratory motion gating (i.e. ventilator-gated MUSIC-MRI). Therefore, the technique is currently only applicable to patients who are under anesthesia/sedation with endotracheal intubation. Our long-term goal is to enable free-breathing MUSIC-MRI scans in children who may not require anesthesia or sedation for MRI. In these cases, an alternative respiratory motion gating strategy will need to be developed and validated, and the image acquisition time will need to be further shortened to ensure high quality studies in these patients. Therefore, in Part II of our study, we will develop such an MRI pulse sequence using a respiratory motion self-gating technique and compressed sensing image reconstruction (self-gated MUSIC-MRI) and we will test the hypothesis that the self-gated MUSIC-MRI pulse sequence is non-inferior to the ventilator-gated MUSIC-MRI in a cohort of children with CHD.

5.1 Benefit to Risk Assessment

Feraheme infusion is associated with risks, which include acute anaphylactoid reactions and hypotension, as well as other types of less severe adverse events such as rash, nausea and vomiting. The investigators are fully aware of the potential risks of Feraheme infusion and the following is our plan to mitigate these risks. As summarized in our Overview of Clinical Studies in Section 4.5, Feraheme® and the control contrast agent, Ablavar®, have a similar and small risk of

hypersensitivity/anaphylactoid reactions in pre-marketing clinical trials and the post-marketing data of Feraheme® safety point to a lower reported incidence of anaphylaxis than pre-marketing data. Furthermore, the elemental iron in its core is indispensible for normal biosynthesis and energy metabolism. Iron deficiency is the most widespread disorder in the world and its prevalence in children is 3-7% in the U.S. (84). It has been shown that the majority of pediatric CHD patients do not meet the recommendations for iron uptake (85). In normal neonates, body iron stores reach a nadir at about six months of age and in order to meet the demands of rapid growth must be appropriately supplemented by a diet rich in iron, such as formula. In sick neonates, body iron stores are frequently low from birth and these patients often require iron supplements (85). In these patients, it seems likely that Feraheme® may offer a nutritional iron boost, over and above its imaging applications. Therefore, for the pediatric CHD patient population in this study, who will need a high quality cardiovascular MRI exam in order to inform surgical decision-making, the Investigators feel the potential benefit for these children greatly outweighs the potential incremental risks associated with participating in this study, especially given the recent reports of gadolinium deposits in the brain tissue after repeated GBCA exposure (17–22).

5.2 Monitoring of Adverse Events after Feraheme® Administration

A careful interview with the patient's legal guardians will be conducted before each study. The pediatric CHD patients will undergo clinical screening procedures for exclusion criteria. In children examined under anesthesia or sedation for MRI at UCLA, the full spectrum of anesthetic and sedative agents is routinely available, as is the full spectrum of pharmacological agents required to treat anaphylactic or other adverse drug reactions. Due to the specific concerns about acute AEs, including hypersensitivity and anaphylactic reactions, awareness of the possibility of such occurrence will be appropriately emphasized among the anesthesiology and NICU staff. The following safety strategies are in place at UCLA to mitigate any risks associated with infusion of Feraheme in our pediatric patients with CHD:

1) All patients will be screened for contraindications to Feraheme administration, including a history of allergy to intravenous iron products and iron overload.
2) Prior to Feraheme infusion, all patients will be sedated (or anesthetized), intubated and ventilated such that, in the case of a serious AE, there will already be in place full airway protection and support of respiration. Anesthesia is routinely employed in most centers for infants and young children undergoing cardiac MRI who cannot reliably follow verbal instructions during imaging.
3) Continuous patient monitoring and recording of vital signs (heart rate, blood pressure, oxygen saturation and ECG) before, during and for at least 30 minutes following Feraheme infusion will be provided by expert and specialized staff, including pediatric anesthesiologists and /or staff from the UCLA Neonatal or Pediatric Intensive Care Units. These vital signs data will be uploaded to the UCLA electronic medical record system. In the case of a clinically urgent or life-threatening AE, changes in vital signs will be recognized immediately and appropriate treatment initiated.
4) The dose of Feraheme will be up to 4 mg /kg (approximately half the single therapy dose), diluted with saline and infused slowly (approximately over 10 minutes). Our infusion rate will be consistent with latest FDA guidelines on a mg/kg/sec basis.

Consistent with institutional clinical practice at UCLA and other Centers of Excellence in pediatric cardiovascular MRI, anesthesia in the pediatric CHD patients will be performed by specialized pediatric anesthesiologists regardless of contrast agent used (Feraheme® vs. Ablavar®) with continuous monitoring of heart rate, blood pressure, pulse oximetry, end tidal CO2, and, where appropriate, anesthetic gas levels (such as sevofluorane). All of the children in our study will have protected airways and mechanical ventilatory support from the start of the procedure to the end. Clinically urgent and life-threatening adverse reactions to any agent may include laryngeal edema and bronchospasm, where prior endotracheal intubation may be lifesaving. Should other events occur involving changes in heart rate, heart rhythm and /or blood pressure, the anesthesiologist can focus his / her attention on the appropriate use of chronotropic or inotropic agents, intravenous fluids and /or vasoactive drugs, without having to secure the airway. All patient physiological monitoring data are, and will continue to be, digitized and transferred in real time to the electronic patient medical record, where they are stored permanently and retrievably. Relevant abstractions from these and other patient safety data (protected health information removed) will be included in our DMC, IRB and IND adverse event reports.

6 STUDY OBJECTIVES

1. To compare the diagnostic efficacy of Ablavar®-based MUSIC-MRI with Feraheme®-based MUSIC-MRI in pediatric patients with CHD. (Study Part I)
2. To determine the diagnostic efficacy of an accelerated self-gated MUSIC-MRI cardiovascular MRI technique for pediatric patients with CHD, which ultimately may not require sedation or general anesthesia. (Study Part II)
3. To summarize the safety of Feraheme® and Ablavar® as an MRI contrast agent in pediatric patients with CHD.

7 STUDY DESIGN

7.1 Study Overview

This is an exploratory, investigator-initiated, prospective, open label, case-control, single center, phase IV study that will examine the diagnostic effectiveness of Feraheme® as an MRI contrast agent in pediatric patients with CHD. A total of 120 patients with suspected or known CHD (age newborn to 6 years) of all ethnicities, females and males, will be enrolled. Total duration of subject participation in the imaging study will be approximately 1.5 hours. In general, we expect the pediatric MRI scans to take no more than about 60 minutes once the scan begins. We will also follow up the children for up to 60 days. Total duration of the study is expected to be 5 years. Our study has two parts:

1) Part I is a comparison study between Feraheme®-enhanced MRI and Ablavar®-enhanced MRI. The goal of Part I is to compare the diagnostic efficacy of these two contrast agents for pediatric CHD using our validated technique MUSIC-MRI (1). We hypothesize that Feraheme® provides superior diagnostic effectiveness than Ablavar® for these patients. The duration of Part I is expected to be 3 years.

2) Part II is expected to commence after the statistical significance of the primary endpoint of Part I is confirmed and the goal is to determine the diagnostic efficacy of an MRI pulse sequence (self-gated MUSIC-MRI), which will leverage advanced image reconstruction techniques and

respiratory motion compensation strategies. Self-gated MUSIC-MRI may ultimately enable high-quality free-breathing cardiovascular MRI for pediatric CHD patients who are able to undergo imaging without sedation or general anesthesia (i.e. children >6 years old who can consistently follow verbal instructions during scanning). The duration of Part II is expected to be 2 years.

7.2 Study Design Part I: diagnostic efficacy of Feraheme vs. Ablavar

For Part I of our study, the legal guardian(s) of all consecutive pediatric patients with suspected or known CHD referred for cardiovascular MRI will be given the option of having their child undergoing an Ablavar®-enhanced MRI or a Feraheme®-enhanced MRI, after the potential benefits and risks of both agents are clearly explained and their questions addressed. The risks and benefits of using each of these agents will be verbally discussed with the patients' legal guardians and referring cardiologists or cardiac surgeons. A copy of the written IRB-approved informed consent will be provided. Because Feraheme®-based imaging is in use at several pediatric specialty centers, including UCLA whereby Feraheme is used more frequently than GBCA-based imaging for children in our study age-group, in the current state of professional equipoise, we judge it appropriate to allow parents to participate in the choice of agent (see Section 11.1.1 for detailed justification). Those choosing to undergo an Ablavar®-enhanced MRI will be considered the "control" group while those choosing to undergo a Feraheme®-enhanced MRI will be considered enrolled in the "test" group. We plan to recruit 40 patients for test group and 40 patients for the control group with consideration of approximately 10% missing or unusable image quality. To avoid performing a study with larger-than-necessary enrollment, the Investigators will request an interim analysis at 50% enrollment for both groups. If statistical analyses, either as part of interim analyses requested by the Investigators, or after full enrollment, shows statistically significant primary endpoint comparison, we will close Part I enrollment and begin Part II enrollment using the superior agent identified in Part I. If statistical significance is not reached for the primary endpoint during interim analyses, the patient enrollment for both test and control groups will continue until full enrollment, at which time another final statistical analyses will be performed with $p<0.025$ considered statistically significant. If statistical significance is not reached at full enrollment, we will seek advice from the DMC regarding Part II of the study, including contrast agent to be used.

See Section 11.1.1 for justification for involving the patient's parents in patient group assignment.

7.3 Study Design Part II: diagnostic efficacy of ventilator-gated MUSIC-MRI vs. self-gated MUSIC-MRI

The Part II study will commence after statistical significance of the primary endpoint of Part I is confirmed. The overall goal of Part II is to develop and validate a more advanced MUSIC-MRI pulse sequence that incorporates image acceleration and compressed sensing reconstruction techniques as well as respiratory motion self-gating methods. Our current MUSIC-MRI pulse sequence is designed for young patients (newborn to 6 years old) who undergo cardiovascular MRI under general anesthesia or sedation per our institutional standard practice. The ventilator air pressure signal is currently used as a respiratory motion gating signal in our ventilator-gated MUSIC-MRI pulse sequence. For older children with CHD who can reliably follow verbal instructions during the MRI examination, undergoing sedation or anesthesia for the purpose of MRI would not be justifiable. Therefore, a free-breathing self-gated MUSIC-MRI pulse sequence

that is capable of acquiring high-quality images of the heart and blood vessels of these patients without anesthesia/sedation or the need for a ventilator gating signal would be highly desirable. To achieve this goal, the investigators plan to first validate such a self-gated MUSIC-MRI technique against the existing ventilator-gated MUSIC-MRI technique in patients undergoing MRI under anesthesia or sedation, a similar setup as Part I. The reasons for testing the new pulse sequence in younger patients under anesthesia/sedation rather than in older patients without anesthesia are two fold: 1) This strategy provides a means for the investigators to verify the diagnostic effectiveness of the self-gated MUSIC-MRI sequence against the "gold-standard' ventilator-gated MUSIC-MRI sequence, which would not be possible in spontaneously breathing patients. 2) Even in patients under anesthesia with mechanical ventilation, potential benefits of self-gated MUSIC-MRI include shorter acquisition time (8-10 min for current MUSIC-MRI vs. approximately 4-5 min for the accelerated self-gated MUSIC-MRI) and improved respiratory motion gating, as the self-gating signal is a more direct measurement of diaphragmatic motion than the ventilator circuit air-pressure signal. Therefore, in the Part II study, we will recruit 40 patients who provide informed consent into the contrast agent group (either Feraheme® or Ablavar®) identified in Part I of the study. In each patient, the current ventilator-gated MUSIC-MRI pulse sequence and the to-be-developed self-gated MUSIC-MRI pulse sequence will be performed sequentially in a randomized order by flipping a coin. Other than the added self-gated MUSIC-MRI pulse sequence and target enrollment differences, all other aspects of study design for Part II, including patient safety monitoring and safety data recording, will be the same as Part I. The additional self-gated MUSIC-MRI pulse sequence will require approximately 5 minutes to perform. At the end of Part II study, we will perform statistical analyses specified in Section 18 to confirm the diagnostic efficacy of the self-gated MUSIC-MRI pulse sequence.

If no statistical significant difference for the primary endpoint was found in Part I, we will seek advice from the DMC regarding whether or not to start Part II of the study. If the DMC decides in favor of pursuing Part II of the study, they will also comment on which contrast agent to be used. In offering advice for the choice of agent in Part II, the Investigators will ask the DMC to take the following factors into consideration:

1. Numerical superiority of one agent in Part I, even if statistical significance cannot be reached.
2. Superiority of one agent for the secondary endpoints in Part I.
3. Differences in safety information observed during Part I, if any, and differences in safety based on the current literature at the end of Part I study, including risks of life-threatening reactions, risks to immature kidneys in the participants, potential long-term risks of gadolinium deposit in neurological tissues.

8 CRITERIA FOR EVALUATION

8.1 Part I – Comparison between Feraheme® and Ablavar®

8.1.1 Primary Endpoint

A statistically significant difference in composite image quality score for 7 important anatomical structures between Feraheme®-enhanced vs. Ablavar®-enhanced ventilator-gated MUSIC-MRI.

8.1.2 Secondary Endpoints

A statistically significant difference in image quality scores in each of the 7 important anatomical structures between Feraheme®-enhanced vs. Ablavar®-enhanced ventilator-gated MUSIC-MRI.

A statistically significant difference in sharpness measurements at the ascending aorta and the interventricular septum.

A statistically significant difference in blood-myocardium CNR measurements.

8.1.3 Safety Endpoints

- Incidence of AE for both the test group and the control group.
- Changes in vital signs recordings (hemodynamic parameters, oxygenation, and ventilator parameters) associated with Feraheme® administration
- Changes in vital signs recordings (hemodynamic parameters, oxygenation, and ventilator parameters) associated with Ablavar® administration

8.2 Part II – Comparison between Ventilator-Gated MUSIC-MRI and Self-Gated MUSIC-MRI

8.2.1 Primary Endpoint

Statistically non-inferior composite image quality score of self-gated MUSIC-MRI for 7 important anatomical structures compared to the ventilator-gated MUSIC-MRI.

8.2.2 Secondary Endpoints

1. Statistically non-inferior image quality scores for the self-gated MUSIC-MRI in each of the 7 important anatomical structures compared with the ventilator-gated MUSIC-MRI.
2. Statistically non-inferior sharpness measurements at the ascending aorta and the interventricular septum using the self-gated MUSIC-MRI compared with the ventilator-gated MUSIC-MRI.
3. A statistically non-inferior blood-myocardium CNR measurements.

8.2.3 Safety Endpoints

- Incidence of AE during and after Feraheme administration.
- Changes in vital signs recordings (hemodynamic parameters, oxygenation, and ventilator parameters) associated with contrast agent administration (Feraheme or Ablavar).

9 SUBJECT SELECTION

9.1 Study Population

Pediatric patients of all ethnicities, age newborn to 6 years, of either gender with known or suspected CHD will be recruited. This specific age population has been chosen because our preliminary experiences suggest that the PDB is greatest in the smallest and sickest children. Furthermore, all patients of ages < 6 years undergoing cardiac MR imaging are routinely examined under anesthesia with controlled ventilation independently of our study and under the direct and

continuous observation of a pediatric anesthesiologist or neonatologist. These elements minimize the relative risk of Feraheme® administration by enabling airway protection and the ability to perform non-breath-held diagnostic studies in potentially fragile patients. Our study population will include patients with any types of CHD who are clinically referred to cardiovascular MRI; however, we expect our patient recruitment to be concentrated in more complex forms of CHD, such as Tetralogy of Fallot, because these are the patients that typically have inadequate echocardiographic exams and are referred for MRI. We do not expect our study population to have eGFR<40 mL/min/1.73m^2, although renal insufficiency is an exclusion criteria for the Ablavar® group of Part I study.

9.2 Inclusion Criteria

1. Male or female pediatric patients (age newborn to 6 years) of all ethnicities with known or suspected CHD with insufficient echocardiographic exams and are referred for cardiovascular MRI for further evaluation of cardiac anatomy and function.
2. Written informed consent obtained from subject's legal representative/guardian(s) and ability for subject to comply with the requirements of the study.

9.3 Exclusion Criteria

1. Standard clinical contraindication to MRI, including subjects with cochlear implants and implanted cardiac devices
2. Subjects with past or current diagnosis of iron overload due to hereditary hemochromatosis or other causes (for subjects receiving Feraheme® injection only).
3. Subjects with known hypersensitivity or allergy to iron oxide particles.
4. Subjects with renal insufficiency defined as estimated glomerular filtration rate (eGFR) < 40 mL/min/1.73m^2 (for subjects receiving Ablavar® injection only).
5. Subjects who are critically ill at the time of MRI and for whom the period of general anesthesia and separation from the critical care nursery or intensive care unit poses added risk as deemed by referring cardiologists, cardiac surgeons or the managing radiologist (for Part II only).
6. Other medical conditions, in the judgment of the clinician investigator, that would increase the risks to the child related to participation in the study.

10 CONCURRENT MEDICATIONS

Concurrent medication is allowed as clinically necessary for the subject.

10.1 Allowed Medications and Treatments

Concurrent medication is allowed as clinically necessary for the subject.

10.2 Prohibited Medications and Treatments

None

11 STUDY TREATMENTS

11.1 Method of Assigning Subjects to Treatment Groups

Refer to Section 7.2 for patient group assignment in Part I of the study. For Part II of the study, all subjects will undergo the ventilator-gated MUSIC-MRI and self-gated MUSIC-MRI in one imaging session in a randomized order by flipping a coin.

11.1.1 Justification for Parents Participation in Patient Group Assignment

We plan to allow parents to participate in the decision about treatment assignment in Part I of this open-label, evaluator-blinded study. Feraheme-based imaging is in use at several pediatric specialty centers, including UCLA whereby Feraheme is used more frequently than GBCA-based imaging for children in our study age-group. In the current state of professional equipoise, we judge it appropriate to allow parents to participate in the choice of agent. There is no *a priori* reason to expect MRI image quality, ventricular or aortic wall definition, or the risks of anaphylaxis or other important AEs to be influenced by parental preferences; thus the generalizability of our scientific conclusions will not be diminished by allowing parents to influence the treatment assignment for their children. Recruitment of children in clinical studies is a challenging process. Based on our clinical investigative experience in the particular patient population at UCLA, many parents and/or legal guardians are more willing to participate in a study without randomization of treatment than one that required randomization of the child's treatment. We expect this route of recruitment will facilitate recruitment and successful completion of the study. In scenarios where the patient's legal guardians defer and ask the managing clinician to make 'the best choice', the decision will be based on the apparent needs of the trial at the time and the patient will be assigned the group that is under-enrolled with reference to the target enrollment. If our patient group assignment method results in a severely distorted imbalance (e.g. nearly all parents elect either Feraheme or Ablavar), the DMC will be convened and asked to advise on a corrective measure, which may include changing the patient group allocation method.

11.2 Blinding

For Part I of the trial, the image readers will be blinded to the patient's information and the contrast agent used. For Part II of the trial, the image reviewers will be blinded to the techniques used. In both cases (Part I and Part II), scores for image quality, sharpness and CNR measurements will remain blinded to the managing clinician and the Investigators and only the study statistician will be able to pair the contrast agent assignment /score (for Part I) and technique assignment /score (for Part II).

11.3 Formulation of Test and Control Products

Formulation of Test Product

Feraheme (Feraheme®) is an FDA approved drug manufactured by AMAG Pharmaceuticals for I.V. administration as an iron supplement for iron deficiency anemia in chronic kidney disease. It is an aqueous colloidal product that is formulated with mannitol. It is a black to reddish brown liquid, and is provided in single use vials containing 510 mg of elemental iron. Each mL of the sterile colloidal solution of Feraheme contains 30 mg of elemental iron and 44 mg of mannitol,

and has low bleomycin-detectable iron. The formulation is isotonic with an osmolality of 270-330 mOsm/kg. The product contains no preservatives, and has a pH of 6 to 8.

Formulation of Control Product

Ablavar® is an FDA approved MRI contrast agent for evaluation of aortoiliac occlusive disease in adults with known or suspected peripheral vascular disease. Ablavar is a sterile, nonpyrogenic, formulation of stable gadolinium diethylenetriaminepentaacetic acid (GdDTPA) chelate derivative with a diphenylcyclohexylphosphate group. Each mL of Ablavar contains 244 mg of gadofosveset trisodium (0.25 mmol), 0.268 mg of fosveset, and water for injection. It contains no preservative and the solution pH ranges between 6.5 and 8.0. Gadofosveset trisodium is chemically trisodium-{(2-(R)-[(4,4-diphenylcyclohexyl) phosphonooxymethyl]-diethylenetriaminepentaacetato)(aquo) gadolinium(III), with a molecular weight of 975.88 g/mol, and an empirical formula of $C_{33}H_{40}GdN_3Na_3O_{15}P$.

Packaging and Labeling

Feraheme® and Ablavar used in this study will be commercial product and will be in the commercial primary container/closure system. Investigational labels, with the required caution statement, "Caution-new drug limited by Federal Law to Investigational Use" will be used to over-label individual vials of commercial product by the UCLA pharmacy before supplying to the investigator for the clinical study.

Dosage/Dosage Regimen

Feraheme will be infused intravenously at a dose level of 4 mg of iron per kg of body weight.

Ablavar is administered by an intravenous bolus at a dose of 0.03 mmol/kg body weight over a period of time up to 30 seconds followed by a normal saline flush.

Administration Instructions

The drug will be administered by a nurse or MRI technologist who is trained on the clinical protocol and will be administered under the direction of the Principal Investigator.

11.4 Supply of Study Drug at the Site

The investigators will obtain Feraheme® and Ablavar® from the UCLA Hospital pharmacy with the investigational labels and the IND caution statement.

11.5 Storage

Feraheme will be stored at 20° to 25°C (68° to 77°F). Excursions permitted to 15° – 30°C (59° – 86°F) and ABLAVAR will be stored up to 25°C (77°F); excursions permitted to 15-30°C.

11.6 Study Drug Accountability

Both Feraheme and Ablavar used in our study will be marketed product and they will be obtained from the UCLA Medical Center pharmacy in pre-packaged sterile vials. Any unused contrast agents will be disposed of according to Standard Procedures at the imaging site.

12 STUDY PROCEDURES AND GUIDELINES

Prior to conducting any study-related activities, written informed consent and the Health Insurance Portability and Accountability Act (HIPAA) authorization must be signed and dated by the subject or subject's legal representative/guardian(s).

12.1 Clinical Assessments

Concomitant Medications

All concomitant medication and concurrent therapies will be documented at the date of study MRI. Dose, route, unit frequency of administration, and indication for administration and dates of medication will be captured.

Demographics

Demographic information (date of birth, gender, race) will be recorded at Screening.

Medical History

Relevant medical history, including history of current disease, other pertinent respiratory history, and information regarding underlying diseases will be recorded at Screening.

Physical Examination

N/A

Vital Signs / Oximetry / Ventilatory Parameters

Continuous heart rate, blood pressure, ECG, pulse oximetry, and end tidal CO_2 will be monitored for the duration of the MRI study and until the subject is recovered or returned to the Intensive Care facility, or for 30 minutes after the infusion, whichever is longer.

Adverse Events

Information regarding occurrence of acute adverse events will be captured throughout the study. Duration (start and stop dates and times), severity/grade, outcome, treatment and relation to study drug will be recorded on the case report form (CRF). Each patient will be followed up via a review of medical record at 60 days after MRI study to capture any additional AEs.

12.2 MRI Procedures

12.2.1 Patient Preparation and Setup

All patients will undergo MRI on an FDA approved, clinical 18-channel Siemens 3.0T Magnetom TIM Trio scanner, and running version B17 software. The appropriate RF receiver coil configuration will be chosen depending on patient size; for example neonates may be studied using a 16 element adult knee coil, infants using an adult head-neck coil combination and older children using surface flex coils or a body array coil. Fixed (spine) coil elements in the patient table may be used in combination with any of the other coils (except the knee coil).

Prior to Feraheme or Ablavar infusion, all patients will be asleep, intubated and ventilated such that, in the case of a clinically urgent or life-threatening AE, there will already be in place full

airway protection, physiological monitoring and support of respiration. Consistent with institutional clinical practice at UCLA, anesthesia in the pediatric CHD patients will be performed by specialized pediatric anesthesiologists regardless of contrast agent used (Feraheme vs. Ablavar) with continuous monitoring of heart rate, blood pressure, pulse oximetry, end tidal CO_2, and, where appropriate, anesthetic gas levels (such as sevofluorane). In the case of a SAE, changes in vital signs will be recognized immediately and appropriate treatment initiated. All of the children in our study will have protected airways and mechanical ventilatory support from the start of the procedure to the end. Serious adverse reactions to any agent may include laryngeal edema and bronchospasm, where prior endotracheal intubation may be lifesaving. Should other events occur involving changes in heart rate, heart rhythm and /or blood pressure, the anesthesiologist can focus his / her attention on the appropriate use of chronotropic or inotropic agents, intravenous fluids and /or vasoactive drugs, without having to secure the airway. All patient physiological monitoring data are, and will continue to be, digitized and transferred in real time to the electronic patient medical record, where they are stored permanently and retrievably. Relevant abstractions from these and other patient safety data (protected health information removed) will be included in our DMC, IRB and IND adverse event reports.

Once asleep and intubated, patients will be positioned in the appropriate receiver coils and monitored with ECG and with MRI compatible pulse oximetry and non-invasive blood pressure measurement (InVivo Medical Solutions, Inc.). The vital signs monitoring equipment will all be FDA approved devices. The end-tidal CO_2 line will be interfaced via a three way plastic stop-cock to the respiratory port of the MRI physiological monitoring unit. In this way, the respiratory signal is split so as to provide both uninterrupted end-tidal CO_2 monitoring and airway pressure signal. The airway pressure trace is available for use as a respiratory gating signal to compensate for respiratory motion artifact without interrupting patient ventilation (1).

12.2.2 Contrast Agent Dose and Injection Rate

12.2.2.1 Feraheme® Dose and Injection Rate

Once patients are positioned and outfitted with receiver coils, venous access lines and physiological monitoring sensors, dilute Feraheme® will be administered by slow intravenous infusion to a total dose of 4 mg /kg (1,83). According to the recent FDA warning issued in March 2015, the entire therapeutic dose (510mg of Fe) of Feraheme should be injected as a slow infusion over 15 min or longer. For an average adult of 75kg body weight, 510 mg corresponds to 7 mg /kg and a 15 min infusion time corresponds to an infusion rate of 0.45 mg /kg.min. We will infuse 4 mg/kg of Feraheme which, at the rate suggested by the FDA, would require 8 min 30 sec to infuse. We will round this time up to 10 minutes for practicality and to ensure we do not exceed the recommended rate. The Feraheme will be diluted before injection for safety as well as practical reasons. The FDA warning dated March 2015 requires that Feraheme not to be injected without dilution to minimize risks of acute adverse reactions. The weight of patients in our study may range from 1kg (neonate) to 20 kg (6 year old). For neonates or small infants, the absolute volume of Feraheme to be used may be < 0.5 ml, and without significant dilution, controlling infusion of this volume over 10 minutes would be technically impractical. The factor by which the Feraheme can be safely diluted will also be influenced by the patient's physiological status. Patients with cardiac failure, a fixed cardiac output or with renal impairment may not tolerate a fluid challenge well.

For precise calibration of dose and delivery rate in the study group, the following considerations apply:

The stock formulation of Feraheme® is an isosmolar solution containing 30 mg elemental iron equivalent per ml. Therefore, 4 mg of iron is contained in 0.13 ml of undiluted Feraheme®. Total administered volume of undiluted Feraheme® is therefore 0.1333 ml /kg * weight in kg. Due to the requirement to infuse over several minutes, the stock formulation will be diluted by a factor of 6-30 with normal saline and infused over 10 minutes (600 secs). This rate corresponds to an 18 minute infusion period for a 500 mg dose of iron in a 70 kg adult and is therefore within the 15 minute infusion guideline set forth by the FDA in March, 2015.

12.2.2.2 Ablavar Dose and Injection Rate

Each patient in the Ablavar group of our study will receive 0.03 mmol/kg of Ablavar, the FDA approved dose for MR angiography. The Ablavar will be diluted up to 8 fold and injected over 15-20 seconds (86). These dose and injection rates are consistent with our current institutional standard practice.

12.2.3 MRI Scanning Procedures

As for all patients, the MRI system will perform automatic adjustment procedures to optimize the homogeneity of the magnetic field, customized to the individual patient. It will also perform standard RF frequency adjustment of the transmitter RF field and calibration of the receiver coil elements. All parameters for RF dose (SAR or specific absorption rate) and gradient switching rates will be within FDA approved limits and monitored continuously by permanent and immutable software and hardware watchdogs (Siemens product specifications on file at FDA).

With the patient being ventilated continuously, the following MRI pulse sequences will be performed:

For Study Part I, each patient (regardless of test or control group assignment) will undergo MRI using the following MRI pulse sequences: 1) Conventional 2D cine imaging for cardiac function assessment and phase-contrast MRI for flow quantification in imaging planes specified by a radiologist or cardiologist at the point of delivery, per our institutional standard practice regardless of our research protocol. These image acquisitions are necessary to ensure that each patient will get a diagnostic MRI study regardless the outcome of our research and will only be performed when the patient is considered by the supervising anesthesiologist to be stable for ventilator-controlled breath-hold maneuvers; 2) The 3D cine ventilator-gated MUSIC-MRI MRI, based on which the Feraheme vs. Ablavar comparison will be performed.

For Study Part II, each patient will undergo the same MRI pulse sequence as Part I of our study. In addition, each patient will undergo the to-be-developed self-gated MUSIC-MRI MRI and the order of the ventilator-gated MUSIC-MRI and the self-gated MUSIC-MRI will be randomized. The self-gated MUSIC-MRI pulse sequence will require an additional 5 min of scan time and anesthesia time without breath holding, which the investigators feel does not add significant additional risk to the patient.

13 EVALUATIONS BY VISIT (ONLY 1 VISIT)

13.1 Visit 1 (Day/Week/Month #)

1. Clinical indication for contrast enhanced cardiovascular MRI established by discussion involving the referring clinician and the Investigators or designee.
2. Clinical rationale for Feraheme vs. Ablavar is discussed with the patient's legal guardian(s) by the Investigators or designee in Part I of the study and the patient's legal guardian(s) are given the choice to choose Feraheme vs. Ablavar as the contrast agent. In Part II of the study, the superior contrast agent as determined in Part I of the study will be used and the benefits and risks of undergoing a combination of ventilator-gated MUSIC-MRI and self-gated MUSIC-MRI will be discussed with the patient's legal guardian(s)
3. Obtain written informed consent and HIPAA authorization and assent, if appropriate by the Investigators or designee.
4. Assign the subject a unique screening number.
5. Record demographics data.
6. Record medical history, including a history of hypersensitivity reactions to iron products and iron overload. Screen for inclusion and exclusion criteria.
7. Procedure for sedation and cardio-respiratory support discussed with the anesthesiologist or NICU staff by the Investigator or designee.
8. Patient undergoes general anesthesia with endotracheal intubation or is transported sedated and intubated from the NICU.
9. Record concomitant medications.
10. Perform and record vital signs.
11. Perform and record oximetry.
12. Perform and record results of blood pressure testing.
13. Contrast infusion.
14. Continue monitoring and recording vital signs and oximetry up to 30 min after infusion
15. MRI exam (ventilator-gated MUSIC-MRI for Part I of the study, and both ventilator-gated and self-gated MUSIC-MRI for Part II of the study).
16. Patient recovers from anesthesia/sedation.

It is noted that one or more of Items 1-through-7 may occur on a day different from (prior to) the day of the MRI study. All of these procedures will be included in 'Visit 1' with relevant dates as appropriate.

14 ADVERSE EXPERIENCE REPORTING AND DOCUMENTATION

14.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a clinical investigation of a patient administered a pharmaceutical product and that does not necessarily have a causal relationship with the treatment. An AE is therefore any unfavorable and unintended sign (including an

abnormal laboratory finding), symptom or disease temporally associated with the administration of an investigational product, whether or not related to that investigational product.

The Investigator will probe, via discussion with the subject, for the occurrence of AEs during each subject visit and record the information in the site's source documents. Adverse events will be recorded in the patient CRF. Adverse events will be described by duration (start and stop dates and times), severity, outcome, treatment and relation to study drug, or if unrelated, the cause.

14.1.1 AE Severity

The National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 should be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant. If the experience is not covered in the criteria, the guidelines shown in Table 6 below should be used to grade severity. It should be pointed out that the term "severe" is a measure of intensity and that a severe AE is not necessarily serious.

Table 6. *AE Severity Grading*

Severity (Toxicity Grade)	Description
Mild (1)	Transient or mild discomfort; no limitation in activity; no medical intervention or therapy required. The subject may be aware of the sign or symptom but tolerates it reasonably well.
Moderate (2)	Mild to moderate limitation in activity, no or minimal medical intervention/therapy required.
Severe (3)	Marked limitation in activity, medical intervention/therapy required, hospitalizations possible.
Life-threatening (4)	The subject is at risk of death due to the adverse experience as it occurred. This does not refer to an experience that hypothetically might have caused death if it were more severe.

14.1.2 AE Relationship to Study Drug

The relationship of an AE to the study drug should be assessed using the following the guidelines in Table 7.

Table 7. *AE Relationship to Study Drug*

Relationship to Drug	Comment
Definitely	Previously known toxicity of agent; or an event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is not explained by any other reasonable hypothesis.
Probably	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is unlikely to be explained by the known characteristics of the subject's clinical state or by other interventions.

Possibly	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to that suspected drug; but that could readily have been produced by a number of other factors.
Unrelated	An event that can be determined with certainty to have no relationship to the study drug.

14.2 Serious Adverse Experiences (SAE)

An SAE is defined as any AE occurring at any dose that results in any of the following outcomes:

- death
- a life-threatening adverse experience
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly/birth defect

Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the subject or require intervention to prevent one of the outcomes listed.

Serious Adverse Experience Reporting

The investigators will document all SAEs that occur (whether or not related to study drug). The collection period for all SAEs will begin after informed consent is obtained and end after procedures for the study visit have been completed. In accordance with the standard operating procedures and policies of the local Institutional Review Board (IRB), the site investigator will report SAEs to the IRB.

14.3 Medical Monitoring

J. Paul Finn, M.D. should be contacted directly at these numbers to report medical concerns or questions regarding safety.

Phone: (310) 825-0958

15 DISCONTINUATION AND REPLACEMENT OF SUBJECTS

15.1 Early Discontinuation

A subject may be discontinued from study treatment at any time if the subject's legal guardians or the Investigators feel that it is not in the subject's best interest to continue. The following is a list of possible reasons for study treatment discontinuation:

- Subject withdrawal of consent
- Subject is not compliant with study procedures
- Adverse event that in the opinion of the investigator would be in the best interest of the subject to discontinue study treatment
- Protocol violation requiring discontinuation of study treatment

- Lost to follow-up
- Sponsor request for early termination of study

If a subject is withdrawn from treatment due to an AE, the subject will be followed and treated by the Investigator until the abnormal parameter or symptom has resolved or stabilized.

15.2 Withdrawal of Subjects from the Study

A subject may be withdrawn from the study at any time if the subject, the investigator, or the Sponsor feels that it is not in the subject's best interest to continue.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents.

15.3 Replacement of Subjects

Subjects who withdraw from the study prior to the recording of the MRI images required for statistical analyses will be replaced.

16 PROTOCOL VIOLATIONS

A protocol violation occurs when the subject, investigator, or Sponsor fails to adhere to significant protocol requirements affecting the inclusion, exclusion, subject safety and primary endpoint criteria. Protocol violations for this study include, but are not limited to, the following:

- Failure to meet inclusion/exclusion criteria

Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation. The Sponsor will determine if a protocol violation will result in withdrawal of a subject.

17 DATA SAFETY MONITORING

A Data Monitoring Committee (DMC) will review data relating to safety and efficacy, will conduct and review interim analyses, and will ensure the continued scientific validity and merit of the study, according to the UCLA Data Safety Monitoring Board Operations Manual. There will be at least 3 members, who will have no other role in the trial, including at least one expert with statistical expertise, one member with clinical specialty of pediatric cardiology, and one member with experience serving on independent data review boards. At the first meeting, held before the trial either face-to-face or via tele-conference, the committee will approve or revise the charter. The chair of the DMC will be appointed by the PI of the study.

18 STATISTICAL METHODS AND CONSIDERATIONS

The primary efficacy analysis of visual imaging quality will be conducted using all usable scans from the patients. All safety analyses will be conducted using a population defined as all patients undergoing study contrast-agent (Safety Population).

18.1 Data Sets Analyzed

All eligible patients who receive Feraheme or Ablavar infusion as part of our study will be included in the data analysis. The results will be reported separately for the two contrast agents.

18.2 Demographic and Baseline Characteristics

The following demographic variables at screening will be summarized by treatment group and study part. While the study is too small to expect statistical significance in sub-groups, the efficacy and safety results will be examined by standard age categories (neonates (0-to-1 month of age), infants and toddlers (1 month to 2 years of age) and children (>2 years of age)) and any clinically important differences reported.

18.3 Definition of Numeric Efficacy Outcomes

Subjective Image Quality Scores: The image quality will be independently scored by 2 board certified blinded readers with >5 years of cardiovascular MRI reading experience using a 1-4 point scale (1). The composite image quality score for each subject will be a number between 7 (all subjective image quality scores are 1=non-diagnostic) to 28 (all subjective image quality scores are 4=clear visualization). The scores will be assessed separately for each of the following seven anatomical structures that are important for imaging pediatric CHD patients: the aortic root, the pulmonary artery (PA), the coronary arteries, the out-flow tracts, the valves, the ventricles, and the atria. If the difference in the composite score is greater than or equal to 4 between two image readers, the readers will read the image together in a consensus reading session and arrive at a consensus for each of the 7 structures. The criteria for assigning scores will be as follows: 1) For **aortic root and PA**, 1=nondiagnostic (vessels not visualized or diagnostically not assessable due to small size and /or motion artifact and /or poor contrast enhancement), 2=vessels visualized but with poor contrast enhancement and /or motion artifact so as to limit confident assessment and measurement of dimensions, 3=vessels visualized with good contrast so as to enable confident assessment of patency but with poor edge definition due to motion artifact so as to limit confident measurement of dimensions, 4=vessels visualized with good contrast and good edge definition such that patency and dimensions are confidently assessable; 2) For **coronary arteries**: 1=nondiagnostic (vessels not visualized or diagnostically not assessable due to small size and /or motion artifact and /or poor contrast enhancement), 2=only origins of main coronary arteries confidently identifiable, 3=origins and proximal course of RCA and left anterior descending (LAD) confidently evaluable, 4=origin, proximal and mid courses of RCA and LAD and proximal takeoff of left circumflex confidently evaluable; 3) For **out-flow tracts and valves**: 1=nondiagnostic (structures not visualized or diagnostically not assessable due to small size and /or motion artifact and /or poor contrast enhancement, 2=annulus and sinotubular junction visualized but borders not sufficiently well defined for confident measurement, 3=annulus and sinotubular junction visualized with well defined borders sufficient for confident measurement, 4=annulus and sinotubular junction clearly visualized with well defined borders sufficient for confident measurement and valve leaflets clearly seen; 4) For the **ventricles and atria**: 1=nondiagnostic (chambers not visualized or diagnostically not assessable due to small size and /or motion artifact and /or poor contrast enhancement, 2=chambers distinguishable but walls poorly defined and only gross features evaluable, 3=chambers clearly distinguishable with well defined septum and free walls confidently evaluable but with poor definition of the papillary

muscles and trabeculae, 4=chambers clearly distinguishable with excellent wall definition and with clear definition of the papillary muscles and trabeculae.

Image Sharpness Values: Objective image sharpness will be measured through the interventricular septum and ascending aorta using a previously described method (1). The image sharpness scores will be measured by drawing a linear signal profile and calculating the slope of the signal intensity curve. The slope is defined as the image intensity difference divided by the distance between the two points at 20% and 80% of the dynamic range, respectively.

Blood-Myocardium CNR: The blood-myocardium CNR will be measured by drawing three regions of interest (ROI), one at the center of left ventricular (LV) cavity, one at the interventricular septum and one in the background air region. The CNR will be calculated as the difference in mean signal intensity between the LV cavity ROI and the septum ROI divided by the standard deviation of the background air ROI signal.

18.4 Primary Efficacy Endpoints

18.4.1 Part I – Difference in the composite image quality score between Feraheme and Ablavar

The primary efficacy endpoint for Part I is the composite image quality score, which is defined as the sum of the subject image quality scores assigned to seven important anatomical structures for pediatric CHD patients: the aortic root, the PA, the coronary arteries, the outflow tracts, the valves, the ventricles, and the atria. The score will be the average scores from all the image readers. The consensus reading will occur if the difference in the composite score from the same image is 4 or greater. The composite image quality score will be tested for normality using the Shapiro-Wilk and Shapiro-Francia tests. For normally distributed data, comparison will be made between the composite image quality scores for Feraheme and Ablavar using a parametric two-sided t-test with an appropriate transformation if needed. If statistical transformation of the image quality score is not possible, a non-parametric Wilcoxon-rank-sum test can be considered. The null hypothesis is that there is no difference in the composite subjective image quality scores between Feraheme and Ablavar. This comparison will be a two-sided test at the overall 0.05 level of significance. Summary statistics of mean, standard deviation and their 95% confidence intervals of each contrast agent and the difference will be used for statistical description. There will be a formal interim analysis and a primary analysis for image quality score. The primary analysis of image quality scores will be performed when 80 CHD subjects are enrolled and the images are scored by the image readers. Scores for subjective image quality, sharpness and CNR measurements will remain blinded from the Investigators and only the study statistician will be able to pair the contrast assignment and the score.

[Interim Analyses]: To avoid performing a study with larger-than-necessary enrollment for Part I of our study, a formal interim analysis for the composite imaging score will be performed at approximately 20 CHD subjects for each of the contrast group (50% of the target number for the primary analysis). Alpha-spending for the interim analyses and the primary analysis will be 0.025 and 0.025 (both two-sided), respectively. The overall alpha for the composite image quality score will be preserved at the two-sided 0.05 significance level. The interim analysis of the composite image quality score will be conducted by an independent statistician under the charter of the DMC.

The interim effect of Feraheme compared to the Ablavar of ventilator-gated MUSIC-MRI based on the average composite score from two image readers will be tested with a two group t-test with an appropriate transformation if needed. Non-parametric Wilcoxon rank sum test will be considered if the data are not normally distributed based on the Shapiro-Wilk and Shapiro-Francia tests and transformation is not feasible.

18.4.2 Part II – Difference in the composite image quality score between ventilator-gated and self-gated MUSIC-MRI

The primary efficacy endpoint for Part II is the composite image quality score defined in 18.4.1. Data will be tested for normality using the Shapiro-Wilk and Shapiro-Francia tests. The composite subjective image quality score will be compared between ventilator-gated MUSIC-MRI and the self-gated MUSIC-MRI using a paired t-test with appropriate transformation if needed. If the data are not normally distributed and transformation is not possible, a Wilcoxon-sign-rank test will be considered. The null hypothesis is that the mean difference in the image quality scores of the self-gated images is smaller than or equal to the mean from ventilator-gated images with the non-inferiority margin of – 1.75 (self–ventilator $\leq$ -1.75). The alternative hypothesis is that the mean difference in image quality score of self-gated images from the ventilator-gated images is greater than the non-inferiority margin of -1.75 (self–ventilator > -1.75) (see the detail of choosing the margin in section 18.7.2.).

Rejection of the null hypothesis will occur if the p-value is $\leq$0.025. Scores for subjective image quality will remain blinded from the Investigators and only the study statistician will be able to pair the MRI pulse sequence and the score. No interim analyses will be performed in Part II of the study.

18.5 Secondary Efficacy Endpoints

18.5.1 Part I – Differences in individual image quality score (per segment or structure), image sharpness values, and CNR measurements for Feraheme vs Ablavar

The secondary efficacy endpoints for Part I are the individual image quality score for each of the seven anatomical structures as defined in 18.3, the image sharpness values for the ascending aorta and the interventricular septum, and the CNR measurements for each contrast agent. Data will be tested for normality using the Shapiro-Wilk and Shapiro-Francia tests. The average image quality scores of each anatomic structure between two readers, the image sharpness values, and the CNR measurements will be compared using a parametric two group t-test with an appropriate transformation if needed. If the data are not normally distributed and transformation is not possible, a non-parametric Wilcoxon-rank-sum test will be considered. Kappa statistics will be reported between two independent scores in each secondary endpoint.

18.5.2 Part II – Differences in individual image quality score (per segment or structure), image sharpness values, and CNR measurements for ventilator-gated vs self-gated MUSIC-MRI

The secondary efficacy points for Part II are the individual subjective image quality score for each of the seven anatomical structures as defined in 18.3, the image sharpness values for the ascending aorta and the interventricular septum, and the CNR measurements for the two MRI techniques. Data will be tested for normality using the Shapiro-Wilk and Shapiro-Francia tests. The average image quality scores of each anatomic structure between two readers, the image sharpness values, and the CNR measurements will be compared using a paired t-test with the margin of non-

inferiority of 0.75 for the image quality scores. If the data are not normally distributed and transformation is not possible, a non-parametric Wilcoxon-rank-sum test will be considered. Kappa statistics will be reported between two independent scores in each secondary endpoint.

18.6 Safety Evaluations

AEs, SAEs, AEs resulting in premature withdrawal from the study, AEs of special interest (hypersensitivity reactions and blood pressure changes requiring intervention), and study related adverse events will be listed and summarized by contrast agent group. For the purpose of safety evaluation, the patients in both Part I and Part II who received the same contrast agent will be included in a single safety report. Due to the limited study cohort size and the small anticipated AE rates, no formal inferential statistical analyses will be performed for AEs. However, while the study is too small to expect statistical significance in sub-groups, the efficacy and safety results will be examined by standard age categories (neonates (0-to-1 month of age), infants and toddlers (1 month to 2 years of age) and children (>2 years of age)) and any clinically important differences reported.

18.7 Sample Size and Power Estimate for the Primary Efficacy Endpoints

18.7.1 Part I – Difference in the composite image quality score between Feraheme and Ablavar

Sample size and power estimates are based on data from an internal pilot analysis. Based on the average composite image quality scores of two independent readers, the mean image quality score was 25.62 with a standard deviation of 1.82 for the Feraheme-enhanced, ventilator-gated MUSIC-MRI group. The difference in the image quality scores between two readers was a mean of -1.96 with a standard deviation of 2.72. We expect the agreement between two independent readings will improve with consensus reading in cases with largely different image quality scores (4 or more absolute difference in the score).

Based on the above preliminary analyses, for the interim analysis, a sample size of 20 in each contrast agent group will achieve a power of 62% to detect a difference of 1.75 (7% reduction) in the composite image quality scores between the Ablavar group and the Feraheme group with an estimated standard deviation of 2.10 in each group and a significance level of 2.5%.

In the primary analysis, a sample size of 36 subjects will achieve a power of 90% to detect a difference of 1.75 (7% reduction) in composite image quality scores between the Ablavar group and the Feraheme group with an estimated standard deviation of 2.10 in each arm and a significance level of 2.5%. Taking into consideration missing data due to unusable image quality or incomplete studies, we plan to enroll 40 CHD subjects in each group (N=80).

18.7.2 Part II – Difference in the composite image quality score between ventilator-gated and self-gated MUSIC-MRI

The quality of self-gated images are expected to be of at least equal quality to ventilator-gated images with a larger standard deviation due to variation in the methods of respiratory motion gating. We choose the non-inferior margin (87) to ensure that the upper bound of the 95% confidence interval (CI) for the difference between ventilator-gated and self-gated (ventilator – self-gated) is less than 1.75. We expect the mean of the composite image quality score for ventilator-gated images to be approximately 25.62, which is the expected upper limit of the self-gated image quality

score. The margin of 1.75 was derived using an expected mean of 24.75 for the self-gated image (~4% reduction in image quality score compared to that achieved with the ventilator-gated method). The non-inferiority comparison will be a one-sided test at a 0.025 level of statistical significance and a -1.75 margin. A sample size of 36 will achieve a statistical power of greater than 90% to detect non-inferiority between ventilator-gated and self-gated MUSIC-MRI using a one-sided t-test with a non-inferiority margin of -1.75 and a true difference of zero with a standard deviation of 3.0 and a significance level of 2.5%. Taking into account missing data due to unusable image quality or incomplete studies, we plan to enroll 40 CHD subjects.

19 DATA COLLECTION, RETENTION AND MONITORING

19.1 Data Collection Instruments

The Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each subject treated with the study drug.

Study personnel will enter data from source documents corresponding to a subject's visit into the protocol-specific electronic Case Report Form (eCRF) when the information corresponding to that visit is available. Subjects will not be identified by name in the study database or on any study documents to be collected by the Sponsor (or designee), but will be identified by a subject number and initials.

If a correction is required for an eCRF, the time and date stamps track the person entering or updating eCRF data and create an electronic audit trail.

The Investigators are responsible for all information collected on subjects enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the Investigators. A copy of the eCRF will remain at the Investigators' site at the completion of the study.

19.2 Data Management Procedures

The data will be entered into a validated database. The Data Management group will be responsible for data processing, in accordance with procedural documentation. Database lock will occur once quality assurance procedures have been completed.

All procedures for the handling and analysis of data will be conducted using good computing practices meeting FDA guidelines for the handling and analysis of data for clinical trials.

19.3 Data Quality Control and Reporting

After data have been entered into the study database, a system of computerized data validation checks will be implemented and applied to the database on a regular basis. All changes to the study database will be documented.

19.4 Archival of Data

The database is safeguarded against unauthorized access by established security procedures; appropriate backup copies of the database and related software files will be maintained. Databases are backed up by the database administrator in conjunction with any updates or changes to the database.

At critical junctures of the protocol (e.g., production of interim reports and final reports), data for analysis is locked and cleaned per established procedures.

19.5 Availability and Retention of Investigational Records

The Investigators will make study data accessible to the monitor, other authorized representatives of the Sponsor (or designee), IRB, and Regulatory Agency (e.g., FDA) inspectors upon request. A file for each subject will be maintained that includes the signed Informed Consent, HIPAA Authorization and copies of all source documentation related to that subject. The Investigators will ensure the reliability and availability of source documents from which the information on the CRF was derived.

All study documents (patient files, signed informed consent forms, copies of CRFs, Study File Notebook, etc.) will be kept secured for two years after the center has been notified that the IND has been discontinued. There may be other circumstances for which the Sponsor is required to maintain study records and, therefore, the Sponsor will be contacted prior to removing study records for any reason.

19.6 Monitoring

Monitoring visits will be conducted by representatives of the Sponsor according to the U.S. CFR Title 21 Parts 50, 56, and 312 and ICH Guidelines for GCP (E6). By signing this protocol, the Investigator grants permission to the Sponsor (or designee), and appropriate regulatory authorities to conduct on-site monitoring and/or auditing of all appropriate study documentation.

19.7 Subject Confidentiality

In order to maintain subject confidentiality, only a subject number and subject initials will identify all study subjects on CRFs and other documentation submitted to the Sponsor. Additional subject confidentiality issues (if applicable) are covered in the Clinical Study Agreement.

20 ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).

Any serious, unexpected suspected adverse reactions, findings from other clinical, animal, or in-vitro studies that suggest significant human risk, and any clinically important increases in the rate of a serious suspected adverse reaction will be sent to the FDA, Division of Medical Imaging Products, and to all investigators no later than 15 calendar days after determining that the information qualifies for reporting [21 CFR 312.32(c)(1)]. Additionally, any unexpected fatal or life-threatening suspected adverse reaction will be reported to the FDA Division of Medical Imaging Products no later than 7 calendar days after initial receipt of the information [21 CFR 312.32(c)(2)]. Each submission will be addressed to the Regulatory Project Manager and/or the Chief, Project Management Staff for the Division.

To maintain confidentiality, all reports, evaluation forms, and other records will be identified by a coded number and initials only. All study records will be kept in a locked file cabinet and code sheets linking a patient's name to a patient identification number will be stored separately in

another locked file cabinet. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the FDA. The Investigator must also comply with all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC).

20.1 Protocol Amendments

Any amendment to the protocol will be written by the Investigators. Protocol amendments cannot be implemented without prior written IRB approval except as necessary to eliminate immediate safety hazards to patients. A protocol amendment intended to eliminate an apparent immediate hazard to patients may be implemented immediately, provided the IRBs are notified within five working days.

20.2 Institutional Review Boards and Independent Ethics Committees

The protocol and consent form will be reviewed and approved by the IRB of the participating center prior to study initiation. Serious adverse experiences regardless of causality will be reported to the IRB in accordance with the standard operating procedures and policies of the IRB, and the Investigator will keep the IRB informed as to the progress of the study. The Investigator will obtain assurance of IRB compliance with regulations.

Any documents that the IRB may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB. The IRBs written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. This approval must refer to the study by exact protocol title and number and should identify the documents reviewed and the date of review.

Protocol and/or informed consent modifications or changes may not be initiated without prior written IRB approval except when necessary to eliminate immediate hazards to the patients or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB and written verification that the modification was submitted and subsequently approved should be obtained.

The IRB must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

20.3 Informed Consent Form

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, US Code of Federal Regulations for Protection of Human Subjects (21 CFR 50.25[a,b], CFR 50.27, and CFR Part 56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA, if applicable), and local regulations.

The Investigator will prepare the informed consent form, HIPAA authorization prior to submission to the IRB. The consent form generated by the Investigator must be acceptable to the Sponsor and be approved by the IRB. The written consent document will embody the elements of informed consent as described in the International Conference on Harmonisation and will also comply with

local regulations. The Investigators will send an IRB-approved copy of the Informed Consent Form to the Sponsor (or designee) for the study file.

A properly executed, written, informed consent will be obtained from each subject prior to entering the subject into the trial. Information should be given in both oral and written form and subjects (or their legal representatives or legal guardians) must be given ample opportunity to inquire about details of the study. If appropriate and required by the local IRB, assent from the subject will also be obtained. If a subject is unable to sign the informed consent form (ICF) and the HIPAA authorization, a legal representative may sign for the subject. A copy of the signed consent form (and assent) will be given to the subject or legal representative or legal guardian(s) of the subject and the original will be maintained with the subject's records.

20.4 Publications

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the study Sponsor and participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

20.5 Investigator Responsibilities

By signing the Agreement of Investigator form, the Investigator agrees to:

1. Conduct the study in accordance with the protocol and only make changes after notifying the Sponsor (or designee), except when to protect the safety, rights or welfare of subjects.
2. Personally conduct or supervise the study (or investigation).
3. Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.
4. Report to the Sponsor or designee any AEs that occur in the course of the study, in accordance with §21 CFR 312.64.
5. Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.
6. Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection with the Sponsor (or designee).
7. Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the clinical study.
8. Promptly report to the IRB and the Sponsor (or designee) all changes in the research activity and all unanticipated problems involving risks to subjects or others (to include amendments and IND safety reports).
9. Seek IRB approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/subjects.
10. Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

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